Science in IBB PAS

Nauka w IBB PAN 2024

70 YEARS

INSTITUTE
OF BIOCHEMISTRY
AND BIOPHYSICS
POLISH ACADEMY
OF SCIENCES

The building at 36 Rakowiecka Street in Warsaw, the headquarters of the Institute from 1963 to 1993
Gmach przy ul. Rakowieckiej 36 w Warszawie, siedziba Instytutu w latach 1963–1993

DIRECTORS of IBB PAS

Dyrektorzy IBB PAN

1954–1966

Prof. dr hab.

Józef **Heller**

1967–1972

Prof. dr hab.

Wacław **Gajewski**

1972–1981

Prof. dr hab.

Lech **Wierzchowski**

1981–1984

Prof. dr hab.

Andrzej **Paszewski**

1984–1987

Prof. dr hab.

Jerzy **Buchowicz**

INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

The complex at 5A Pawińskiego Street in Warsaw, the current headquarters of the Institute
Kompleks przy ul. Pawińskiego 5A w Warszawie, obecna siedziba Instytutu

1987–1990

Prof. dr hab.

Kazimierz **Kleczkowski**

1990–2007

Prof. dr hab.

Włodzimierz **Zagórski-Ostoja**

2007–2019

Prof. dr hab.

Piotr **Zielenkiewicz**

2019–present

Prof. dr hab.

Jarosław **Poznański**

"...that it would be better to stretch activity in many directions. I was thinking about the future on staff education in many areas of biochemistry."

„...że lepiej będzie rozciągnąć działalność na wiele kierunków. Myślałem o przyszłości, o wykształceniu kadr w wielu dziedzinach biochemii."

prof. Józef Heller

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INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

Warsaw, 2024

Pawińskiego 5a, 02-106 Warszawa, Polska
e-mail: secretariate@ibb.waw.pl
www.ibb.waw.pl

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Introduction

The Institute of Biochemistry and Biophysics of the Polish Academy of Sciences is more than just a scientific institute. This is an institution that has been integrating and coordinating the activities of hundreds of people for decades. For many employees, work at the Institute has been and is a source, not only of scientific inspiration, but also contributes to their life choices. On the other hand, IBB PAS is an institution whose shape, achievements and position in the world of science result from the actions of each employee and each doctoral student that has worked there.

Managing an Institute with a 70-year-old tradition is not easy and must take into account the past, present and future prospects for further development. The vision of the Institute's development must take into account the dynamic balance of several elements: maintaining a high quality of scientific research, obtaining funds for research, promoting research results and continuing and expanding the Institute's involvement in numerous national and international collaborations. The scientific position of the Institute has been shaped for decades, resulting in the highest category (A+) in the ranking of Polish scientific institutions.

The aim of this publication is to present the current scientific, innovative and human resources potential of the Institute in the form of descriptions of the achievements of individual organizational units. We also include a brief outline of the Institute's history in the form of a condensed calendar and a number of individual stories of employees, those who have already retired and those who have recently started working at IBB PAS. We sincerely thank them for sharing their own experiences.

We hope that the way we have chosen to present the Institute is interesting, inspiring and encouraging to contact and cooperate with us in all possible forms and on all possible levels.

Because IBB is a magical place....

Editors

INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

Wstęp

Instytut Biochemii i Biofizyki PAN to więcej niż instytut naukowy. To instytucja od dziesiątek lat integrująca i koordynująca działania setek osób. Dla wielu pracowników praca w Instytucie była i jest źródłem nie tylko inspiracji naukowych, ale przyczynia się także do dokonywania przez nich wyborów życiowych. Z drugiej strony, IBB PAN jest instytucją, której kształt, osiągnięcia oraz pozycja w świecie nauki wynikają z działania każdego pracownika oraz każdego doktoranta.

Zarządzanie Instytutem o 70-letniej tradycji nie jest proste i musi uwzględniać przeszłość, teraźniejszość oraz perspektywę dalszego rozwoju. Wizja rozwoju Instytutu musi uwzględniać dynamiczną równowagę kilku elementów: utrzymanie wysokiej jakości badań naukowych, zdobywanie funduszy na badania, promowanie wyników badań oraz zaangażowanie Instytutu w liczne współprace krajowe i międzynarodowe. Pozycja naukowa Instytutu kształtowana była przez dziesiątki lat, czego skutkiem jest najwyższa kategoria (A+) w rankingu polskich instytucji naukowych.

Celem niniejszego wydawnictwa jest przedstawienie aktualnego potencjału naukowego, innowacyjnego oraz kadrowego Instytutu w formie opisów dokonań poszczególnych jednostek organizacyjnych. Zamieszczamy także krótki zarys historii Instytutu w formie skondensowanego kalendarium oraz szereg indywidualnych historii pracowników, tych, którzy są już na emeryturze oraz tych którzy niedawno rozpoczęli pracę w IBB PAN. Serdecznie im dziękujemy za podzielenie się ich własnymi doświadczeniami.

Mamy nadzieję, że wybrany przez nas sposób prezentacji Instytutu jest interesujący, inspirujący i zachęcający do kontaktów i współpracy z nami we wszystkich możliwych formach i na wszystkich możliwych płaszczyznach.

Bo IBB to miejsce magiczne....

Edytorzy

INSTYTUT BIOCHEMII I BIOFIZYKI
POLSKIEJ AKADEMII NAUK

70 years of IBB PAS History in a Nutshell

1954

Resolution of the Scientific Secretariat of the Presidium of the Polish Academy of Sciences (Resolution No. 26/54 of February 9, 1954) on the establishment, as of May 1, 1954, of the Department of Biochemistry of the Polish Academy of Sciences as an auxiliary scientific and research unit. The Department was located on the premises of three institutions: the National Institute of Hygiene in Warsaw, the Medical University of Warsaw and the Warsaw University of Life Sciences. Five departments were established: Department of Evolutionary Biochemistry (head: Dr. Irena Mochnacka), Department of Biophysics (head: Dr. Dawid Shugar), Department of Cytochemistry (head: Dr. Aleksander Szenberg), Department of Immunochemistry (head: Andrzej Koziński, MD), Department Plant Biochemistry (head: Prof. Ignacy Reifer). The management of the Department of Biochemistry was taken over by the corresponding member of PAS prof. Dr. Józef Heller.

1956

Resolution of the Presidium of the Polish Academy of Sciences (Resolution No. 10/56 of January 10, 1956) on the establishment of the Institute of Biochemistry and Biophysics as an independent scientific and research facility of the Polish Academy of Sciences.

1957

Approval of the resolution of the Presidium of the Polish Academy of Sciences of January 10, 1956 by Resolution No. 85/57 of the Council of Ministers of the Polish People's Republic on March 14, 1957.

1960

Establishment of the structure of the Institute, which includes the departments of Evolutionary Biochemistry, Plant Biochemistry, Biophysics, and Microbial Biochemistry, located in Warsaw, and the Pathological Biochemistry laboratory, established in 1959 and located in Gdańsk. Research work at IBB concerns, among other things: protein synthesis, radiochemistry and enzymology of nucleic acids and their derivatives, biochemistry of cellular respiration, insect metabolism, plant nitrogen metabolism and kidney biochemistry.

1963

Moving of IBB PAS to the building of the Fermentation Institute at Rakowiecka St. 36 in Warsaw.

Incorporation of the Department of Genetics into the Institute and separation of the Department of Protein Biosynthesis from the Department of Comparative Biochemistry (previously the Department of Evolutionary Biochemistry). In later years, further organizational units were established.

70 lat Historii IBB PAN w Pigułce

1954

Uchwała Sekretariatu Naukowego Prezydium Polskiej Akademii Nauk (Uchwała Nr 26/54 z dn. 9 lutego 1954r.) w sprawie utworzenia z dniem 1 maja 1954r. Zakładu Biochemii PAN jako pomocniczej jednostki naukowo-badawczej. Zakład mieścił się na terenie trzech instytucji: Państwowego Zakładu Higieny w Warszawie, Akademii Medycznej w Warszawie oraz Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie. Utworzono pięć pracowni (org. oddziałów): Oddział Biochemii Ewolucyjnej (kierownik: doc. dr Irena Mochnacka), Oddział Biofizyki (kierownik: dr Dawid Shugar), Oddział Cytochemii {kierownik: dr Aleksander Szenberg), Oddział Immunochemii (kierownik: lek. Andrzej Koziński), Oddział Biochemii Roślin (kierownik: prof. Ignacy Reifer). Kierownictwo Zakładu Biochemii objął członek koresp. PAN prof. dr Józef Heller.

1956

Uchwała Prezydium Polskiej Akademii Nauk (Uchwała Nr 10/56 z dn. 10 stycznia 1956 r.) w sprawie powołania Instytutu Biochemii i Biofizyki (IBB) jako samodzielnej placówki naukowo-badawczej Polskiej Akademii Nauk.

1957

Zatwierdzenie uchwały Prezydium Polskiej Akademii Nauk z 10 stycznia 1956 r. Uchwałą Nr 85/57 Rady Ministrów Polskiej Rzeczpospolitej Ludowej w dn. 14 marca 1957.

1960

Ukonstytuowanie się struktury organizacyjnej Instytutu, która obejmuje zakłady: Biochemii Ewolucyjnej, Biochemii Roślin, Biofizyki, Biochemii Drobnoustrojów mieszczące się w Warszawie oraz pracownię Biochemii Patologicznej, powstałą w 1959 r. i mieszczącą się w Gdańsku. Prace badawcze w IBB PAN dotyczą m.in.: syntezy białek, radiochemii i enzymologii kwasów nukleinowych i ich pochodnych, biochemii oddychania komórkowego, metabolizmu owadów, metabolizmu azotowego roślin i biochemii nerki.

1963

Przeprowadzka IBB PAN do budynku Instytut Fermentacyjnego przy ul. Rakowieckiej 36 w Warszawie.

Włączenie do IBB PAN Zakładu Genetyki i wydzielenie Zakładu Biosyntezy Białka z Zakładu Biochemii Porównawczej (poprzednio Zakład Biochemii Ewolucyjnej). W późniejszych latach powstawały kolejne jednostki organizacyjne.

1979 Obtaining a building permit for the IBB PAS premises in Ochota district, at Pawińskiego St. 5A in Warsaw.

1987 Establishment of an Advisory Group at the Scientific Council of the IBB PAS, composed of scientists from foreign institutions and former IBB PAS employees working scientifically abroad.

1991 Opening of the Oligonucleotide Sequencing and Synthesis Laboratory of IBB PAS. The laboratory was intended to provide services to the Institute's employees. This decision of the then IBB PAS Director, Prof. Włodzimierz Zagórski enabled the Institute's subsequent participation in sequencing of the yeast genome, the Paramecium genome and joining in the global potato genome sequencing consortium.

1992 Moving to the Institute's own premises. Establishing close cooperation (including providing access to the IBB PAS space) between IBB PAS and the Faculty of Biology of the University of Warsaw (1992–1994).

Initiating the European Scholarship Fund, which covered a total of 114 researchers (120 person months of scholarships) from Central and Eastern Europe, mainly from Belarus, Ukraine and Russia.

1993 Establishment of the Polish-French Center for Plant Biotechnology with its headquarters at IBB PAS, which was joined by 20 Polish and 33 French scientific institutions.

1994 Establishment of the IBB PAN branch at the University of Gdańsk on the initiative of Prof. Karol Taylor. The laboratory operated until April 2023.

1996 The Institute establishes a Doctoral Study – The School of Molecular Biology.

1997 The first Science Festival in Poland, organized in Warsaw on the initiative of Prof. David Shugar. Representatives of IBB PAS actively participate in its organization. In the subsequent years, the Science Festival is becoming a nationwide annual event in which the IBB PAS employees actively participate.

1998 The first international conference "Protein kinase inhibitors". IBB PAS hosted 10 conferences in this series in a two-year cycle.

IBB PAS wins the first competition for the European Union Center of Excellence for research institutions from countries newly admitted to the EU.

1979 Uzyskanie pozwolenia na budowę siedziby IBB PAN w dzielnicy Ochota, przy ul. Pawińskiego 5A w Warszawie.

1987 Utworzenie Grupy Doradczej przy Radzie Naukowej IBB PAN, w której skład weszli naukowcy z instytucji zagranicznych oraz dawni pracownicy IBB pracujący naukowo za granicą.

1991 Otwarcie Pracowni Sekwencjonowania i Syntezy Oligonukleotydów IBB PAN. Pracownia z założenia miała prowadzić działalność usługową dla pracowników Instytutu. Ta decyzja ówczesnego Dyrektora, Prof. Włodzimierza Zagórskiego umożliwiła późniejszy udział IBB PAN w sekwencjonowaniu genomu drożdżowego, genomu pantofelka oraz w ogólnoświatowym konsorcjum sekwencjonowania genomu ziemniaka.

1992 Przeprowadzka do własnej siedziby Instytutu. Nawiązanie ścisłej współpracy (także lokalowej) pomiędzy IBB PAN i Wydziałem Biologii Uniwersytetu Warszawskiego (1992–1994).

Zainicjowanie Europejskiego Funduszu Stypendialnego, który objął łącznie 114 pracowników naukowych (120 osobomiesięcy stypendiów) z Europy Środkowo-Wschodniej, głównie Białorusi, Ukrainy i Rosji.

1993 Powołanie Polsko-Francuskiego Centrum Biotechnologii Roślin z centralą w Instytucie, do którego przystąpiło 20 instytucji naukowych polskich i 33 francuskie.

1994 Powstanie filii IBB PAN na Uniwersytecie Gdańskim z inicjatywy Prof. Karola Taylora. Pracownia funkcjonowała do kwietnia 2023 r.

1996 W IBB PAN powstaje Studium Doktoranckie – Szkoła Biologii Molekularnej.

1997 Pierwszy w Polsce Festiwal Nauki - zorganizowany w Warszawie z inicjatywy Prof. Davida Shugara. W jego tworzeniu aktywny udział biorą przedstawiciele IBB. W kolejnych latach Festiwal Nauki staje się ogólnopolską coroczną imprezą, w której aktynie uczestniczą pracownicy IBB PAN.

1998 Pierwsza międzynarodowa konferencja z serii „Inhibitory kinaz białkowych". W IBB PAN odbyło się 10 konferencji z tej serii w cyklu dwu-letnim.

IBB wygrywa pierwszy konkurs na Centrum Doskonałości Unii Europejskiej dla placówek badawczych z krajów nowo przyjętych do UE.

2002 IBB PAS becomes the Marie Curie Training Center - Education and Research in Molecular Biology (under EU FP5).

2007 Establishment of the Ochota Biocentrum Consortium, which included 6 research institutes located on the Ochota Campus, including IBB PAS.

2008 Establishment of The Preclinical Research and Technology Center Consortium (CePT) bringing together 10 institutions (universities and research institutes), including IBB PAS.

2010 Opening at IBB PAS of a modern-equipped Phytotron and Greenhouse Complex.

2012 As of January 1, 2012, the Department of Antarctic Biology, operating in the years 1992-2011 as an independent scientific unit of the Polish Academy of Sciences, was incorporated into the structure of the IBB PAS (Resolution No. 53/2011 of the Presidium of the Polish Academy of Sciences of November 29, 2011).

2018 The Institute co-organizes and hosts for the first time a conference on the occasion of Rare Disease Day. The conferences have since been held annually at IBB PAS on the last day of February as part of the celebration of World Rare Disease Day.

2019 The Doctoral School of Molecular Biology and Biological Chemistry is established at IBB PAS. It replaces the Doctoral Study – The School of Molecular Biology.

2021 Limiting the hierarchical structure of the scientific division at IBB PAS by creating separate research laboratories and establishing a number of new laboratories headed by young researchers.

2024 The scientific structure of the Institute includes 38 research laboratories, 2 departments, and 6 service and research laboratories (facilities). IBB PAS has approximately 250 employees, including over 180 in the scientific division. At IBB PAS, over 110 PhD students are educated.

2002 IBB PAN staje się Centrum Treningowym Marie Curie - Edukacja i Badania w Biologii Molekularnej (w ramach 5PR UE).

2007 Utworzenie Konsorcjum Biocentrum Ochota, w skład którego weszło 6 instytutów naukowych zlokalizowanych na Kampusie Ochota, w tym IBB PAN.

2008 Utworzenie Konsorcjum Centrum Badań Przedklinicznych i Technologii (CePT) zrzeszającego 10 jednostek (uczelni i instytutów naukowych, w tym IBB PAN).

2010 Oddanie do użytkowania w Instytucie nowocześnie wyposażonego Kompleksu Fitotronowo-Szklarniowego.

2012 Włączenie od 1 stycznia 2012 r. w strukturę IBB PAN Zakładu Biologii Antarktyki, funkcjonującego w latach 1992-2011 jako samodzielna jednostka naukowa Polskiej Akademii Nauk (Uchwała Nr 53/2011 Prezydium PAN z dnia 29 listopada 2011 r.)

2018 Instytut współorganizuje i gości po raz pierwszy konferencję z okazji Dnia Chorób Rzadkich. Konferencje odbywają się odtąd corocznie w IBB w ostatnim dniu lutego jako część obchodów Światowego Dnia Chorób Rzadkich.

2019 W IBB PAN powołana zostaje Szkoła Doktorska Biologii Molekularnej i Chemii Biologicznej, która zastępuje Studium Doktoranckie - Szkołę Biologii Molekularnej.

2021 Ograniczenie struktury hierarchicznej działu naukowego w IBB PAN poprzez wydzielenie odrębnych pracowni naukowych oraz powołanie szeregu nowych pracowni kierowanych przez młodych naukowców.

2024 Struktura naukowa Instytutu obejmuje 38 pracowni naukowych, 2 zakłady, 6 pracowni usługowo-naukowych. W IBB PAN zatrudnionych jest około 250 pracowników, w tym ponad 180 w pionie naukowym. W IBB kształci się ponad 110 doktorantów.

Past and Future of IBB PAS

Past of IBB PAS from individual perspectives

We are deeply grateful to the senior Professors who have long been part of the Institute for sharing their personal stories. Each has significantly contributed to the Institute's development and success, through scientific research, leadership, outreach, and collaborations. Their collective dedication has truly shaped the Institute into what it is today. Special thanks to Andrzej Paszewski, whose text highlights the pivotal role of the Institute's director during martial law.

Jacek Karol BARDOWSKI

Personal memories

I was lucky and happy when, in September 1974, I was accepted by prof. Tomasz Dobrzański, our chief and research and didactic mentor in the Department of Pharmaceutical Microbiology at the Medical Academy (now Warsaw Medical University) in Warsaw, and was incorporated into the group headed by dr Witold Kozak. It was he from whom I learned scientific and practical microbiology, from medical and pharmaceutical to biotechnological and applied. At that time, I also met dr. Piotr Cegłowski, who was the "right hand" of prof. W. T. Dobrzański.

My first contact with the Institute of Biochemistry and Biophysics of PAS was in the '70s of the 20th century. At that time, the Department of Pharmaceutical Microbiology of the Medical Academy, where I had already worked, had developed research connections with scientists from IBB PAS, in respect to scientific collaboration, evaluation of research (KNiT grants!) and training courses. I was impressed by the extremely high level of scientific knowledge and depth of experimental research analysis demonstrated by scientists from the Institute. Anytime and anywhere they assisted us with their scientific experience. I had never dreamed that I would become a member of the IBB scientific team, one day. But it happened!

After my return from a 6,5-year post-doc at INRA in Jouy-en-Josas in France, in the Laboratory of Microbial Genetics (headed by prof. S.D. Ehrlich), I was employed by prof. Włodzimierz Zagórski-Ostoja (the highest gratitude to dr Piotr Cegłowski) at IBB PAS starting September 1994 and became a member of his team at the Department of Microbial Biochemistry, headed by prof. Danuta Hulanicka. It was not 'easy-peasy'. Firstly, prof. Zagórski came on a visit to INRA in Jouy-en-Josas, where he met prof. S.D. Ehrlich, and they had a scientific appointment, in which I also participated. Next, I went to Poland, where I was 'tested', or rather, interviewed by a committee con-

Piotr Cegłowski and Jacek Bardowski, FP5 Grant Meeting, Torremolinos, Malaga, Spain, 2002

sisting of prof. W. Zagórski-Ostoja, prof. D. Hulanicka (Head of the Department of Microbial Biochemistry), dr M. Hryniewicz (Group Chief) and dr. Piotr Cegłowski (Group Chief). Finally, I passed this evaluation and got a research position at IBB PAS in the Team headed by dr P. Cegłowski. Uffffff!

In fact, my recruitment was part of a strategic, long-term research vision of our Director, prof. W. Zagórski (and the team of Board of Directors), who undertook an effort to complement scientific staff with highly trained scientists with new research visions and having worked abroad in '90s of the 20th century. I was one of several of 'lucky winners' of this recruitment strategy.

The management of IBB PAS accepted my proposal to develop research on lactic acid bacteria (LAB) under the condition that I would successfully apply for research grants from the respective authority institutions. This strategy of the management, to give formal permission for innovative, complex, systemic and interdisciplinary research activities of teams, if kept within the mainstream of the scientific interest of IBB, has been sustained by the following Boards of Directors.

Thus, feeling free in my research choices, I (we) successfully applied for research grants, individually and in collaboration with Polish (e.g. Warsaw University, University of Warmia and Mazury in Olsztyn, Warsaw University of Life Sciences – SGGW, Warsaw Medical University, Lodz University of Technology, University of Life Sciences in Lublin, Biolacta Poland, Piątnica Regional Dairy, JHJ sp. z o.o.-Nowa Wieś) and foreign institutions (e.g. Groningen University from The Netherlands, INRA France in Jouy-en-

-Josas, IPLA-CSIC in Villaviciosa in Spain) – both scientific and industrial partners, including those within EU FP5 and FP7. As a result, a group devoted to research on LAB had been established, expanded (over 30 researchers during the 'golden' period) and scientifically activated at IBB. We were honoured with several national and international awards, including those from the respective Polish ministries and universities as well as international bodies (e.g. Palmes Academiques de Premier Ministre de France pour prof. Jacek Bardowski). I feel exceptionally grateful to three colleagues of mine – Tamara Aleksandrzak-Piekarczyk, Magdalena Kowalczyk and Agnieszka Szczepankowska – who accepted my invitation for them to work with me during their university studies and who next developed their own scientific careers, becoming Associate Professors before my retirement.

In general, we were interested in deciphering gene/genome structure and function with respect to microbial cell biology, its host physiology, interactions within complex natural microbial populations and adaptation to their biological environments, and biotechnological applications. We deposited several patents, of which some resulted in incomes to IBB PAS. Several years ago, we were developing medical aspects of LABs' biological activities, including for human and animal health and wealth being. With great success! Scientific and applied.

Finally, I direct my gratitude and thanks to all of my supervisors and mentors, who introduced me to and aroused my curiosity in science and my thirst for knowledge, and mentored me during my scientific life, as well as my collaborators, especially those who I didn't mention here, solely due to lack of text space.

Magdalena FIKUS

My IBB

I got my degree at the University of Warsaw's Faculty of Biology in 1958. My longest and deepest working relationship – dating back to 1960 – was with Professor David Shugar. It was not until 1976 that I started working with IBB.

I worked under David Shugar at (in this order) the National Institute of Hygiene, the Institute of Oncology and the University of Warsaw (PhD, habilitation). Working in close collaboration with, and under the supervision of, Kazimierz Lech Wierzchowski (IBB), I investigated the impact of ultraviolet radiation on nucleic acid derivatives (cytosine, uracil). In the 1960s, Shugar received a strain of bacteria from France, along with a formula with which to prepare from them an enzyme synthesising ribo-polynucleotides. At the University of Warsaw's Institute of Oncology and Department of Biophysics, we investigated the characteristics of the ribo--polynucleotides we synthesised and of their constituent nucleic analogues – fluorouracil, xanthine and isoguanine. In 1966, David Shugar was appointed Head of the University of Warsaw's Department of Biophysics, Poland's first biophysics unit. The Department hired me. When I started, it was based in an empty building space on Banacha Street. Together with Ewa Sztumpf-Kulikowska, we put together a biochemistry teaching lab. We had to furnish the rooms, string together equipment and come up with a teaching programme. I remember that biochemistry was looked down upon by students from the Faculty of Physics (physics being considered by them the greatest of sciences).

In 1968, I went to the US for a year--long fellowship at Stanford University. After coming back to Poland, I continued in my teaching and research positions at the Department. Around that time, I had my daughter Marta, who would grow up to become a doctoral student at IBB. After passing my habilitation test before the Scientific Council of IBB PAS, I was hired at IBB.

At the Council session following the test, Professor Irena Chmielewska (University of Warsaw) is said to have remarked that "this person clearly needs to work in a teaching role as well". It was not until around 1985 that I came to the same conclusion.

However, the Institute's Director, prof. Wierzchowski, made my teaching position conditional on one thing: I had to set up a genetic engineering lab. This marked another stage in my life where I ventured into a professional field that was completely new to me. I was assigned with the supervision of two doctoral students, Bożenna Rempoła and Jola Kulik. The Institute gave me a small room and hired Teresa Bąk – a marvellously experienced technician – to support me. It was at that time that Professor Wacław Gajewski, along with a team of thirty-odd well-educated microbiologists and geneticists, embarked on his "genetic engineering" research at IBB's and the University of Warsaw's Institutes of Genetics. I stood no chance.

Gradually, the team expanded to include Elżbieta Grzesiuk, a PhD graduate from Gdańsk, Elżbieta Stępień, Hanna Kozłowska, Piotr Naimski (a chemist) and Piotr Pawłowski (a physicist). I am very grateful to Andrzej Bierzyński (IBB) and Jarek Poznański (IBB), with whom I established a strong collaborative partnership and found an affinity of thinking. In 1980, Jerzy Zieliński – a lecturer with the Institute of Electrical Engineering in Międzylesie – contacted me to suggest I search for a research topic related to the impact of various electric fields on living objects.

We learned how to obtain biological materials (enzymes, plasmids, phages) required for genetic manipulation. We investigated the first stage of the interactive impact of two restriction enzymes on restriction sequences in various DNA (Eco RI and HapII, pBR322 and phage PM2; P. Naimski's doctoral dissertation). I approached the Institute of Electrical Engineering with a proposal for research into the impact of variable and impulsive fields on spherical cells, *N. crassa*. After several years of research, we were able to identify a number of such impacts and came up with a theoretical model for these changes. We termed it bioelectrology (P. Pawłowski's doctoral dissertation on the design of an original variable field generator, a physical model of field-cell interaction).

Bożenna Rempoła and Jola Kulik defended their doctoral dissertations, on the mechanisms of sulphur metabolism regulation in yeast and bacteria and the cloning of the human epidermal growth factor gene, respectively. The former was a contribution to the extensive research conducted by prof. Andrzej Paszewski's and Professor Danuta Hulanicka's teams in their fellow IBB institutes. I should also mention here the invaluable consultations provided by prof. Michał Bagdasarian, prof. A. Putrament and dr. Jan Cybis.

After establishing contacts with prof. Anna Inglot from the Institute of Immunology and Experimental Therapy in Wrocław, Hania Kozłowska and I made attempts at cloning the human interferon gene. Gene cloning was beyond our capabilities, but we

were able to investigate interactions between interferon inhibitors synthesised in Wrocław and polynucleotides and DNA.

The writing of this text has allowed me to take another look at my research journey in hindsight. Evidently, I proved effective at dealing with staff and equipment shortages thanks to my collaborative partnerships with multiple research establishments, so when Professor Shugar – together with my former Department colleagues Maciek Geller and Bogdan Lesyng – approached me in 1995 with the unprecedented task of devising a concept for and organising Poland's first Science Festival in Warsaw, it seemed I could be the right person for the job. Festivals like this were only beginning to spring up in Europe. Shugar saw one of them in Edinburgh and thought it a good idea to put together a couple of such lectures and demonstrations here in Poland.

IBB's then Director Włodek Zagórski backed me up in this and assigned me to the project, relieving me from my individual research obligations. Maciek Geller (University of Warsaw) became the Science Festival's Director and I was his Deputy. We complemented each other so well, bringing science closer to the public in Poland and Europe in our own unique ways. David Shugar would continue working on the popularisation of science until his last days. I was also involved in the establishment of the Copernicus Science Centre (Poland's first Science Centre). IBB was always the biggest supporter of our endeavours, with across-the-board backing from the management, the institutes and all our colleagues. I retired after the 14th edition of the Festival, which is still running strong after another successful edition (26th) in 2023. The Copernicus Science Centre is visited by about a million people every year.

I believe that the most important and valuable thing I did in my life was to advance different types of, and approaches to, the popularisation of science based on my own understanding of what science is and who needs it. This would not be possible without the cooperation and support of numerous and diverse research teams in Poland and beyond. I want to thank them from the bottom of my heart.

Witold (Witek) FILIPOWICZ

Traversing the RNA World: early days in Warsaw

After studying medicine at Łódź Medical University, in 1969 I moved to Warsaw to work toward a PhD at the Institute of Biochemistry and Biophysics. I joined its Department of Protein Synthesis, headed by Przemyslaw Szafranski. In the late 1960s, RNA bacteriophages represented a simple system to define the basic mechanisms behind the regulation of gene expression. I began, together with other members of the lab, to study the *Escherichia coli* RNA phage f2. My objective was to assess the usefulness of methoxyamine to modify the secondary structure of f2 RNA and so to study the possible involvement of RNA higher structure in the initiation of protein synthesis. The choice of methoxyamine as an RNA-modifying agent was based on its specificity for cytosine (C) residues; hence, AUG initiation codons were not affected. Research on RNA phages was then a very hot area, but our competition with a couple of leading American and British labs was a rather exasperating experience. We were very ambitious and planned to beat them all but it did not always turn out that way. Still, we managed to publish a couple of reasonable quality papers and present our data at some international meetings.

Scientific frustrations during my postgraduate studies were more than compensated by a very important "up". In 1970, I met and later married Aleksandra (Ola) Wodnar, who was a biochemist working in the same laboratory at that time. We worked together for many years (till 1984; much too long from her perspective) and published a dozen papers together. Ola eventually got her own lab and later became Professor of Experimental Hematology at the University of Basel, after our move to Switzerland.

Despite many difficulties such as shortages or poor quality of reagents, and delayed access to scientific journals (due to the censors going through them; it must have been torture for the censors to read every

1977- This team was researching the f2 phage at the IBB PAS Department of Protein Biosynthesis.
From left to right: Włodek Zagórski, Ania Szkopińska, Ola Wodnar-Filipowicz, Wisia Zagórska, Witek Filipowicz

issue of the *Journal of Biological Chemistry*!), I have very good memories of my PhD time. We worked very long hours, often at night, acting, for example, as fraction collectors replacing the not very reliable automatic ones. The PhD students entering IBB in 1969, including Jurek Barankiewicz, Jarek Kusmierek, Agnieszka Siwecka, and others, were part of a PhD School, going through a special program of lectures. We were also partying a lot, of course with the purpose of talking science. Szafranski's Department was full of interesting individuals. In addition to Ola, I shared a lab and sometimes a bench with Wlodek Zagorski, Wisia Passent (later Chroboczek), Wisia Zagorska (later Zimniak), or Piotr Chomczynski. Together with Andrzej Rabczenko and a few others, we were trying to animate scientific life at IBB by organizing brain-storming discussion group meetings in the evening. We also had close contacts with other groups studying different aspects of gene expression: Eugeniusz Gasior in Lublin, Mieczyslaw Chorazy in Gliwice, and Andrzej Legocki and Jerzy Pawelkiewicz in Poznan.

I defended my PhD thesis in 1973 and moved with my family in early 1974 to the US for postdoctoral studies in the laboratories of Severo Ochoa and Aaron Shatkin,

first at the New York University Medical School and then at the Roche Institute of Molecular Biology (RIMB) in Nutley. I was purifying translation initiation factors and investigating the role of the m7G cap, which had just been discovered in Aaron's lab. With two such famous mentors as Ochoa and Shatkin, my time in the US was very rewarding. Identification of the first protein activity specifically recognizing the m7G cap was certainly the most important finding of mine. The time in the US was also very fruitful for Ola, performing the experimental part of her PhD thesis on cholera toxin at the RIMB.

We arrived back in Poland in early 1976, full of ideas how to procced with our research. At this point, both myself and Wlodek Zagorski were encouraged by Prof. Przemyslaw Szafranski to establish our own independent groups in his department. This was an exceptional development in a still rather hierarchical Polish science organization. I was greatly indebted to Prof. Szafranski for his decision and for his continuing support.

We had decided to continue the study of eukaryotic translation. The start was stymied by the refusal of most of the *in vitro* extracts I used routinely in US to display any activity in Poland. This was due the impurity of the ordinary salts purchased from local suppliers; recrystallization of the salts solved the problem. We relied largely on self-prepared research tools. We purified RNase inhibitor from placentas obtained from obstetrics clinics and prepared reticulocyte lysates from hydrazine-injected rabbits. We carried out enzymatic decapping of mRNAs using nucleotide pyrophosphatase purified locally from potatoes, in collaboration with Halina Sierakowska's group. Our early work focused primarily on the requirement for the m7G cap in the translation and the mechanism of 5'-UTR scanning for the AUG codon by ribosomes. We used plant viral RNAs as mRNA models because at that time working with animal viruses or cells was too expensive.

Experimentation with the tobacco mosaic virus (TMV) RNA was particularly rewarding. Its long ~70-nt 5′-UTR is devoid of G residues between the cap and the AUG. Using RNase T1, we could isolate this fragment (referred to as Ω) in pure form and use it not only for translational studies but also as a substrate for RNA ligases which should convert the linear form of the decapped Ω to the circular RNA. To address some of these questions, we initiated collaborations with Anne-Lise Haenni at the Institute Jacques Monod in Paris and Hans Gross at the Max-Planck-Institute for Biochemistry in Martinsried. Hans had just reported the sequence of the potato spindle tuber viroid (PSTV) RNA, the first example of a molecularly characterized circular RNA. In 1979, Maria (Magda) Konarska, a PhD student in my lab, moved to Martinsried to take advantage of Hans's experience with RNA circles which initiated long-lasting cooperation with his lab.

The collaborative work with Hans's lab resulted in characterization of a novel type of RNA ligase, originally identified in wheat

germ extracts, which joins two RNA ends via an unorthodox 2′-phosphomonoester, 3′,5′-phosphodiester linkage. We were all very proud of this work. The two 1981/1982 papers by Konarska *et al.* in Nature and PNAS provided the first mechanistic insights into eukaryotic RNA ligation. The finding that ligation depended upon the 2′,3′-cyclic phosphate ends represented the first example of the "anabolic" function of the terminal cyclic phosphate. It wasn't easy to carry out experiments in the Warsaw of 1980/1981. The Solidarność movement had been at the peak of its activity, opening the first cracks in the communist system. But Hans's generosity, with repeated shipments of reagents to Warsaw, kept our science alive.

In October 1981, I left Warsaw for a sabbatical year in Aaron Shatkin's laboratory in the US, accompanied by Ola and our two children. Ola took up a post-doctoral position in Bernard Horecker's lab. Unfortunately, political events in Poland caught up with us almost immediately, with martial law being declared in Poland on December 13. Despite that, returning to Aaron's lab was like a welcome homecoming, with the lab again full of interesting people. I decided to investigate the mechanism of tRNA splicing in HeLa cells, particularly the ligation of the two tRNA halves that resulted from intron excision, and whether exon ligation followed the 2′-phosphomonoester, 3′,5′-phosphodiester-forming pathway as in plants. We quickly found that HeLa cell extracts contain activity distinct from that operating in plants, joining the RNA ends via an orthodox 3′,5′-phosphodiester, and that animal cells – in contrast to plants and yeast - use this ligation pathway during tRNA splicing. The requirement for 2′,3′-cyclic phosphate ends was common to both plant and animal pathways. Intriguingly, we discovered that cleavage by cyclizing endonucleases is not the only way to produce RNAs with 2′,3′-cyclic ends. They can also form in an ATP-dependent reaction by the RNA 3′-terminal phosphate cyclase, a novel enzyme that we identified in HeLa cells and *Xenopus* oocytes, and later also in *E. coli*. We continued to investigate this enzyme for nearly two decades, first in Warsaw and then in Basel.

In late 1982, I gave a talk at the Rockefeller University in New York, where I was asked by Prof. Ed Reich, working at this university, whether I would be interested in setting up a lab at the Friedrich Miescher Institute (FMI) in Basel, Switzerland. Reich was about to take over the directorship there. As I did not plan to leave Poland and my Warsaw lab permanently, I investigated the possibility of a part-time or temporary appointment in Basel, arguing that a commute between the two countries would have advantages for Polish science. Indeed, the Polish Academy of Sciences agreed and granted me, in writing, a leave of absence.

With this official permission in my hand, I returned to Warsaw in March 1983 to spend some time in my laboratory at IBB and to plan details of the upcoming Swiss-Polish adventure. Unfortunately, once in Warsaw, I was repeatedly denied a passport to travel to Basel, in fact to any symposium or seminar

In a good company (late 1970s). From left to right: Andrzej Legocki, Przemyslaw Szafranski, Eugeniusz Gasior, Mieczyslaw Chorazy, Witold Filipowicz, Anne-Lise Haenni.

abroad. Ola and the children arrived back in Warsaw in June. Over the next year, many people in Poland and abroad tried to help, but to no avail, and it became more worrying when I was even refused permission to travel to Moscow, where I was organizing a virology symposium as part of the June 1984 FEBS Congress. However, the Moscow incident turned out to be the key to resolving my year-and-a-half detention in Warsaw. Anybody interested in more detail about our final quite dramatic departure from Poland in July 1984, and in my scientific activity at the Friedrich Miescher Institute in Basel, can consult my "Reflections" article, which the *Journal of Biological Chemistry* invited me to write in 2017 (ref. 1).

After departure, I could not travel to Poland for the next seven years. I felt very bad about abandoning my students in Warsaw, but my worries dissipated somewhat since Magda Konarska departed for a postdoc at MIT with Phil Sharp and when Kazio (Tyc) Tycowski, another brilliant Warsaw student, left shortly afterward to join Joan Steitz's lab at Yale. My connection with IBB got revived in 1991, with frequent visits and seminars, association with its Scientific Advisory Board, and talks at different symposia, the last one commemorating Prof. David Shugar, a scientific role model for many of us working at IBB. I am very happy to be able to join IBB for the festivities associated with its 70th anniversary. I wish the Institute a lot of success in the future and look impatiently forward to the celebration of its 100th anniversary.

Filipowicz W (2017) Traversing the RNA World. J Biol Chem 292:8122- 8135.

Elżbieta GRZESIUK

New knowledge to the life history

A long time ago, in 1979, I moved from the Department of Microbiology of the University of Gdańsk to IBB due to personal reasons. Prof. Kazimierz Wierzchowski, director of the Institute and leader of the Department of Biophysics, hired me and I joined the lab of Magda Fikus within this department. At this time the 25th Anniversary of IBB took place and every IBB employee was honored with a very nice medal. I received one two, but prof. Wierzchowski was not happy with this because in his opinion, as a new member of the IBB community, I didn't deserve it.

Together with Bożenna Rempoła and Teresa Rak, I was taking part in research projects that were being carried out by the group of Magda Fikus. I also performed DNA/RNA hybridization (a method I had been using previously, at the University of Gdańsk) to recognize PM2 phage transcription. In the meantime I was offered a postdoc position in the group of Dana Carroll at the Department of Biochemistry, University of Utah, Salt Lake City, USA. I spent there two years (1985-1987) but in spite of my success there, there was no place for me at the IBB Biophysics Department after my return. The new IBB director, prof. Jerzy Buchowicz, saved me by offering me the option to join any IBB department I wish to work in. My choice was prof. Celina Janion's lab in the Department of Molecular Biology led by prof. David Shugar. In this department there were labs, led by: Celina Janion, Irena Pietrzykowska, Tadeusz Kuligowski, Zofia Zarębska, and Jarosław Kuśmierek. Prof. Janion was interested in alkylating agent-induced mutagenesis in *E. coli*. As a team, we were searching for the influence of polymerase V, the DnaQ subunit of DNA polymerase III, and base excision repair (BER), on the levels of MMS-induced mutagenesis. We published several papers, succeeded in securing NCN grants, and even won a third-degree PAS Award. Since 1996 I have been the leader of the group "Indu-

ced and adaptation mutations". Around the year 2000, prof. Janion found that the AlkB dioxygenase was a very interesting protein, involved in repairing alkylating damage to DNA/RNA. We prepared an "AlkB" proposal and got an NCN grant.

Apart from the project mentioned above, we also searched for an influence of the myoelectrical migration complex (MMC) on bacterial growth and on the adherence of several species of bacteria: pathogens and probiotics, to intestinal cells. This was being carried out in cooperation with prof. Grzegorz Pierzynowski from the Lund University (Sweden) and prof. Romuald Zabielski from SGGW, Warsaw.

Around 2003, dr. Joanna Krwawicz, a former PhD student of prof. Pietrzykowska, informed me about the possibility of obtaining a Polish-Norwegian grant. As a group leader, I prepared a project concerning the role of AlkB in prokaryotes and together with Joanna we recruited four groups of scientists from the Oslo University Hospital and three from IBB: led by myself (Joanna Krwawicz, Anna Sikora, Damian Mielecki, Michał Wrzesiński, Aleksandra Chojnacka, Jan Piwowarski), Barbara Tudek (quite big group with Elżbieta Speina), and Jarosław Kuśmierek (Agnieszka Maciejewska). We received the grant, which amounted to about a million €! During the next 5 years (2004-2009) we have published over 20 publications. In 2013 we again applied for a Polish-Norwegian Grant, but this time we concentrated on eukaryotic ALKBHs and their role in cancerous transformation. Prof. Jarosław Poznański (IBB) and prof. Romuald Zabielski (SGGW) were also part of the team now. Also this time we obtained the funding and the project was carried out from 2013 to 2017, and again, we reported it successfully.

In 2004, on the occasion of the 50th Anniversary of IBB, I was honored with a Silver Medal.

In the meantime, in cooperation with the Department of Hydrobiology of the Warsaw University and in the frames of an NCN grant, we were investigating the influence of alkylating agents that are contaminating the aquatic environment on the development of *Daphnia magna*. We added new knowledge to the life history as well as proteome and transcriptome analysis of *Daphnia magna* living in contaminated environments.

Summing up, I am the author of over 100 publications, 4 book chapters, and abstracts at international conferences. I have supervised 10 PhD dissertations and several dozens of MSc theses.

Grażyna JAGURA-BURDZY

From bacterial genetics to broad host plasmids

I graduated from Warsaw University's Faculty of Biology in 1976. My interests in bacterial genetics were ignited by prof. Wacław Gajewski, a superb scientist and an enthusiastic mentor. My research career was initiated in the same year at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw where I started working on the PhD degree under the supervision of prof. Danuta M. Hulanicka on the regulation of cysteine synthesis in *Enterobacteriaceae*.

After my dissertation in 1980, due to my supervisor having established a collaboration with an American colleague, I could join the research group of prof. Nicholas M. Kredich at Duke University Medical Center in Durham, North Carolina. I spent almost three years there continuing digging into the function of the regulatory CysB protein. It was the beginning of the use of restriction endonucleases, recombinant DNA techniques and DNA sequencing. Only a few restriction enzymes could be obtained in the limited quantities from friendly laboratories, where they were purified by PhD students or postdoctoral researchers. So I was very lucky to do this pioneering work at DUMC.

In 1989, when I started working with prof. Christopher M. Thomas at the University of Birmingham, United Kingdom, we had a freezer filled up with the hundreds of commercially available restriction enzymes. New cloning techniques, PCR, fluorescence microscopy, protein overproduction and purification methods, as well as collaboration with the biophysical laboratories facilitated our research on the bacterial plasmid genetics. Due to the regularly scheduled international Plasmid Biology conferences I had the privilege to get to know the famous founding fathers of Molecular Biology and Plasmidology, for example F. Crick, D.R. Helinski, W.T. Szybalski, R.P. Novick, S.J. Austin, B.E. Funnell, D.K. Chattoraj, and F. Hayes.

After getting back to Poland in 1997, I was offered a position in the IBB PAS by its visionary director, prof. Włodzimierz Zagórski-Ostoja. I was astonished by the change the IBB had undergone, becoming a modern, very well equipped and high quality research facility. I established my research group with the support of prof. Piotr Cegłowski, head of the Department of Microbial Biochemistry. I could pursue my love of plasmid biology, and in parallel to initiate a new project on genome segregation in pathogenic bacteria, financed for six years by Wellcome Trust Collaborative Initiative grants and later by NCN grants.

My fascination with the broad host range plasmids, the perfectly organized, tiny genetic entities with incredible abilities to adapt to the changing environment, has not disappeared over the years. The studies on the bacterial chromosome segregation with the large-scale analyses available at IBB, i.e. genome sequencing, transcriptomics and proteomics, led to a better understanding of this vital process. During these years I was lucky to supervise fifteen enthusiastic PhD students, the majority of whom were honoured with distinctions for their PhD theses. I am proud that several of them have continued the adventure with the science.

Andrzej JERZMANOWSKI

Unconventional approach to collaboration in science

When I began my studies at the Faculty of Biology at the University of Warsaw in 1964, the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (IBB PAS) had been in existence for just a decade. It was located in a building on Rakowiecka Street, a ten-minute walk across the Mokotowskie Field from the then headquarters of the University Department of Biochemistry on Banacha Street, where I received my Master's degree in 1969.

I established contacts with the IBB quite early, working as an assistant at the Institute of Biochemistry at the University of Warsaw. This was all the more easy and natural because Professor Wacław Gajewski, who worked at the University of Warsaw and with whom I completed a genetics course, also had a department at the IBB. Moreover, right next door in the same building on Banacha Street was the Department of Biophysics of the University, led by Professor Dawid Shugar, who, like Gajewski, also headed a department at the IBB. We quickly established fruitful cooperation with Professor Shugar's team.

After the historic political breakthrough at the end of the 1980s and my return from a scientific internship in the USA, I began working in 1992 at the Institute of Experimental Plant Biology at the University of Warsaw, where I organized the Laboratory of Plant Molecular Biology. This was also an important period in the history of the IBB, as the Institute was moving to a newly built modern headquarters on Pawińskiego Street. A lot was happening at that time. The then director of the IBB, Professor Włodzimierz Zagórski, was seeking new goals for the Institute, wanting to reformulate and modernize its research profile to adapt it to the opportunities opened up by the technological revolution in genomics and structural research. It was then that he proposed I collaborate in developing modern molecular biology and genomics of plants

along with researchers from the IBB, offering space in the Institute to create a joint research laboratory. This laboratory was established in cooperation with the IBB's Protein Biosynthesis Department in 1994 and operated successfully for the next 30 years. Its PhD students were recruited both from the Faculty of Biology at the University and from the IBB, forming a cohesive research team together.

Among the scientific fruits of this initiative, I primarily count the new original results concerning the structure and function of chromosomes in plants obtained through the application of large-scale analyses, including transcriptomics and proteomics, and the extensive use of bioinformatics. These achievements were the result of the intermingling of ideas and concepts, made possible by daily interactions with research teams at the IBB (one example was the fruitful cooperation with the Department of Bioinformatics led by Prof. Piotr Zielenkiewicz). These interactions also resulted in numerous joint grants for funding research and infrastructure projects, as well as the participation of IBB staff in teaching students and doctoral candidates at the Faculty of Biology of the University, especially in the area of computer applications.

This story, while an important part of my life, is just a small example of the positive and synergistic effects that can come from an unconventional approach to collaboration in science, including breaking down barriers created by belonging to formally separate organizational units of science. However, this requires boldness and a readiness to take risks associated with venturing beyond the beaten path. This is particularly important now, as the situation in science is changing very rapidly, and in biology, a completely new era of research is opening up based on systems utilizing artificial intelligence capabilities.

Andrzej PASZEWSKI

A small memoir of a director of IBB PAS during martial law

With the outbreak of martial law, our institute became one of those that drew special attention from the authorities.

On Monday, December 14, 1981, the second day of martial law, a delegation from the Second Division of the Polish Academy of Sciences (PAN) arrived at the institute. However, they could not provide a specific reason for their visit. I believe, considering the institute's political reputation, they expected a strike or some other form of protest. They didn't observe anything of the sort.

The next day, an inspector from the Supreme Audit Office (NIK) came to the institute with the task of conducting a thorough inspection of its operations. After two months of work, she prepared a report, which NIK sent to us.

This rapid NIK inspection was most likely a consequence of a speech by Wojciech Jaruzelski, in which he mentioned institutes where the ratio of trips to Western countries versus those to Eastern Bloc countries (RWPG) was 50:1. Of course, our institute belonged to such a category, and the NIK inspection was meant to confirm this.

However, the inspector, a doctor of chemistry, seemed unaware of the real purpose of her visit. She primarily checked finances, but I was confident in this area, knowing our chief accountant. Her report contained minor remarks, such as the too-easy access to the photocopier, but not a word about international exchanges. The reaction of our newly appointed personnel officer was amusing; after reading the report, he said, "This is a panegyric."

Nevertheless, NIK sent a revised final report, which addressed the issue of international trips. My response was straightforward: "We allocate 100% of the funds we receive from PAN for trips to RWPG countries. Our trips to the West are made by invitation, with the inviting party covering the costs.

We will continue this practice, as it benefits Polish science." And that was the end of the correspondence with NIK!

In February 1982, I traveled to the United Kingdom for a meeting of the Editorial Board of the *Journal of General Genetics*. The Academy particularly supported this trip, as it was meant to serve as a sign of the normalization of relations in Poland. I was also asked to contact our embassy, as was customary for official trips. My phone call to the embassy caused some commotion, and I was asked to visit them. It turned out that they had letters from British scientists protesting my alleged arrest, which had been reported in the press. They asked me to call these people, which I did, informing them that I was fine.

In documents detailing the activities of the PZPR (Polish United Workers' Party) cell at PAN and its cooperation with the Security Service (SB) during martial law, there was a proposal to replace the directors of IBB and the Institute of Physics. I don't know what happened at the Institute of Physics, but I was not replaced, probably because the party cell at our institute had fallen apart, and it was difficult to find a successor. They only sent us a new, party-affiliated administrative director, with whom cooperation went well.

Kazimierz Lech WIERZCHOWSKI

My path to scientific competence in molecular biology and my role in managing the Institute

I have been associated with the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in various ways for almost as many years. I presented the history of my scientific activities with my colleagues in detail in a book published on the occasion of the 50th anniversary of the Institute (IBB PAN 1954-2004). Here, I will limit myself to memories about my path to scientific competence in molecular biology and my role in managing the Institute.

In the spring of 1955, I moved from the Department of Inorganic Chemistry at the University of Warsaw, where I was an assistant to its head, prof. Wiktor Kemula, to the Laboratory of Biological Physics and Chemistry of Dr. David Shugar, which was a part of the Department of Biochemistry of the Polish Academy of Sciences, established in 1954. The laboratory was renamed the Department of Biophysics after the Department of Biochemistry was transformed into the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (1956).

I was prof. Kemula's first post-war master's student in the field of photochemistry and molecular spectroscopy, and David Shugar was looking for someone with experience in this field for his planned pioneering research on photochemical damage to nucleic acids. David previously dealt with protein photochemistry and tautomerism of diketopyrimidines in leading biological laboratories in France and Belgium. Nucleic acid photochemistry became our collective focus for almost 20 years, alongside rapid progress in understanding the structure and function of nucleic acids. David Shugar was the supervisor of my doctoral thesis (1960) and a scientific mentor until I became independent after obtaining my habilitation (1965).

I started working at the Department of Biochemistry during the period of great acceleration in the post-war development of methods and research leading to the de-

scription of the world of biology at the molecular level - proteins, nucleic acids and lipid membranes. The new tools included methods based on the use of chemical compounds labelled with stable and radioactive isotopes of elements. The use of nuclear energy for war and peaceful purposes forced research into the mechanisms and effects of damage to living organisms by ionizing radiation. In Poland, the organization of the course on "The use of isotopes in biological sciences" in the fall of 1955, on the initiative of David Shugar with help of the Institute of Nuclear Research, contributed significantly to the interest in this issue. As a physical chemist by education, I participated in the organization and implementation of the course and the editing of course materials in the form of a script (1956). Further development of radiobiological work in Poland was facilitated by the launch of a strong source of gamma radiation at the National Institute of Hygiene, the so-called "cobalt bomb" (60Co), on the initiative of David Shugar. At the beginning of 1957, I was delegated to the Ministry of Health in Moscow to agree with manufacturers in the USSR on the technical details of the "cobalt bomb" ordered there.

In 1957, I completed a 3-month scientific scholarship at the Carlsberg Laboratory (Copenhagen) in the laboratory of prof. Kaj Ulrich Lindenstrom-Berg to learn about the densimetric method he developed for measuring the relative content of hydrogen isotopes H and D in water, we wanted to use it in our studies on the photohydration of pyrimidines. My stay at Carlsberg Laboratory was extremely educational for me. In the 1950s, the Department of Chemistry became one of the most important centers for protein research, often visited by leading researchers in this field from around the world. Among visitors I met there were Harold Scheraga from Cornell University (USA) and Walter Kauzman from Princeton University (USA). Harold Scheraga studied dynamics of bovine pancreatic ribonuclese by the method of H/D exchange. Walter Kautzman, who introduced the concept of hydrophobic bonds in 1955, dealt with the theory of intramolecular interactions stabilizing protein structures, replacing the previously proposed concept of "hydrophobic bonds" with "hydrophobic interactions" of a thermodynamic nature. Reading related works in the field, listening to seminar presentations of the results of current research and accompanying discussions, introduced me to the previously little-known, fascinating world of protein research. I later used the physicochemical research and interpretation approaches I learned then in my own work on the stabilization of helical structures of nucleic acids by hydrogen bonds and hydrophobic interactions.

During my stay in Copenhagen, the Institute authorities entrusted me with the purchase of laboratory equipment. The amount of 10,000 US dollars was transferred to our embassy. I collected them in cash, and purchased equipment from a list received from David Shugar in the well-known Copenhagen company of Rassmussen. It included, among others: 3 spectrophotome-

ters from Unicam (UK), several high-speed centrifuges from Spinco (USA, the company had to obtain consent from the American authorities to re-export them to Poland), several pH meters and a Titrator-pH-stat, produced by the Danish company Radiometr in cooperation with specialists from the Carlsberg Laboratory and some smaller laboratory equipment (including the famous Carlsberg calibrated micropipettes, made by the glassmaker Mr. Pedersen from the Laboratory). This was the first major purchase of basic laboratory equipment for the Institute.

After returning to Poland, I launched the densitometric measurement method I had learned in Denmark in our laboratory and used it to study the mechanism and kinetics of the reversible photochemical reaction of the attachment of a water molecule to cytosine and uracil residues in nucleosides, RNA oligo- and polynucleotides. I used these results in my doctoral dissertation (1960).

One-year post-doctoral research stay (1960/1961) in the laboratory of prof. Paul Doty at the Faculty of Chemistry at Harvard University in Cambridge (USA) introduced me to the then mainstream research on confirming the validity of Crick and Watson's model of DNA structure (1953, Nobel Prize 1962) and learning about its functional properties. At the turn of the 1950s and 1960s, research by Julius Marmur, Joseph Eigner, Carl Schildkraut and Paul Doty on thermal dentauration and renaturation of DNA using biological (transformation of bacteria) and physicochemical methods (among others buoyant density in a CsCl gradient), showed the double-stranded structure of the DNA molecule, contributing significantly to confirming the credibility of the Crick-Watson model of the structure of the DNA molecule as a double-stranded helix. It was also shown that there is a linear relationship between the basic composition of DNA (GC/AT) and the thermodynamic stability of DNA (melting temperature Tm) and its buoyant density in a CsCl density gradient. The method of equilibrium sedimentation of macromolecules in a cesium chloride density gradient demonstrated the formation of hybrid double-stranded DNA molecules from single chains derived from the DNA of different but genetically similar organisms and the possibility of using this process to determine the degree of genetic homology of these organisms.

When I got there, prof. Doty suggested that I join the work of doctoral student Carl Schildkraut and Dr. Julius Marmur on the DNA hybridization of T-even (T-2,-4,-6) and T-odd bacteriophages (T-1,-3,-5,-7) of *Escherichia coli* bacteria in order to determine the degree of their homology in each of them groups, and comparisons with taxonomic data. Our research showed a varying degree of homology between phages of the T-even group and its complete lack between phages of the T-even and T-odd groups, confirming their adopted taxonomy and proving the usefulness of this method in taxonomic research. I presented my preliminary results in the spring of 1961 at a symposium on the latest research in nucleic acids, organized at the University of North Carolina in

Knoxville. Their full results were published a year later in Virology.

Prof. Doty gave his co-workers a lot of freedom in planning and implementing experiments. Weekly seminars with his active participation, often organized on Saturday evenings, were a permanent forum for meetings and presenting closed fragments of research and sharing information from reading current publications. The professor was then a scientific advisor to President J.F. Kennedy and spent a lot of time in Washington. He also gave lectures on the physicochemistry of biological macromolecules for PhD students of the Faculty of Chemistry, based on the latest research results; he engaged his co-workers to prepare sets of questions for colloquia in the form of tests. It was the first time I encountered this form of testing students' knowledge. At the Faculty of Chemistry, University of Warsaw, tests were always oral.

Prof. Doty tried to ensure that his non-US post-doctoral students could also become acquainted with scientific life in the US outside their home university. Therefore, he funded their participation not only in specialized scientific symposia, where they could present their research results, but also in national scientific meetings, and also facilitated visits to other laboratories. In my case, it was the symposium at the University of North Carolina, the annual meeting of the Federation of American Societies for Experimental Biology (FASEB), and a visit to the photophysical and photochemical laboratory of Dr. Antonio A. Lamola at the famous Bell Laboratories.

At the beginning of September, on the recommendation of prof. Doty, I unexpectedly became a scientific advisor to the USSR delegation to the meeting of the UN Scientific Committee on the Effects of Atomic Radiation in New York. It was chaired by prof. Anatol M. Kuzin, who knew Doty from meetings of the Pugwash Conference, an organization of the anti-nuclear movement. My role as an advisor consisted of participating in meetings of this Committee devoted to summarizing the current state of knowledge about the effects of radiation on nucleic acids, cells and organisms, and assisting in the editing of the final report.

After returning from Harvard (1962), continuing our earlier research with Shugar on the photochemistry of cytosine and 5-methylcytosine, I discovered a new photochemical ring-opening reaction of cytosine in its 2,6-methyl derivative leading to the formation of alpha-cyanodiimine. Work on this transformation and the characteristics of dark products formed the basis of my habilitation thesis (1965). I then received the position of assistant professor, became a member of the Institute's Scientific Council and started creation of my own research group in the Department of Biophysics. In 1971, I received the title of professor and in 1973, I took over the management of the Department of Biophysics after separating from it the Department of Molecular Biology under the management of David Shugar.

My participation in organizational and administrative work for the Institute was manifold. The Institute's laboratories, located in several locations at universities in Warsaw, only in the early 1960s received the administrative building of the departmental Institute of Fermentation Industry at 36 Rakowiecka Street as their common headquarters. I then became a delegate of the Scientific Council to coordinate construction and equipment works on its adaptation for laboratory purposes, and then, together with dr. Zofia Lassotowa, consolidating the relocation in 1963.

Due to the introduction in the early 1970s of the subject financing of scientific research based on 5-year core research programs, the Institute was entrusted with organizing the core problem entitled "Research on genetic information in microorganisms, plants and animals" (1971-1975) and coordination of work with the participation of several dozen scientific institutions. Its manager was prof. Wacław Gajewski, director of the Institute since 1967. I participated in the work on its establishment and administration as deputy director for scientific affairs (since 1969) and director of the Institute (1972-1981). The Research Planning and Coordination Department was established under the supervision of Dr. Danuta Dębczyńska. In the following years, this program was continued under the changed name "Molecular basis of life processes in microorganisms and higher organisms" under the same management. The research projects of scientific teams submitting to the programme were reviewed, as were the reports on the work performed and presented at annual symposia of thematic groups. In this way, a wide network of mutual connections and scientific contacts was formed, conducive to increasing the methodological level and cognitive value of research work. The experience gained from the period of conducting core problems and the positive assessment by the scientific community of this form of cooperation allowed the Institute to undertake the Central Research and Development Program "Molecular foundations of biotechnology" in the years 1986-1990, on similar principles, under my leadership in cooperation with prof. Przemysław Szafrański and prof. Andrzej Paszewski. The co-coordinators of this Program were: the Center for Molecular and Macromolecular Research of the Polish Academy of Sciences (prof. Wojciech J. Stec), the Institute of Human Genetics of the Polish Academy of Sciences (prof. Antoni Horst), the Central Laboratory of Sera and Vaccines (prof. Michał Korbecki), and the Institute of Physical and Organic Chemistry of the University of Technology in Wrocław(prof. Andrzej Zabża). Most of the molecular biology and genetics research teams with which the Institute had previously cooperated participated in this Program, as well as many teams dealing with the chemistry and synthesis of nucleic acids, genetic engineering of proteins, chemistry and biochemistry of biologically and therapeutically active peptides, obtaining enzymes for industrial

applications and improving vaccines. The cooperation of scientists representing such different cultures and research approaches in this Program has undoubtedly created better conditions for the development of modern biotechnology in Poland in the following years,

Currently, retired for 25 years, I am observing with satisfaction the further development and scientific flourishing of the Institute as a member of its Scientific Council, using the privilege of members of the Polish Academy of Sciences.

Newest laboratories & group leaders

In the last few years, a number of new groups have been formed in the Institute, an important aspect of building for IBB's future. Each lab is led by an internationally recognised researcher in their respective field, some of whom are taking their first step as independent group leaders, whereas others are already established as independent investigators. All of these groups have acquired substantial research funding and established their research teams at IBB PAS, in which their work complements and strengthens existing research themes.

Agata STAROSTA

Laboratory of Translatomics,
established 2021

Agata Starosta completed her PhD in the laboratory of Prof. Daniel Wilson at Gene Center, Ludwig-Maximillians University in Munich, Germany. Later, she was awarded an AXA Research PostDoc Fellowship to study the role of translational factor EF-P in protein biosynthesis, and a Marie Skłodowska-Curie Individual Fellowship to study the regulation of sporulation in Bacillus subtilis on the translational level.

After receiving a First Team grant from the Foundation for Polish Science and EMBO Installation grant, she moved to Poland as a Principal Investigator at Maria Curie-Skłodowska University in Lublin to start her own group, researching translation regulation and specialized ribosomes in Bacillus subtilis. In Spring 2021, she re-located to the Institute of Biochemistry and Biophysics of the Polish Academy of Science in Warsaw to start new projects on the regulation of gene expression in antibiotic-producing soil bacteria.

Her work has resulted in almost 40 international peer-reviewed research articles and a collaboration with a Nobel Prize laureate Prof. Tom Steitz. She has experience in combining biochemistry with structural biology, genetics and high-throughput approaches, especially next-generation sequencing techniques to study complex processes in bacterial cells, especially protein synthesis, antibiotics targeting translation, antibiotic resistance mechanisms and translation regulation.

Michał TUREK

Laboratory of Animal Molecular Physiology, *established 2021*

The Laboratory of Animal Molecular Physiology was established by Michał Turek at the beginning of 2021. Prior to joining IBB, Turek completed his PhD at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. His thesis focused on systems neurobiology, specifically the transcriptional control of sleep in the model organism, the nematode Caenorhabditis elegans. Subsequently, he returned to Poland and explored the interaction between the proteasome and mitochondria, initially at the International Institute of Molecular and Cellular Biology and later at the University of Warsaw.

Following this, he was awarded a SONATA grant from the NCN to investigate the regulation of large extracellular vesicle formation, a project he began at IBB. Shortly thereafter, he secured a second grant, SONATA BIS, also from NCN, to study the epigenetic regulation of EV formation. Turek's work leverages his extensive experience in various biological disciplines to understand the regulation of EVs on multiple levels, from intracellular to inter-organismal. He employs C. elegans, a workhorse of modern biology, as the primary model organism for his studies. Given the multidisciplinary nature of all IBB groups and its conducive environment for knowledge sharing, the institute provides an ideal setting for projects that embrace such a comprehensive approach, as exemplified by Turek's laboratory.

Tomasz TUROWSKI

Laboratory of Transcription Mechanisms, *established 2021*

Turowski, a junior group leader recruited by IBB in the 2020 call, returned to Warsaw after 7 years as a postdoc at the University of Edinburgh, where he worked in the group of Prof. David Tollervey at the Wellcome Centre for Cell Biology. His previous research focused on RNA biology, particularly the transcription and maturation of non-coding RNAs like tRNAs and rRNAs. Turowski secured external funding from NAWA and NCN to investigate transcription in coronaviruses and humans.

Formed over the summer of 2021, the Turowski Lab is housed in a newly refurbished laboratory at IBB. The lab's establishment was greatly facilitated by the supportive IBB administration and the scientific community of the Institute. The group's research is concentrated on understanding how the biophysics of transcription influences gene regulation, with a focus on applying this knowledge to address issues such as SARS--CoV-2 transcription and RNA polymerase III-related leukodystrophy.

The lab employs a diverse range of methodologies, including in vitro biochemistry, UV-based techniques for studying RNA--protein interactions, and bioinformatics. These approaches complement and integrate seamlessly with the cutting-edge laboratories already present at IBB, opening up new avenues for independent scientific inquiry and collaborative opportunities with other research groups at the Institute.

Kevin WALDRON

Laboratory of Metalloprotein Biology, *established 2022*

Waldron is the most recent addition to IBB, arriving in late 2022 to establish the Laboratory of Metalloprotein Biology. He moved to Warsaw from Newcastle University in the UK, where he was based for the prior 10 years as principal investigator. During that time, thanks to fellowship funding from the UK Royal Society and the Wellcome Trust, he established his laboratory as a leader in the study of the functional roles of essential metal ions in bacteria, and published a series of high-profile discovery papers.

Securing a MAESTRO grant from NCN in 2022, Waldron re-located to Warsaw and has built a new team and equipped a newly refurbished laboratory space in IBB. His group studies how protein structures control the reactivity of their essential metal cofactors, how this regulates the metal specificity of catalysis by metalloenzymes, and how metal specificity can evolve over time. His research thus blends protein biochemistry, biophysics and structural biology, as well as computational approaches, making IBB an ideal home due to its considerable infrastructure for such studies. Furthermore, given that almost half of all enzymes require metals to function, making metalloproteins crucial to every biochemical process and pathway, his work offers exciting opportunities to collaborate with other groups in the Institute with diverse research interests on innovative new projects.

Michał WANDEL

Laboratory of Intracellular Immunity, *established 2021*

Michał Wandel moved to the IBB in 2021 to become an independent researcher and establish the Laboratory of Intracellular Immunity. He returned to Warsaw from Cambridge in the UK, where he completed his PhD at the University of Cambridge and post-doc at the renowned MRC Laboratory of Molecular Biology (MRC LMB). During this time, thanks to a PhD Studentship and Postdoctoral Fellowship from the Medical Research Council (MRC), he made significant contributions to the understanding of host-pathogen interactions, in particular of innate immunity and programmed cell death in response to bacterial infection. Michał profited tremendously while working at the world-leading molecular biology institute, MRC LMB, where a culture of scientific excellence has supported work awarded 12 Nobel prizes.

Wandel's research achievements allowed him to acquire generous funding from EMBO (Installation Grant), NAWA (Polish Returns) and NCN (OPUS, SONATA) and join IBB due to the Institute's interest in strengthening research in the area of host-pathogen interactions. As his research employs molecular and cell biology as well as biochemistry in a quest for comprehensive analysis of host-pathogen interactions, he has accommodated well in the IBB environment. In a newly equipped and refurbished laboratory, he endeavours to continue to pursue high standard and high-impact research aimed to further understanding of intracellular anti-microbial mechanisms of innate immunity and inflammation and directly impact on the development of science in Poland.

Scientific Departments, Laboratories and Facilities

Scientific Departments, Laboratories and Facilities

Departments and Laboratories

- Laboratory of Biological Chemistry of Metal Ions
- Laboratory of Molecular Basis of Biological Activity
- Laboratory of Lipid Biochemistry
- Laboratory of Metalloprotein Biology
- Laboratory of Chemoinformatics and Molecular Modeling
- Department of Antarctic Biology
- Department of Bioinformatics
- Lab. of Environmental and Evolutionary Systems Biology
- Laboratory of Fungal Bioinformatics
- Laboratory of Mass Spectrometry
- Laboratory of Genome Engineering
- Lab. of DNA Replication and Genome Stability
- Laboratory of Genetic Stability Mechanisms
- Lab. of DNA Segregation and Life Cycle of Proteobacteria
- Laboratory of Bacteriophage Biology
- Lab. of Mutagenesis and DNA Damage Tolerance
- Laboratory of Bacterial Drug Resistance
- Laboratory of Fungal Biology
- Laboratory of White Biotechnology
- Laboratory of Applied Microbiology
- Laboratory of Lactic Acid Bacteria Biotechnology
- Laboratory of Plant Protein Phosphorylation
- Laboratory of Plant Pathogenesis
- Laboratory of Plant and Microbial Biology
- Laboratory of Plant Protein Homeostasis
- Laboratory of Seeds Molecular Biology
- Laboratory of Non-coding RNA and Genome Rearrangements
- Laboratory of tRNA Transcription
- Laboratory of RNA Biology
- Laboratory of Transcription Mechanisms
- Laboratory of RNA Maturation and Degradation
- Laboratory of Translatomics

Laboratory of Genetic Basis of Human Diseases

Laboratory of RNA Metabolism in Immune Response

Lab. of Bioenergetics and Mitochondrial Disease Mechanisms

Laboratory of Gene Expression Regulation

Lab. of Molecular Basis of Aging and Rejuvenation

Laboratory of Animal Molecular Physiology

Laboratory of Intracellular Immunity

Laboratory of Yeast Genetics and Moleclular Biology

Facilities

Fluorescence Microscopy Facility

Cell Culture and Protein Production Facility

Mass Spectrometry Facility

Biological NMR Facility

DNA Sequencing and Synthesis Facility

Microarrays Analyses Facility

Laboratory of Biological Chemistry of Metal Ions

Prof. dr hab.
Wojciech Bal

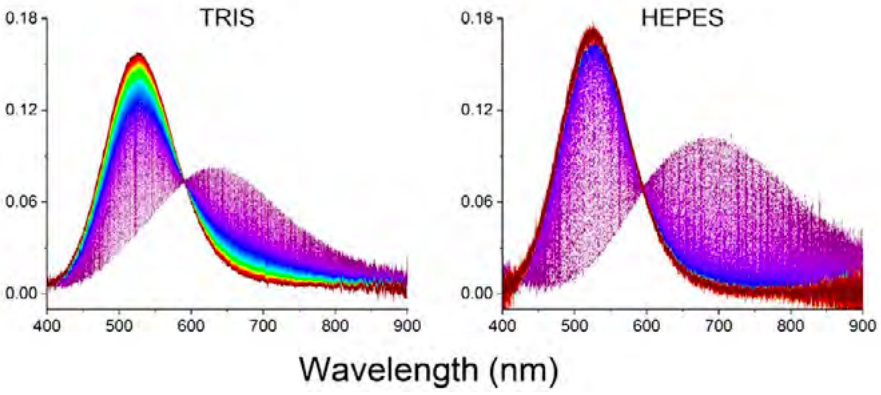
Research in LBCMI is focused on creating and validating molecular models for metal ions physiology and toxicity. Particular attention is paid to quantitative aspects of interactions. Stability constants provide data for modelling distributions of metal species in biological systems, and reaction rates help match models with physiological cycles. Medicinal chemistry and theoretical biology projects are also conducted.

The Laboratory of Biological Chemistry of Metal Ions (LBCMI) originated from the former Department of Biophysics (DB), one of the founding pillars of IBB PAS. The research on biological roles of transition and heavier metal ions, such as Co(II), Ni(II), Cu(II)/Cu(I), Zn(II) and Cd(II) was brought to DB by the group PI when he joined IBB in 2002, following his doctoral and habilitation studies at the University of Wrocław and postdoctoral research at the University of London (with Prof. Peter J. Sadler), the US National Institutes of Health (with the late Dr. Kazimierz S. Kasprzak) and University of Karlsruhe (with Prof. Andrea Hartwig). This research theme, new at IBB, landed on a friendly soil of research on calcium binding proteins performed by prof. Andrzej Bierzyński, the contemporary head of DB. The main objective of the decade 2002-2012, stemming from the PI's postdoctoral studies, was the elucidation of mechanisms of carcinogenesis of metal species, primarily nickel. Several novel indirect molecular mechanisms were proposed, including the assault of Ni(II) ions on zinc finger (ZF) transcription factors and the sequence-specific hydrolytic cleavage of peptide bonds, which could affect the DNA access regulation at the histone H2A C-terminal chain. This research was later extended to include the putative role of said reaction in nickel allergy, and also yielded a patented protein purification method, based on the clean cleavage of affinity tags. This research was strongly supported by a TEAM grant from Fundacja na rzecz Nauki Polskiej (FNP) and a large institutional equipment purchase campaign under the auspices of the Centrum Badań Przedklinicznych i Technologii

(CePT). The ongoing interest of the PI in the participation of reduced and oxidized glutathione (GSH and GSSG) in metal ion trafficking resulted in a characterization of their Zn(II) and Ni(II) complexes. This research line provided strong indications of the participation of ternary GSH complexes in the mobile cellular Zn(II) pool. This notion was independently confirmed in biochemical experiments. Currently, the GSH research is being extended into Cu(I) transport processes. Research on albumin as metal ion transport protein, initiated during the PI's postdoctoral stay in London, is another continuous thread in LBCMI. Its current

Automatic peptide synthesizer at work (with Ms. Dobromiła Sudzik, Ph.D. student)

Left to right: Joanna Ziemska-Legięcka, Adam Mieczkowski, Anand Mohan, Dobromiła Sudzik, Lesia Kolomiiets, Magdalena Sokołowska-Piechocińska, Tomasz Frączyk, Wojciech Bal, Krzysztof Drabikowski, Marcin Grynberg



The rainbow representation of stopped-flow kinetics of the final step of Cu(II) ion binding to a peptide modelling biological copper binding sites, recorded with a diode array spectrophotometric detector. The presented experiment highlights the effect of the choice of buffer to maintain the reaction pH.

focus is on albumin's role in copper transport in blood and delivery to cells. Research on β-amyloid peptides as key molecules in the pathology of Alzheimer's disease (AD), initiated by in-house collaboration with prof. Michał Dadlez, was a dominant theme of the 2012-2022 decade. The most important result in this area of LBCMI activity is the rediscovery of the importance of N-truncated $A\beta_{4-x}$ β-amyloid variants as major Cu(II) binding molecules in the central nervous system. Our original concept, of $A\beta_{4-x}$ peptides as molecules positively engaged in brain physiology by scavenging superfluous Cu^{2+} ions, recently gained support by discovery of their Cu(II) complexes in cerebrospinal fluid. Unsolved details of this concept turned our interest towards a broader issue of physiological and pathological aspects of extracellular transport and cellular uptake of copper ions. The research on albumin mentioned above has been complemented by studies of interactions of Cu(II) ions with and within the low molecular weight ligands and with the extracellular part of the Ctr1 cellular copper transporter. Relevance of this research is powered by epidemiological observations of copper distribution abnormalities in various diseases, including AD. Its latest aspect is the search for Small Copper Carrier, a putative molecule enabling distribution of copper from blood to tissues. The traditional approach focused on establishing the equilibrium states cannot provide a comprehensive picture of biorelevant copper chemistry. Therefore, we resorted to methods of fast kinetics, primarily the stopped-flow technique. Unexpectedly, we found that the formation of final complexes is often too slow to be completed within time windows of specific physiological processes. This opens up a possibility for intermediate kinetic species formed transiently to be actual biological copper carriers and effectors.

This concept is currently being developed vigorously in LBCMI. Biochemical experiments must be performed at constant pH, to reflect the physiological conditions. This issue is particularly troublesome in Cu(II) studies, because apparently all buffers form relatively strong Cu(II) complexes. To enable control over this issue, we performed comprehensive studies of Cu(II) complexes of HEPES and Tris, two most common buffers, and formulated recommendations about their usage in quantitative studies (see image). Recent years witnessed the explosive development of silver nanoparticles (AgNPs) as antimicrobial materials in medicine and daily products, but evidence from cell cultures and experimental animals points to significant toxicity of AgNPs, and Ag^+ ions formed upon the AgNP dissolution in body fluids. Together with Prof. Artur Krężel we demonstrated that ZF domains are primary targets of silver toxicity. Ag(I) ions easily replace native Zn(II) ions and destroy the ZF structure and function. These studies are continued with a focus on the possible protective role of GSH in silver toxicity. Our work is performed in broad collaboration with laboratories in Europe, USA and Korea.

Biochemistry of metal ions is the main focus of LBCMI activity, but other research directions are also independently pursued within its structure (listed in alphabetical order): Dr. Krzysztof Drabikowski works on biocidal materials and physiological models implemented in *Caenorhabditis elegans* nematode, a model multicellular organism; Dr. Tomasz Frączyk pursues interrelationships of metal ion interactions with biological targets with post-translational modifications; Dr. hab. Marcin Grynberg realizes a broad program of theoretical studies of low complexity sequences in proteins; and Dr. hab. Adam Mieczkowski runs medicinal chemistry projects focused on design, synthesis and biological testing of novel anticancer drugs.

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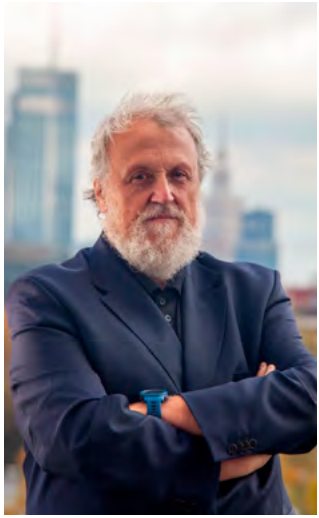
Laboratory of Molecular Basis of Biological Activity

Prof. dr hab.
Jarosław Poznański



We study the general structure-activity relationship. We analyse the structures in the context of available experimental data (biological activity, biochemical parameters, kinetics, thermodynamics, etc). Specific types of interactions or their combinations that explain the observed effect are identified. A significant area concerns the balance of hydrophobic interactions and halogen bonding in protein-ligand systems.

We are trying to identify relationships between the structure and biological function of proteins. Our research is primarily based on the analysis of structural data determined both experimentally (X-ray) or via *in silico* modelling (e.g., by homology) in the context of available (literature or obtained by us) experimental data (biological activity, biochemical parameters, kinetic data, thermodynamic measurements, spectroscopic measurements, statistical analyses). Specific types of interactions or their combinations that explain the observed effect(s) are identified.

One of the key aspects of our research is the application of the perturbation theory in practice. We slightly modify the model protein-ligand systems to study the thermodynamic contribution of individual atoms to the interaction between a ligand and its molecular target. We use the catalytic subunit of protein kinase CK2 as a model protein and halogenated heterocyclic compounds as molecular probes. This practical approach allows us to sample the effect of small environmental changes (e.g., pH, buffer composition, solvent isotopic composition, temperature), the target protein (numerous rationally designed replacements of particular amino-acid residues in the protein sequence) or the ligand properties. We have designed and synthesized dozens of such molecular probes carrying various combinations of halogen atoms (F, Cl, Br, I) and studied their interactions with the molecular target using a broad spectrum of modern thermodynamics techniques, including low-volume differential scanning fluorimetry (DSF), microscale thermophoresis (MST) or isothermal titration calorimetry (ITC). On the way we proposed, synthesized and

Left to right: Maria Winiewska-Szajewska, Marta Onuk, Jarosław Poznański, Sławomir Kasperowicz, Agnieszka Maciejewska

further optimized a new CK2 bi-substrate inhibitor of moderate activity. We designed new state-of-art procedures for data analysis, which are more efficient and more effective than the commercial ones. Among others, we have presented the first global analysis of the MST data for the two independent binding sites.

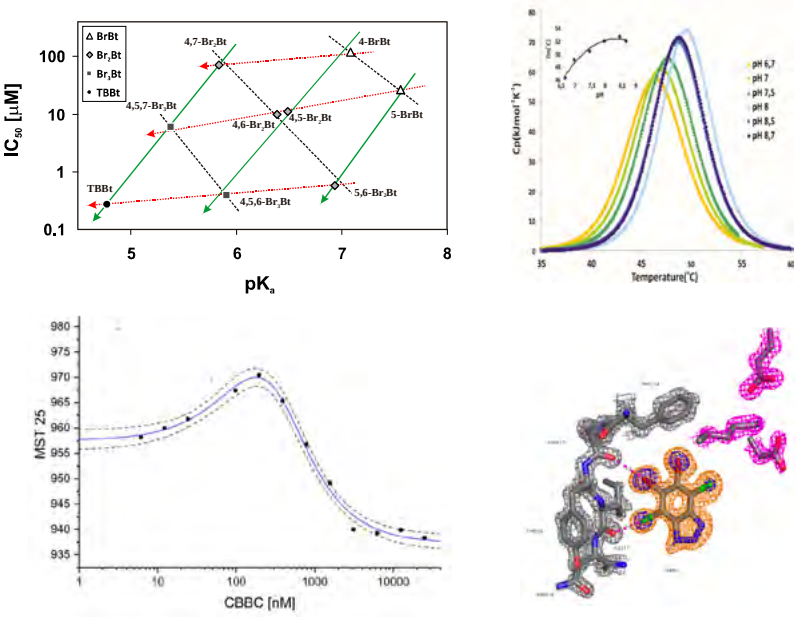
Our research has led to the development of a new hydrophobicity scale. We applied density measurements to obtain a deeper view into the organization of the solvation shell (i.e., the solvent region surrounding the solute whose organization differs from that of the bulk solvent) by studying solute-solvent (mainly water) interactions with density measurements. Each molecule affects the organization of the proximal solvent, and higher hydrophobicity of the solute is associated with a lower density of water in the solvation shell. We use this parameter to assess the hydrophobic contribution to the free energy of ligand binding. Using the same approach we are studying the solvation of ions commonly present in biological samples. On the way we have for the first time demonstrated the asymmetry of the isotope effect in diluted H_2O/D_2O binary mixtures.

Protein kinases are the main target of our study. They are the enzymes that catalyze the phosphorylation of various proteins, including those from signaling pathways, which makes them a key player in regulating cell functions. The disruption of expression or function of a particular protein kinase may perturb signaling pathways, resulting in cancer formation. Because of that, protein kinases have become an attractive thera-

peutic target in cancer therapies and are of interest to many research groups. They constitute the largest family of enzymes – over 500 human protein kinases have already been identified, comprising almost 2% of all proteins encoded in the human genome. We are employing biophysical (structural and thermodynamics) and biochemical tools to understand better the mechanisms of substrate recognition and activity regulation in protein kinases. Starting with the well-described protein kinase CK2, we study the so-called CMGC-insert (a distinctive segment in one of kinase's groups) and the ATP-binding site in the context of allosteric regulations of activity and selectivity. Using

Evidence of millimeter-scale hydrophobic interactions.

^{19}F nuclear magnetic resonance spectroscopy, we discovered a cross-talk between the CMGC insert and the ATP-binding site, as well as the CK2 alpha/CK2 beta interface. As the determined mechanism appears more



Small perturbations in practice: Subtle changes in the bromination pattern of benzotriazole affect its inhibitory activity; Small pH changes affects the thermal stability of protein-ligand complex monitored by Differential Scanning Calorimetry; Chlorine-bromine replacement in perhalogenated benzotriazoles drives the binding affinity monitored by Microscale Thermophoresis, and ligand orientation at the target protein binding site

general, our study is being extended to other protein kinases.

We also study the Ada operon's role in repairing exocyclic DNA damage. We monitor the mutagenic properties of some acrolein and chloroacetaldehyde (common environmental mutagens) adducts to bases of nucleic acids and their repair by bacterial AlkB dioxygenase and AlkA glycosylase. AlkB acts according to a previously unknown DNA repair mechanism by removing modifications from alkylated DNA bases via oxidative dealkylation, leaving the unaltered DNA. We experimentally proved that AlkB preferentially recognizes and repairs positively charged substrates. We now focus on the biological function of AidB dehydrogenase, characterizing DNA-AlkB-AidB, RNA-AlkB-AidB, and RNA-AlkB complexes and monitoring *in vitro* repair of the exocyclic RNA adducts. Such a multidisciplinary approach should provide deeper insights into fundamental aspects of cellular homeostasis maintenance.

We use our expertise to answer essential questions in molecular biology or medicine. Among others, we are building structural models of proteins whose coding genes were identified as altered for a particular patient. Such an approach that may support doctors in finding the therapy the patient needs as a part of personalized medicine. We also study interactions of pathogens with host proteins, designing targeted modifications that may prevent penetration by a pathogen. The data analysis methods we have developed can be easily transplanted to other biological, biochemical and biophysical problems. We have been involved in numerous fruitful scientific collaborations.

In summary, our group is quite unique, not only in the Institute. We are successfully combining physical chemistry, medical chemistry, molecular biology, and medicinal biology to make science and even more.

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Laboratory for Lipid Biochemistry

Prof. dr hab.
Ewa Kula-Świeżewska

Our studies focus on the biosynthesis, cellular function, and biotechnological applications of isoprenoids. We seek to elucidate the biochemical pathways that lead to the formation of polyisoprenoid lipids and prenylated proteins and understand consequences of aberrations of these processes in model organisms, including *Arabidopsis thaliana, Saccharomyces cerevisiae, Paramecium tetraurelia,* and mammalian cells.

Our research focuses on polyisoprenoid lipids, which are common components of all living cells. There are two variants of polyisoprenoids in nature: polyprenols and dolichols. Plants are good models for our research because they produce polyprenols in their leaves and dolichols in their roots at the same time.

Our ongoing research

(a) routes leading to formation of polyisoprenoids

Formation of the polyisoprenoid molecule is catalysed by the enzyme called *cis*-prenyltransferase (CPT). In the model plant *Arabidopsis thaliana* we characterized 5 such CPT enzymes (still another 4 potential CPTs remain uncharacterized) and found that each of them produces different polyprenols. At the same time, in the protista *Paramecium tetraurelia*, we characterized two CPTs, only one of which is essential for *P. tetraurelia* survival. Furthermore, some polyprenols produced by particular Arabidopsis CPTs are immediately converted to dolichols by polyprenyl reductase (PPRD; two PPRDs were characterized by us in Arabidopsis) while some others are not. Interestingly, shortage of only selected dolichols of Arabidopsis is essential for plant survival while the absence of other dolichol species is not deadly but affects plant development, e.g. the growth of roots. In *P. tetraurelia*, on the other hand, the lack of dolichols is lethal. In turn, polyprenols that are found in plant leaves and remain unconverted to dolichols are also biologically important since they affect photosynthesis. Furthermore, a deficiency of these polyprenols is fatal to young plants when they are grown at elevated temperatures (heat stress).

Left to right: Liliana Surmacz, Marta Zajbt-Łuczniewska, Marta Hoffman-Sommer, Zuzanna Bechler, Ewa Kula-Świeżewska, Karolina Skorupińska-Tudek, Natalia Piłka, Aleksandra Weremczuk, Karolina Sztompka

A separate line of our research is focused on the initial steps of polyisoprenoid biosynthesis, namely formation of the building blocks used by CPTs to form polyprenols. In plants there are two pathways which operate in parallel and our studies show that the biosynthetic route of polyprenols and dolichols are different.

Interestingly, while in Arabidopsis a shortage of essential dolichols is lethal, in humans an aberration in the formation of dolichols result in a fatal genetic disease (Congenital Disorder of Glycosylation I) that manifests as intellectual disability and ophthalmological and neurological abnormalities. Dietary supplementation with dolichol and dolichol-enriched plant products is planned as a possible therapeutic strategy for the individuals suffering from this disease.

Collectively, our results show that polyisoprenoids have a variety of biological functions; while some polyisoprenoids are essential for plant and animal survival, other polyisoprenoid species help organisms adapt to adverse environmental conditions. Dolichols also have potential pharmacological applications as components of the human diet.

(b) factors affecting formation of Polyprenols and Dolichols

In addition to molecular and biochemical characterization of the key enzymes responsible for the formation of polyisoprenoid molecules, CPTs and PPRDs, we are also trying to unravel the cellular machinery responsible for regulation of the activity of these enzymes. There are several steps which might be involved in such regulation and we found that:

- Not all, but only selected CPTs of Arabidopsis need protein partners for catalytic activity. Besides the one which was characterized some years ago (named Lew1) we are also looking for new candidates. In the current project, the involvement of Rubber Elongation Factor (REF) known to participate in the formation of natural rubber (which is structurally homologous to polyprenols and dolichols) is being

analysed. In Paramecium, CPTs require a protein named Partner of CPT1 (POC1) to perform catalytic activity;

- The regulation of the activity of CPT, which synthesizes polyprenols in Arabidopsis leaves, is achieved through the interaction of its mRNA with a specific protein (called Heat Shock Transcription Factors A1), and results in a significantly higher amount of polyprenols being accumulated in leaves when plants are exposed to heat, compared to normal temperature;
- CPT activity is also regulated by its phosphorylation - this is the subject of our current project.

Taken together, our obtained results so far described above expand the basic knowledge of the cellular processes leading to the formation of biologically important compounds, polyprenols and dolichols.

(c) Dolichols and intracellular vesicular transport

In a parallel project, we were able to demonstrate a link between increased dolichol accumulation and dysfunction of several basic cellular processes (e.g., autophagy) when vesicular transport is impaired in the cell.

(d) biotechnological application of polyprenol derivatives

A specific line of our research is devoted to the use of chemically generated derivatives of polyprenols as components of vaccines or gene transfer enhancers, such as during gene therapy. The project, supported by EU structural funds, was successfully implemented by the research consortium under the IBB PAS leadership.

Some of the Researchers who have studied POLYISOPRENOIDS (clockwise from bottom right): Tadeusz Chojnacki, Józefina Hertel, Ewa Ciepichał, Katarzyna Gawarecka, Adam Jóźwiak, Agata Lipko, Agnieszka Onyśk, Przemysław Surowiecki, Małgorzata Gutkowska, Daniel Buszewicz

In conclusion, understanding the mechanisms that affect the cellular content of polyprenols and dolichols makes it feasible to build biotechnology platforms aimed at producing high-value biotech products - polyprenols, dolichols, dolichol-enriched plants on the one hand or generating plant varieties with increased adaptability to environmental stresses on the other.

Future perspectives

Among numerous questions in the field of polyprenology that have not been addressed yet the most appealing are: (1) the molecular mechanism underlying degradation of polyisoprenoids; (2) the signalling pathway(s)

regulating the cellular level of dolichols. We hope to find answers to these questions in the near future.

Our fundamentals

The projects summarized above stood on the shoulders of our predecessors - pioneer studies on polyisoprenoids were initiated at IBB PAS in the early 1970s by Professor Tadeusz Chojnacki. Initially, due to broad screening analysis of dozens of plant species obtained from botanical gardens and natural habitats of various geographical regions, the diversity of polyisoprenoid structures was elucidated. A huge set of data collected at that time made IBB PAS a reference centre in the field of polyprenology. It also gave the foundation to the 'Collection of Polyprenols' (established in 1976) offering polyisoprenoids to numerous researchers either within the frame of scientific collaboration or on commercial basis.

Subsequent broadening of our expertise with molecular biology and mass spectrometry methods was achieved due to the expansion of our team as well as due to the collaborative projects. Extensive scientific collaboration was and is currently conducted with numerous research groups from IBB PAS and other institutions in Poland and abroad, including Prof. Jarosław Poznański IBB PAS, Prof. Witold Danikiewicz, IChO PAS, Prof. Michel Rohmer, Louis Pasteur University, Strasbourg, Prof. Gustav Dallner, University of Stockholm, Dr Kariona Grabińska, Yale School of Medicine, New Haven and many others. Implementation of the research projects would not be possible without the involvement of current and previous members our Laboratory, just to mention a few of those who have already changed their field of interest: Drs Małgorzata Gutkowska, Daniel Buszewicz, Ewa Ciepichał, Adam Jóźwiak, Katarzyna Gawarecka, Agata Lipko, Przemysław Surowiecki, Agnieszka Onyśk and Mrs Jozefina Hertel.

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Laboratory of Metalloprotein Biology

Dr hab.
Kevin Waldron, Prof. IBB PAS

The Laboratory of Metalloprotein Biology studies the structure and function of metalloproteins and metalloenzymes. Their research aims to determine why metalloproteins function best with one preferred metal cofactor, and how this metal specificity evolves over time. They also study the mechanisms of cellular metal homeostasis and how this ensures selective binding to the 'correct' metal by metalloproteins *in vivo*.

Proteins perform the essential functions of living cells, catalysing chemical reactions that enable metabolic pathways, anabolic synthesis of biological macromolecules, and replicating DNA. The structure of proteins determine their function, arranging their polypeptide chain into a defined three-dimensional folded state that brings together specific residues to form a catalytic 'active site'. However, all proteins are composed of just five elements, carbon, hydrogen, nitrogen, oxygen and sulfur, which limits their catalytic chemistry. To overcome this limitation, evolution also selected a small number of essential metals for utilisation by a subset of proteins and enzymes to expand the elemental composition of life. These metals (magnesium, calcium, manganese, iron, cobalt, nickel, copper, zinc and molybdenum) are utilised as cofactors by specific groups of proteins and enzyme, termed metalloproteins and metalloenzymes. These metals expand the range of chemistry that can be catalysed by living systems. Although the selection of biologically essential metals has varied somewhat over geological time, mediated for example by changes in oxygen abundance in the atmosphere, nonetheless the same small group of metals are essential for almost all extant life on Earth.

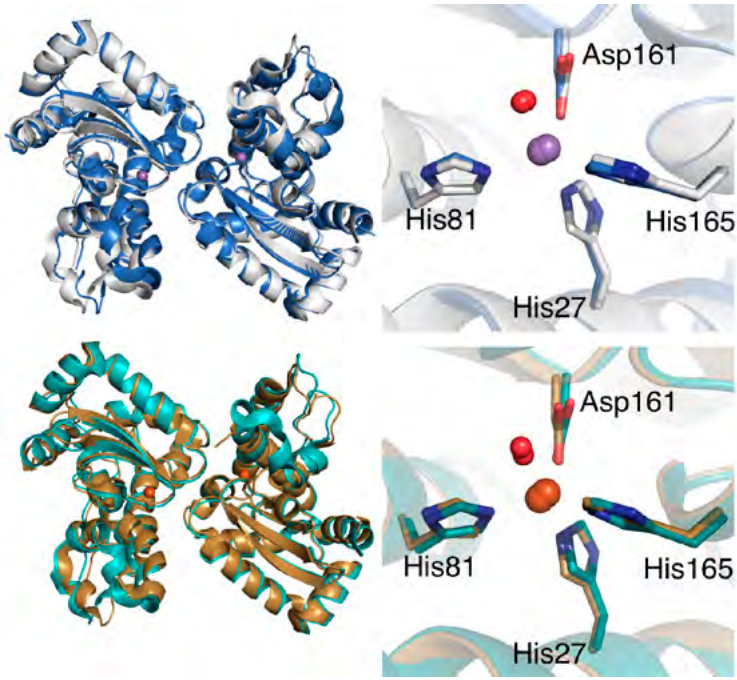
Metals can perform a number of different functions within metalloproteins. They can directly participate in an enzyme's chemical reaction, for example acting as redox active cofactors, gaining and donating electrons during the catalytic cycle, or as Lewis acids, activating inert substrates such as water molecules to facilitate the reaction. In other cases, metal cofactors can play purely structural roles, with the metal ion coordinating

Left to right: Mariam Esmaeeli, Rafał Mazgaj, Kevin Waldron, Lorna Nikolić, Natalia Kwiatos

amino acid ligands from different regions of the polypeptide sequence and holding together the three dimensional fold of the protein, for example in the common regulatory zinc-finger proteins.

Most metalloproteins exhibit a preference for one specific essential metal. For example, a metalloenzyme will exhibit maximal catalytic activity with the metal it binds *in vivo*, and less (or even no) activity when associated with the other essential metals *in vitro*. This metal specificity is especially common among metal-dependent oxidoreductases, where the metal ion performs an essential redox role during catalysis. This is due to the need for each metalloenzyme to manipulate the metal's reduction potential, which is inherent to each element, in order to optimise it for the redox reaction the enzyme functions to catalyse. Such 'redox tuning' is common among redox metalloenzymes and is controlled by both the metal ion's primary (directly coordinating ligand shell) and secondary (amino acid residues that are spatially close to the metal, but make no direct contacts with it) coordination sphere, as well as through manipulation of hydrogen bonding networks within the protein structure. Nonetheless, despite the observed preferences of metalloenzymes, combined bioinformatic and biochemical analyses of diverse metalloprotein families demonstrates unambiguously that metal specificity can change over evolutionary time. This shift in metal preference can either enable the newly evolved isozyme to perform the same chemical reaction but utilising an alternative metal cofactor, or alternatively it can enable the evolved isozyme to perform an entirely new catalytic function.

The mechanisms by which a metal ion's second sphere interactions can control the reactivity of the cofactor and thus regulate the metal preference of catalysis are poorly understood. To study this phenomenon, our lab is exploiting a remarkable model system: the family of iron- or manganese--dependent superoxide dismutases (SODs). These enzymes, which play an important role in cellular defence against oxidative stress, perform a reaction that detoxifies the reactive oxygen species, superoxide radical (O_2^-). The iron- or manganese-dependent SODs are widely distributed, found in the genomes of organisms from all three of the



Overlaid crystal structure models of two related SOD enzymes from *Staphylococcus aureus*, SodA and SodM, each loaded with either (upper panels) manganese or (lower panels) iron. The left panels illustrate the complete structures of the homodimeric protein forms, whereas the right panels show a zoomed in view of the catalytic active sites.

classical branches of life (Bacteria, Archaea and Eukarya), and their evolutionary origin likely occurred before the advent of the oxygen-rich atmosphere on Earth.

This SOD family have traditionally been considered to come in three distinct types, previously thought to represent phylogenetic sub-families: MnSODs are specific for manganese, and lack activity with iron; FeSODs are specific for iron, and lack activity when loaded with manganese; and cambialistic SODs exhibit catalysis when loaded with either manganese or iron. This latter class of SODs, which exhibit cofactor flexibility, are remarkably rare examples of metalloenzymes that are not highly specific for a single metal cofactor, despite the fact that they utilise their cofactor for redox activity. This raises questions about how they have evolved to apparently optimise their active site for the manipulation of two distinct metal cofactors which have distinct reduction potentials.

In recent studies, we have demonstrated that these three 'types' of SOD are not actually discrete. In fact, the family of iron- or manganese-dependent SODs shows little correlation between phylogeny and metal-preference. Furthermore, we have demonstrated that SODs are able to undergo switching of their metal-preference – from manganese-

-preferring to iron-preferring or vice versa, or from either metal-preference to become cambialistic – over very short evolutionary timescales. Indeed, we have identified numerous examples of such SOD metal-preference switching across the tree of life. One such switch occurred very recently, during the evolution of the Gram-positive mammalian pathogen *Staphylococcus aureus*. Its non-pathogenic relatives possess only a single SOD, which is highly specific for manganese, but *S. aureus* possesses two SODs; one manganese-specific and the other cambialistic. The cambialistic SOD likely emerged through an ancient duplication event, whereby the gene encoding the manganese-dependent SOD was copied and the new gene underwent neofunctionalisation, gaining a new ability to catalyse its reaction with iron as well as with its ancestral manganese cofactor.

Our lab in IBB is investigating the molecular mechanisms by which such evolutionary changes in SOD metal preference can occur. Using a suite of SOD isozymes, covering multiple sub-families of SODs and from organisms across the tree of life, we are uncovering the details of the processes that underpin this change in biochemical property in natural evolution. Furthermore, we are leveraging the close sequence and structural similarity between the recently evolved pair of SODs from *S. aureus* as a key tool with which to study the processes by which protein structures control the reactivity of their metal cofactors in unprecedented resolution at the atomic and even the electronic scale. Finally, we will use the SODs, as well as numerous other metalloprotein model systems, to study the metal binding selectivity of metalloproteins, to determine how cells overcome universal chemical principles, such as the Irving-Williams series, to ensure that each metalloprotein and each metalloenzyme becomes associated only with the correct metal cofactor, that for which their function has been optimised by evolution, inside living cells. The latter relies on accurate cellular metal homeostasis, observed in all living cells and a focus of much of my prior research, which ensures that metal availability to nascent proteins is precisely regulated to enable correct metal-protein associations *in vivo*.

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Cheminformatics & Molecular Modeling Laboratory (CHEMM)

Dr hab.
Paweł Siedlecki

The primary focus of the group is to investigate the interactions among biomolecules, specifically binding events and their affinities. A significant challenge lies in predicting the affinity of prospective, unknown complexes. The integration of machine learning, deep learning, and cheminformatics introduces novel approaches and also raises important questions for the dynamically evolving field of drug discovery.

Cheminformatics, molecular modeling, and computational chemistry are integral to understanding biological systems, particularly the multi-faceted interactions between proteins, nucleic acid and ligands. These methodologies are crucial not only in structure- and ligand-based drug discovery but also have significant implications in medicine, agriculture and environmental science.

The CHEMM group is dedicated to refining the sensitivity and specificity of affinity predictions, especially in the context of *in silico* high throughput screening campaigns. We strive to develop novel representations of biomolecular complexes. We apply advancements in machine learning (ML) and deep learning (DL) to enhance the precision of binding affinity predictions. We explore novel compounds using a variety of theoretical and experimental methods, prioritizing their potential applicability as a key consideration.

One of the fundamental challenges in *in silico* screening is the reliance on sub-optimal conformations of molecules. In collaboration with Pedro Ballester from CNRS, Marsellis, our team has developed an effective model, RFScore-VS, trained to predict affinity values from such sub-optimal, noisy data. What sets our model apart is its utilization of negative data, which constitutes about 97.5% of our training dataset. Our approach has demonstrated significant improvements in distinguishing between active and inactive compounds at the top of ranking lists. The method has been recognized with multiple citations and featured in the list of 100 most-read articles in Scientific Reports.

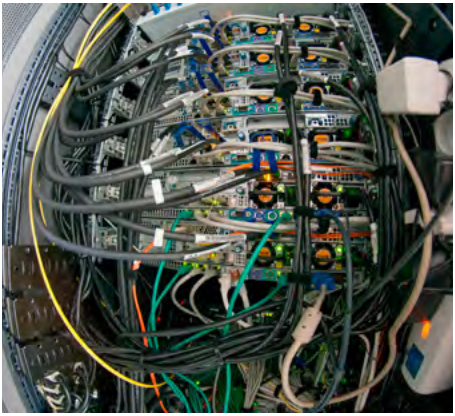
In our pursuit of a representation that is both rich in information and explainable, we explored the field of interaction fingerprints (IFP). Fingerprints are a key concept in cheminformatics, allowing molecules to

Left to right: Łukasz Milewski, Paweł Siedlecki, Jakub Poziemski

be effectively represented by fixed-length vectors of Booleans or integers. Our development, the PLEC FP (Protein Ligand Extended Connectivity Fingerprint), builds upon the ECFP fingerprint by utilizing atom environments rather than defined functional groups or substructures. This method has shown high efficiency in predicting binding affinity even in simple linear models and has addressed the lack of a general, descriptive, and easily interpretable solution in the field.

Neural networks offer distinct advantages, such as the ability for the model itself to select elements crucial for the prediction of interactions. We aimed to create a network capable of autonomously determining the affinity value for a ligand-receptor complex, which could be utilized for *in silico* screening. Our custom-designed convolutional neural network was trained using a unique grid representation of the ligand-receptor complexes. A thorough evaluation revealed enhanced accuracy without human-knowledge intervention, marking our model one of the first applicable to virtual screening. This work has garnered over 400 citations and has influenced the current methodologies of building scoring functions.

Moreover, our recent studies have integrated molecular dynamics (MD) simulations with ML to assess both predictive performance and limitations. MD simulations provide a dynamic perspective by depicting the temporal interactions within protein-ligand complexes, which can contribute additional insights into affinity and specificity estimates. By generating and analyzing over 800 unique protein-ligand MD simulations, we identi-



Welcome to the CHEMM Innovation Factory

fied specific and generalizable features that enhance predictive accuracies, suggesting new methods to augment current in silico affinity prediction pipelines.

The CHEMM group focuses on the practical application of its *in silico* methodologies. One pressing issue in agriculture is the impact of novel compounds on pollinators. With the global decline of bee populations posing significant risks to agriculture, biodiversity, and environmental stability, we introduced ApisTox, a comprehensive dataset that explores the toxicity of pesticides to honey bees (Apis mellifera). This dataset combines data from existing sources into an extensive, consistent, and curated collection, surpassing previous datasets. ApisTox serves as a crucial tool for environmental and agricultural research, aiding in the development of policies and practices to minimize harm to bee populations, and is a valuable resource for benchmarking molecular property prediction methods on agrochemical compounds.

In our ongoing search for novel, therapeutically promising molecules, our laboratory focused on discovering microbiome-derived molecules with therapeutic potential, specifically targeting colorectal cancer (CRC), a prevalent and challenging public health issue. Together with our industry partner, we have analyzed lactic acid bacteria (LAB) strains from dairy and fermented foods for CRC treatment and prevention. A blend of experimental, bio- and cheminformatics techniques revealed a specific strain capable of arginine deiminase (ADI) release

into the culture supernatant under gut-like conditions. This release significantly reduced epithelial cell growth, leading to decreased c-Myc levels, reduced phosphorylation of p70-S6 kinase, and cell cycle arrest. These results demonstrate for the first time an anti-proliferative effect from a cell-free supernatant (CFS), independent of bacteriocins or other small molecules. ADI, derived from a Generally Recognized as Safe (GRAS)-designated strain of *Lactococcus lactis*, shows anti-proliferative effects in cell lines with different argininosuccinate synthetase 1 (ASS1) levels, highlighting its broad therapeutic potential. With this discovery we underscore the value of environmental LAB strains in developing new cancer treatments.

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Department of Antarctic Biology

Dr hab.
Robert Bialik, Prof. IBB PAS

The Department of Antarctic Biology is responsible for the scientific program that is undertaken at the Arctowski Polish Antarctic Station, providing expert opinions about Antarctica for numerous Ministries of the Republic of Poland. We specialize in physical oceanography, marine biology, glaciology, and meteorology, with a particular focus on biology, including conservation biology, ecology, and microbiology.

The activities of the Department of Antarctic Biology can be divided into the following five main areas:

Remote sensing of polar regions

We sought to quantitatively estimate the amount and type of suspended matter that is delivered from glaciers on the South Shetland Islands to the marine environment using a wide spectrum of remote sensing methods to assess turbidity variations in the glaciomarine environment. We also developed new methods to obtain digital data using unmanned aerial vehicles that record information in various spectral ranges and using a terrestrial network of automatic camera traps and telemetry transmitters. In addition, in our work we proved that satellite remote sensing offers great possibilities over spatial and temporal scales and addresses the need for a modern animal census technique in Antarctica. For instance, we have shown that with the use of 31 cm resolution satellite images we can distinguish southern elephant seal males from females or seals in general from landscape elements of analogous form and size.

Ecology of polar microorganisms

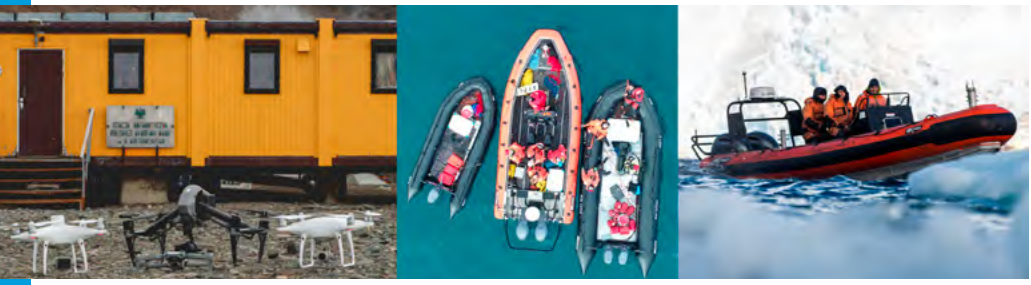
Our research seeks to broadly understand the ecology of polar microorganisms and address the following issues: (i) decomposition of avian guano and marine macroalgae in the Arctic and Antarctic, (ii) the impact of global warming and glacier retreat on the composition of postglacial soil microbiocenoses, (iii) changes in microbial communities on the surface of glaciers in both hemispheres, (iv) study of microbiota that are associated with Antarctic flowering plants and lichens, and (v)

Left to right: Katarzyna Tołkacz, Małgorzata Korczak-Abshire, Julia Brzykcy, Jakub Grzesiak, Robert Bialik, Stanisław Cukier, Joanna Plenzler, Kornelia Wójcik-Długoborska, Katarzyna Fudala

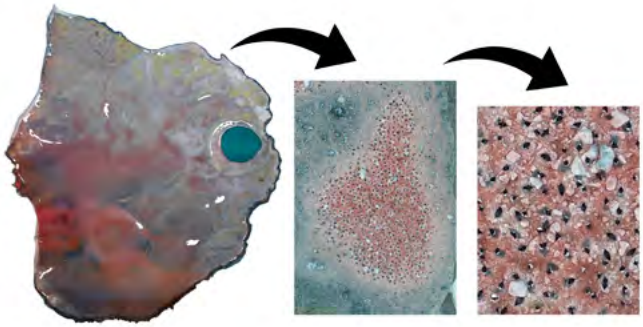
characterisation of psychrophiles, including stress survival, genetics, and biotechnology.

Law related to protection of the environment in Antarctica

It is our responsibility to adapt scientific and logistical activities that are conducted at the Arctowski Polish Antarctic Station to comply with environmental protection standards that result from the Antarctic Treaty System. We supervise monitoring of the natural environment in the vicinity of the Arctowski Station and take care of laboratory and research equipment at the Station. Two team members are Polish Representatives of the Environmental Protection Committee (CEP), which is an advisory body of the Antarctic Treaty Consultative Meeting (ATCM). The research interests of the team primarily focus on conservation biology and the impact of climate change on wildlife distribution and the physiology



Selected drones and boats equipped by the Department of Antarctic Biology



Inter-seasonal fluctuations in the abundance of selected bird species-censuses using remotely piloted aircraft system (RPAS)

and behaviour of animal breeding on King George Island. Apart from employing a fundamental research approach, the results of our investigations constitute recognizable input into managing the commercial harvesting of Antarctic marine life resources in the scope of work by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) and Antarctic conservation issues in the face of rapid climate change. As an example, using remote piloted aircraft systems (RPAS), an inventory of the Destruction Bay area at King George Island was performed and the quantity of Antarctic shag nests found allows for the classification of the area of Cape Melville as an Important Bird and Biodiversity Area (IBA). Among the 175 currently known colonies of Antarctic shags in Antarctica, this is the fifth largest.

Parasites of Pygoscelis penguins

One of the aims of our team is to compare the current extent that three Pygoscelis spp. penguin species that nest in the South Shetland Islands are infected with blood parasites and quantify their infestation with ectoparasites. As there is currently little to no systematic knowledge about hemoparasites of penguins and their vectors in this area, there is no baseline for evaluating changes in the community of blood parasites in their host populations. Furthermore, the influence of climate change is increasing the risk that local animal populations may be exposed to non-native species of parasites and their vectors. We would like to provide an insight into the epidemiology of vector-borne pathogens on Antarctic seabirds, especially considering their potential implications for the health and conservation of these species under climate change.

Hydro-glaciological monitoring

The glaciers of King George Island are also in our area of interest. During the Antarctic winter, we monitor the ice thickness of the Warsaw Dome located in close proximity to the Arctowski Station. These observations are made using ground penetrating

radar (GPR). Our Department also runs a project called King George Island Glacial History (KNIGHT). This project aims to investigate the terrestrial deglaciation chronology of Admiralty Bay, King George Island, South Shetland Islands, since the Last Glacial Maximum (LGM). Terrestrial deglaciation chronologies for many areas in the Antarctic Peninsula region have yet to be constrained. This lack of knowledge is problematic as understanding the past behaviour of the Antarctic Ice Sheet is key for predicting its future behaviour in response to current warming. As part of these activities, we also look after four automatic weather stations, which are installed within Admiralty and King George bays. The stations provide data on weather conditions and allow us to directly assess the rate of climate change taking place.

Some of our other main achievements are as follows:

1. We implemented novel technology solutions for research on krill-dependent indicator species, which led to the location and identification of threatened Antarctic fauna populations in isolated and hard-to-access polar regions.
2. We discovered several succession-related phenomena within the microbial community that resided on the surface of Arctic and Antarctic glaciers.
3. We estimated overwintering habitat use by three species of Pygoscelis penguins from colonies that are widely distributed across the Antarctic Peninsula.
4. We propose the following recommendations to policy makers and the scientific community that the area of Cape Melville should be classified as an Antarctic Important Bird and Biodiversity Area and that the threshold value (based on >1% of species) to establish an IBA for Antarctic shags should be changed to 122 to reflect the increased estimate of the global population of Antarctic shags.

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Department of Bioinformatics

Prof. dr hab.
Piotr Zielenkiewicz

The bioinformatics Department of IBB grows from a small group of theoretical biophysicists since 20th century eighties. The experience in application of by then existing computers and programs to molecular biology problems resulted in recognition of the group as the Polish center of bioinformatics expertise, nomination as national node of EMBnet by the Ministry of Science (in 1993) and creation of the first Bioinformatics Department in Poland (2000). Today, the members of the department continue to deal with a large variety of bioinformatics topics, often in close cooperation with experimental groups from and outside Poland.

The (first in Poland) Department of Bioinformatics was created on Jan. 1st, 2000. The Department evolved from a laboratory of theoretical molecular biophysics supervised by Prof Andrzej Rabczenko existing since 1980 in the Department of Biophysics (Head Prof. K.L. Wierzchowski). The scientific interests of this group concentrated on subjects classified nowadays as belonging to the bioinformatics domain such as sequence-structure relationships, protein-protein recognition, DNA conformation (pseudorotation of the furanose ring) etc.

Nowadays, we continue to apply *in silico* approaches to address molecular biology problems. Current scientific interests of today's Department of Bioinformatics include, but are not limited to:

- Protein-protein recognition and the design of protein-protein interaction inhibitors
- Macromolecular crowding
- Modeling and modifying the regulation of basic cellular processes and metabolic pathways
- Protein structure modeling
- Analyses of genome sequence data (particularly ? the search and characterisation of regulatory elements and phylogenetic analysis)
- Drug design and the development of drug design methodologies
- Analyses of protein interaction networks
- Molecular dynamics simulations of macromolecular structures.
- Literature mining

Left to right: Hubert Salamaga, Piotr Pawłowski, Kamil Steczkiewicz, Aizhan Rakhmetullina, Norbert Odolczyk, Szymon Kaczanowski, Piotr Zielenkiewicz, Tomasz Włodarski, Leszek Pączek, Piotr Włodzimierz, Mehmet Özhelvaci

In the recent years :

- We established the general methodology for the construction of protein-protein interaction peptide inhibitors and successfully applied it to a number of biologically meaningful examples
- We discovered novel potent deltaF508-CFTR correctors as potential drugs against Cystic Fibrosis (CF)
- We proposed a comprehensive, genome-wide model of translation.
- We described the role of plant miRNAs in the anti-inflammatory properties of selected edible plants and suggested possible roles of plant miRNAs in human genes regulation

We have licenses and use the following software tools for:

- Sequence and structure analysis (e.g., EMBOSS)
- Visualization and structure modelling (e.g., Chimera, YASARA, Swiss-PDB-Viewer, PyMol)
- Pathway and metabolic pathway modeling and analysis (e.g., Cytoscape, IPA, Gepasi)
- Docking (e.g., DOCK, Schroedinger).
- Molecular dynamics (e.g., AMBER, GROMACS)
- application of both (i) ligand-based and (ii) target-structure-based virtual screening for drug searches - home-developed AI tools: Kalasanty, PLEC, Pafnucy, DeCAF, RF-Score-VS, ODDT, tools4mirs

The Department employs 10+ researchers holding a PhD degree.

The dedicated infrastructure includes 1152 CPU cores (processors Intel Xeon

X5650 and AMD Opteron 6174, 2MB RAM/core), oraz 2), over 12000 cores available on GPU Nvidia Tesla K20 and Intel Phi 5110 coprocessors. The cluster includes 6 servers for CPU calculations (Eterio 217 RZ2, 2x AMD Epyc7763, 128 physical cores in total, 256GB RAM), 6 servers for GPGPU calculations (Eterio G127 RZ2, 1x AMD Epyc 7763, 512 GB RAM, 4x RTX3080 10GB) and 1 server dedicated to AI/Tensorflow calculations (Eterio G127 RZ2, 1x AMD Epyc 7763, 1TB RAM, 2x A100 40GB) and a master server (HP ProLiant DL380 G7, 2xIntel Xeon 5650, 96 GB RAM). All machines are equipped with IB HDR (100Gbit/s) and emergency network cards 10/1Gbps LAN links. The server has remote management compatible with IPMI 2.0 v 1.1 with a dedicated network port. The servers are connected to the IB Nvidia MSB7890-ES2F switch and the SX350X-24-K9-EU Ethernet switch.

Selected current international collaborations:

- Freie Universitaet Berlin, D
- INSERM, FR
- Cambridge University, UK
- Leiden University, NL
- Institute Pasteur, Paris, France
- Sussex University, UK
- EU-OPENSCREEN ERIC, EU
- Max Planck Institute of Plant Breeding Research, D

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Laboratory of Environmental and Evolutionary Systems Biology

Dr hab.
Urszula Zielenkiewicz

The main focus of our research is comparative and evolutionary genomics. We investigate various microbial consortia from natural and experimental environments and explore the history and evolution of apoptotic machinery. We look for novel antibacterial therapies based on the targeted inhibition of protein-protein interactions. We also search for possible links between plant miRNA and human health.

The group, established in 2021, derives directly from the former Department of Microbial Biochemistry, continuing dr. Piotr Cegłowski's research on plasmid biology. For many years, studies were focused on bacterial toxin-antitoxin (TA) systems, especially ε-ζ system of the pSM19035 plasmid from the clinical *Streptococcus pyogenes* strain. Their activation causes growth inhibition or cell death and contributes to effective plasmid inheritance. Our review paper, one of the first focused on plasmid addiction TA systems is still widely cited. We functionally characterized the ε-ζ system, demonstrating that ζ toxin is bactericidal for Gram-positive, bacteriostatic for Gram-negative bacteria, and toxic for yeast. We showed that the ε antitoxin is degraded by ClpXP protease. We also showed an *in vivo* interaction between ε and ζ *proteins*. The ε-ζ TA family members have been found on resistance plasmids in major human pathogens. Toxin-antitoxin systems are considered potential candidates for the development of targeted antibacterial drugs. The knowledge gathered from the ε-ζ system allowed us to develop a sensitive fluorescence polarisation assay that evaluates the effectiveness of protein-protein interaction inhibition. This test is currently used to search for molecules that may be useful for the development of therapies against strains that carry antibiotic resistance plasmids. The methodology of designing short peptides which potentially abolish selected protein-protein interactions is also applied in other approaches aimed at peptide-based therapeutics invention.

My interest in TA systems found at present a continuation in research related to theories of the origin of apoptosis, where

one of the proposed paths of its evolution is the addiction process hypothesis, which derives the origin of apoptosis directly from plasmid's toxin-antitoxin systems.

Actually, in cooperation with prof. S. Kaczanowski, we are exploring the history and evolution of apoptotic machinery. Apoptosis, often referred to as programmed cell death, is a fundamental process crucial to the development of multicellular organisms. This process, or a primordial form of it, is also observed in single-cell eukaryotes like yeast and protists. Phylogenetic studies have noted that apoptosis-initiating factors generally have a bacterial protomitochondrial origin. We performed ancestral state reconstruction through phylogenetic analysis of the genomic data and found that protomitochondrial apoptosis contained both caspases and meta-caspases, four types of apoptosis induction factors, fungal and animal mitochondrial proteases, and various DNases. To shed further light on the evolution of apoptosis, we investigated the functional conservation of apoptotic factors through a yeast complementation test. Each of the four apoptotic genes in yeast was replaced with bacterial and other eukaryotic orthologs. We found that distantly related proteins from protists, plants, animals, slime molds, and bacteria were largely able to functionally substitute for the original yeast proteins. The functional replacement of yeast genes by diverse orthologs provides empirical evidence that apoptotic mechanisms have likely been conserved since the time of mitochondrial domestication.

We hypothesize that an ancient evolutionary arms race between protomitochondria and host cells led to the establishment of the currently existing apoptotic pathways and we postulate that regulated cell death, oxidative respiration, and multicellularity in eukaryotes may have a co-evolutionary relationship that plays a crucial role in the evolution of complex life.

We are also interested in the overall mechanism of gene evolution by examining the results of experimental "in the lab" yeast evolution. The standard approach includes parallel evolutionary experiments, whole genome sequencing, mutation detection, and results confirmation. Through the specialised statistical analyses of different mutational events, we are looking for general principles of fixation of mutations. Recently, we showed that fixed or highly frequent gene loss-of-function mutations are almost exclusively adaptive. Our results pointed out that accumulating such mutations could be adaptive and selected in response to implemented conditions. This finding aligns with the classic "less is more" hypothesis, which states that loss-of-function mutations are fundamental for rapid adaptation to ecological conditions and carries important implications for interpreting other evolutionary experiments.

Our second line of research focuses on environmental studies, in particular on the structure and biodiversity of naturally occurring biofilms and specialised microbial consortia. We analyse metagenomes and the biodiversity of unique environments,

Multiplexed self-made chemostat (with controlled temperature, medium and air flow).

including post-mining and agricultural soils, sediments, historical silk textiles, and selected microorganism genomes.

We also investigate possible links between plant miRNA and human health. Recently, the possibility of cross-kingdom gene expression regulation by miRNAs from other species ("xenomiRs"), specifically from plants, has acquired scientific meaning. Following bioinformatic analyses, we evaluated by real-time qPCR the presence of five food-derived miRNAs in human breast milk. We confirmed that one of these plant miRNAs (miRNA172a) is able to modulate the expression of the human FAN (Factor Associated with Neutral Sphingomyelinase Activation) protein in eukaryotic cells. The potential of miR172a to mitigate the inflammatory process was tested in a mouse model of rheumatoid arthritis. Animal studies showed decreased oedema of inflamed paws in mice with rheumatoid arthritis model induced after treatment with miR172a. This subject will be developed more in the coming years.

The group closely collaborates with Prof. J. Rademann from Institute of Pharmacy, Freie Universität Berlin, Germany.

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Laboratory of Fungal Bioinformatics

dr hab.
Anna Muszewska, Prof. IBB PAS

The Laboratory of Fungal Bioinformatics studies evolutionary histories of multiple fungal traits. We associate trait evolution with knowledge of fungal ecology and adaptive capabilities. We work on publicly available information stored in diverse biological databases and use a wide set of computational tools to analyse them. We discovered ancestral traits such as cobalamin and fucose metabolism to be present in fungi.

The Laboratory of Fungal Bioinformatics studies the genome content and genome organisation, focusing on fungal trait history among diverse fungal lineages.

We live in the genomic era, which means that the whole genetic information of many species has been read. The genomic content is shaped by various factors, including the way how particular organisms live, reproduce and get their food. The adaptation of the organism to a given lifestyle shapes its genomic composition. Fungi are a diverse group of organisms; they are interesting for specialists but also important for the health and economy as pathogens of crops, and fermenting organisms. So far fungal studies focused on evolutionarily younger fungi, yeasts and moulds grouped together into Dikarya. Non-Dikarya fungi include flagellated aquatic ones, terrestrial fungi associated with insects and nematodes, many types of saprotrophs as well as arbuscular mycorrhiza plant symbionts.

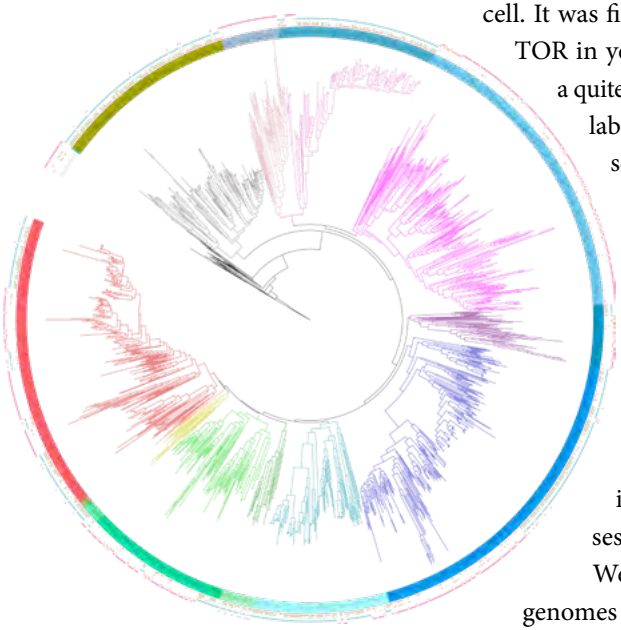
We describe how different groups of fungi cope with their environments and find the adaptative genomic features which separate one type of fungi from the other. We navigate the fungal tree of life keeping in mind that different fungal lineages group organisms living in different ways. During this journey, we look for genes and groups of genes which are specific to ancient fungi. We also describe their metabolic potential by predicting secondary metabolite candidate gene clusters. For instance, we predicted that all sequenced Mucoromycotina fungi possess a complex set of natural product gene clusters (for example terpenes). In consequence, the easy-to-culture and grow Mucoromycotina representatives could be

Left to right: Marianna Krysińska, Magdalena Płecha, Anna Muszewska, Aleksander Kossakowski i Drishtee Barua

employed for natural product screening and later may be used as biofactories. We have shown that some of the genes involved in lipid and sphingolipid/ceramide metabolism are multiplicated in lipid-producing fungi. Currently, there are attempts to use lipid-producing fungi to obtain valuable unsaturated fatty acids. Our results expand the list of candidate taxa and products which might be obtained from fungi, including sphingomyelin.

We described which genes are present and shared with animals in evolutionarily old fungi. We discovered the conservation of cobalamin (B12) and fucose metabolism, Fanconi anaemia DNA repair pathway and mTOR regulatory pathway among fungi. Previously, vitamin B12 was thought to be produced in bacteria and accumulated in animals and algae. According to the textbooks, plants and fungi are devoid of vitamin B12. Our findings contradict this knowledge and show a complete set of genes associated with cobalamin usage in a range of fungal lineages.

Fucose, a simple sugar, is a part of the sugar decorations of human red blood cells. In fungi, it is a component of the cell wall. However, the way it is produced, transported and degraded in fungi was unknown. Our proposed metabolic pathway shows ancestral similarities between animals and fungi. We found a difference between fucose usage in Dikarya and non-Dikarya fungi.



Maximum likelihood phylogenetic tree showing the diversity of A1 peptidases across Fungi.

Dikarya use fucose as a source of nutrients for metabolism, while the non-Dikarya depended on fucose as a building block and signalling compound.

The Fanconi anemia pathway was described based on genomic data obtained from patients suffering from a debilitating disease, which was further associated with a deficiency in repairing errors in DNA. The pathway is known to be present in animals, partially in plants and other organisms, but information on the fungal one was incomplete. Our analyses showed that the pathway is the most complete in non-Dikarya fungi and therefore could be functional.

Mammalian target of rapamycin (mTOR) is a key pathway for the energetics of the cell. It was first described with the name TOR in yeasts and later discovered in a quite distinct form in humans (and labelled mTOR). We have analysed almost two hundred fungal proteomes and surprisingly found that most non-Dikarya and many Dikarya have more in common with the mammalian version of the pathway rather than with the yeast one. This finding may have broad consequences in the biotechnological processes using fungi.

We sequenced and analysed genomes of fungi living together with associated bacteria. Such interactions may facilitate their survival in unfavourable environmental conditions. Together with the Laboratory of Fungal Biology and Julia Pawlowska's group at the University of Warsaw, we characterized several strains of Mucoromycotina and showed their broad potential to degrade sugars, lipids and proteinaceous substrates, most probably due to the associated bacterial activity.

Reports on the diversity and occurrence of low complexity regions (LCR) in Eukaryota are limited. Some studies have provided a more extensive characterisation of LCR proteins in prokaryotes. There is a growing body of knowledge about a plethora of biological functions attributable to LCRs. In terms of fungi, most studies are limited to model yeasts. However, it is hard to determine to what extent

observed phenomena apply to other fungi. We performed a survey of LCR regions in protein sequences across diverse fungi. We showed that the number of LCR regions and their abundance in protein sequences are positively correlated with proteome size. We observed that, even though most LCR are present in proteins that contain protein domains, they do not overlap with the domain regions. The observed amino acid distribution in LCRs deviates from the average amino acid distribution for the entire proteome. In LCRs there is a clear overrepresentation of amino acids with functional groups, predominantly negatively charged. Moreover, we discovered that each lineage of fungi favours distinct LCRs expansions. This again, as other traits, points towards a different evolutionary trajectory of each fungal group. Symbiotic fungi have LCRs in proteins linked to their ecology. For instance, cellulosome components in anaerobic rumen fungi harbour homopolymers.

We have also identified novel and missing components in the genomes of arbuscular mycorrhiza fungi. These fungi form interactions with approximately 70% of all plants on the Earth. These fungi have genomes with lots of genomic repetitive elements and we have discovered a novel class of those, which we named Glomhoppers. We have also detected that mycorrhiza fungi lack some of the key genome maintenance processes. Maybe the loss of some genomic control steps enables them to have multiple non-identical nuclei in a single cell.

Our results shed new light on how we understand the relationship between organisms' lifestyles and genome organization. On the practical side, we found novel fungal metabolic capabilities, which could have biotechnological applications.

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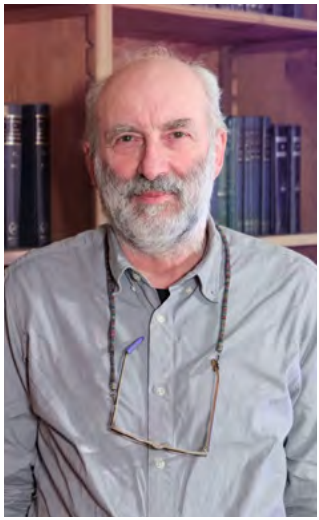
Laboratory of Mass Spectrometry

Prof. dr hab.
Michał Dadlez



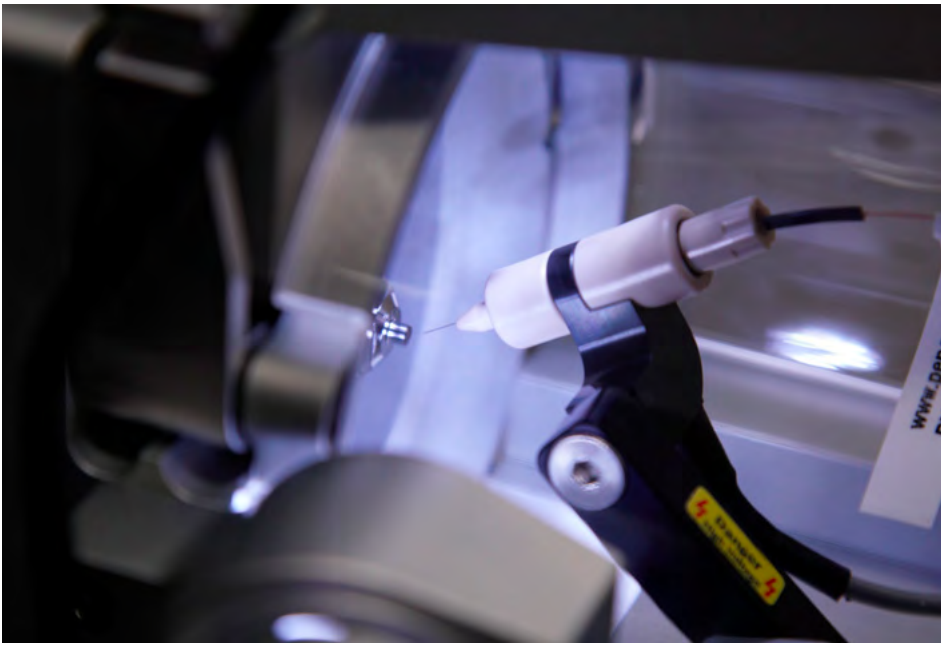
Mass spectrometry-based methods are used to provide structural information on protein systems, which due to their high dynamics cannot be analysed by traditional methods like X-ray or cryoEM. The major technique used is proton-deuterium exchange (HDX) monitored by mass spectrometry (MS), which gives unique insight into the realm of relatively unstable protein regions. Multiple protein systems are studied by the group and new HDX-MS data analysis tools are developed.

In recent years the dynamic aspects of protein structures became adequately appreciated, mainly due to the discovery of a class of highly dynamic proteins named intrinsically disordered region/proteins, which have revolutionised our understanding of new aspects of the protein "structurome". These extremely unstable proteins are not a rare exception, they constitute significant fraction of the protein world. It is common practice to exclude these regions in classic structural approaches, like X-ray or cryoEM, because regions of high dynamics are not tractable by these methods. Classical methods thus narrow our field of study, with obvious loss of knowledge of important aspects of protein biology, as regions of high dynamics were proven to have a set of distinct functions. Moreover, they are not structurally neutral, impacting structural properties of neighbouring regions, as well as stable regions. Therefore, it was important to develop new methods to obtain insight into the 'dynamic axis' of the protein structurome, which spans from complete disorder, through several levels of partial order, to very stable proteins. One of the most fruitful approaches, providing insight into the dynamic character of a protein main chain in native conditions, are measurements of the rates of exchange of protons to deuterium added to a solution (HDX). These measurements probe the stability of protein main chain hydrogen bonding networks, which become broken more often in unstable regions than in stable regions, along with their solvent accessibility. Therefore, they allow to map the stability of protein structural elements in their native context, either in a protein's 'apo' form or in the presence of various interactors. Experi-

Left to right: Bianka Świderska, Michał Kistowski, Ewa Sitkiewicz, Jacek Olędzki, Weronika Puchała MD, Michał Dadlez, Magdalena Bakun, Jakub Karczmarski, Emilia Samborowska, Mariusz Radkiewicz

mentally, the easiest analytical method that allows us to measure the kinetics of such exchange is mass spectrometry, constituting the methodology of HDX-MS. Also, it allows us to study proteins of full length, a condition very often not fulfilled for classic methods, with loss of biologically important information, as it is known that highly dynamic regions play important structural and functional roles.

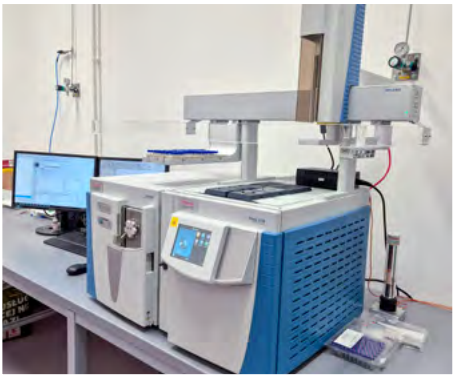
The Lab specialises in the application of HDX-MS for the studies of various protein complexes/assemblies, either as a method of choice or a method complementary to other, more classical approaches. During the experiment, a protein or complex is incubated in native buffer conditions in the presence of D_2O. At selected times, the exchange is blocked and the number of deuterium atoms that exchanged for main chain amide protons during the incubation period is measured precisely and reproducibly by MS. The results are then mapped onto the protein sequence or structural models, indicating regions of high stability (low exchange) and regions of more and more dynamics (faster and faster exchange). Differences in exchange between different states (for instance, between apo protein versus protein with ligand or protein partner) indicate possible regions of interaction. Results are also often interpreted by combining the fragmentary knowledge obtained before by classical methods for shorter, truncated protein variants.



The miracle of transfer of molecules from solvent to the gas phase by electrospray happens here – the ion source is a critical element of a mass spectrometer

Numerous protein systems/assemblies have been and are being studied in the group. In the case of the receptor of advanced glycation end-products (RAGE), we were able to map the interaction sites to the transmembrane region using a full-length protein variant, which enabled construction of a multimeric complex in the presence of one of its many ligands, S100B protein. For a ternary eEF1B translation initiation complex, we mapped interaction regions between each pair of the three components, revealing a unique multi-GEF domain assembly pattern. For intermediate filaments, again being able to work with full length protein versions of vimentin, we expanded existing fragmentary structural knowledge by mapping the regions engaged in structural formation of filaments, first at the stage of unit length filament and further on mature filaments. This led to a new model of pairing of vimentin tetramers in higher order structures, with very short elements engaged in their stabilisation. Similarly, we extended structural knowledge on centriole proteins Gorab-Sas6-Ana2, elements of histone pre-mRNA processing, chaperone-cochaperone-substrate complex, etc.

An important part of our Lab's activity is developing new software for HDX data analysis. Main-chain amide proton exchange in protein regions of different levels of stability proceeds with complex kinetics. To fully explore the datasets, and extract



Upper: A combination of two systems coupling liquid chromatography separation with mass spectrometry (LC-MS) provides enormous capacity of 24 h a day, 7 days a week automated sample analysis for proteomics, thanks to efficient auto-samplers (orange block in front).
Lower: Also molecules from the gas phase, volatile compounds can be analyses in GC-MS instruments.

all information, the shape of the deuterium uptake curves by time needs to be analysed in detail. For that purpose, we have designed and developed a set of freely accessible programs (https://hradex.mslab-ibb.pl/; https://compahradex.mslab-ibb.pl/) that characterise uptake in each protein region by the composition of fast, intermediate and slow regimes. The software proved to be very useful in interpreting HDX data in greater detail than routinely exercised.

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Laboratory of Genome Engineering

Prof. dr hab.
Matthias Bochtler

The group is interested in DNA, RNA and chromatin modifications, and their biological roles. For prokaryotes, we focus on the discovery of biosynthesis pathways and sensor domains of modified bases. For eukaryotes, we study the 5-methylcytosine oxidation for methylation reversal and the co-option of DNA repair pathways in this process, as well as biochemical mechanisms of positive epigenetic memory.

Genetic information is stored using a four-base alphabet, with the letters guanine (G), adenine (A), cytosine (C) and thymine (T). Both prokaryotes (bacteria) and eukaryotes (complex creatures like us) have learnt to modify this alphabet for other purposes, without compromise to its coding ability.

In prokaryotes, modifications of the adenine and cytosine bases by methyl groups are used to control the initiation of DNA replication, and a pathway of DNA repair known as mismatch repair, which corrects errors while genomic information is replicated. Moreover, nucleobase modifications are also used for the distinction between self- and non-self in the battle between bacteria and their viruses (bacteriophages). Canonically, the DNA modification is used as a mark of "self", its absence as a mark of "non-self", and the latter licenses DNA destruction. As always in warfare, any strategy of one adversary triggers countermeasures by the other. Hence, it is not surprising that in some instances the role of methylation is reversed, so that it serves as a mark of "non-self" and a license for destruction.

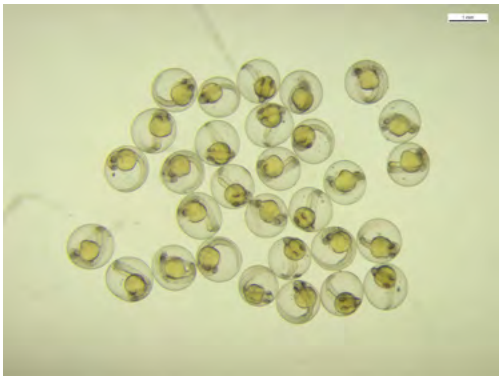
For the defence to be efficient, bacteria have evolved proteins or their fragments (domains) that very specifically bind modified DNA. In the last decade, we have extensively characterized such domains recognizing modified bases in the context of assembled DNA. Our work has uncovered protein modules that have not previously been implicated in the detection of modified nucleobases. We have also shown that many of the detector domains are more related to each other than previously anticipated.

More recently, the group has also started to work on the characterization of bio-

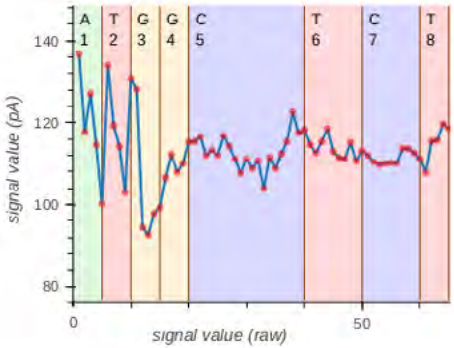
Left to right: Dominik Rafalski, Maciej Migdał, Honorata Czapińska, Matthias Bochtler, Magdalena Klimczak, Igor Helbrecht

synthesis pathways for more exotic DNA modifications, which are used by phages as a defensive measure against prokaryotic immune mechanisms. In the case of momylation, a DNA modification that has remained enigmatic for almost 50 years, we showed that phages can hijack host protein biosynthesis and harvest activated raw components to introduce DNA modifications that benefit the invader at the expense of the host. In the future, we intend to complement the work on DNA modification-based immunity in prokaryotes with studies of 'specific' immunity mediated by CRISPR systems and other non-restriction-modification defence systems.

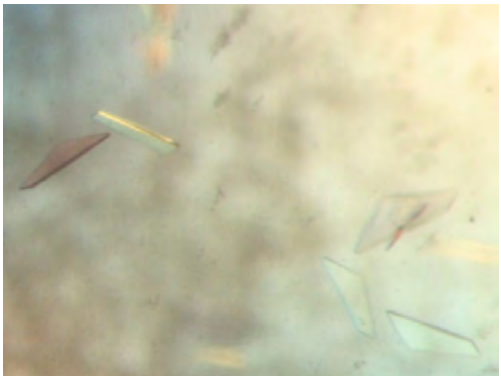
Only one of the DNA modifications present in bacteria, 5-methylation of cytosine, plays a significant role in higher eukaryotes. Together with histone marks, it is used to control gene expression, i.e. to determine which genetic information is transcribed at any given time. 5-methylation of cytosine has long been considered as an irreversible modification, which is acquired as cells commit to only one fate, to never come back. It is now clear that this picture is incorrect and that there are pathways for reversal of the methylation. In replicating cells, the reversal is relatively straightforward: failure to maintain the modification leads to its loss. This is called 'passive demethylation'. In terminally differentiated cells that have stopped dividing, passive demethylation is not an option. Instead, cells found a way to undo the methylation, by an 'active demethylation' process. Nature's strategy for active DNA demethylation is to co-opt, to the maximal extent possible, DNA repair pathways. In the first step, variants of enzymes that repair



The group uses zebrafish as an animal model



The group uses nanopore sequencing to directly detect DNA modifications



The group uses X-ray crystallography and cryo-EM to solve macromolecular structures

DNA, oxidize the 5-methylcytosine bases, to make them look like 'damaged' DNA bases. In subsequent steps, the 'damaged' bases are excised, leading to the eventual restoration of unmodified DNA. The lab is interested in all steps of this process. How are targets for oxidation identified? What are the relative contributions of DNA sequence and histone marks? And once oxidation has occurred, what are the pathways that replace the oxidized base (with its 'scaffold' 2′-deoxyribose) by an unmodified base (also with 2′-deoxyribose)?

While most of our work in eukaryotes is on DNA, we have also done some work on RNA modifications. Specifically, we were intrigued by RNA editing, which relies on adenine deamination to inosine to alter messenger RNA (coding for proteins) after it has been made by transcription. In mouse and man, this editing primarily plays a role in the distinction between an own and invading RNA, and hence experimental interference with the editing causes an immune phenotype. Intriguingly, one of the editing enzymes (ADAR) is expressed very early in the development of fish, long before it could play a role in immunity. In collaboration with the group of dr. hab. Cecilia Winata, we characterized the editing in early fish embryos, and could show that it plays a role in patterning. It is currently unclear whether the new role of RNA editing is unique to fish, or whether it is more general and also relevant for other organisms.

In the last five years, the lab has also extended the scope of its interests from

nucleobase to histone modifications. In eukaryotes, histones are the 'scaffolding' on which DNA is wrapped. The histones also double as an information storage unit. Chemical modifications to the histones, such as methylation or acetylation, serve to store biological information about the cellular state. Together with cytosine methylation, they control whether or not a given fragment of DNA should be transcribed. For cells to maintain their identity, it is critical that transcription promoting marks in so-called promoters and enhancers, which control gene expression, are accurately maintained. This requires feed-forward loops. Our work has uncovered the sensing part of the loops. We could show that so-called PHD domains recruit the COMPASS-like complexes, which deposit activating methylation mark on a specific lysine residue in histone 3 (H3K4), to maintain active promoters and enhancers.

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Laboratory of DNA Replication and Genome Stability

Prof. dr hab.
Iwona Fijałkowska

Mechanisms that maintain high-fidelity DNA duplication and repair are critical to handle the malfunction of replication forks or DNA damage. While mutations are essential for evolution or antibody diversity, in the short run they are deleterious and may cause human disease. Our research aim is to identify mechanisms and factors that ensure efficient and highly accurate DNA replication and influence genetic stability.

Our laboratory's bacterial team investigates factors that ensure high accuracy of DNA replication using *Escherichia coli* as a model organism. Apart from the well-known mechanisms of correct base pairing and the proofreading activity of the replicase as well as mismatch repair, replication fidelity also depends on replicase dissociation and subsequent DNA polymerase exchange. However, high DNA replication fidelity means choosing not only the right base but also the right sugar. Ribonucleotides are misincorporated into DNA much more frequently than mismatched nucleotides and the complete mechanism of their removal by ribonucleotide excision repair (RER) was first described only in the previous decade.

Recently, we have been investigating how two cellular endoribonucleases, RNases HI and HII, that excise poly- and monoribonucleotides from DNA, respectively, influence DNA replication fidelity. We have shown that the repair of ribonucleotides incorporated into both DNA strands in *E. coli* is unequal. The leading DNA strand is mostly subject to the RNase HII-dependent RER mechanism, while on the lagging strand this pathway cooperates with RNase HI-dependent RER and nucleotide excision repair to efficiently remove ribonucleotides from DNA. We propose that the engagement of RNase HI on the lagging DNA strand, which results in removal of single ribonucleotides despite RNase HI lacking such activity *in vitro*, is a consequence of its participation in Okazaki fragment maturation. Our conclusions are in line with previous results from our group showing that the DNA lagging strand is replicated with higher final fidelity than the leading strand. Notably, under certain

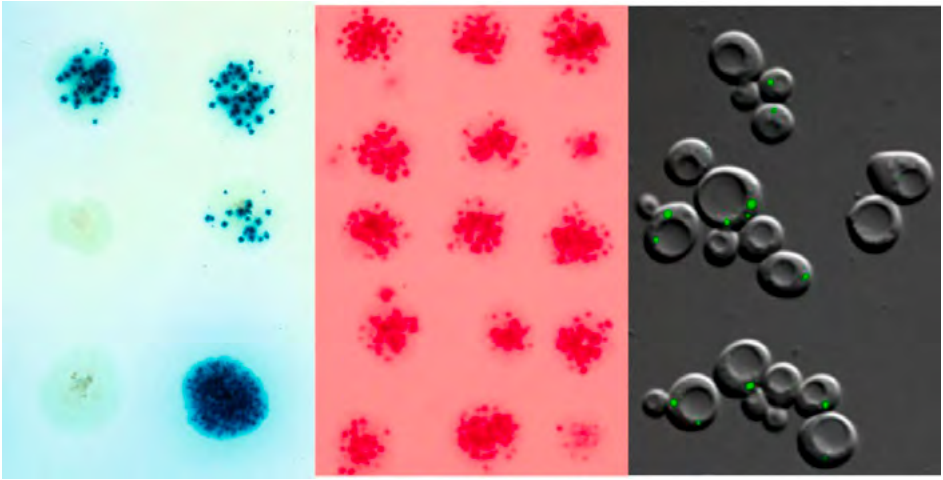
Left to right: Deepali Chaudhry, Michał Dmowski, Iwona Fijałkowska, Alina Krasilia, Karolina Makieła-Dzbeńska, Krystian Łazowski

conditions, high RER activity can decrease mutation rates in living cells.

Another important activity of RNase HI in the cell is removal of undissociated transcripts in front of the replication fork (R-loops). Lately, we have been investigating novel roles of RNase HI in controlling the mechanism and fidelity of leading-strand synthesis that is related to efficient repair of R-loops.

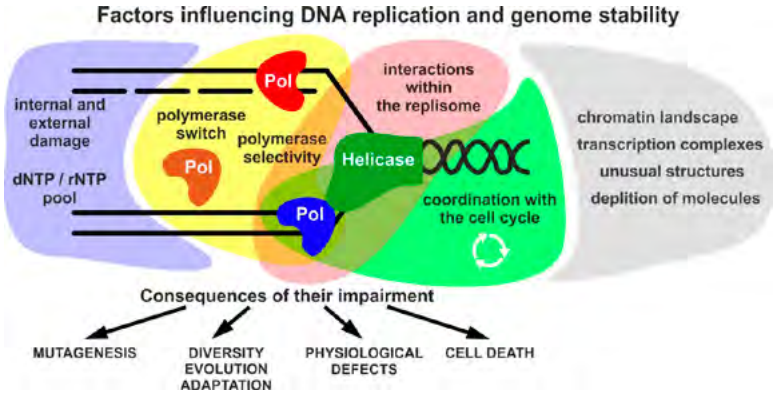
Our studies are enabled by the fact that, unlike in eukaryotes, in *E. coli* both DNA strands are replicated by the same replicase. Thus, we are able to study factors influencing the fidelity of DNA replication that stem from the fundamental differences in the mechanism of leading- and lagging-strand synthesis, which are universal for all living cells. Hence, our work has potential to be translated into eukaryotic research.

We also use the yeast *Saccharomyces cerevisiae* as the model organism to investigate factors involved in the maintenance of genome stability in eukaryotes. We are particularly focused on non-catalytic subunits of the replisome – the multiprotein complex responsible for DNA replication. Its essential components are the helicase which unwinds the DNA, and three highly specialized DNA polymerases. The replisomes act in tight coordination with the cell cycle. Our analyses of yeast cells with defective variants of non-catalytic subunits of the CMG helicase or DNA polymerase epsilon (Polε) show that impaired interactions within the replisome are the source of increased spontaneous mutation rates, instability of the DNA repeat tracts, and slower progression through the cell cycle. Recently, using specific genetic tests, we demonstrated



Left: Visual scoring of Lac+ *Escherichia coli* papillae growing on the surface of a colony on XPG plates; Middle: *E. coli* colonies with red-colored papillae (Gal+ revertants) on McConkeyGal plates; Right: *Saccharomyces cerevisiae* cells expressing *YFP* gene in translational fusion with *RFA1* encoding a subunit of RPA which binds to single-stranded DNA (ssDNA).

that defective functioning of CMGE or Polε alters the canonical division of labor between particular replicative DNA polymerase. In consequence, Polδ, which is the main lagging DNA strand replicase, replaces more often Polε, which performs the bulk of replication of the leading DNA strand. Finally, detailed transcriptomic analysis of yeast cells entering a new cell cycle demonstrated significant changes in the expression of numerous genes



Schematic presentation of issues that are the subject of research in the Laboratory DNA Replication and Genome Stability

in cells with impaired Polε subunit. The list includes genes encoding cyclins and other proteins involved in the regulation of the cell cycle and DNA replication. This analysis also enabled identification of genes whose expression, after entry into a new cell cycle, increases faster or slower compared to wild-type cells. Therefore, we have shown that defective functioning of non-catalytic subunits of the replisome has severe effects on genome stability and cell physiology.

Since replisome components are highly conserved in eukaryotes from yeast to humans, our results provide insight into the understanding of DNA replication mechanisms in human cells. Importantly, recent studies reported correlation of mutations or changed expression of genes encoding non-catalytic subunits of the replisome with human disorders, especially cancer. Therefore, recently, we extended our investigations to human cells in order to characterize the consequences of altered expression of and mutations within genes encoding helicase and polymerase non-catalytic subunits in human cells. This will allow us to comprehensively analyze the effects of defective functioning of these subunits, a putative target in cancer therapies, and will provide new insights into our understanding of their involvement in carcinogenesis and other diseases.

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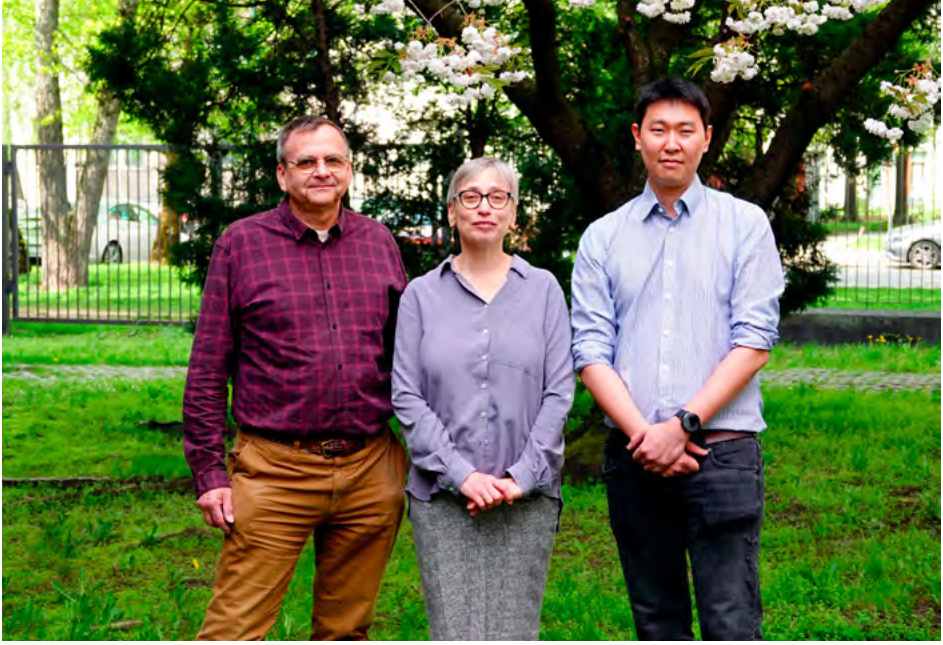
Laboratory of Genome Stability Mechanisms

Dr hab.
Adrianna Skoneczna, Prof. IBB PAS

Genome stability mechanisms are crucial for the proper functioning of every cell, ensure DNA damage repair, avert chromosomal aberrations and prevent aging. These mechanisms' dysfunction in humans causes aging, cancer, or neuronal and congenital diseases. During stress, efficient genome maintenance mechanisms secure survival. The same applies to all cells, so we use the budding yeast as a cell model in our research.

The integrity of the genome is constantly challenged by exogenous or endogenous threats. Endogenous stress can result from cellular metabolism byproducts (e.g., reactive oxygen species generated by dysfunctional mitochondria); it can also result from the dysfunction of molecular mechanisms that are responsible for cell protection, including damage recognition, stress signaling, stress response, checkpoint activation, damage repair, damaged molecules removal, autophagy induction, and finally if there is no chance for cell functions' to recover, execution of apoptosis.

Despite the long history of genome stability-linked research, many undiscovered mechanisms involved in genome maintenance remain to be revealed. Our previous work permitted us to show the existence of genome maintenance mechanisms that are specific for cells of particular ploidy (i.e., the number of complete sets of chromosomes in a cell). We employed functional genomics methods to investigate the causes of the genome instability of *Saccharomyces cerevisiae* diploid cells and cellular mechanisms protecting the cellular genome against double-strand break stress. The use of a yeast knock-out collection and Agilent platform-based microarray technology allowed us to provide evidence of the involvement of vesicular transport in the maintenance of genome stability. We proved that the role of vesicular transport is not limited to the control of uptake and detoxification of genotoxic compounds. Vesicular transport also influences many pathways that ensure genome maintenance. These include the transduction of signals of DNA damage, the activation of cellular

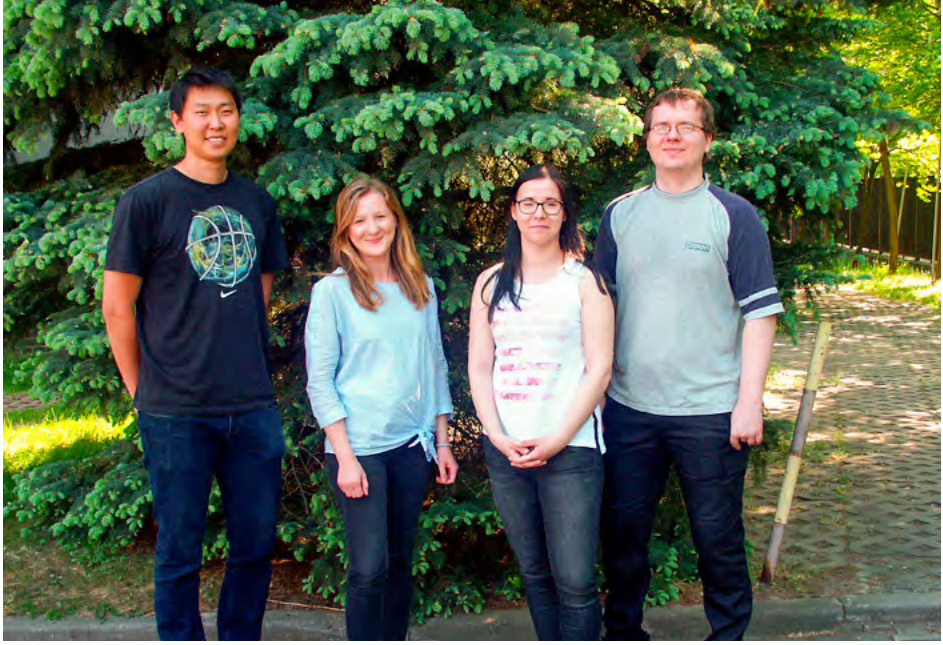


Left to right: Marek Skoneczny (the permanent collaborator, IBB PAS), Adrianna Skoneczna, Tuguldur Enkhbaatar

defense mechanisms, regulation of the efficiency of DNA damage repair, and exit from cell cycle arrest. We also showed that the main problem of diploid cells during genotoxic stress is maintaining ploidy, avoiding genome rearrangements, and sustaining the length of repetitive sequences present in the genome (e.g., rDNA region encoding rRNA genes or telomeres).

Our results showed that the published information does not always fully agree with the biological truth. For example, we showed that not only the shortening of telomeres, which was already known to limit cellular lifespan, but also increased telomere length may cause physiological defects. We showed that cell ploidy can shift with time. The results we obtained contradicted the previously believed rule that the number of extra-chromosomal rDNA circles (ERCs) increases with time and thus might be used as a hallmark of aging. We actually showed that while rDNA repeat numbers might shift with time, especially during stress conditions, the number of ERCs does not always correlate with a shortened lifespan. We also revealed that cells might choose to use illegitimate recombination instead of faithful homologous recombination's sub-pathways when the DNA damage level is high and finishing the replication is unlikely; even such repair does not assure long existence or even ensure proficient next division.

In a research line concerning chronological aging, we detected the importance of initiating replication mechanisms for lifespan and proved that lowered expression of genes encoding proteins involved in the initia-



The current and previous lab members, left to right: Tuguldur Enkhbaatar, Anna Długajczyk, Justyna Antoniuk-Majchrzak, Kamil Król

tion of replication results in chronological lifespan elongation. This might be partially explained by the delay in the respective cell cycle phases, likely linked to observed DNA repair system imperfections in the tested strains. However, we also noticed significant changes in the chemical molecule content of the analyzed cells.

Our observation that the cells having problems with the replication start overproduce Rad51 recombinase under genotoxic stress conditions led us to discover that such cells accumulate replication-borne double-strand breaks and preferably use Rad51-dependent illegitimate recombination to allow cells survival. These results prompted us to further research how the Rad51 recombinase level is regulated. We proved the posttranslational modifications of Rad51 recombinase with ubiquitin and SUMO not only determine the protein half-life in the cell, as Rad51 is degraded via ubiquitin-dependent proteolysis by 20S proteasome but also influence recombinase activities during homologous recombination repair. The preliminary data suggest the regulation of Rad51 recombinase half-life and the way of its control might vary depending on the stress applied.

Presently, we focus on the indication of the amino acid residues within Rad51 recombinase that, when post-translationally modified, affect Rad51 recombinase molecular functions, e.g., its DNA binding properties, ATPase activity, or abilities to build the filamentary structure.

Asking questions about various mechanisms of control of homologous recombination sub-pathways usage, we are exploring

the regulation of Srs2 protein level, i.e., DNA helicase/Rad51 translocase, as the balance between Rad51 and Srs2 protein is believed to decide on the homologous recombination sub-pathway choice. Our data suggest that the control of Srs2 protein levels occurs at different expression levels, among others, through posttranscriptional regulation.

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Laboratory of DNA Segregation and Life Cycle of Proteobacteria

Prof. dr hab.
Grażyna Jagura-Burdzy*

Our research group is interested in the intricate mechanisms governing DNA segregation, cell division, and survival in *Pseudomonas aeruginosa*. Through a combination of genetic, molecular biology, and next-generation sequencing techniques, we aim to uncover novel insights into bacterial adaptation, resistance mechanisms, and potential therapeutic targets, ultimately advancing antibacterial strategies.

Single bacterial cells harbor complete instructions for thriving in diverse environments, including the human body, necessitating faithful DNA transmission during cell division. Exploring the interdependence between DNA segregation, bacterial cell division, and cellular adaptation is a compelling research area. Our lab focuses on exploring the molecular mechanisms involved in DNA segregation, cell division and survival of *Pseudomonas aeruginosa*, an opportunistic pathogen known for its extreme adaptation potential and intrinsic antibiotic resistance. Alongside other ESKAPE group pathogens, *P. aeruginosa* is a leading cause of hospital-acquired infections, posing particular risks to immunocompromised individuals and cystic fibrosis patients. Our studies focus on understanding of its resistance and adaptation potential associated with multilayered regulation, including the components that are also required for the faithful transmission of DNA material.

Historically, the research in the group began more than 20 years ago with investigations of the mechanisms involved in maintenance of plasmids, which are small, circular DNA molecules, replicating independently of the chromosomal DNA. In this line of research, the mechanisms that plasmids use to distribute between the daughter cells during division, spread between bacteria, and adapt to phylogenetically distant hosts were characterized. This contributed to the understanding of the process of horizontal-gene transfer the process by which genetic material is transferred between organisms, separate from the vertical inheritance of

* Prof. Jagura-Burdzy has been retired since July 2023. The laboratory is currently under supervision of the Director of Science IBB PAS

Left to right:Weronika Czekała, Barbara Domańska, Aneta Bartosik, Adam Kawałek, Shweta, Filip Pośnik, Asha Ajithakumari Sobhanakumar

genes from parent to daughter cell. Simultaneously, research into the principles of *P. aeruginosa* chromosome segregation during cell division was initiated, and has been the main interest of the lab over the years.

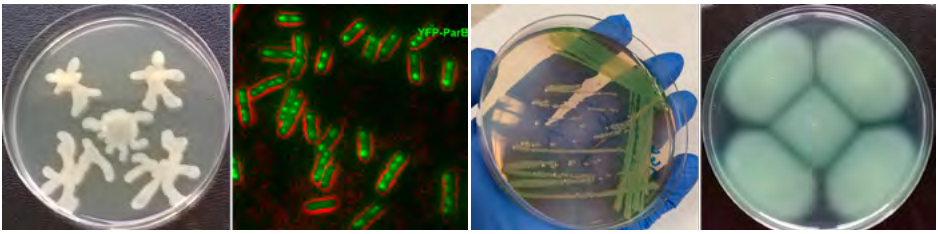
P. aeruginosa possesses a single, circular chromosome, which encodes an active partitioning system that is homologous to plasmidic Class IA systems. It consists of ParA (a Walker-type ATPase), ParB (a DNA binding protein) and *parS*s, centromere-like sequences that are recognized and bound by ParB. Numerous aspects of ParAB-*parS* functioning in *P. aeruginosa* have been studied within our group. *P. aeruginosa* mutants lacking *par* genes, or *parS* sites, were constructed and the consequences of these changes for cell biology were analyzed. Additionally, interactions of ParA and ParB with proteins involved in the cell cycle, cell division, metabolism, motility, antibiotic resistance, and virulence were also identified and studied. Our recent analyses confirmed that MksBEF proteins become crucial for DNA segregation in cells lacking a ParAB-*parS* system. We have shown that the action of this enigmatic condensin--like complex involves cooperation with topoisomerase IV, explaining its role as the second DNA segregation mechanism in *P. aeruginosa*.

A significant part of the research conducted within the group also involves use of next-generation sequencing-based techniques. ParB binding to the genome was analyzed using chromatin-immunoprecipitation and sequencing (ChIP-seq), which demonstrated ParB nucleoprotein formation around *parS*-sites. This analysis also showed, for the first time, binding of

ParB proteins to numerous half-*parS* sites, containing a sequence motif representing one arm of the *parS*. Using transcriptome analyses, we demonstrated the ability of ParB to directly repress the expression of certain operons and postulated a broad, but indirect role for ParB in gene regulation. Using chromosome conformation capture with sequencing (3C-seq), we showed multifaceted influence of the ParAB-*parS* system on the spatial organization of the *P. aeruginosa* chromosome. ParB was critical for loading of SMC condensin (structural maintenance of chromosomes protein) on DNA, whose function is to bring together opposite arms of the circular chromosome. The genome structure analyses also showed the presence of a ParB-dependent loop bringing together the origin of replication (the region where the DNA replication starts, also including the *parS* sites) and the region containing the operon encoding a toxin--antitoxin system, critical for formation of antibiotic-tolerant subpopulations of cells called 'persister' cells. This finding suggests an intricate relationship between DNA organization and cellular processes crucial for bacterial survival.

The absence of Par proteins triggered significant expression changes of numerous genes encoding uncharacterized proteins with predicted roles in gene expression regulation. An extensive analysis of five transcriptional regulators encoded by PA2121, PA2577, PA3973, PA3458 and PA3027 was conducted, in order to understand their biological function and possible link with DNA segregation. The genes under their control were established, and extensive phenotypic analyses were performed, including for example phenotype microarrays. Strikingly, the analyzed proteins were involved in regulation of diverse vital cellular processes, including the tricarboxylic acid cycle, glycerolipid and amino acid metabolism, and transport or osmoprotection functions.

Another independent research line was devoted to characterization of Nudix proteins from *Pseudomonas* species to decipher their role in virulence and metabolism of bacteria. Participation of Nudix hydrolases in intracellular redox balance maintenance and in response to oxidative stress, resistance to β-lactam antibiotics and virulence of *Pseudomonas* spp., was demonstrated.



Collection of images showcasing our research model

Currently, our research focuses on:

1. Further characterization of the mechanisms involved in *P. aeruginosa* chromosome segregation and their connections with other processes in the cell.
2. Adaptation of the CRISPR interference (CRISPRi) method for functional analysis of *P. aeruginosa* genes and use in studying mechanisms of bacterial resistance to antimicrobial compounds.
3. Characterization of genes encoding drug/ /metabolite transporters (DMTs) and selected transcription regulators to shed light on their role in physiology, drug resistance and virulence of *P. aeruginosa.*

Our long-term goal is to identify potential targets that, when inactivated, limit the survival of bacteria and could be used in antibacterial therapies.

In our research we have adapted and use various genetic and molecular biology techniques, e.g. phenotype analyses, fluorescence microscopy, RT-qPCR, RNA-seq, ChIP-seq, 3C -seq, Tn-seq or CRISPRi-seq. The last technique has not previously been used in this species. In our lab, vectors and protocols were optimized for CRISPRi-mediated gene silencing in *P. aeruginosa*. The performance of the system was determined by silencing of the reporter genes, essential genes, and genes involved in antibiotic resistance. Recently, a protocol for highly efficient preparation and analysis of pooled *P. aeruginosa* CRISPRi libraries was also established. As CRISPRi-seq offers a powerful platform for quantitative assessment of the significance of essential cellular processes, we are expecting to uncover essential genes and pathways in which, for example, inactivation enhances the killing by known antibiotics.

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Laboratory of Bacteriophage Biology

Prof. dr hab.
Małgorzata Łobocka

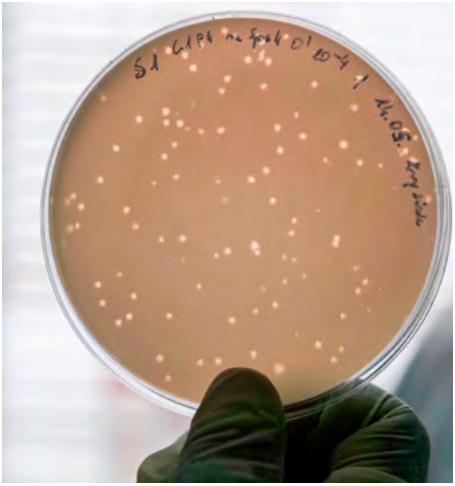
The main objects of our studies are bacteriophages that can infect several strains of taxonomically different bacteria. We try to elucidate what underlies the wide host range of these phages, what are their strategies to propagate in various hosts, and to evade phage-defense mechanisms. We are especially interested in phages of therapeutic use and in P1 phage of human microbiota that can spread virulence traits.

The object of our studies are bacteriophages - viruses that specifically infect bacteria. They are the most numerous entities on our planet. Their largest and the most ancient group, which evolved before the diversification of bacteria and archaea, are phages of complex virion structure. They are composed of a tail, and a head which encapsidates double-stranded DNA. The sizes of their genomes vary between a dozen to over 300 kb. The biology of these phages and their interaction with bacteria in natural environments have started to be uncovered only recently. Certain of them can only propagate by lytic development killing their bacterial host at the release of progeny phages, and could be used as antibacterial agents in the therapy of infections. Certain others can alternatively remain in a cell in the form of DNA that is either integrated with a chromosome or maintained as a plasmid. They can encode features adaptive for bacteria, including virulence or antibiotic-resistance determinants, and are the main factors of horizontal gene transfer between bacterial strains.

Our interest are bacteriophages of wide host range, especially those that infect pathogenic bacteria of the ESKAPE group, which are known for their rapid acquisition of antibiotic resistance and pose a serious risk to public health. We characterized, at the level of genomic sequence, host specificity, and certain other properties, more than 30 phages of wide host-range that infect *Staphylococcus*, *Enterococcus, Pseudomonas*, *Bacillus* or enterobacterial strains, and verified potential applicability of these phages or their selected proteins to antibacterial therapies. Despite genomic similarities between phages within the same genera, many

Left to right: Aleksandra Krysiak, Małgorzata Łobocka, Łukasz Kałuski, Stephen Amankwah

of them differ in their strain specificity, lytic efficacy and other types of interaction with their hosts. Our attempts to identify these differences in detail and to understand the mechanisms that are responsible for them is based on comparative studies of infectivity of similar phages to bacterial strains of genomically diversified panels and on comparative studies of phage-bacteria interactions. The external environment for these interactions in the case of human or animal pathogens are the bodies of infected organisms. Thus, some comparative studies of phage therapeutic efficacy are performed under *in vivo* conditions, in the *Caenorhabditis* model of staphylococcal infection that was originally developed in our laboratory. One of our main goals is to correlate the observed differences between phage physiology and phage-bacteria interactions with the genetic differences between phages as well as between their various hosts. This could help in future in the prediction of bacterial susceptibility to particular phages, and in the design of phage genome modifications in response to therapeutic needs.



Plaques formed by a phage on the layer of *Staphylococcus* cells

Among major objects of our interest are staphylococcal obligatorily lytic phages of wide strain range within the *Staphylococcus aureus* species. Those of large (~140 kb) genomes belong to the *Kayvirus* genus, while those of small (~17 kb) genomes belong to the *Rosenblumvirus* genus. We demonstrated for the first time that the strategy of these phages to protect their bacterial hosts from

Stephen Amankwah, Aleksandra Krysiak, Łukasz Kałuski

eradication caused by phage-mediated lysis is the formation of so called phage carrier state populations (PCSP). In PCSPs, phages coexist with unstable phage-resistant bacteria and at least a small fraction of phage-sensitive bacteria that serve as a constant source of newly released phages. We showed that the ability of staphylococcal phages to form PCSPs does not preclude their therapeutic efficacy in the nematode infection model. Moreover, major factors that determine the resistance of bacteria to phages of *Kayvirus* and *Rosenblumvirus* genera differ, providing a rationale for the use of combinations of these phages in antistaphylococcal therapies. The correlation of a *Rosenblumvirus* infectivity for various *S. aureus* strains with certain DNA sequence features allowed us to demonstrate the avoidance of restriction sites for staphylococcal type I restriction-modification (R-M) systems as the main basis of wide strain-specificity of this phage. To the contrary, the genomes of *Kayvirus* genus phages contain numerous sites recognized by the type I R-M systems. We identified the genes of these phages that may encode antirestriction functions. They are currently under studies.

Functions of over half of *Kayvirus* phages genes are unknown, which is a main obstacle in the wide therapeutic use of these phages. Thus, we have undertaken systematic studies aimed to identify these functions. We demonstrated that the only kayviral protein similar in structure to an *S. aureus* virulence factor, autolysin IsaA, does not contribute to virulence of *S. aureus* cells that express its gene. Instead, it is involved in phage lytic development and functions as an additional phage endolysin. Our ongoing studies on kayviruses aim to functionally characterize a subset of kayviral genes of unknown function and to identify the genomic determinants of *S. aureus* strains that are responsible for the resistance of certain of these strains to particular kayviruses. By analogy to other phages, the kayviral genes that are expressed early during phage development should encode host takeover functions, and functions responsible for overcoming bacterial anti-phage defense.

We cloned over 40 kayviral early genes in a plasmid. A two-component vector system developed in our laboratory allows one to introduce them to clinical *S. aureus* isolates of different clonal complexes. Testing the phenotype of *S. aureus* cells that express them will help to identify those that encode proteins targeting essential staphylococcal functions redirecting bacterial metabolism towards the production of progeny phages.

A temperate phage of wide-host range whose genomic sequence we determined in the past is P1. This large phage of 95 kb genome can infect various strains of *Enterobacteriaceae* and *Rhizobiaceae* family and is maintained in lysogens as a circular plasmid. Thus, it was included to a separate group of mobile elements designated as phage-plasmids (P-Ps). Recent metagenomic studies revealed the common presence of P1-derived plasmids and P-Ps in environment, in plants, and in the microbiota of humans and animals. Moreover, P1-derived elements appear to be one of the main factors responsible for the spread of resistance to new-generation of beta-lactams among enterobacterales, making P1 a phage of clinical importance. We developed a simple method of efficient targeted mutagenesis of the P1 genome. Phenotypic analysis of P1 mutants depleted of potential lytic genes and their combinations allowed us to identify new lytic enzymes encoded by P1 and to show that the complexity of P1 lytic functions plays a role in the adaptation of this phage to infect various hosts. The initiation of productive phage infection is associated with the redirection of bacterial metabolism and intracellular stress. Phage-encoded mechanisms that prevent the stress-induced apoptosis of infected cells are essential for infection spread and contribute to the phage host range. Putative components of anti-phage defense avoidance systems that were predicted by us in the proteomes of analysed phages will be studied in detail in the near future in parallel with studies on relevant bacterial stress-response mechanisms that are part of anti-phage defense.

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Laboratory of Mutagenesis and DNA Damage Tolerance

Prof. dr hab.
Ewa Śledziewska-Gójska

We investigate processes that affect DNA stability by regulating DNA damage tolerance pathways via translesion synthesis (TLS), with a special focus on TLS DNA polymerases in yeast and mammalian cells. We also investigate DNA damage avoidance via recombination mechanisms. Within the scope of our interest are also processes involved in maintaining the stability of mitochondrial DNA.

The integrity of genetic information is constantly threatened by DNA damage, which often remains unremoved before the start of DNA replication. The maintenance of genome functionality, despite the presence of DNA damage or defects in DNA replication machinery, depends on the so-called DNA damage tolerance processes. These processes are conserved in all organisms and prevent genome rearrangements that are responsible for the acceleration of aging or can lead to cancer development and premature death.

The leading role in DNA damage tolerance is played by DNA polymerases (Pols), which are capable of translesion synthesis (TLS). Among TLS Pols, the main roles are played by the Y-family Pols and Polζ, a B-family member. For several years, we have investigated the regulation of the flag member of the Y-family Pols, Polη. Polη is responsible for the tolerance of a number of DNA lesions, especially those that are caused by ultraviolet radiation, and its activity in human cells protects against XP-V cancer predisposition. Polη (as well as other TLS Pols) can function in an error-prone manner. Both decrease and increase in Polη abundance cause genetic instability and the regulation of this enzyme is important. A method that was developed by our group for the detection of the native form of this Pol in yeast cells allowed us to establish that the cellular abundance of Polη is regulated in the cell cycle at the level of protein stability, leading to Polη accumulation in the G2 phase. Our recent results have shown that this regulation is modulated differently depending on which DNA damage response sub-pathway is activated by a given genotoxic

Left to right: Martyna Latoszek, Aneta Kaniak-Golik, Agnieszka Hałas, Ewa Śledziewska-Gójska, Justyna McIntyre, Ebru Yazici, Shadi Setayeshi.

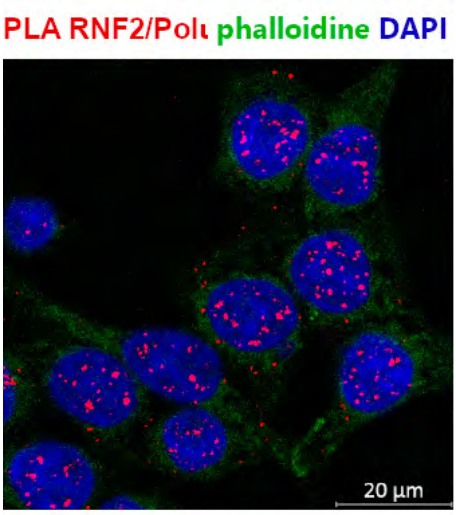
stress, and that this alternative regulation is common for all Y-family Pols.

In human cells, in addition to Polη, functions also its closest paralogue, Polι. It is the most mutagenic of all known mammalian DNA Pols. Our goal is to discover a specific cellular role of Polι and determine the mechanisms lying beyond the regulation of this enzyme. We have recently revealed that Polι deficiency leads to alleviated levels of spontaneous mutagenesis, indicating that in normal conditions Polι is an error-prone enzyme and underlining thus the necessity of its strict control. In our research we identified several regulatory enzymes that interact with Polι and modify the protein, and/or affect the cellular level of the polymerase. We have shown that Polι not only interacts with the p300 acetyltransferase, but is also a subject of p300-dependent acetylation. Interestingly, this acetylation is highly specific for Polι, suggesting that it can be a key factor in the discovery of a specific cellular function(s) of this enzyme. We have identified ubiquitin ligases that interact with Polι and can directly or indirectly affect its cellular destiny. One of them, RNF2, protects Polι from destabilization, however, the cellular significance and consequences of this relation require further investigation.

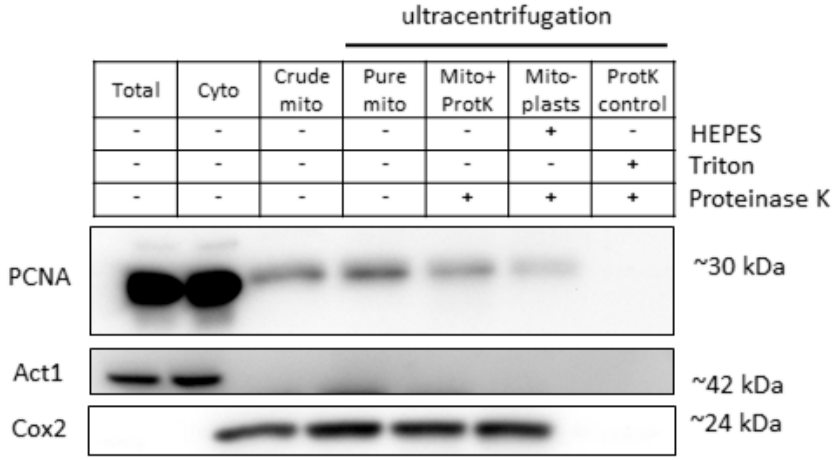
Another TLS polymerase that our research focuses on is Polζ. The activity of this polymerase is responsible for a part of spontaneous and the majority of DNA damage-induced mutagenesis in eukaryotic cells. The lack of Polζ leads to embryonic death. We analyzed the functional consequences of a REV3L-T2753R mutation that was identified in a child with developmental delay, hypotrophy and dysmorphic features. This is the first point mutation that had been identified in the gene that

encodes the enzymatic subunit of Polζ with specific disease consequences in humans. Using a yeast model, we have shown that an equivalent mutation increases the lethal effect of DNA-damaging agents and causes an instability of mitochondrial DNA.

We analyze also the mode of action of TLS polymerases in mitochondria (mt). It is well established that upon UV-induced damage TLS Pols participate in a non--mutagenic bypass of lesions in mtDNA. Recruitment of TLS Pols to DNA in the nucleus requires ubiquitination of K164 of the DNA replication processivity factor, PCNA. However, the bulk of mtDNA replication is mediated by the replicative high-fidelity Polγ that does not require PCNA for replication processivity and none of the many mitoproteome investigations showed a presence of PCNA in mitochondria so far. We have recently shown that, contrary to expectations, yeast PCNA is localized in mitochondria and the K164R substitution in PCNA leads to an increase of UV-induced point mutations in mtDNA.



Visualisation of interaction between endogenous Polι and RNF2 by proximity ligation assay (PLA).



PCNA is located in the mitochondrial matrix. Cellular fractionation of mitochondria by ultracentrifugation. Cyto – cytoplasmic/non-mitochondrial fraction; mito – mitochondrial fraction; protK – proteinase K

This strongly suggests the role of PCNA in the antimutator activity of mitochondrial TLS Pols. Consistently, our recent results indicate an involvement of PCNA and Polη in the same pathway of error-free tolerance of UV-induced damage in mtDNA. Additionally, our results indicate that PCNA and RNAseH1 function in two independent pathways protecting mtDNA against mutations induced by ribo-photoproducts.

As an alternative to TLS, DNA damage can be tolerated by damage avoidance via a template switch (TS). This process is initiated by polyubiquitination of PCNA by the Mms2-Ubc13-Rad5 complex. Our study showed that the activity of this complex was not limited to the TS. We have found that it stimulates general gene conversion and that PCNA SUMOylation inhibits this recombination process. Additionally, our genetic studies suggested that the proteins of this complex play additional roles in controlling genetic stability. We discovered a novel, non-canonical function of Mms2 that is linked to a new pathway that controls spontaneous mutagenesis. We have shown that Mms2, previously known only as a co-factor of Ubc13, can independently of Ubc13 cause a reduction in the cellular abundance of replicative Polδ, leading to more frequent replacement of this polymerase by the error-prone Polζ and, in consequence, an increase in mutagenesis. We identified ubiquitination enzymes and other factors affecting this process. The mechanisms that are involved in this new process are currently investigated.

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Laboratory of Bacterial Drug Resistance

Dr hab.
Izabela Kern-Zdanowicz

Our research goes in two directions. First, we analyze bacterial plasmids responsible for the dissemination of antibiotic-resistance genes in populations of clinically important bacteria. We characterize the conjugation systems and plasmid impact on bacterial hosts. Second, we characterize the genes of *Streptococcus anginosus*, a human commensal that is very diverse in terms of gene content, which enable them to become invasive.

Infections caused by antibiotic-resistant bacteria are a severe problem of contemporary medicine. It is estimated that by 2050, up to 10 million people a year could die due to practically incurable infectious diseases unless fundamental progress is made. The rise of resistance to therapeutically important agents and its spread among bacteria forced the World Health Organization to set a list of antibiotic-resistant "priority pathogens" according to the urgency of the need for new antibiotics. The most critical group comprises Gram-negative bacteria resistant to antibiotics used in therapies against multiresistant strains: *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* family bacteria (e.g. *Escherichia coli*) resistant to carbapenems, as well as *Enterobacteriaceae* producing the extended-spectrum β-lactamase (ESBL) and therefore resistant to the newer β-lactams and cephalosporins. All mentioned bacteria acquired their resistance (resistance genes) due to the mobility of plasmids.

Plasmids are DNA molecules widespread in bacteria, which can replicate in host cells independently of the bacterial chromosome. They bear genes encoding antimicrobial resistance, virulence or metabolic pathways. They are part of a mobile gene pool available for bacteria, which can be moved in a specific manner called conjugation. DNA of a conjugative plasmid is transferred from a donor to a recipient cell after physical contact between them is set, due to the activity of a specialized proteic machinery called a conjugative transfer system. Transfer of conjugative plasmids greatly enhances their impact on the genetic plasticity, metabolic potential, and environmental adaptability of

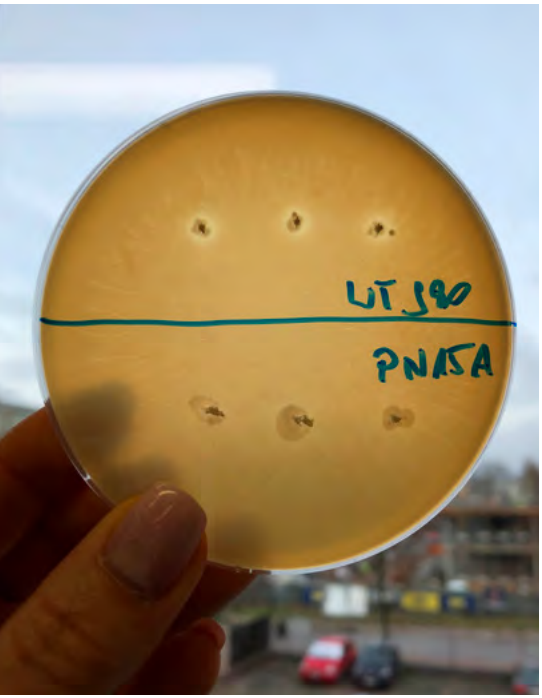
Left to right: Izabela Kern-Zdanowicz i Aleksandra Kuryłek

bacteria. It is critically important in hospitals, where large amounts of various antibiotics are used, and the selection of bacteria resistant to these antibiotics is ongoing. Thus conjugative plasmids are a source of serious clinical and epidemiological problems.

Conjugative transfer systems encoded on the majority of large plasmids present in bacteria recognize each plasmid-free cell as a potential plasmid recipient. There are three types of the conjugative transfer system in Gram-negative bacteria: F-, P- and I-type. The first two are well known for their organization and regulation. The third, I-type, is poorly characterized, and its regulation is practically unknown. A single bacterial cell can bear plasmids of several types. A conjugative plasmid can "lend" its conjugation machinery to another non-conjugative plasmid possessing certain specific traits, and mobilize it for transfer.

Genes encoding extended-spectrum β-lactamases (ESBLs) are often encoded within conjugative plasmids, which in large part are responsible for resistance of *Enterobacteriaceae* species to newer β-lactams. The epidemiology of ESBLs was dominated in the 1990s by the extremely rapid and worldwide spread of bacteria that produce enzymes of the CTX-M family, both in nosocomial and community environments. In Poland, the first gene of the $bla_{\text{CTX-M}}$ type, $bla_{\text{CTX-M-3}}$, was identified in 1996 in *Citrobacter freundii* and *E. coli* clinical isolates in Warsaw. It was associated with a conjugative 90-kb plasmid, named pCTX-M3, that conferred resistance to penicillins, cephalosporins, aztreonam, and aminoglycosides. Later, the rapid spread of $bla_{\text{CTX-M-3}}$ among *Enterobacteriaceae* species was observed all over the country, and it has been mainly attributed to the conjugative transfer of this plasmid.

Only a few major plasmid groups are responsible worldwide for antibiotic resistance gene dissemination in *Enterobacterales*. One of them is an IncM group, to which pCTX-M3 belongs. IncM plasmids are vectors of not only $bla_{\text{CTX-M-3}}$, but also $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, $bla_{\text{IMP-34}}$ (encoding metallo-β-lactamase, carbapenemase, and imipenemase, respectively), the aminoglycoside resistance gene *armA*, and others.



Virulence factors of *Streptococcus anginosus* – among other factors enabling virulence, bacteria produce DNases, DNA-degrading enzymes. High molecular weight (hmw) DNA was added to the solid medium, the medium was inoculated by stabbing with the *S. anginosus* clones. After the bacteria had grown, hmw DNA was precipitated with trichloroacetic acid. If the bacteria in the streak have degraded hmw DNA, there is a transparent halo around the streak (upper range). From the transposon insertion library, we have selected and tested *S. anginosus* clones unable to degrade hmw DNA (lower range).

We have sequenced the pCTX-M3 plasmid (AF550415) and analysed its conjugative transfer system. This system is encoded in two regions, *tra* and *trb*, and comprises a specific sequence where the DNA transfer begins (*oriT*). It is classified as the I-type. pCTX-M3 can be transferred by ca. 10% of an *E. coli* donor cell population under optimal conditions on a solid surface, and less efficiently in liquid culture. We made a deletion analysis of 27 *tra* and *trb* genes and found important differences with another I-type plasmid, suggesting differences in the organization and function of conjugation machinery encoded by these two plasmids. We have identified two regions encoding transcriptional regulators of conjugative transfer genes. We determined the host range of the pCTX-M3 replicon which is limited to *Enterobacteriaceae* (part of Gammaproteobacteria), and the very broad range of recipients of its conjugation system, comprising not only representative of Alpha-, Beta-, and Gammaproteobacteria but even Firmicutes (*Bacillus subtilis* and *Lactococcus lactis*). The research on the plasmid's impact on the host is ongoing.

DNA transfer tools based on conjugative plasmids enable gene manipulation, even in strains of clinical or environmental origin, which are often difficult to work with. Therefore, using pCTX-M3 transfer genes, we constructed a biotechnological tool – a helper plasmid and helper *E. coli*

strains that mediate the mobilization of $oriT_{pCTX\text{-}M3}$-bearing plasmids, with an efficiency up to 1,000-fold higher than the efficiency achieved with native pCTX-M3. This tool has many possible applications.

The other line of our research concerns analyses of the virulence of *Streptococcus anginosus* which, together with *Streptococcus intermedius* and *Streptococcus constellatus*, constitutes the Streptococcus anginosus group (SAG), which have long been considered to be commensals that inhabit mucosal membranes of the human body. However, in recent years, much data appear to indicate links between SAG members and health problems in humans, including abscess formation and pulmonary infections, especially in immunocompromised patients. *S. anginosus* has been associated with dental, brain, liver, and spleen abscesses, pulmonary infections in patients with cystic fibrosis, and empyema. Therefore it can be recognised as an opportunistic pathogen. We have constructed a library of an invasive *S. anginosus* strain isolated from blood. This is the collection of *S. anginosus* clones where each strain contains a single-site transposon insertion in its chromosome. First, we have determined the set of genes that are essential for living in *S. anginosus* under optimal lab conditions by analysing the transposon-integration sites in the whole libraryusing transposon directed insertion sequencing (TraDIS). We have looked for the genes not represented in the TraDIS approach. Then, the library was challenged with complete human blood to determine which genes are necessary for survival in such conditions. The results were depleted by the set of essential genes. As a result, four *S. anginosus* genes were identified. The mutants in each of these genes were constructed and challenged in blood – each of them survived in blood ca. two times less efficiently than the original strain. The mutants will be verified for their virulence capacities, in tissue cultures, in *Galleria mellonella* larvae, and finally in mice. The library was also challenged with human endothelial cell culture. The genes important for adhesion were identified. Those mutants will be constructed and analysed.

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Laboratory of Fungal Biology

Prof. dr hab.
Joanna Kruszewska

Our research is in three main subjects. First, we are looking for factors that enable the transition of the fungus *Candida albicans* from a commensal to a pathogenic form in order to find molecules that that can prevent it. Second, we conduct environmental research related to fungal microbiomes, their modification and their impact on crops and the environment. Third, we are also developing possibilities of using fungal vaccines for waste disposal.

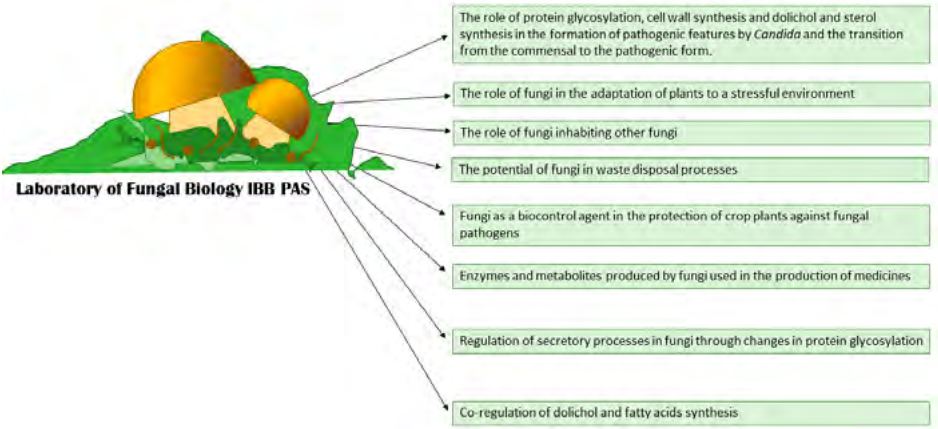
Candida albicans transition from commensal to pathogen

C. albicans is a common component of human microflora and the most common cause of opportunistic fungal infections in immunocompromised patients, with a mortality rate as high as 30–50%. Better understanding of the vital processes in Candida that contribute to its pathogenicity should improve our knowledge of fungal pathogenesis with obvious therapeutic benefits.

The pathogenic potential of C. albicans is attributed to several its features including expression of adhesins (heavily glycosylated proteins), the yeast-to-hyphae transition, and biofilm formation. Our study revealed that all these processes can be affected by changes in protein glycosylation, cell wall synthesis, and stress resistance. The participation of these processes in the transition of Candida from a commensal to a pathogenic form underscores the importance of studying them in detail. When Candida attacks a host, the fungus changes from its yeast-like state to hyphae and forms a biofilm. We have found that transition from commensal to pathogen is influenced by protein glycosylation and synthesis of dolichol.

DPM synthase from Saccharomyces cerevisiae is a complex of two proteins

In a wide range of organisms, dolichyl phosphate mannose (DPM) synthase is a complex of three proteins, Dpm1, Dpm2 and Dpm3. However, in the yeast S. cerevisiae it was believed to be a single Dpm1 protein. The function of Dpm3 is performed in S. cerevisiae by the C-terminal transmembrane domain of the catalytic subunit Dpm1. The regulatory Dpm2 protein has not been found



in S. cerevisiae. In our study we showed that in fact the Yil102c-A protein interacts directly with Dpm1 in S. cerevisiae and influences its DPM synthase activity. Deletion of the YIL102c-A gene is lethal and this phenotype is reversed by the dpm2 gene from Trichoderma reesei. Functional analysis of Yil102c-A revealed that it also interacts with glucosylphosphatidylinositol-N-acetylglucosaminyl transferase (GPI-GnT), similarly to DPM2 in human cells. Taken together, we have shown that Yil102c-A is a functional homologue of DPMII from T. reesei and DPM2 from humans. We also proposed a new structure of DPM synthase in S. cerevisiae.

Environmental fungi

Native and alien Antarctic grasses as a habitat for fungi

Biological invasions are now seen as one of the main threats to Antarctic ecosystems. An example of such an invasion is the non-native grass Poa annua which recently colonized the H. Arctowski Polish Antarctic Station area, previously occupied only by native plants like the Antarctic hair grass Deschampsia antarctica. To adapt successfully to new conditions, plants cooperate with soil microorganisms, including fungi. The aim of this study was to determine which fungi cooperate with D. antarctica, and which ones cooperate with the novel alien grass P. annua. We also investigated whether the cooperating fungi aided the grass adaptation to the difficult Antarctic environmental conditions. We found that D. antarctica recruits selectively a subset of fungi from the surrounding soil (preferably Ascomycota), while P. annua recruits indiscriminately the locally dominant fungal species. The fungi grown directly from the roots of Poa hydrolyzed proteins more effectively liberating a large nitrogen pool, compared with the fungi grown from the Deschampsia roots. In addition, three fungi recruited by Deschampsia have earlier been found to be pathogenic. Our study suggests that extensive cooperation with the

recruited fungi could be the reason for the P. annua's success in effectively colonizing the Antarctic environment.

Summer truffle (Tuber aestivum Vittad), a microhabitat, hosting bacteria and filamentous fungi

Truffles belonging to the Ascomycota phylum form ectomycorrhizae with trees. Some Tuber species produce edible fruiting bodies with a unique flavor and texture that are commercially valuable. We presented a microbiome analysis of the gleba of T. aestivum fruiting bodies harvested in southern Poland. Fungal and bacterial communities residing inside fruiting bodies have been proposed to contribute to the characteristic flavor, which may vary quite substantially between specimens.

Practical use of fungi

Biocontrol activity

Some Trichoderma spp. have natural abilities to reduce plant fungal diseases through their mycoparasitic and antagonistic properties. We have created new strains of Trichoderma atroviride with increased antifungal activity by increasing protein glycosylation activity at various stages of this process.

Left to right: Anna Janik, Urszula Perlinska-Lenart, Joanna Kruszewska, Sebastian Piłsyk

A protective effect of Trichoderma on bean germination and bean seedlings growth in soil infected with Pythium ultimum. (P1 – control -T. atroviride P1; new strains – T. atroviride P1 mutant with elevated glycosylation of secretory cellulases) resulted in higher cellulolytic activity. The enhanced biocontrol effect against Pythium, which has cellulose in its cell wall, is due to the increased cellulolytic activity of Trichoderma mutants. The higher activity of cellulases secreted by the mutants resulted from increased glycosylation of secretory proteins.

Sewage utilization

Municipal sewage is treated in treatment plants. The end products are water returned to the environment and the sediment that is produced as waste and must be disposed of. So far, the dominant and cheapest method was agricultural development and use for recultivation and sending to landfills. Since 2016, a storage ban has been in force. The National Waste Management Plan indicates thermoutilization as the preferred method of management, but it generates high costs because sludge, even dewatered mechanically, contains approximately 80% water. Sludge can become a valuable biofuel, but it requires drying, which requires additional energy. We proposed the use of fungal inoculations to grow sewage sludge and change its structure, which will enable natural drainage.

Enzymes and metabolites

Fungal environmental isolates, inhabiting feces of ruminants, exhibit significant and unique enzymatic activities that degrade polysaccharides other than cellulose. These fungi are also a valuable source of secondary metabolites. Our research has shown that these metabolites have antibacterial, antifungal and oncostatic properties.

We also conduct research on the use of fungi to produce valuable pharmacogenic substrates, which are too scarce on the market, which limits the production of some drugs.

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Laboratory of White Biotechnology

Dr hab.
Anna Sikora, prof. IBB PAS

The scientific scope of the Laboratory of White Biotechnology focuses on

- **anaerobic digestion (AD) as a source of gaseous biofuels, and other bioproducts in the light of sustainable development within basic research, R&D, and implementation works; and**
- **analogies between AD and human gut microbiomes as a reflection of the ubiquity of microbial interactions and fermenting activity in anaerobic environments.**

Anaerobic digestion (AD) is a four-step (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) microbial process for conversion of organic matter to biomethane (bioCH_4) and carbon dioxide in anaerobic environments. Smart approaches allow the recovery of biohydrogen (bioH_2) and other valuable bioproducts in addition to biogas. We have selected and characterized bioH_2- and bioCH_4-yielding anaerobic microbial communities and elaborated an innovative two-stage system for bioH_2 and bioCH_4 production based on AD of by-products from the sugar industry. In the first stage, a bioH_2-rich fermentation gas is produced in an anaerobic packed-bed reactor (PBR) by acidic fermentation (mainly dark fermentation, DF) of the substrate. The bioreactor filling package facilitates the formation of biofilm and granule-type structures by microorganisms, which increases the efficiency of fermentation processes. Subsequently, the acid effluent from dark fermentation processes is methanized in an up-flow anaerobic sludge blanket (UASB) bioreactor.

The main problem in the implementation of dark fermentation as a method of bioH_2 on an industrial scale is the lack of stability of the process. Our system allows molecular analyses of AD microbiomes including interactions between microorganisms and metabolic pathways of bioH_2 and bioCH_4 production. We showed that optimal conditions for bioH_2 production require a specific balance between dark fermentation bacteria and lactic acid bacteria (LAB) to induce the transformation of lactate and acetate to butyrate, bioH_2, and carbon dioxide, an important reaction of bioH_2 production. It is a phenomenon

Left to right: Marta Paul, Anna Sikora, Elżbieta Grzesiuk, Anna Detman-Ignatowska

called cross-feeding of lactate recognized in healthy human gut microbiomes resulting from symbiotic interactions between LAB and butyrate-producers. Using Clostridium butyricum as a new model, we have proposed an updated metabolic scheme of lactate and acetate conversion to butyrate that involves a flavin-based electron bifurcation mechanism. The source of inoculum, bioreactor design, package material, organic loading rate, and hydraulic retention time determine the balance between individual bacterial groups and a stable bioH_2 production process.

Within the search for factors improving bioH_2 production during acidogenesis, we demonstrated the beneficial effect of biochar on DF efficiency. Biochar ensures the provision of an active surface for the development of microorganisms, the release of macro- and microelements enriching the culture medium, and the absorption of elements adversely affecting the development of micro-organisms.

We identified yeasts as factors inhibiting bioH_2 production during acidogenesis. They cause metabolic shifts towards the synthesis of lactic acid and ethanol, decrease pH, and secrete metabolites inhibiting the growth of DF bacteria. We isolated from the DF bioreactor and characterized a new strain of Kazachstania humilis (K. humilis MAW1), including its genome sequence and physiological aspects. The revealed ability to inhibit bacterial growth has not been previously recognized in this species.

We also developed a new model to study the effects of acidogenesis products on the course of acetogenesis and methanogenesis:

processing of artificial substrates imitating the products of different types of acid fermentations by methane-yielding microbial communities in a continuous system. Lactate and acetate determined the acetotrophic pathway whereas butyrate and propionate the hydrogenotrophic pathway of CH_4 synthesis with a moderate influence on the final bio-CH_4 yield. The results are relevant since lactate, acetate, butyrate, and propionate are universal products of acidogenesis, regardless of the feedstock. A multi-omics approach allowed for the reconstruction of metabolomes and characterizing their metabolic potential in terms of the ability to methanize lactate, butyrate, propionate, and acetate in bioreactors.

We found the final digestate after anaerobic digestion of by-products of the sugar beet industry as a valuable product. We evaluated its fertilizing properties with particular attention paid to the plant growth indicators, plant and soil elements status, soil microbiome dynamics, and activity in terms of nitrogen cycling. The main hypothesis of this research assumes that the use of this digestate as a soil fertilizer will positively affect soil microbiome and soil nutrients availability, finally accelerating plant growth.

The two-stage system of bioH_2 and bioCH_4 producing based on AD of by-products from the sugar industry has been demonstrated in three different scales, on three different technology readiness levels (TRL): bench scale TRL III, 10-fold enlarged TRL IV, and quarter-technical TRLV. The latter two were done in cooperation with the industrial partner, Krajowa Grupa Spożywcza S.A. (KGS S.A.) in Dobrzelin Sugar Factory. The developed know-how based on biological knowledge deepened by the research team of the Laboratory of White Biotechnology IBB PAS and the ready-made infrastructure built by the entrepreneur (KGS SA) offers the prospect of implementing a new technology for producing alternative energy sources from the sugar beet industry by-products.

As a part of the Laboratory of White Biotechnology, activity was performed in cooperation with KGS S.A. towards the evaluation of methanogenesis processes in the anaerobic digester of a wastewater treatment plant in the Dobrzelin Sugar Factory and modernization works to use biogas for energy production. The produced biogas is agricultural biogas, meeting all the parameters required for the proper operation of the cogeneration engine. The gained energy ensures energy self-sufficiency of the wastewater treatment plant.

Various options for bioreactors used in anaerobic digestion studies.

Finally, we notice (i) the versatility of fermentation processes and analogies between anaerobic digester and the gut microbiome; (ii) the importance of fermentation processes in digestive systems for health and diseases. Therefore we have been starting a new research scheme in our laboratory concerning healthy microbiome and dysbioses. There are studies on the dynamics and metabolic activity of the fecal microbiota outside the gut in in vitro cultivation and elaboration of the conditions for rapid laboratory screening tests supporting the diagnosis and treatment of inflammatory bowel diseases (IBD). Many diseases are accompanied by dysbioses and consequently changes in the metabolic activity affecting the host organism.

Future plans include:

(i) expanding the range of substrates used for bioH_2 and bioCH_4 production;

(ii) adding a photo-fermentation node as an additional source of bioH_2 to the two-stage system for bioH_2 and bioCH_4 production;

(iii) studies on mechanisms maintaining a specific balance in the microbial community favoring bioH_2 production and explanation of mechanisms of biochar on stimulation of bioH_2 production;

(iv) development of work on the application of the final digestate after anaerobic digestion of waste from the agri-food industry contributing to zero-waste and circular economy;

(v) research on inhibitory properties of yeasts against bacteria and interactions between bacteria and yeasts, this subject is relevant to food technology, human health (probiotics), and antibacterial therapies;

(vi) studies on fermenting potentials of fecal-derived microbial communities from healthies and patients suffering from IBD and other diseases such as diabetes, obesity, allergies, and neurological or psychiatric disorders.

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Laboratory of Applied Microbiology

Dr hab.
Tamara Aleksandrzak-Piekarczyk

The Laboratory of Applied Microbiology focuses on the applications of bacteria in biotechnology and to improve human and animal health. We actively search for strains and their metabolites that exhibit beneficial properties, aiming to harness them for both biotechnological innovation and improvements in health outcomes, and finally translating these discoveries into practical solutions.

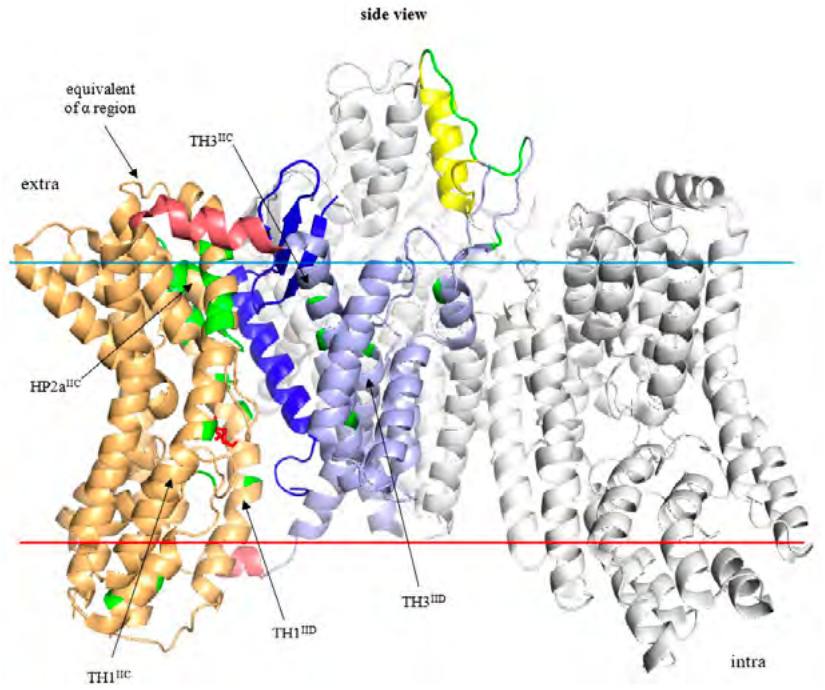
Among our diverse research initiatives, we devote considerable attention to investigating the biodiversity of natural bacterial strains and bacteriophages, probiotic features and molecular mechanisms of bioactivity of lactic acid bacteria (LAB) on model tissue cultures and animals, identification of novel compounds with antipathogenic, antitumor and/or immune system-stimulatory activity, genetic modifications of bacteria for organic acid bioproduction, LAB plasmidomes and designing vectors for heterologous protein expression.

For a number of years, our studies included the molecular mechanisms of sugar metabolism in lactic acid bacteria (LAB), characterization of their plasmidomes, bacteriophages infecting LAB and more recently, the biology of toxic bacterial peptides—bacteriocins. The last subject includes studies on bacteriocin purification, functional genetics, gene regulation and quorum sensing, receptor identification, and research on their mechanisms of action and resistance development. Recently, we have focused on understanding how bacteriocins interact with receptors on target cells and how these interactions lead to bacterial destruction. The gathered knowledge is important for the development of bacteriocins for safe and effective applications, both as food preservatives and as new drugs. We are increasingly interested in applied research, particularly the development of bacteriocins as drugs to combat antibiotic-resistant pathogens and as anti-tumor agents. In our study, we demonstrated that the mannose-specific phosphotransferase system (Man-PTS) is involved in the interaction of a number of structurally and functionally distinct bacteriocins, refuting the previously prevailing thesis that non-homologous bacteriocins with diffe-

Left to right: Anna Santo, Tamara Aleksandrzak-Piekarczyk, Agnieszka Szczepankowska

rent activity spectra interact with distinct membrane receptors of bacterial cells. We postulate that this interaction is possible due to differences in amino acid sequence/ /Man-PTS structure, which determine the selectivity of binding of specific bacteriocin groups. We are currently investigating the molecular basis of bacteriocin resistance induction among susceptible strains. We have shown that the presence of bacteriocins can induce mutations in four-component systems, resulting in increased resistance (so-called gain-of-function mutations). These mutations also lead to cross-resistance to peptide antibiotics, but in some cases have the opposite effect, leading to susceptibility of the strain to the antibiotic. Our group is constantly searching for new bacteriocins and unravelling their molecular mechanisms of action. By elucidating the intricate interplay between bacteriocins and bacterial targets, we aim to identify potent antimicrobial agents with broad-spectrum activity, offering promising solutions for the development of novel therapeutic interventions to combat multidrug-resistant pathogens.

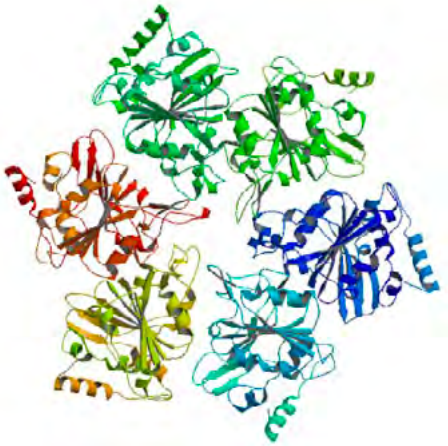
Another aspect of our research is broadly understood molecular microbiology, biochemical and genetic mechanisms related to the probiotic features in bacteria and their potential influence on humans/animals. These interests are primarily in the area of probiogenomic characterization of newly isolated environmental strains of lactic acid bacteria, including identification of key metabolic pathways, adherence to biotic and abiotic surfaces, resistance to salinity and acidification, bacteriocin production and antimicrobial activity, antibiotic resistance, and genetic identification of bacteria



Localization of Man-PTS Lactococcus lactis residues (in green) required for sensitivity to bacteriocin GarQ (navy blue)

isolated from animal gastrointestinal tracts and other natural environmental niches. By screening the most beneficial probiotic strains on model cell lines in vitro, we have identified strains which hold promise for the development of novel therapeutic strategies targeting colorectal cancer. This work is part of the framework of comprehensive analyses of the potential of bacterial strains in the development of innovative microbial therapeutic products. Research on the mentioned strains resulted in a Polish patent (P.436821) and a European patent application.

Our current plans aim to investigate the complex interplay between bacteria and



Homo-hexamer of Lactococcus phage F13 DNA helicase GP46 protein (generated by Swiss Model)

other gastrointestinal or immune-related disorders, such as celiac disease. Through probiogenomic analysis, we have recently identified specific bacterial species and enzymes that may contribute to the pathogenesis of celiac disease, offering valuable insights into the underlying mechanisms of this autoimmune disease.

Beyond our focus on human health, we also explore the biotechnological potential of bacteria, using the metabolic capabilities of lactic acid bacteria for the production of propionic acid. This application highlights the versatility of bacterial systems in bioprocessing and chemical production, offering sustainable alternatives to traditional manufacturing methods.

Lastly, we explore multiple aspects of bacteriophage biology, starting from infection, through anti-phage defense systems to functional analysis of unexplored phage functions. By tracking the dynamic interactions between bacteria and phages, we examine the intricate systems and proteins involved in lytic phage replication. Our work in this field has resulted in identification and molecular characterization of a DnaB-like helicase protein, the first of its kind to be described for lytic Lactococcus phages (Photo 4).

Collectively, our multifaceted research initiatives underscore our dedication to advancing scientific knowledge and translating discoveries into tangible benefits for human health and biotechnological innovation.

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Laboratory of Lactic Acid Bacteria Biotechnology

Dr hab.
Magdalena Kowalczyk

Our laboratory's research focuses on biodiversity and environmental interactions within complex ecosystems, with a special emphasis on lactic acid bacteria (LAB). We are interested in LAB interactions with other microbes and different environments they colonize. The long-term objective is to create knowledge-based probiotics beneficial to human health and bacterial cultures for various biotechnological purposes.

The study of biodiversity in complex ecosystems, particularly focusing on bacterial interactions within the environment, is a pivotal aspect of microbial ecology and has practical applications in selecting strains for probiotic purposes and industrial processes. The use of both culture-dependent and -independent methods allows for a comprehensive analysis of microbiota in food samples. The aim of our research is to characterize LAB isolated from various environments in terms of their possible use as starter cultures/production strains in industry and probiotics.

Our former research on various food ecosystems, including raw milk, kefir grains, traditional Polish cheese - Oscypek - and different wholegrain sourdoughs, has led to the isolation and genetic characterization of numerous LAB strains. These strains have been deposited in the Central Collection of Strains of the IBB PAS "COLIBB" and are used to screen bacteria for potential probiotic and protechnological properties. The analysis of wholegrain sourdough samples, for instance, has led to the selection of specific LAB strains optimized for the production of functional bread. These strains not only enhance the bread's nutritional value but also inhibit the formation of resistant starch by producing exopolysaccharides (EPSs), thereby improving the bread's overall quality. The development of starter cultures and the associated sourdough and wholemeal bread technologies were awarded four patents.

Our recent research on *Weissella* sp. isolates led to the selection of the *Weissella cibaria* strain IBB3394 with particular industrial traits: exceptionally high efficiency of dextran production without cytotoxicity, acidifying activity, activity against spore-forming bac-

Left to right: Julia Kopczyńska, Magdalena Kowalczyk, Olha Kostiuchenko

Bioreactor for controlled growth of bacteria

teria undesirable in bread (*Bacillus cereus*, *Bacillus brevis*, *Lysynibacillus fusiformis*) which was used to compose starter culture for sourdough bread production demonstrating beneficial technological activities. The same strain was also characterized in terms of health-promoting properties for probiotic and anticancer applications. The IBB3394 strain, apart from dextran production, was shown to present anti-staphylococcal activity, anti-proliferative activity against human colorectal adenocarcinoma cells (Caco-2), the ability to survive in conditions imitating the digestive tract and confirmed safety of use (acceptable level of antibiotic resistance, no antibiotic resistance genes, no virulence genes). These results were considered innovative and the appropriate patent application was sent to the Patent Office.

The adaptation of LAB to various niches within the host organism is a complex process influenced by their ability to resist stress, actively metabolize in the host environment, and colonize by adhering to the intestinal mucosa. Through *in vitro* and *in vivo* analyses of the IBB477 strain, undertaken some time ago, we developed bacterial adhesion assays and identified potential factors that

contribute to bacterial adherence in the first *Lactococcus cremoris* strain with adhesive and muco-adhesive properties to be sequenced. The strain IBB477 was proposed and selected as a model of Gram-positive transient food-borne bacteria in the gastrointestinal tract (GIT) to study interactions between exogenously applied bacteria and nano-sized titanium dioxide (TiO_2).

The probiotic properties, including the ability to survive in the GIT, were the subject of our interest in various studies. The comprehensive screening approach on the set of LAB strains including three-hundred lactobacilli strains revealed that several LAB strains may be particularly suitable for food-based applications to support the microbiota equilibrium in the gut and reinforce the host immune system by varying cytokine modulation. These results led to the commercialization of five thoroughly characterized LAB strains to Olimp Labs Sp. z o.o.

Similar research is currently performed towards characterization of lactococcal and leuconostoc strains, unraveling molecular mechanisms of adhesion and probiotic potential of the most promising strains in the *in vivo* irritable bowel syndrome (IBS)-mouse model. Apart from the screening for strains with good persistence in the GIT (high adhesion; low pH and bile salts resistance; production of EPSs; degradation of mucin sugars), bacteria were tested for production of specific metabolites (lactate, SCFAs, vitamins) and subjected to safety assessment of potentially probiotic strains.

The characterization of probiotic strains is enriched with the whole genome sequencing and probiogenomic analyses,

Our group taking part in popularization of science event (Julia Kopczyńska, Magdalena Kowalczyk, Olha Kostiuchenko)

whereas the effect of a LAB strain on the host or environment is evaluated using cell lines and animal models. We are interested in the role of probiotics in reinforcing the gut barrier and a healthy gut microbiome thus preventing gut dysbiosis and disease development.

Our research plans consider analysis of the role of LAB in preventing development of other diseases, such as inflammatory bowel disease (IBD) or neurodegenerative disorders. We are also interested in the influence of LAB strains on plant growth and in the possibility of their application in agriculture.

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Laboratory of Plant Protein Phosphorylation

Prof. dr hab.
Grażyna Dobrowolska

Our laboratory's scientific interest is focused on the signaling pathways involved in plant development and response to environmental stresses. We are particularly interested in elucidating the role and mechanisms of regulation of protein kinases belonging to the SNF1-related protein kinases type 2 family. In our research we apply genetics, biochemistry, molecular biology, microscopy, and plant physiology methods.

The SNF1-related protein kinases type 2 family (SnRK2s) are plant-specific kinases involved in plant acclimation to harsh environmental conditions, primarily osmotic stress caused by drought and salinity. They are strongly and transiently activated in response to these stressors. In *Arabidopsis thaliana*, which is our scientific model, ten SnRK2s have been identified. Based on phylogenetic analysis, SnRK2s have been classified into three groups. This classification correlates closely with their sensitivity to the plant hormone, abscisic acid (ABA). Group 1 consists of SnRK2s that are not activated in response to ABA, group 2 consists of those that are not or only weakly activated by ABA, and in group 3 belong kinases strongly activated by this phytohormone. Numerous data show an essential role of ABA-activated SnRK2s in plant development and tolerance to environmental stress. They regulate the expression of various stress-response genes and the activity of several proteins engaged in plant tolerance to harmful conditions.

Our group's primary scientific interest is in a relatively unexplored area of research - the role of kinases belonging to group 1 of the SnRK2 family. Our studies revealed that ABA-unresponsive SnRK2s regulate plant responses to cadmium ions, drought, and salinity stress in part through regulating reactive oxygen species homeostasis, as well as control of RNA metabolism. We showed that one of the kinases studied, SnRK2.10, protects photosynthetic machinery in *Arabidopsis* by mitigating the adverse effects of salinity and the accompanying oxidative stress. Additionally, we found that SnRK2.10 modulates the expression of four hub WRKY transcription factor genes in a stress-specific

Left to right: Olga Sztatelman, Maria Bucholc, Anna Kulik, Lidia Polkowska-Kowalczyk, Grażyna Dobrowolska, Mateusz Jan Olechowski, Dominika Cieślak, Paulina Stachula

manner in response to salinity and oxidative stress.

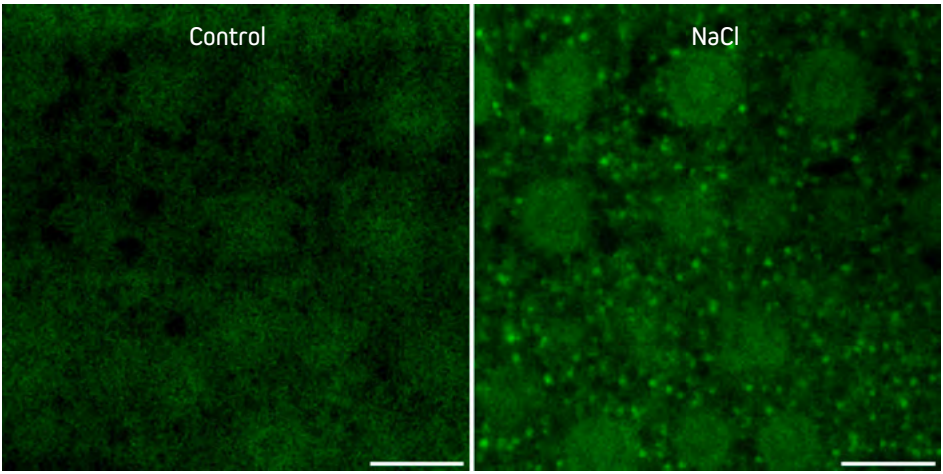
Using an integrated approach, by combining phosphoproteomic, biochemical, molecular biology, and plant physiology tools, we identified several potential targets of ABA-unresponsive SnRK2s, e.g. dehydrins [ERD10 and ERD14] and numerous RNA-binding proteins. Our results revealed that the phosphorylation of ERD14 by SnRK2s impacts its subcellular localization. The analysis of one of the RNA-binding proteins (glycine-rich RNA binding protein 8, GRP8) showed that its phosphorylation at S27 in the RNA-recognition motif by SnRK2s significantly affects the structural dynamics of GRP8, facilitates its dimerization, and subsequent liquid-liquid phase separation leading to its sequestration into stress granules (SGs) upon salinity and heat stresses.

One of our research goals is to determine the role of ABA-unresponsive SnRK2s in the regulation of developmental processes in plants. We found that two kinases of this group, SnRK2.4 and SnRK2.10, act redundantly, positively regulating developmental leaf senescence *via* regulation of ABA biosynthesis and controlling the expression of many senescence-associated genes (SAGs), including key transcription factor genes. We found that two of these transcription factors are direct targets for SnRK2s. Moreover, our results revealed that ABA-unresponsive SnRK2s are involved in

seed development, secondary dormancy, and longevity, indicating their important role also in seed biology.

Another issue studied in our lab is the integration of stress signals in plants. This part of our research focuses on the interplay between light perception *via* photoreceptors and the response to stress factors that evoke osmotic stress. We found that light-induced chloroplast movements are inhibited by salt stress, and we are studying the role of SnRK2s in this process. We identified several proteins involved in chloroplast movements as SnRK2 targets. Now, we study their role in plant cells to understand how phosphorylation can affect their function. One of those proteins is PLASTID MOVEMENT IMPAIRED 1 (PMI1), a plant-specific protein involved in light-directed chloroplast movement. We identified SnRK2-interacting regions in PMI1, as well as its lipid-binding domain, which is responsible for PMI1 localization to the membranes. In order to identify regions of the protein crucial for its function, we created plants missing defined parts of the PMI1 using the CRISPR-Cas9 technique. Using those plants we confirmed the role of PMI1 in chloroplast movements and observed that both the lipid binding domain as well as SnRK2-interacting regions are critical for this process.

Part of our study is devoted to understanding the regulation of SnRK2 activity. We found that clade A PP2Cs (ABI1, PP2CA) phosphatases, as well as okadaic acid-sensitive phosphatases of the PPP fa-



GRP8 is recruited into stress granules in response to salinity stress
Confocal images of GRP8 in 7-day-old roots of transgenic plants expressing eYFP-GRP8 under control and salinity stress conditions. Scale bar = 10 μm.

mily, dephosphorylate and inhibit some members of group 1 SnRK2s. Moreover, we identified a novel negative regulator of SnRK2s that appears to be a plant-specific calcium-binding protein, SCS (SnRK2-interacting calcium sensor). We identified two forms of SCS in *Arabidopsis* (SCS-A and SCS-B) and described the mechanism responsible for inhibiting SnRK2 activity by these proteins. Physiological studies showed that both forms of SCS regulate plant resistance against water deficit.

In conclusion, the main goal of our research is to expand knowledge about the mechanisms responsible for plant development and stress tolerance. Detailed knowledge of these mechanisms will be crucial in selecting and breeding the crops and other plants needed to cope with upcoming climate change.

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Laboratory of Plant Pathogenies

Prof. dr hab.
Jacek Hennig

The aim of our laboratory is understanding the mechanisms of plant-microbe interactions and developing innovative solutions for disease control in the 21st century. We are also focused on the characterization of a novel calcium binding protein, which belongs to the annexin family, that is involved in pollen development, with a special emphasis on its role in ribosomal RNA metabolism.

The Laboratory of Plant Pathogenies (LPP) was established in 2004 and is located in building F of IBB PAS complex. The LPP is studying many aspects of the molecular mechanisms that underlie the viral infection process and the plant response to infection. Our methodologies include various plant models that belong to the *Solanaceae* family (*Solanum tuberosum*, *Nicotiana tabacum*, and *Nicotiana benthamiana*) as well as *Arabidopsis thaliana* plants, different systems for gene isolation and characterization and its expression in prokaryotic or eukaryotic cells, resistance gene enrichment sequencing (RenSeq) and Pacific Biosciences Single-Molecule Real Time Sequencing (PacBio SMRT), live-cell imaging of green fluorescent protein (GFP) in plants, the use of GFP to study viral infections, CRISPR/Cas9-mediated targeted mutagenesis of host plants, and a live-tissue quantitative assay for assessing the hypersensitive response (resistance).

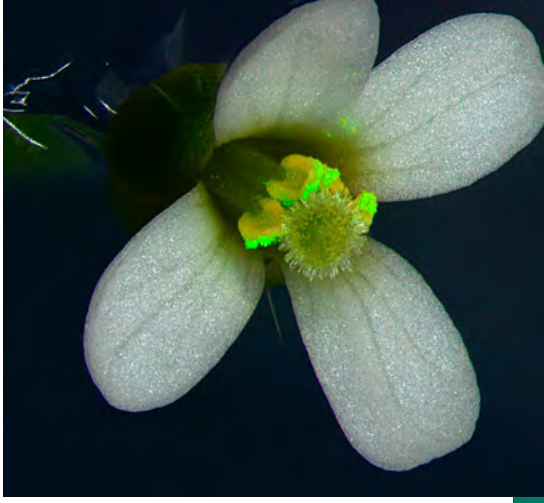
In response to virus infection, plant cells initiate a signalling cascade that leads to changes in expression profiles of many genes and consequently the reprogramming of cellular metabolism. To establish a successful defence strategy, this response must occur quickly and effectively. Therefore, the recognition of pathogen-derived signals appears to be a critical process. Interactions between potato and its pathogen Potato Virus Y (*PVY*) are the principal, although not exclusive, model systems used. We applied a technique that combines target enrichment (TE) and next-generation sequencing (NGS) to isolate and characterize the Ry_{sto} gene that is responsible for the recognition of PVY upon infection that in turn initiates the activation of a pathway that leads to extreme resistance. The Ry_{sto} gene encodes

a protein forming the multimeric scaffold of the cytoplasmic TNL class of receptor. In parallel studies, we identified PVY coat protein (CP) as an avirulence factor in *Ry*$_{sto}$-triggered immunity. To precisely map a minimal CP fragment that is sensed by Ry$_{sto}$ receptor, we created a series of constructs that encode truncated CP variants. We found that any deletion that affected the CP core abolished virus perception, indicating that an intact CP core was required for the Ry$_{sto}$-dependent resistant response. The long-term goal in this research is to use the knowledge gained about the molecular basis of plant-pathogen interactions to develop plants with increased natural resistance to diseases. Such plants would require fewer applications of pesticides producing economic and environmental benefits while providing food for consumers with less pesticide residue.

The study of the physiological role of plant annexin 5 (ANN5) represents the second strand of our research interest. We found that downregulation of a gene encoding annexin 5, a pollen-specific member of the calcium binding protein family of *Arabidopsis thaliana*, significantly reduced the pollen viability and the rate of pollen tube growth in the style. A localization of ANN5 in the nucleolus and plastidial nucleoids, the sites of active ribosomal RNA metabolism; its

Left to right: Izabela Barymow-Filoniuk, Angelika Fiodor, Jacek Hennig, Małgorzata Lichocka, Marta Grech-Baran

Potato tubers infected with PVY virus.



Pollen specific expression of the annexin 5 in *Arabidopsis thaliana* flower.

interaction with the translation factor EF--Tu; and abnormalities in rRNA processing observed in the plants with the manipulated ANN5 levels, all suggest that ANN5 might be involved in ribosome biogenesis and/or protein synthesis. To test this hypothesis we designed a series of experiments to define how ANN5 influences cellular translational machinery in the developing pollen grains and under stress conditions exerted by the excess of manganese and zinc ions. We believe that the research outcome of our studies will significantly contribute to the current knowledge of the pollen physiology and will improve our understanding of the regulation of translational machinery in plants.

Current work of the LPP relies on diverse experimental approaches involving methods derived from the fields of biochemistry, cell biology, forward and reverse genetics, molecular biology, plant breeding, plant pathology, and structural biology.

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Plant and Microbial Biology Laboratory

Dr hab.
Magda Krzymowska, Prof. IBB PAS

Our group investigates an interplay between plant and pathogenic microorganisms. In particular, we are interested in i) mechanisms used by plants to detect the presence of invaders and initiate defense signaling, and ii) ingenious methods employed by pathogens to dampen the host's immunity.

Our research focuses on understanding a molecular dialogue between host and pathogenic microorganisms. We put a special emphasis on virulence strategies of bacteria threatening plants. The main model of our studies is *Pseudomonas syringae*, a causative agent of various devastating diseases of crops and ornamental plants. *P. syringae*, like many other gram-negative pathogenic bacteria, delivers proteinaceous effectors directly into host cells *via* the type three secretion system. Some effectors are involved in nutrient acquisition, but the majority are tailored to target key components of the plant defense system. Therefore, their examination not only provides insight into virulence mechanisms of bacteria but additionally may shed a new light on the function of the host's cellular machinery.

The aim of our studies is to elucidate the function of some *P. syringae* effectors. To this end, we adopt a multidisciplinary approach that combines plant molecular biology, cutting-edge biochemistry, microscopic imaging, bioinformatics and phytopathology assays. We search for enzymatic activities of the effectors studied, their virulence targets and interaction

Plant & Microbial Biology Laboratory (PMBL) was formally detached from Plant Pathogenesis Laboratory in 2021, but independent research projects have been led by its current leader for more than 20 years.

Left to right: Tomasz Krępski, Chablina Hazarika, Magda Górecka, Magda Krzymowska. Patrycja Zembek, Juan Ochoa Cabezas, Martyna Jonak

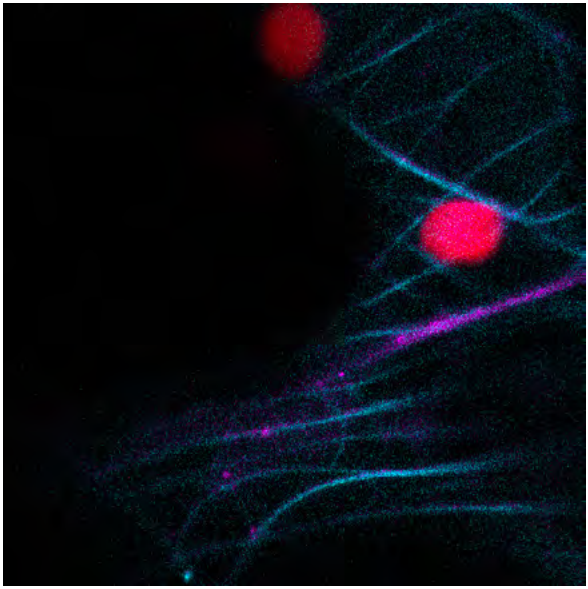
partners, and their sites of action inside the host cell. All these aspects allow us to picture a *modus operandi* of a given effector.

HopQ1 (for Hrp outer protein Q) from *P. syringae* promotes disease development in bean and tomato plants. However, *P. syringae* cells producing the effector are sensed by plants of *Nicotiana* spp., which have evolved the Roq1 receptor. Activated Roq1 adopts a tetrameric conformation and exerts NADase activity, catalyzing production of different signaling molecules (pRib-AMP/ /ADP and di-ADPR/ADPr-ATP) using NAD^+ or NAD^++ATP as substrates. Strains of *P. syringae* infecting *Nicotiana* plants eliminated HopQ1 from their genomes. We have described two novel mechanisms that the bacteria evolved to avoid the recognition mediated by HopQ1. The first involves processing of the N-terminus of the effector. We found that the presence of serine 87 and leucine 91 renders HopQ1 derived from pathovar tomato DC3000 susceptible to plant proteases. The second strategy is based on the cooperation of HopQ1 with HopR1, another *P. syringae* effector.

We have also shown that HopQ1 contains an atypical calcium binding site, whose affinity depends on pH. It mediates unfolding of the effector under mildly acidic conditions corresponding to the pH of the bacterial cytoplasm and refolding upon

calcium binding in the host cell. Therefore, we propose that the presence of the non-canonical calcium binding motif provides a mechanism that enables unfolding of the effector prior to passing through the needle of the type three secretion system and then maturation once the effector reaches the host cell. Our experiments revealed that HopQ1 undergoes a specific phosphorylation upon delivery into a plant cell. This modification enables binding of host proteins from the widely conserved 14-3-3 family. One molecule of HopQ1 assembles in the plant cytoplasm with a 14-3-3 dimer. This interaction increases the steady state level of HopQ1. Additionally, it regulates nucleocytoplasmic partitioning of HopQ1, since binding of 14-3-3s changes the effector's translocation towards the nucleus from active to passive.

HopBF1 is another effector whose mechanism of action we are studying. We have shown that it acts as a general toxin. HopBF1 is an atypical protein kinase that inactivates the host HSP90 chaperone by transferring a phosphate group to the highly conserved serine residue. This leads to inhibition of the ATPase activity of HSP90 resulting in cell death. Fascinatingly, we noticed that expression of the wild-type HopBF1, but not the kinase-inactive variants, in the lower parts of *Nicotiana benthamiana* led to malformation of the upper leaves, distant from the infiltration site. Young systemic leaves displayed a significantly altered phenotype, including crinkling, shortening, and the formation of necrosis-like changes on the veins, leading to a retardation of plant growth. Interestingly, we could detect neither bacteria car-



Confocal image showing an interaction of one of *Pseudomonas syringae* effectors with the cytoskeleton of *Nicotiana benthamiana* epidermal cells. Photo: Magda Górecka

rying *hopBF1* nor the HopBF1 protein itself in the systemic tissue. Similarly, in plants infected locally with *P. syringae* expressing HopBF1, the systemic leaves developed symptoms and were free of the bacteria. Our transcriptomic profiling studies revealed upregulation of genes associated with defense response, oxidative stress, protein degradation, and autophagy in the systemic leaves displaying abnormalities compared to the control tissue. Further experiments will help us to elucidate the underlying mechanism, and the nature of the long-distance signal that triggers changes in the systemic leaves.

We also investigate the virulence properties of HopAG1 and HopR1. To this end, we apply a pipeline we have established comprising bioinformatic and biochemical analyses, imaging and pathoassays.

Climate changes, expansion of monoculture farming, continuous introduction of new cultivars out of phytosanitary control, all result in phytopathogenic bacteria seriously threatening crop productivity in Poland. We expect that the results obtained in our studies will contribute to deeper understanding of the virulence mechanisms of bacteria which may help in future find out new ways to control crop diseases.

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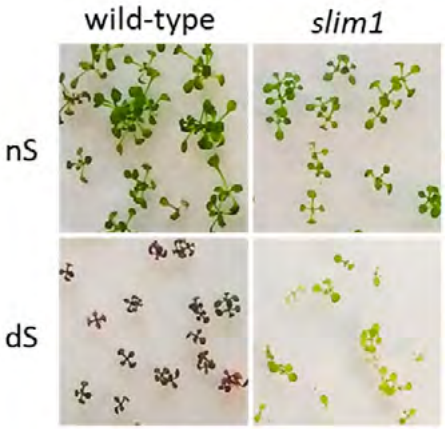
Laboratory of Plant Protein Homeostasis

Prof. dr hab.
Agnieszka Sirko

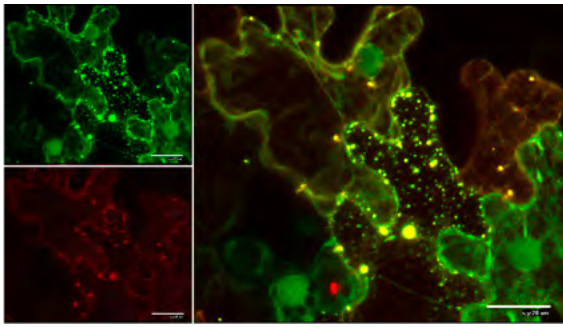
Proper response to abiotic stresses and rapid regeneration after their disappearance are crucial for plants' survival during the climate change era. We study these processes by deciphering changes in transcriptome, metabolome, and proteome during plants' stress response, adaptation, and recovery. We search for novel plant stress response regulators and examine their crosstalk with phytohormones and autophagy.

We are interested in the molecular details of stress response regulation in plants and study them by the combination of the methods of molecular biology, biochemistry and genetics. Nutrient deficiencies are an increasingly common cause of low crop productivity and quality. A short-term sulfur (S) starvation results in extensive gene expression changes. We identified many genes encoding proteins of unknown function among those that showed differential expression under S-deficient conditions. We selected some of them for detailed characterisation expecting to identify novel regulators important for plant response to nutrient starvation stress. The most intriguing were the members of the plant-specific LSU (Upregulated by Low Sulfur) family, which are about 100 amino acids long and have a coiled-coil structure. In *Arabidopsis thaliana* there are four LSU proteins (LSU1-4) capable of forming homo- and hetero-dimers and involved in interaction with numerous proteins. Further studies of the functions of LSU proteins and their interactomes led us to the conclusion that their function extends far beyond the regulation of the plant response to S starvation. Since then, our interest has evolved from S metabolism and regulation of plant response to S starvation to the role of selective autophagy in plant stress response and its crosstalk with abscisic acid (ABA) and other plant hormones. Below is a short description of the main projects ongoing in our laboratory.

- **Response to S starvation at the level of transcription.** Analysis of the shoot and root transcriptomes in S-sufficient and S-deficient conditions revealed distinct



Growth phenotype of 12-day-old *Arabidopsis thaliana* seedlings in normal sulfate supply (nS) and sulfate deficiency (dS). In contrast to the wild-type, the slim1 mutant does not produce anthocyanin under sulfur-deficiency stress.



Co-localization of NBR1-mCherry and LSU1-YFP transciently co-expressed in *Nicotiana benthamiana* cells. The individual signals are on the left-hand side, while overlay of both signals on the larger image on the right-hand side.

sets of differentially expressed genes, depending on the plant parts. Regulation of S-starvation responsive genes is complex but a direct involvement of only one transcription factor (TF), SLIM1, is well documented. SLIM1 belongs to the plant-specific EIL family of TFs. Our collaborative results indicated that SLIM1 is also involved in sugar signalling during S starvation and that its C-terminal part is necessary for S-starvation response. Based on the assumption that the occupancy of SLIM1-binding sites differs in S-sufficient and S-deficient conditions, we will compare the genome-wide binding sites of SLIM1 in both conditions. We also examine the conditions affecting the SLIM1 stability and search for the factors modulating its degradation under both S-sufficient and S-deficient conditions.

- **LSUs modulate S starvation response in plants.** The increased amount of LSUs during S-starvation suggests that they might be an important element of S-starvation response. We obtained a series of *Arabidopsis* lines with single and multiple deletions of *LSU* genes. Their morphological and phenotypic analysis indicated that LSUs are not essential for plant growth and development since the quadruple *lsu* mutant is viable. However, we observed that the gene expression pattern and the amount of S-containing metabolites (cysteine, glutathione) in the quadruple *lsu* mutant differs from the parental line in S-sufficient conditions and mimics the changes caused by S-starvation in the parental line. Based on these observations we concluded that LSU facilitates the assimilation of inorganic S.

From the left: Abdel Aziz Gad, Marzena Sieńko, Konrad Jurczewski, Anna Góra-Sochacka, Marcin Olszak, Agnieszka Sirko (Group Leader), Jerzy Brzywczy. Anna Wawrzyńska, Justyna Piotrowska

Subsequently, we confirmed the direct interaction of LSUs with the enzymes from this pathway and demonstrated that *in vitro* LSUs stimulate enzymatic activity of sulfite reductase.

- **Protein partners of LSUs.** In search of other molecular functions of LSUs, we decided to build an interaction network consisting of all known LSUs partners (identified by us and available in public databases) and their direct partners. We failed to detect an enrichment in any particular molecular function or cellular process. Instead, we conclude that LSUs probably interact with complexes rather than with a single protein and many proteins from the network are linked to tubulin-based transport. Among many enzymes interacting with LSU1 are all three isoforms of catalases, CAT1, CAT2 and CAT3 and iron-dependent superoxide dismutase FSD2. Therefore, one of the functions of LSUs is direct interaction with antioxidant enzymes, their stabilization and protection against oxidative damage.
- **Role of LSU1-NBR1 interaction in the modulation of the function of each partner.** We have identified NBR1, a selective autophagy cargo receptor as one of the strongest interactors of LSU1. The exact role of this interaction remains elusive, however some data indicates that LSU1 is not an autophagy cargo recognized by

NBR1. Instead, LSU1 binding to some extent prevents autophagic degradation of NBR1.

- **Selective autophagy and phytohormones signaling in plant stress response and recovery.** We have observed changes in phytohormones levels and signaling (particularly ABA and cytokinins) in *nbr1* mutants and NBR1-overexpressing lines. Interestingly, the main ABA-responsive TFs (ABF3, ABI3, ABI4 and ABI5) directly interact with NBR1 and seem to be targeted to autophagic degradation by NBR1.
- **Approaches and tools used for DNA vaccines and for heterologous production of useful proteins.** This category includes small projects performed mostly in collaborations that aim towards development of various expression plasmids for the production of antigens or recombinant proteins having biotechnological or therapeutic significance in heterologous hosts. The projects are tailored to the needs of our collaborators and they are based on our previous experience with experimental DNA vaccination of laboratory animals against avian flu, and our expertise in recombinant technology and knowledge of molecular biology tools. One of the recent projects deals with heterologous production of L-gulonolactone oxidase (GULO), an enzyme catalyzing the production of the precursor to ascorbic acid (vitamin C), which spontaneously converts to the vitamin itself. The GULO enzyme is non-functional in primates and its loss is responsible for the inability of humans to enzymatically synthesize vitamin C.

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Laboratory of Seeds Molecular Biology

Dr hab.
Szymon Świeżewski, Prof. IBB PAS

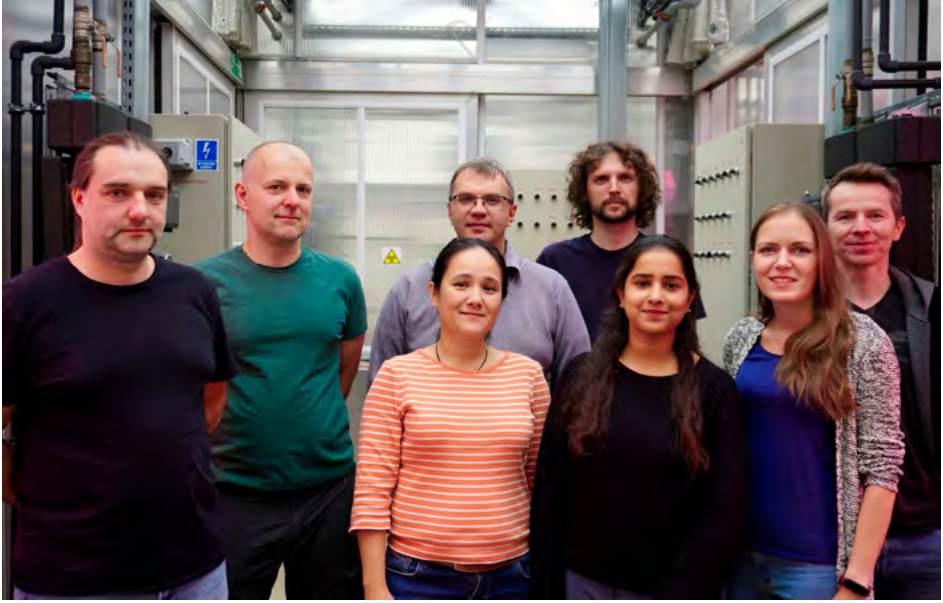
Seed dormancy reflects the ability of plants not to germinate despite favorable conditions. Seed dormancy is a fascinating seed property that underlies seed-plant ecological success and forms the overall basis of agriculture. Much is known about seed dormancy physiology and genetics. Our laboratory focuses on molecular mechanisms, with a focus on a conserved key regulator of seed dormancy, the DOG1 gene.

Seeds allow plants to colonize new places, by traveling long distances. But seeds can also travel in time. A special state, called seed dormancy, enables plants to postpone their germination despite favourable conditions. In agriculture, dormancy is also an important seed trait as it is responsible, for example, for seed germination synchrony. Seed dormancy is therefore subject to strong selection and in *Arabidopsis thaliana*, the *Delay of Germination 1* (*DOG1*) gene has been selected by nature to align the dormancy strength with local conditions. We study different aspects of *DOG1* regulation including regulation by a non-protein coding transcript.

An antisense transcript 1GOD, transcribed from within the DOG1 locus, supresses dormancy by inhibiting DOG1 expression in cis. DOG1 antisense is itself negatively regulated by ABA in seedlings and by DOG1 alternative polyA site selection in seeds.

PUPPIES are a group of sense lncRNA transcripts transcribed from the DOG1 promoter that activate DOG1 expression in freshly harvested stratified seeds in response to the presence of salt, delaying germination. PUPPIES activate DOG1 expression by enhanced pausing of RNA polymerase II, slower transcription and higher transcriptional burst size.

Seed dormancy strength is usually analysed as the percentage of seeds in a seed lot that germinate or postpone germination. We are analysing the variability among individual seeds, focusing on the transcriptional variability between individual seeds. To do this we developed a single seed RNA sequencing method that allows us to interrogate transcriptomes of hundreds of seeds



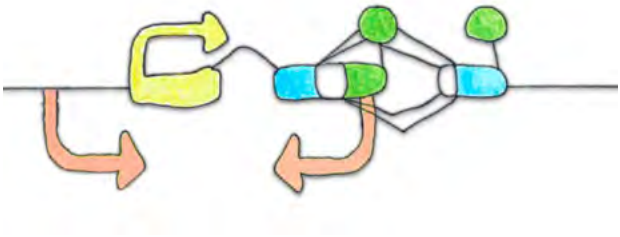
Left to right: Krzysztof Kokoszka, Szymon Świeżewski, Lien Brzeźniak, Ruslan Yatusevich, Michał Krzysztoń, Veena Mavatkar, Magdalena Wrona, Sebastian Sacharowski

at the same time. This allowed us to describe transcriptional variability among individual seeds that could underlie the physiological variability in dormancy strength among seeds from an individual seed lot.

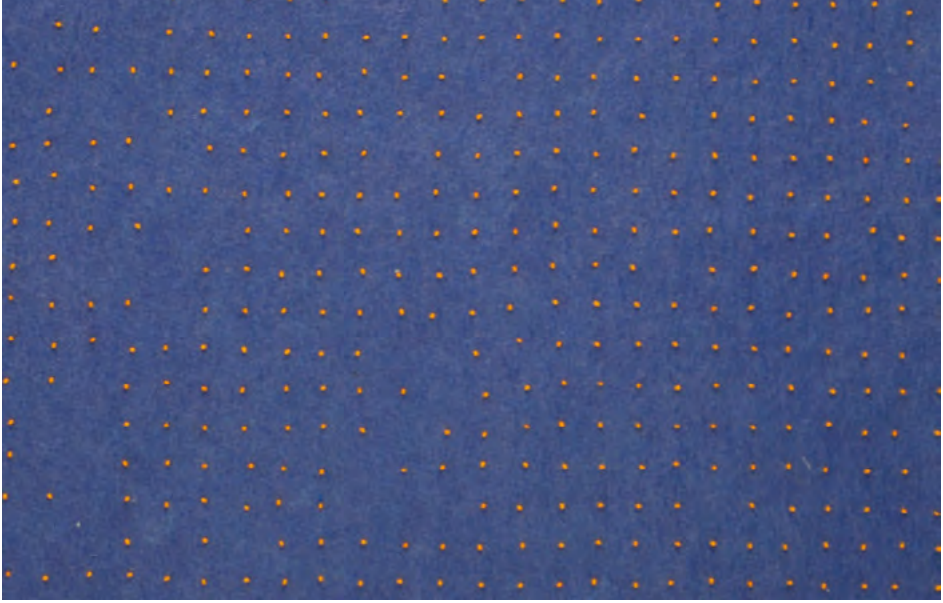
We found that alternative polyadenylation results in production of two DOG1 transcripts: a shorter two exonic short DOG1(shDOG1) and longer three exonic long DOG1 (lgDOG1). Our work showed that the shDOG1 transcript is evolutionary conserved at the level of amino acids, translated and sufficient to complement dog1 mutant.

DOG1 alternative splicing

The second intron of DOG1 is subject to alternative splicing, generating four different isoforms. By studying the regulation of this alternative splicing, we described a tight coupling between transcription and alternative splice site selection. We showed that mutations in transcription



DOG1 gene schematic. Exons are shown as thick lines, exon1 in yellow, exon 2 and 3 in blue, short *DOG1* unique exon 2 sequence in green and alternative splicing in white. Arrows represent transcription start sites, with pink arrows for lncRNA and lollipops indicating transcription termination sites.



Arabidopsis seed dormancy test

elongation factor TFIIS lead to selection of proximal splice sites, not only on DOG1 but also at the majority of tested genome wide target, suggesting the existence of a kinetic coupling between polymerase (Pol) II elongation and splicing in plants. We have also shown that alternative splice sites are active players in Pol II elongation control. We hypothesized that alternative splicing may locally pause the elongating Pol II using a chromatin-based mechanism that is centered around the spliceosomal disassembly factor NTR1. We have also helped to describe a mechanism where alternative splicing control by light is mediated by changes in Pol II speed that is dependent on the TFIIS elongation factor.

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Laboratory of Non-coding RNA and Genome Rearrangements

Dr
Jacek K. Nowak

Research performed in the laboratory aims to understand the mechanisms responsible for the synthesis of developmentally regulated non-coding RNAs and their role in programmed genome rearrangements. The key question that we address is whether ncRNA synthesis and metabolism in eukaryotes requires a specific composition of the transcription machinery as well as modification and transport pathways.

Nearly all kinds of genomic regions in eukaryotes have been reported to have some transcriptional activity, and give rise to a variety of non-coding transcripts (ncRNAs). The important question in the field is whether ncRNA synthesis requires a particular composition of the transcriptional machinery. To answer this, we use the ciliate *Paramecium tetraurelia*, a model unicellular organism in which different types of ncRNAs are implicated in the regulation of developmental genome rearrangements. At each sexual cycle, during the development of the somatic nucleus (macronucleus; MAC) from the germline nucleus (micronucleus; MIC), the *Paramecium* genome is massively rearranged through the reproducible elimination of germline-specific sequences, including multi-copy transposons and thousands of short, single-copy, non-coding internal eliminated sequences (IESs). According to the current model proposed for *P. tetraurelia*, these programmed genome rearrangements involve a specialized, RNA interference-related machinery and is mediated by different types of non-coding RNA molecules: a special class of developmentally regulated small RNAs from the MIC (scnRNAs) and longer, non-coding transcripts produced by the maternal MAC as well as by the new MAC. 25-nt scnRNAs are produced from the entire germline genome, bound by PIWI proteins and transported to the maternal somatic nucleus, where selection of scnRNAs corresponding to germline-specific sequences is thought to take place. Selected scnRNAs are then translocated again into the developing somatic nucleus guide the elimination of transposable elements and IES sequences. Epigenetic control of genome

Left to right: Yuni Gao, Julita Gruchota, Jacek K. Nowak, Katarzyna Nowak, Seyma Duran

rearrangements in *P. tetraurelia* by RNA-mediated, homology-dependent mechanisms provides evolutionary insight into the diversity of non-coding RNA pathways involved in genome plasticity.

Our research involves in-depth studies of factors specifically responsible for epigenetic control of developmentally programmed DNA elimination mediated by non-coding transcripts in *Paramecium*. We focus our study on the characterization of proteins influencing transcription, including families of the TFIIS, Spt5 and Spt4 elongation factors as well as other components of the RNA polymerase II complex. We are testing the hypothesis concerning the possible role of these proteins in initialization of genome-wide non-coding transcription. Our research interest also includes other proteins involved in RNA metabolism – PIWI proteins and its associated factors responsible for RNA-RNA and protein-chromatin interactions as well as selective degradation of scnRNAs.

Sequencing and annotation of the largest somatic chromosome and establishment of the whole genome sequence of Paramecium tetraurelia The DNA Sequencing and Oligonucleotide

„Paramecium room". Left to right: Katarzyna Nowak, Seyma Duran, Julita Gruchota

Jacek Nowak operating Narishige/Olympus injection system

Synthesis Laboratory (currently DNA Sequencing and Synthesis Facility) from IBB PAS took part in a pilot project for the random sequencing of the genome of *Paramecium* (1999-2000) and sequencing of the entire chromosome of the macronucleus (2001-2003). The largest *Paramecium* chromosome we sequenced, one-million base pair in length, was the longest DNA sequence established in Poland at that time and the first ciliate chromosome ever sequenced. The data obtained in these projects were instrumental for the macronuclear genome sequencing (2003-2006).

Functional study of genes essential for sexual processes in Paramecium In a subsequent project (2004-2010), we applied a global strategy to monitor gene expression profiles of the genes carried by the largest somatic chromosome. Transcriptome analysis involving dedicated DNA arrays revealed four major patterns of gene expression, including two successive waves of gene induction. Functional analysis of 15 upregulated genes revealed four that are essential for vegetative growth, one of which is involved in the maintenance of MAC integrity and another in cell division or membrane trafficking. Two additional genes, encoding a MIC-specific protein and a putative RNA helicase localizing to the old and then to the new MAC, are specifically required during sexual processes.

Discovery and characterization of TFIIS4 elongation factor involved in synthesis of long non-coding RNA In the course of our studies (2009-2014) we identified an autogamy-specific essential function of the TFIIS4 putative elongation factor that is involved in regulation of genome rearrangements and transcription in the new MAC. We obtained the first evidence for the existence of germline-specific non-coding transcripts at an early stage of MAC development and proved that TFIIS4 plays an important role in their production. We postulated that TFIIS4 may be involved in transcription of an entire genome, providing

an interaction RNA scaffold for small RNAs. Our work demonstrated, for the first time in any eukaryote, that a TFIIS homolog is an essential factor for the production of regulatory non-coding zygotic transcripts.

Discovery of a role of Spt5-Spt4 complex in production of development-specific small ncRNAs Spt5-Spt4 complex is a component of the RNAPII elongation complex. We found that the developmentally up-regulated Spt5 homolog, in cooperation with Spt4 factors, is necessary for sexual reproduction and production of meiosis-specific short non-coding RNAs - scnRNAs. Spt5m and Spt4 proteins are also responsible for correct genome rearrangements in the new MAC. Mass spectrometry analysis following affinity purification of FLAG-tagged proteins confirmed interaction between Spt5m and Spt4mA/Spt5mB/Spt4vA as well as between Spt4mA and Spt5m/Spt5v, leading to the conclusion that different configurations of DSIF complex may exist in *Paramecium*. These studies established a novel connection between non-coding transcription and the control of genome plasticity.

Discovery of the role of the PIWI--interacting protein Gtsf1 in the selective degradation of small RNAs We provided important mechanistic insights into the scnRNA selection pathway by identifying a *Paramecium* homolog of Gtsf1 as essential for the selective degradation of scnRNAs corresponding to retained somatic sequences. We also showed that Gtsf1 is localized in the maternal somatic nucleus where it associates with the scnRNA-binding protein Ptiwi09. Furthermore, we demonstrated that the scnRNA selection process is critical for genome elimination. We proposed that Gtsf1 is required for the coordinated degradation of Ptiwi09-scnRNA complexes that pair with target RNA via the ubiquitin pathway.

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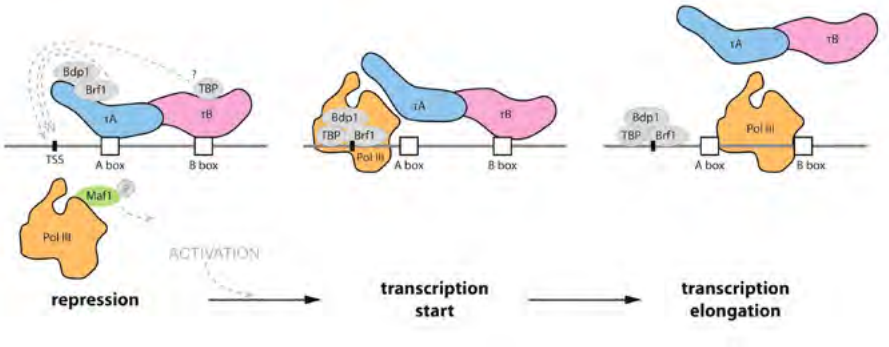
Laboratory of tRNA Transcription

Prof. dr hab.
Magdalena Rakowska-Boguta

The Laboratory studies the regulation of tRNA transcription by RNA polymerase III (Pol III). We identified Maf1, a general negative Pol III regulator, and deciphered the mechanism of Maf1-mediated repression. We also proposed the mechanism of Pol III repression by general transcription factor TFIIIC. Next, we discovered RNA-binding protein Rbs1, implicated in Pol III assembly by a possible co-translational mechanism.

In all eukaryotes, transcription of nuclear DNA is carried out by three different RNA polymerases (Pols), designated Pol I, II and III. Our scientific interest in RNA polymerase III (Pol III), specialized in the high level synthesis of small protein-noncoding RNA, mostly 5S rRNA and tRNA. The 17-subunit Pol III complex is assembled, at least in part, in the cytoplasm and then imported to the nucleus with the help of auxiliary factors.

In-depth analyses of the yeast Pol III transcription system have revealed a cascade of protein-DNA and proteinprotein interactions leading to the recruitment of Pol III to its target tRNA genes: binding of the six-su-



Model presenting the molecular mechanism of TFIIIC-mediated recruitment of TFIIIB and control of tDNA transcription by RNA polymerase III.

Left to right: Malak Farhat, Aleksandra Łopusińska, Magdalena Rakowska-Boguta, Małgorzata Cieśla, Alicja Armatowska

bunit general TFIIIC factor to the intragenic promoter, TFIIIC-directed recruitment and assembly of the three-subunit general factor TFIIIB, and subsequent recruitment of the Pol III enzyme.

Pol III is under control of a global and general negative regulator, Maf1 protein, which was initially discovered in our laboratory. Maf1 is essential for repressing Pol III transcription in yeast and mediates both RAS and TOR signaling pathways. We found that Maf1 inhibits tRNA transcription via a mechanism that depends on its phosphorylation status. Under the repressive conditions, dephosphorylated Maf1 binds to Pol III and impairs recruitment of Pol III to tRNA genes. Maf1 is also a target of the casein kinase II (CK2). Upon transfer of *Saccharomyces cerevisiae* from repressive to favorable growth conditions, CK2 activity is required for the release of Maf1 from Pol III bound to a tRNA gene and for subsequent activation of tRNA transcription. In a yeast strain lacking Maf1, CK2 inhibition showed no effect on tRNA synthesis, confirming that CK2 activates Pol III via Maf1. Additionally, CK2 was found to associate with tRNA genes, and this association is enhanced in absence of Maf1, especially under repressive conditions.

We characterized overall Pol III transcription in yeast by accurate mapping of the location of Pol III along all nascent transcripts under permissive and restrictive growth conditions. This revealed strikingly uneven polymerase distributions

across transcription units, generally with a predominant 5′ peak. This peak was higher for more heavily transcribed genes, suggesting that initiation site clearance is rate-limiting during Pol III transcription. Down-regulation of Pol III transcription under stress conditions was found to be uneven; a subset of tRNA genes showed low response to nutrient shift or loss of the major transcription regulator Maf1, suggesting potential "housekeeping" roles. Many tRNA genes were found to generate long, 3′-extended forms due to read-through of the canonical poly(U) terminators. The degree of read-through was anti-correlated with the density of U-residues in the nascent tRNA, and multiple, functional terminators can be located far downstream. The steady-state levels of 3′-extended pre-tRNA transcripts are low, apparently due to targeting by the nuclear surveillance machinery.

We also investigated function of the yeast Pol III initiation factor, TFIIIC, in activation and repression of tRNA gene transcription. We reported the interplay between TFIIIC and Maf1 in controlling Pol III activity upon the physiological switch of yeast from fermentation to respiration. TFIIIC directly competes with Pol III for chromatin occupancy as demonstrated by inversely correlated tDNA binding. The association of TFIIIC with tDNA was stronger under unfavorable respiratory conditions and in the presence of Maf1. Induction of tDNA transcription by glucose-activated protein kinase A (PKA) was correlated with the down-regulation of TFIIIC occupancy on tDNA. The conditions that activate the PKA

Trip of the group to the highest located shelter in Tatra Mountains (Schronisko Pięciu Stawów)
Monika Hejnowicz, Magdalena Rakowska-Boguta, Dominika Foretek, Tomasz Turowski, David Płonka (accompanied person), Marta Płonka, Rafał Bazan, May 2012

signaling pathway promoted the binding of TFIIIB subunits, Brf1 and Bdp1, with tDNA, but decreased their interaction with TFIIIC. Association of Brf1 and Bdp1 with TFIIIC was much stronger under repressive conditions, potentially restricting TFIIIB recruitment to tDNA and preventing Pol III recruitment. Altogether, we propose a model in which, depending on growth conditions, TFIIIC promotes activation or repression of tDNA transcription.

A consequence of Pol III transcription repression is a degradation of the largest Pol III catalytic subunit, C160. We detected ubiquitylated forms of C160 and demonstrated that C160 protein degradation is dependent on proteasome activity. A comparable time-course study of Pol III repression upon metabolic shift from fermentation to respiration shows that the transcription inhibition is correlated with Pol III dissociation from chromatin but that the degradation of C160 subunit is a downstream event. We postulate that the degradation of C160 is activated under stress conditions to reduce the amount of existing Pol III complex and prevent its *de novo* assembly.

Another interesting protein involved in Pol III control is Rbs1, characterized in our laboratory as an assembly/import factor for Pol III complex. We selected the Rbs1-encoding gene as a multicopy suppressor of the *rpc128-1007* mutation which has severe consequences for the assembly of the active Pol III complex and hence for tRNA transcription activity and growth. Rbs1 was found to overcome these defects and to interact physically with a subset of Pol III subunits, AC19, AC40 and ABC27/Rpb5. Additionally Rbs1 interacts with the Crm1 exportin and shuttles between the cytoplasm and the nucleus. Recently we found that Rbs1 is a poly(A)mRNA-binding protein which interacts with their 3' regulatory sequences. Importantly, Rbs1 binds directly to 3'UTR of mRNA encoding Rpb10, a small subunit common to RNA polymerases, and modulates Rpb10 expression. According to the current model, Rbs1, by interaction with mRNA, brings together Rpb10, Rpc19 and other subunits of Pol III complex during translation process. This emerging picture suggests a role of Rbs1 in co-translational assembly of polymerase complex.

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Laboratory of RNA Biology

Dr hab.
Roman Szczęsny, Prof. IBB PAS

Our laboratory is interested in mechanisms that control the quality, quantity, and processing of RNAs, with a particular focus on mitochondrial RNA in humans. Our main goal is to decipher the molecular machinery responsible for mitochondrial RNA surveillance and decay. We also investigate mechanisms that maintain and regulate expression of the mitochondrial genome and how they respond and adapt the cell to different stress conditions.

MITOCHONDRIAL RNA EXPRESSION AND SURVEILLANCE

Mitochondria are unique organelles in human cells because their function depends on crosstalk between two genomes: nuclear and mitochondrial. Human mitochondrial DNA (mtDNA) contains only a few genes, but all of them are essential. mtDNA exists in multiple copies per cell, and the expression of mitochondrial genes can be regulated by controlling the gene copy number.

Although the human mitochondrial genome was one of the first to be sequenced, the way it functions is still not entirely understood. Our studies focus on unravelling mitochondrial gene expression mechanisms. To date, we have been mainly interested in post-transcriptional mechanisms, but our ongoing and future studies concern the mechanisms that regulate mitochondrial gene copy number. To this end we have performed a genome-wide siRNA screening to identify new mtDNA maintenance factors. Their role in mtDNA maintenance is currently under investigation. We also intend to investigate the processing of individual mitochondrial transcripts and the ways in which mitochondrial nucleic acid metabolism responds to stresses (e.g., viral infection). We also plan to explore the recent discovery that mtDNA expression leads to the formation of double-stranded RNA. Finally, we investigate a so-called "dark mitoproteome", i.e. proteins with a potential dual intracellular localization, with the major form residing in the nucleus or the cytoplasm and the alternative isoform targeted to mitochondria.

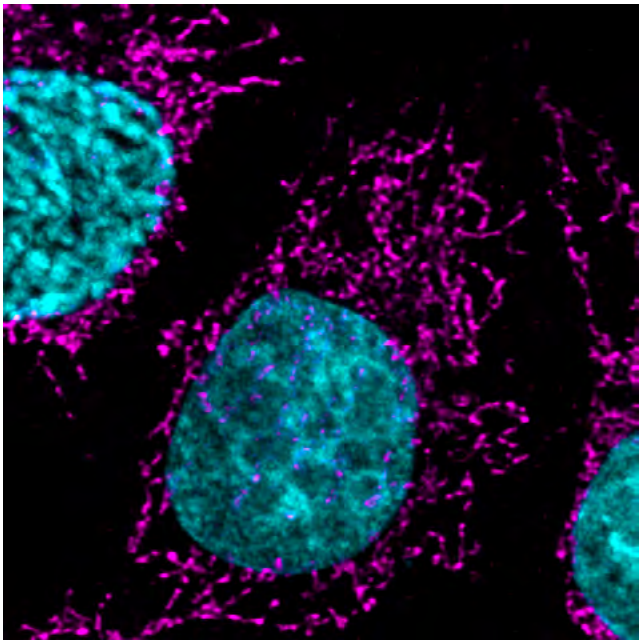
RNA decay and surveillance

The regulation of human mitochondrial gene expression at the initiation of mtDNA transcription appears to be limited. Thus, post-transcriptional processes, including RNA decay, are critical for shaping the mitochondrial transcriptome. We contributed significantly to the identification of an RNA-degrading complex in human mitochondria. This complex—the mitochondrial degradosome—consists of RNA helicase SUV3 and the ribonuclease PNPase. We showed that the degradosome is essential for the surveillance and decay of human mtRNAs, particularly antisense transcripts. Our recent studies revealed that short mtRNAs that are generated by mtRNA processing and decay machinery are removed by REXO2 oligoribonuclease, proving that REXO2 controls short mtRNAs.

Mitochondrial RNA binding proteins

RNA molecules can form different structures, many of which involve non-canonical base pairing, such as the case of G-quadruplexes (G4s). Mitochondrial genomes of vertebrates exhibit extraordinary GC skews (i.e., high guanine content on one strand). Therefore, the transcription of human mtDNA results in the synthesis of G-rich RNAs that are prone to form G4s. Such mtRNAs, mostly antisense RNAs, are transcribed at high rates, but their steady-state levels are extremely low. We described a mechanism by which G4-containing

Left to right: Przemysław Surowiecki, Saakshi Lakhwani, Marcelina Kurowska, Roman Szczęsny, Łukasz Borowski, Elżbieta Speina, Giulia Santonoceto, Aneta Jurkiewicz



Human cells with labeled mitochondria (magenta) and nuclear DNA (cyan).

antisense mtRNAs are efficiently degraded in humans. We showed that the RNA-binding protein GRSF1 melts G4s in mtRNAs, facilitating degradosome-mediated decay. Based on phylogenetic analyses, we proposed that GRSF1 appears in mitochondria when genomes undergo a G4-poor to G4-rich transition. This evolutionary adaptation enabled the control of G4 mtRNA levels. To expand our knowledge on mtRNA binding proteins we have captured mitochondrial proteins bound to RNA and identify them using mass-spectrometry. Selected hits are under further functional studies.

Double-stranded RNA in mitochondrial biology

The importance of degradosome-dependent mtRNA surveillance was underscored by our studies that were performed in collaboration with the Proudfoot laboratory (Oxford University, UK) and others, showing that SUV3 and PNPase are major regulators of mitochondrial dsRNA (mt-dsRNA). We revealed that mtDNA transcription is a significant source of dsRNA in humans. We showed that the depletion of SUV3 or PNPase leads to the accumulation of mt-dsRNA, and in the case of PNPase, to the release of these species into the cytosol. Remarkably, once in the cytosol, mt-dsRNA triggers an innate immune response through induction of the interferon pathway. Our work identified a new mechanism by which mitochondria contribute to cell fate and human health. Currently, we are investigating the role of other proteins in the regulation of mt-dsRNA levels.

Mitochondrial genome expression in stress

The maintenance of mitochondrial gene expression is crucial for cell homeostasis. Stress conditions may lead to a temporary reduction of mtDNA copy number, raising the risk of the insufficient expression of mtDNA-encoded genes. We applied a quantitative proteomic screen to search for proteins that sustain mtDNA expression under stress conditions. We found that the novel mtRNA-binding protein MTRES1 is elevated in cells with perturbances in mtDNA expression. Our study showed that MTRES1 prevented mtRNA depletion during transcription arrest. Which other proteins are involved in response to stress conditions is a subject of our future research.

NUCLEAR-ENCODED RNA SURVEILLANCE AND PROCESSING

Regulation of double-stranded RNA in the nucleus

Transcription of the nuclear genome has the potential to produce long dsRNA species. However, under normal conditions, such RNAs are hardly detectable, suggesting their tight control. To identify proteins that are involved in this regulation, we performed a loss-of-function screen. This screen indicated a pathway, the inhibition of which led to the upregulation of nuclear dsRNA. The molecular mechanism that underlies dsRNA accumulation is the subject of our ongoing studies.

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Laboratory of Transcription Mechanisms

Dr hab.
Tomasz W. Turowski

Our group studies RNA transcription mechanisms, particularly non-coding RNA (e.g., tRNA, rRNA) biogenesis. These RNAs are vital for translation, enzymatic functions, and nucleus organization. Despite their importance, they have been largely overlooked in research. Recent insights linking genetic disorders to non-coding RNA biogenesis have underscored the need for in-depth investigation into their biology.

The genetic code, stored within DNA, serves as the blueprint for life's processes. However, it is the dynamic interplay of RNA and proteins that truly brings information from the DNA to life. RNA molecules, with their versatile roles, regulate gene expression and contribute significantly to the spatial organization of cells, particularly within the nucleus, where the intricate process of RNA production, or RNA biogenesis, takes its initial steps.

At the heart of gene expression lies RNA transcription, a tightly regulated process essential for translating genetic information into functional molecules. While only a tiny fraction of our DNA comprises protein-coding genes (2%), the genome also harbors a multitude of non-coding genes, each playing pivotal roles in various cellular functions. Among these, ribosomal RNA (rRNA) and transfer RNA (tRNA) stand out as the most abundant non-coding RNAs, synthesized by specialized enzymes known as RNA polymerases I and III, respectively.

The transcription of non-coding RNAs serves multiple critical purposes within the cell. Firstly, it generates abundant functional molecules necessary for translation, such as rRNAs and tRNAs, which are indispensable for protein synthesis. Additionally, non-coding RNAs carry out various enzymatic and regulatory functions, contributing to the intricate orchestration of cellular activities. Despite the importance of non-coding RNA biogenesis, our understanding of this process lags behind that of protein-coding genes, highlighting the importance of further investigation.

From left: Ahmed Eisa Elhage, Raymi Edgar Goitia Camacho, Tomasz W. Turowski, Katarzyna Grelewska-Nowotko, Jan Mikołajczyk

Following transcription, newly synthesized RNA molecules undergo processing and maturation to become fully functional. Some of these processing events occur simultaneously with transcription, a phenomenon known as co-transcriptional processing. This synchronized activity ensures that RNA molecules are efficiently prepared for their diverse roles within the cell.

While we have detailed knowledge of the biogenesis of protein-coding genes, the mechanisms governing non-coding RNA biogenesis remain less explored. Given the myriad enzymatic and regulatory functions non-coding RNAs perform within the cell, unraveling the intricacies of their biogenesis promises to deepen our understanding of cellular processes and uncover new avenues for therapeutic intervention.

Mechanism of RNA transcription

RNA transcription progresses through three main stages: initiation, elongation, and termination. Initiation involves various transcription factors and chromatin remodelers that modify the chromatin structure, making DNA accessible for the transcription machinery. Once the chromatin is accessible, transcription begins, with RNA polymerase initiating transcription of the first nucleotides.

While our understanding of transcription initiation is advanced, the process of sequence-specific transcription elongation is

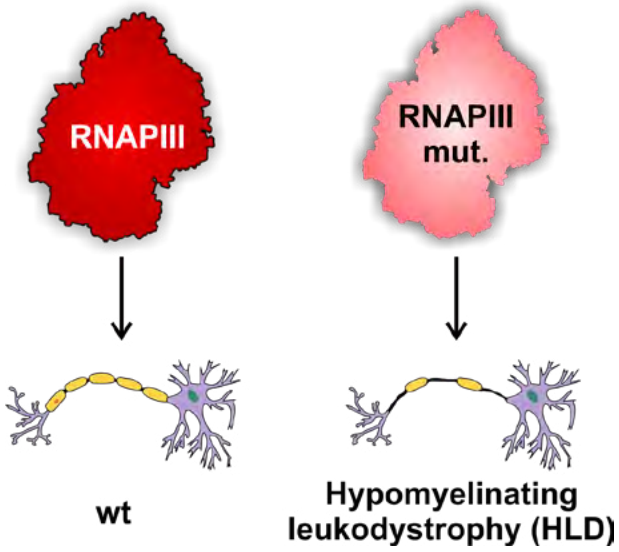
more complex and diverse. Elongation rates vary, impacting RNA folding, termination, alternative polyadenylation, and splicing. All these processes determine the fate of mature transcripts.

The synthesis of non-coding transcripts also undergoes kinetic regulation, affecting processes like prerRNA cleavage or tRNA gene termination. We prioritize understanding transcription dynamics, using a unique approach to identify new regulatory elements by characterizing the biophysical properties of DNA and RNA. This methodology extends to studying less-understood transcription systems like SARS-CoV-2 RNA polymerase and RNA polymerase III in humans. Furthermore, we aim to enhance the design of synthetic genomes through our research.

Molecular mechanisms linking RNA polymerase III mutations to neurodegenerative diseases

We are deciphering the molecular mechanisms that link RNA polymerase III (RNAPIII) mutations to a group of neurodegenerative diseases called hypomyelinating leukodystrophies (HLD). These disorders are characterized by the loss of myelination, leading to the degeneration of white matter in the brain due to defects in the myelin sheath around axons. Myelin, crucial for efficient nerve signal transmission, is produced by oligodendrocyte cells during their differentiation from progenitor cells.

Our goal is to understand the molecular profile of these disorders by mapping actively transcribing RNAPIII and assessing the pool



Mutation in RNA polymerase III can lead to hypomyelinating leukodystrophy, a genetic disease characterized by the loss of myelination in the brain's white matter, resulting in disruptions to the myelin sheath surrounding axons.

of tRNA available for translation. Additionally, we aim to understand how RNAPIII transcription influences the expression of surrounding protein-coding genes.

By doing so, we hope to gain insights into the role of RNAPIII mutations in HLD. Specifically, we aim to identify transcripts directly affected by compromised RNAPIII activity or those particularly sensitive during oligodendrocyte differentiation. Our findings will guide future investigations and may pave the way for novel therapeutic approaches to address RNAPIII-related HLD.

Research methodology

We use a combination of high-throughput techniques, molecular biology, *in vitro* biochemistry, and computational approaches, including bioinformatics, mathematical modeling, and machine learning. This diverse toolkit allows us to tackle complex biological questions with precision and depth.

We specialize in several high-throughput methods, including CRAC (UV cross-linking and analysis of cDNA), which provides detailed maps of RNA-protein interactions, shedding light on the dynamics of RNA transcription. Additionally, we utilize chromatin immunoprecipitation (ChIP) to investigate DNA-protein interactions at high resolution, and RNA-seq to explore both canonical and non-canonical transcriptomes.

We utilize *in vitro* biochemistry to purify large complexes for assays, such as RNA polymerases and pre-ribosomes. We conduct *in vitro* assays for RNA polymerase I and II, studying processes like elongation and backtracking. Moreover, we perform cleavage assays for PIN-domain endonucleases like Nob1 and conduct *in vitro* transcription of RNA-dependent RNA polymerase of the SARS-CoV-2 virus.

For data analysis and mathematical modeling, we specialize in analyzing RNA polymerase dynamics and modeling dynamic processes, with a focus on transcription. We analyze protein-bound transcriptomes, including motif analysis, and parametrize DNA and RNA sequences for thermodynamic features such as folding energy and melting energy. Additionally, we employ a data science approach to cross-compare various datasets, enhancing our understanding of complex biological systems.

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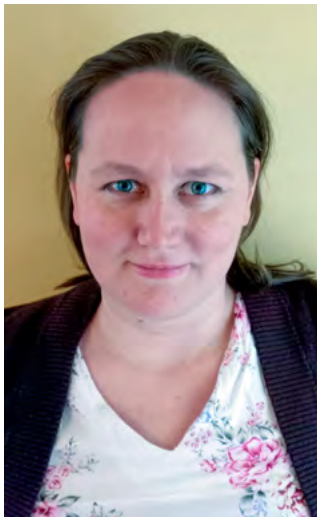
Laboratory of RNA processing and decay

Dr hab.
Agnieszka Tudek



The laboratory explores various aspects of RNA metabolism. We study the dynamics of mRNA 3'end polyadenosine tail shortening in the cytoplasm. We are also the first group to have identified the human TTC33-associated network core (TANC) complex, and are describing the complexes' putative role in nucleic acids metabolism. Lastly, we are developing a pipeline for functional characterization of human pathogenic mutations.

The laboratory of RNA processing and decay was opened in January 2022. The main focus of the group is to characterize the various pathways of RNA biogenesis in the cell, both in normal conditions and in pathogenic states. The group currently explores three main lines of research using as model organisms budding yeast *Saccharomyces cerevisiae* and various human cell lines.

A major line of research conducted by the group investigates the metabolism of the 3'end RNA polyadenosine tail (pA-tail). This important conserved modification to protein-coding messenger RNAs (mRNAs) dictates major steps in the transcripts' cellular metabolism; from defining the mRNA stability to its aptitude for translation. The latter, a role of mRNA as template to protein production, is the main function of protein-coding transcripts. Therefore the study of the dynamics of the 3'end polyadenylation is key to understanding basic mechanisms of gene expression, which can be used to better the design of pharmaceuticals, notably mRNA vaccines. The group focuses on budding yeast as a model organism. The group, in collaboration with other research Institutions (International Institute for Molecular and Cell Biology and Aarhus University), has shown that the newly-made pA-tail, synthesized by Pap1, is around 60 adenosines long, and that the nuclear pA-binding protein Nab2 is the factor defining the pA-tail length. We have also shown that in the nucleus this length can be further regulated by the 3'-5' exoribonuclease exosome complex and its co-factors, the Trf4/5 polyA-polymerases, which to-date were mostly shown to target non-coding RNAs. After its synthesis in the nucleus the mRNA is transported to the cytoplasm to

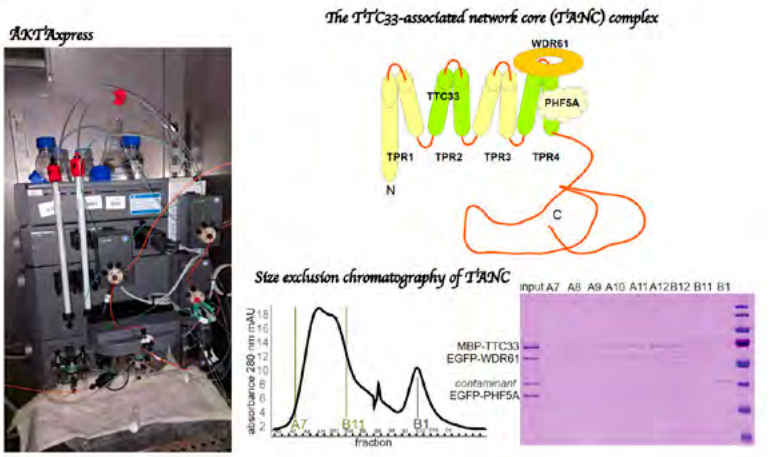
Left to right: Małgorzata Drabko, Rafał Tomecji, Małgorzata Siek, Agnieszka Jabłońska

serve as template for protein production. The nuclear polyA-binding protein Nab2 is replaced by its cytoplasmic counterpart, Pab1, which was shown *in vitro* to form multimers with one molecule binding to 20-30 adenosines. We showed that the steady-state pA-tail length is on average 20-30 adenosines long (corresponding to binding of one Pab1 molecule). The pA-tail is shortened in a process called deadenylation by the two main cytoplasmic deadenylases, the Pan2/3 and Ccr4-NOT complex.

Our current efforts aim at modeling of the deadenylation process in the cytoplasm. To this end, in collaboration with prof. Jarosław Poznański, we developed a numerical model rooted in a modified gamma distribution that allows for calculation of the transcriptomic deadenylation rate. Independently, we devised a simplified method for distinguishing deadenylation rates of individual mRNAs. Using this method we showed that mRNAs that undergo fast deadenylation are also degraded rapidly. Those analyses confirmed that deadenylation is a factor dictating the onset of 5'end decappping, which is the ultimate license for mRNA degradation. We also showed that the deadenylation rate can be modified in response to acute stress; also accelerating mRNA decay. Our data indicate that Pab1 is a master regulator of mRNA stability with the protein being required for deadenylation when bound to the pA-tail as a multimer but promoting decapping on short-tailed tran-

scripts. Though the causative link between deadenylation and mRNA decay is a longstanding paradigm in molecular biology we identified an important group of transcripts coding for ribosomal protein subunits that partially evade this interdependence, and our future aim is to decipher the exact mechanism of turnover of those mRNAs. In sum our results show that the mechanisms governing mRNA decay can be transcript-specific. In respect to deadenylation this specificity of decay speed can be partially dictated by the speed of deadenylation. We showed that the two main deadenylase complexes can have different substrate specificity, with Pan2/3 targeting mostly mRNAs of high abundance and Ccr4-NOT preferring to deadenylate mRNAs expressed at low levels. This discrepancy can be explained by the mode of recruitment of each deadenylase. While Pan2/3 is recruited to the pA-tail and Pab1, which is alike for all transcripts, Ccr4-NOT has a collection of sequence specific co-factors; the Puf1-5 proteins. Our future goal is to decipher the contribution of each Puf1-5 protein to Ccr-4-NOT deadenylase activity. We also plan to dissect the function in the deadenylation process of each Ccr4-NOT complex subunit, which is a large protein assembly containing seven proteins.

Another important line of research conducted by the group is the first biochemical and functional characterization of human TTC33 protein, which is only found in bony vertebrates. Our unpublished data shows that TTC33, WDR61 and PHF5A form a trimeric complex, which we coined the TTC33-associated complex core (TANC). The TANC complex is a platform for recruitment of uncharacterized structural protein CCDC97, the PPP2R2A-containing isoform of PP2A phosphatase and UNG DNA glycosylase.



Using the ÄKTAxpress equipment the laboratory of RNA processing and decay showed that TTC33, WDR61 and PHF5A form a novel trimeric complex; the TTC33-associated network core (TANC).

The immediate goal of the group will be to pinpoint the biological role of TANC. Our current working hypotheses are based on the known functions of the TANC component or its interactors. We predict a role of TANC in gene expression regulation because the core TANC component, PHF5A, is also a subunit of the SF3A/B subcomplex of the U2 splicing factor and a chromatin binding protein. We will also evaluate the potential role of the complex as an auxiliary factor in DNA repair due to its robust binding to UNG; an enzyme that is part of the Base Excision Repair system; one of the major DNA repair pathways. The study of TANC function is one of the main future research directions for the group. However, it is worth noting that many such uncharacterized proteins are found in the human genome. Our group intends to perform the first analysis of protein interaction networks of a collection of such uncharacterized proteins, which will be an excellent resource for future functional inquiries.

The last research topic is the functional characterization of Single Nucleotide Polymorphisms (SNPs). SNPs are mutations abundantly found in any healthy person, which mostly define individual differences between population members. However, some SNPs are variants associated with pathogenic states. There is a plethora of such mutations that cause well-studied genetic disorders; other SNPs alone are benign but only result in disease when combined with additional genetic variants. At times, genetic diseases are identified in children, which cannot be explained by any known mutation, but such patients often present with SNPs that can be the source of the disease. Currently, our group investigates two such mutations that result in changes to the amino acid sequence of CSTF3; a subunit of the complex responsible for transcription termination of mRNAs, and SP3, a transcription factor. We aim to develop a pipeline that will efficiently characterize the functional consequences of those mutations, by defining the impact of the SNP on cellular proliferation and the protein-protein interaction network.

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Laboratory of Translatomics

Dr hab.
Agata Starosta, Prof. IBB PAS

We are interested in gene expression regulation on a translational level in various bacteria, such as *Bacillus subtilis* or *Myxococcus xanthus*. Using Next Generation Sequencing techniques (including ribosome profiling) in combination with high-resolution microscopy, genetics and molecular biology methods, we study how translation of mRNAs can be modulated by the ribosome itself and/or associated translation factors.

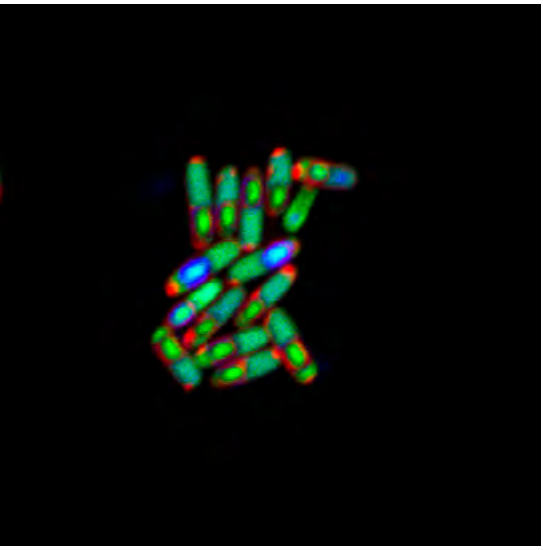
Protein biosynthesis is a fundamental process in every living cell and is performed by the ribosome and associated factors. For decades ribosomes were considered homogeneous macromolecules carrying an unchangeable set of ribosomal RNAs and proteins and the role of the ribosomes in gene expression regulation was neglected. Over time, experimental evidence suggested that ribosomes may take a role of regulatory elements. Regulation of gene expression at the translational level allows for a rapid and transient response to a variety of environmental stimuli without the need to change the transcriptome. Rather, what changes is the rate at which mRNA is engaged by the ribosome leading to the altered translatome and, as a result, differing protein levels. Due to the high-complexity of the translational machinery, the ribosomal adaptation could be achieved by different means including varying stoichiometry of ribosomal proteins, paralogs and post-translational modifications of the r-proteins or variations in rRNA sequences. Specialized translation factors are also known to help the protein biosynthetic machinery to respond to aberrant growth conditions.

In our lab, we employ the sporulation process in *Bacillus subtilis*, an endospore forming bacterium, as a model to study translation regulation. Upon starvation, the bacterial cell develops a survival form – a spore – in a process of asymmetric cell division. This process is tightly regulated on the transcriptional level, however, little is known about how translation is involved in the regulation of this process. Once the cell commits to sporulation, it stops dividing and enters an hours-long, tractable process to become a spore, which is an excellent model in which to study the involvement of

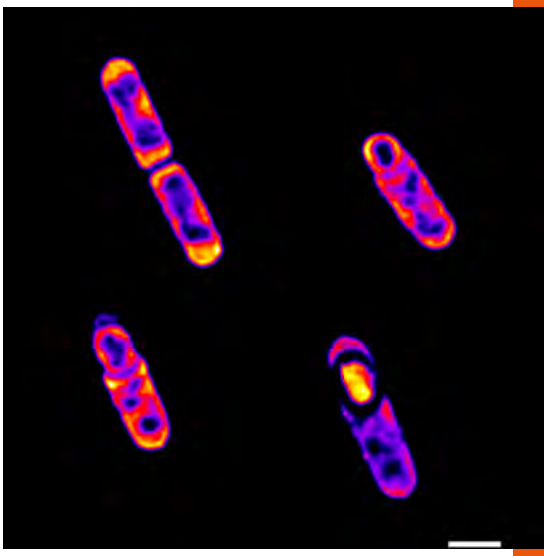
Left to right: Olga Iwańska, Przemysław Latoch, Agata Starosta, Mariia Kovalenko

the translational apparatus in the regulation of cellular development. In our research we look for factors which regulate translation during sporulation, e.g. factors determining ribosomal specialization. We use state-of-the-art approaches including Next Generation sequencing methods such as RNA-seq and ribosome profiling, supported by in-depth bioinformatical analyses. We also apply fluorescent microscopy techniques, including super-resolution structured illumination microscopy (SIM) imaging and click-chemistry mediated biochemical assays. In our toolkit we also have various genetic, molecular biology and biochemistry based assays.

In our research we have described the sequential changes in the translatome of *B. subtilis* during sporulation. Translation and translational machinery are temporally and spatially organised in *B. subtilis* during the process of sporulation, which is especially important during asymmetric septation. During sporulation, translation undergoes two silencing events, first prior to the asymmetric septation and then at the end of sporulation, in preparation for mother cell autolysis. At the first translation silencing event, the ribosomes are transiently arrested and are translating at very low levels. This translational halt lasts until the two asymmetric compartments (spore and the mother cell) are formed and transcription is



Localisation of translational machinery during sporulation in *B. subtilis* expressing GFP-tagged ribosomes, RpsB-GFP. Confocal microscopy



Localisation of translational machinery during sporulation in *B. subtilis* expressing GFP-tagged ribosomes, RpsB-GFP. SIM, super-resolution microscopy

separated based on dedicated sigma factors. Interestingly, the ribosomes do not dissociate from the mRNA, but instead are paused for a while on the mRNA template awaiting their new subcellular destination. We have also discovered that ribosomes are transported into the spore after the chromosome is fully translocated and during peptidoglycan rearrangement at the asymmetric septum. The peptidoglycan of the asymmetric septum is partially degraded with the aid of the SpoIIDMP protein complex, allowing for membrane migration and new peptidoglycan synthesis and thus, for successful spore engulfment. In our research we showed that the asymmetric septum plays an important role in the spatial organisation of the ribosomes and serves as a hub in the cell's developmental control, including translation regulation. The process of ribosome translocation into the spore is thus dependent on the peptidoglycan rearrangement of the asymmetric septum and, without it, the spores do not mature.

We have also investigated the role of three zinc independent paralogs of ribosomal proteins during sporulation in *B. subtilis*. The triple deletion strain showed no growth difficulties in optimal, nutrient-rich conditions but at the same time exhibited defects during cellular stress – sporulation caused by nutrient limitation. Such phenotype indicates that the investigated protein factors are necessary for specialization of

the bacterial cell. Indeed, although expressed at low to moderate levels, the three investigated proteins make up a subpopulation of ribosomes which is important for timely and effective sporulation. The lack of all three proteins results in delayed sporulation and reduced germination efficiency. This is most probably a result of dysregulated translation of key sporulation and metabolism related genes and especially, of the delayed and inefficient translation silencing occurring prior to asymmetric septation, as compared to WT.

In the future we plan to focus on specialized translation factors associated with the biosynthesis and action of antibiotics produced by *Myxococcus xanthus*. The interplay between translation and antibiotic biosynthesis is an attractive research subject. Biosynthesis of antimicrobials targeting translation is, with a doubt, imposing a burden on the bacterial translational machinery. Thus, by investigating the putative antibiotic biosynthetic gene cluster encoded in the *M. xanthus* genome, as well as expression of the paralogs of translation factors, we would like to describe how and when antibiotic biosynthesis is switched-on in bacteria and how the cell is protected from self-intoxication. Such research could not only help to provide new insights into the action of the translational machinery, but also to characterize putative novel determinants of antibiotic resistance.

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Laboratory of Genetic Basis of Human Diseases

Dr hab.
Beata Burzyńska, Prof. IBB PAS

Our laboratory focuses on the relationship between genetic diversity and diseases. We aim to: (1) establish genetic biomarkers that are associated with cardiovascular disorders at the mRNA and miRNA levels, (2) conduct research to determine genetic determinants of hereditary hemolytic anemia, and (3) perform functional analysis of rare genetic variants of unknown significance (VUS).

Molecular Biomarkers of Cardiovascular Disease

Heart failure (HF) is the most common cause of morbidity and mortality in developed countries. Cardiac gene expression plays an important role in the pathogenesis and clinical manifestations of HF. The main aims of our project were to establish alterations of gene expression patterns in leukocytes that are associated with acute myocardial infarction (AMI) and to identify distinct biomarkers that correlate with the development of HF. We developed and patented the method related to biomarkers for individual risk assessment of the development of post-infarction heart failure, as well as a method for optimizing the treatment of cardiac patients. The obtained results allow diagnosis and/or determination of the prognosis for the patient based on the expression of selected mRNA transcripts determined in RNA samples.

We also sought to identify prognostic microRNAs (miRNAs) that are associated with left ventricular (LV) dysfunction following AMI. miRNA profiles were determined in plasma and serum samples from patients on the first day of AMI. This analysis indicated that an elevated level of miR-30a-5p is a good predictor of the future development of LV dysfunction and HF symptoms, following AMI. A bioinformatic analysis indicated that miR-30a-5p may regulate genes that are involved in the pathogenesis of cardiovascular disease.

Recently, we focused on the identification of novel circulating serum miRNAs involved in the process of atrial fibrillation (AF). AF is the most common and persistent cardiac arrhythmia that increases the risk of death,

Left to right: Karolina Maciak, Beata Burzyńska, Monika Góra, Konrad Wasilewski

stroke, and other thromboembolic incidents. Atrial fibrillation patients more frequently develop heart failure and have a higher rate of hospitalization. Approximately one-third of patients may not experience symptoms of arrhythmia. Such an asymptomatic course impedes and delays diagnosis and the implementation of proper treatment. Early diagnosis provides an opportunity to prescribe treatments that can protect against such devastating consequences. On the basis of the duration of an arrhythmia episode, AF is classified as first-diagnosed, paroxysmal, persistent, long-standing persistent, or permanent. In recent years, there has been a growing body of evidence that miRNAs play an important role in AF development and progression. Sequencing data from our study group revealed a putative novel miRNA sequence, put-miR-25, to be significantly downregulated in serum of patients with persistent AF, as compared with individuals with paroxysmal AF. Bioinformatic target gene prediction indicated that put-miR-25 may be involved in metabolic processes relevant to pathogenesis of cardiovascular diseases.

Genetic Background of Inherited Hemolytic Anemias in Polish Patients

The main objective of this project is to determine the molecular background of congenital hemolytic anemias (CHAs) in selected

patients with anemia of unclear etiology. The term CHAs encompasses a heterogeneous group of hereditary disorders that are associated with mutations of more than 70 genes and characterized by the destruction of abnormal red blood cells (RBCs), with presentations that range from asymptomatic to severe and life-threatening. CHAs are associated with ineffective erythropoiesis and the dysregulation of iron metabolism. The pathogenesis of CHAs is still only partially understood. An accurate diagnosis of CHAs is often challenging because the clinical presentation of genetically distinct diseases can be quite similar. This study investigates genetic determinants of CHAs that are caused by impairments in enzyme activity in RBCs, disturbances in membrane proteins, and abnormalities in the structure and synthesis of hemoglobin. We perform whole-exome sequencing for selected patients with hemolytic anemia who lack an accurate diagnosis. Moreover, the analysis of previously uncharacterized variants, both novel and already reported, using bioinformatic tools and molecular modelling was performed. An interesting example of this research may be the study of a group of patients with severe anemia of unknown etiology. Nine Polish patients with severe hemolytic anemia but normal pyruvate kinase activity were found to carry mutations in the *PKLR* gene encoding PK, five already known mutations and a novel one (c.178C > T). We characterized two of the known variants by molecular modeling (c.1058delAAG) and minigene splicing analysis (c.101-1G > A). We proposed mechanisms of pathogenicity of two previously uncharacterized variants and contribute to a better understanding of the genotype - phenotype relationship in PK deficiency.

Functional analysis of Gene Variants of Unknown Significance

Variants of unknown significance (VUS) are mutations that have unknown or unclear effects on protein function. Depending on the gene(s) studied, the frequency of VUS varies. The unclear interpretation causes

Droplet Digital™ PCR (ddPCR™) Bio-Rad which allows ultrasensitive nucleic acid detection and absolute quantification.

anxiety for patients diagnosed with VUS-type variants and difficulties for clinicians in charge of clinical management.

The ACMG-AMP (American College of Medical Genetics - Association for Molecular Pathology) guidelines recommend that when VUS variants are detected, so-called family segregation studies or functional tests should be performed. In cooperation with various clinical centers, we try to determine the pathogenicity of VUS detected in patients with various rare genetic diseases. For example, in a Polish family with a lethal form of short-rib thoracic dysplasia (SRPS) we identified a novel intronic variant in the *NEK1* gene, in the homozygous state. We used bioinformatics tools, minigene assay and other methods to assess the harmfulness and functional impact of this variant.

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Laboratory of RNA metabolism in Immune Responses

Dr
Damian Graczyk

Our research focuses on how RNA polymerase III is regulated in mammalian cells and whether altered levels of its products affect cell physiology. Particularly, for several years, our research projects have mostly revolved around the role of Pol III in innate immune responses and, recently, in cancer. We would like to know if this enzyme could be a therapeutic target for treating immune diseases, infection and cancer.

In all eukaryotes examined, RNA Polymerase (Pol) III synthesizes several essential components of the protein biosynthetic machinery, including tRNA, 5S ribosomal RNA (rRNA), 7SL RNA and a subset of small noncoding RNAs required for the maturation of other RNA molecules (U6 RNA). These untranslated RNAs are essential for cell growth and proliferation. To maintain cell size, cellular growth, or mass accumulation, *de novo* translation is required with each cell cycle. Consequently, cell proliferation is proportional to protein synthesis in almost all cell types. Thus, highly proliferating cells, like cancer cells, greatly depend on translational machinery and, therefore, on Pol III. The rate of Pol III transcription is tightly regulated in response to changing conditions. Notably, the deregulation of Pol III transcription has been implicated in various human diseases, including immune diseases, cardiovascular disorders and cancer. Our laboratory's research scope encompasses the role of Pol III in innate immune responses, infection and cancer.

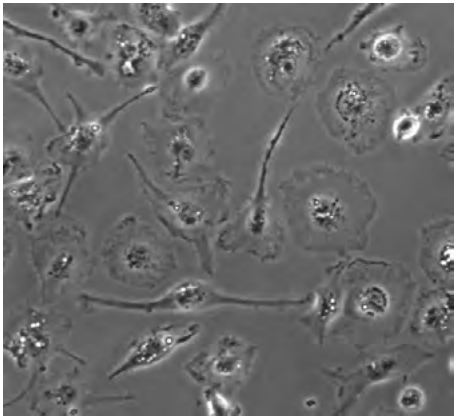

Figure 1. Mouse bone marrow-derived macrophages.

In mammalian cells, Pol III transcription is directly activated by c-Myc, mTORC, ERK and NF-kB, and repressed by RB, p53 and MAF1. Indirect regulation of Pol III activity involves MAP kinase pathways mainly through modulation of Pol III transcription factor subunit levels. Using macrophages, professional phagocytic cells of the innate immune system (Figure 1), we showed that Pol III activity is upregulated during lipopolysaccharides treatment, which mimics bacterial infection. We also showed that regulation of Pol III in these conditions involves MAF1, a general regulator of this enzyme; NF-kB, a key transcription factor mediating inflammatory signals; and MAP kinases (Figure 2). Our data show that modulating Pol III activity in macrophages affects their response to pro-inflammatory stimuli, including interferon signalling, thus pointing to Pol III as a potential target to modulate immune responses. This is of great interest since macrophages often contribute to pathological states such as inflammatory bowel disease, neurodegeneration, arthritis, fibrosis, obesity and diabetes or macrophage activation syndrome. Furthermore, growing evidence indicates that macrophages present in tumours (called tumour-associated macrophages, TAMs), instead of being effective in host-defence, actually contribute to cancer progression by stimulating cancer cell proliferation, angiogenesis, metastasis and suppression of adaptive immunity.

Another aspect of our research investigates the link between Pol III and colorectal

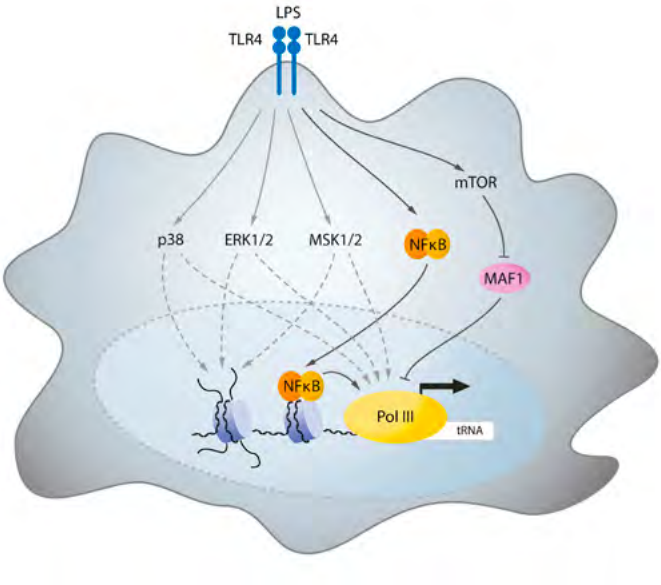

Figure 2. A schematic diagram of RNA polymerase III regulation in macrophages stimulated with lipopolysaccharides (LPS).

cancer (CRC). CRC is the third most commonly diagnosed cancer and the fourth leading cause of cancer deaths worldwide. It is very heterogeneous at the molecular level, and various causative genetic aberrations have been identified, including mutations, loss of heterozygosity and epigenetic changes. The molecular events that underlie the initiation and progression of CRC are still being discovered. Because of the high incidence of CRC, there is an urgent need to uncover the mechanisms that cause the progression of this disease fully and to find drug targets to treat this malignancy. TNFα is vital for apoptosis, cell survival, inflammation, and immune responses, possessing both tumour-promoting and antitumor properties. TNFα can trigger epithelial-to-mesenchymal transition (EMT), increasing metastasis risk. Elevated in tumours, TNFα often encounters resistance from cancer cells, aiding their growth and spread. Restoring TNFα sensitivity in tumours is a potential cancer therapy. Our data show that inhibiting Pol III enhances the cytotoxic effects of TNFα, blocks TNFα-induced migration in colorectal cancer cells, and alters EMT marker expression. Mechanistically, Pol III inhibition affects TNFα-induced nuclear localization of the NF-κB subunit p65, a transcription factor involved in inflammation, proliferation, survival, and migration. Thus, targeting Pol III may sensitize cancer cells to TNFα.

RNA polymerase I, which produces ribosomal RNA (rRNA), and Pol III are highly conserved, consisting of multiple subunits. Some of the subunits are common to both polymerases. For example, a shared subunit POLR1D, a 16 kDa protein, is crucial in both Pol I and III assembly. Interestingly, POLR1D is frequently upregulated in colorectal cancer (CRC). We and others showed that POLR1D downregulation inhibits CRC cell proliferation and migration. Moreover, we found an unexpected link between POLR1D and the mammalian target of rapamycin (mTOR), an essential nutrient sensor and a major regulator of cell growth and proliferation. We also found that POLR1D can self-regulate its expression. Our data reveal an unexpected cytoplasmic role for a subunit of RNA polymerases I and III and unveil a previously unanticipated molecular circuit that may be highly relevant to human health.

Our experience in working with RNA and knowledge regarding immunity provides valuable insight into collaborative projects beyond our central research theme. We are part of a commercial consortium with Dr Roman Szczęsny (IBB PAN), Adamed Pharma, and the University of Gdańsk that aims to develop a platform for RNA vaccine synthesis. In partnership with Prof. Marcin Nowotny (Internationa Institute of Molecular and Cellular Biology, Warsaw) and Dr Roman Szczęsny, we investigated a DNA:RNA hybrid sensor specifically targeting R-loops. Our role in this research involved the bioinformatics analysis of data obtained from large-scale sequencing, providing crucial insights into the structure and function of these hybrids. This collaboration aimed to enhance understanding of R-loops and their implications in various biological processes.

In summary, our research links very important areas of biology – RNA metabolism and regulation, immune cell function and cancer. Our long-term goals consist of fully deciphering the role of Pol III in innate immune responses, infection, and cancer. We study the mechanisms whereby Pol III products impact pro-inflammatory responses of macrophages and innate immune signalling in cancer.

We combine biochemical, bioinformatics, genetic tools, and large-scale transcriptomic approaches to achieve our scientific goals. Our primary experimental model is *in vitro* cultured mammalian cells. We have extensive experience working with mouse primary bone marrow-derived macrophages, human peripheral blood-derived macrophages, and cancer cell lines. In our lab, we have successfully applied several mammalian cell manipulation techniques (transfection, gene silencing using siRNA/shRNA, retroviral/lentiviral transduction, and CRISPR/Cas9 system). On a day-to-day basis, we readily use standard modern molecular biology techniques such as Western blotting, quantitative RT-PCR, Northern blotting, ELISA, chromatin immunoprecipitation, and fluorescent microscopy. Finally, our IncuCyte S3 Live-Cell Analysis System enables us to perform experiments that allow real-time monitoring of various parameters of cultured cells—including proliferation, cell death, and migration—over periods ranging from hours to days. Thus, we are, for example, able to test the response of cells to various drugs or treatments.

Our research was possible thanks to funding from several sources, as listed below.

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Laboratory of Bioenergetics and Mitochondrial Diseases Mechanisms

Prof. dr hab.
Róża Kucharczyk

We focus on ATP synthase biogenesis, mechanism of function, dysfunctions in diseases and regulation by the ampylase Fmp40. The unique expertise of the team is in site-directed mutagenesis of mtDNA in *Saccharomyces cerevisiae*. We are using yeast as a model to determine the molecular basis of rare neurodegenerative diseases caused by mutations in the nuclear *VPS13A-D* and *DHCR7* and mitochondrial *ATP6* and *ATP8* genes.

Mitochondria are double membrane-surrounded, very dynamic organelles. The main, but not the only, function of mitochondria is production of cellular energy in the form of ATP. Energy generation is performed by the multi-subunit complexes localised in the inner membrane of mitochondria, forming the oxidative phosphorylation system (OXPHOS). The enzyme which synthesize ATP, ATP synthase, is the main object of the group's research. In addition to its central metabolic role, this enzyme is also the core of the mitochondrial mega-channel, which, once formed, leads to programmed cell death (PCD).

Mitochondria possess their own DNA, mtDNA, encoding only a few proteins – subunits of the OXPHOS complexes, 8 in yeast and 13 in humans. ATP synthase is built from 17 subunits, constructed into the catalytic F1 and membrane FO domains, linked by the external stalk. Three ATP synthase subunits in yeast (Atp9, Atp6, and Atp8) and two in humans (ATP6 and ATP8) are encoded in mtDNA, while the remaining (14 in yeast or 15 in humans) are encoded in the nuclear DNA. The biogenesis of the ATP synthase is one of the research areas of the group. We contradicted a model widely accepted by researchers in the field. We discovered that the control of expression of the mitochondrial ATP synthase genes by the F1 domain presence in the mitochondrial matrix is effectuated at the level of Atp9 subunit expression. The F1 is indispensable for Atp9 synthesis and its assembly into the ring (formed by 10 Atp9 peptides in yeast and 8 in humans). The mechanism of signalling involves Atp2 subunit of the F1 which interacts with the two accessory *ATP9* expression factors Aep1 and Atp25-C-terminal part, which switch

Left to right: Suchismita Masanta, Marta Sipko, Aneta Więsyk, Róża Kucharczyk, Kierutheile Thou, Joanna Kamińska

on Atp9 synthesis. Then the assembly of the whole complex proceeds, while the complex lacking subunit 6 or 6/8 signals the levels of synthesis of these subunits. We also characterised the role of a new accessory subunit of ATP synthase, Mco10, which is especially involved in the mega-channel formation by ATP synthase.

The unique and world-leading expertise of the team is in site-directed mutagenesis of mtDNA in *S. cerevisiae*. A very important effect of our research is construction of a system, named the Big-Mito-Split GFP. This permits detection, though fluorescence of the green fluorescent protein (GFP) molecule, the matrix pool of the protein localised dually, for example in the cytoplasm or nucleus and the mitochondria. We integrated in the mitochondrial DNA of the Big-Mito-Split strain a fragment of the *GFP* gene that does not fluoresce, while the missing fragment of the *GFP* gene we cloned into plasmid DNA, into which any gene can be cloned in fusion with this fragment. The fluorescent signal will be created only by the matrix pool of the studied protein, after its location to mitochondria. Self-assembly of both GFP parts gives fluorescence and because their synthesis is separated by the two mitochondrial membranes, the system does not give artefacts. We share the system with the scientific community interested to use it in the research.

Development of high-throughput DNA sequencing methods has made it possible to analyse the specific coding sequences of the human genome in diagnostic practice. This has led to the identification of gene sequence variants whose effect on the functionality of the proteins they encode is unknown. In order to determine the functionality of the altered proteins, it is necessary to conduct multiple analyses on different models. Despite being simple single-celled organisms, the yeast *S. cerevisiae* is often used to study human diseases. This experimental approach takes advantage of the fact of evolutionary conservation of cellular function among eukaryotes, as well as the presence of homologous genes in the

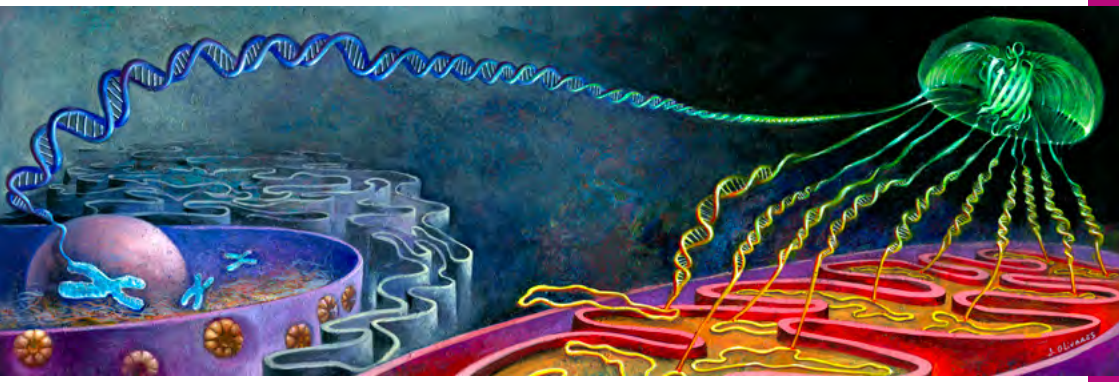
human and yeast genomes. Using yeast as a research model makes it possible to discover new aspects of disease pathogenesis.

Mutations in the ATP synthase genes lead to untreatable neurodegenerative diseases. We therefore focused on the study of the ATP6 and ATP8 variants found in patients to define their pathogenicity or polymorphism and understand the disease mechanism. To date we defined the character and the pathogenicity mechanisms, at the molecular level for some, for 24 mtDNA variants in ATP6. These studies let us to provide not only experimental data permitting understanding of the disease, but very importantly, to understand the mechanism of functioning of ATP synthase molecular machine, because the Atp9-ring forms the proton channel with subunit Atp6. The rotation of the Atp9-ring is driven by the proton gradient across the inner mitochondrial membrane. How the protons pass through the proton channel formed between the Atp9-ring and the Atp6 subunit is not well understood. Our research on the pathogenic variants have filled this gap and

Prof. Joanna Rytka

provided experimental data to understand proton translocation.

We also have created models to determine the molecular basis of rare diseases caused by mutations in the nuclear genes, *VPS13A-D* and *DHCR7*. Mutations in the *VPS13A-D* genes, encoding lipid transporters, have been linked to neurodegenerative diseases. Mutations in the *DHCR7* gene encoding 7-dehydrocholesterol reductase are associated with Smith-Lemlie-Opitz syndrome. As well as functional studies in yeast models, we conduct screens of genetic or chemical factors that compensate for defects caused by mutations.



An artistic vision of the BiG Mito-Split-GFP system by © James Olivares (eLife cover)

Such studies make it possible to propose new therapeutic targets. We have proposed calcineurin, calcium-dependent phosphatase and iron metabolism as two potential targets in diseases caused by *VPS13A-D* genes, and compounds from the flavonoid and copper ionophore groups as potential therapeutic compounds. Further work focuses on testing the selected compounds in another research model, HeLa cells with one of the *VPS13A-D* genes silenced. We will use these approaches to search for a potential therapy for Smith--Lemlie-Optiz syndrome.

Finally, a further area of research concerns two uncharacterised mitochondrial proteins: the ampylase Fmp40 and the depalmitoylase Ynl320w. Both are involved in the regulation of redox homeostasis in the mitochondria in yeast cells, as the ATP synthesis process is accompanied by reactive oxygen species production. We revealed an essential role for Fmp40 in regulation of the redox states of mitochondrial peroxiredoxins Prx1 and Trx3. Prx1 is the only protein shown to neutralize H_2O_2 in mitochondria, with the oxidation of mitochondrial glutathione and Trx3 directly involved in the reduction of Prx1. Deletion of *FMP40* impacts a cellular response to H_2O_2 treatment, at the level of up or down regulation of genes encoding redox enzymes, including mitochondrial redoxins Prx1, Trx3, and Grx2. This ultimately perturbs the reduced glutathione and NADPH cellular pools and impacts the yeast cell's survival. We demonstrated that Fmp40 AMPylates Prx1, Trx3, and Grx2 *in vitro* and interacts with Trx3 *in vivo*. AMPylation of the threonine residue 66 in Trx3 is essential for this protein's proper endogenous level and its precursor forms' maturation under oxidative stress conditions. Based on the results we proposed that Fmp40, especially in the conditions of respiratory growth, through control of the reduction of mitochondrial redoxins, regulates the H_2O_2, GSH and NADPH signaling influencing the PCD execution.

Future research will focus on the Fmp40 and Ynl320w role in the regulation of the ATP synthase and other mitochondrial proteins.

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Laboratory of Gene Expression Regulation

Prof. dr hab.
Tomasz Sarnowski

We are interested in deciphering of the evolutionarily conserved and diversified molecular properties of SWI/SNF chromatin remodelling complexes in various models including human, plants and shrimps. Our long-term goal is to understand better the role of epigenetics in important regulatory processes and exploit gathered knowledge to design advanced, innovative and safe treatments of various diseases including cancers.

The SWI/SNF-type ATP-dependent chromatin remodelling complexes (CRCs) are machineries present in cell nuclei of all eukaryotic organisms. There are several classes of SWI/SNF CRCs which differ by their subunit composition. They are responsible for switching on and off genes in a proper moment, tissue or developmental stage. They achieve this due to the control of the access of transcription factors or other regulatory proteins to DNA present in the chromatin. Their function is frequently affected in human cancer cells due to mutations and/or alterations caused by other factors. Partial inactivation of SWI/SNF CRCs leads to the development of various syndromes in human (e.g. Coffin Siris Syndrome, Nicolaides-Baraitser Syndrome, non-syndromic intellectual disability) and such severe developmental alterations in plants like dwarfism, sterility, abnormal root, leaf and flower development and sometimes lethality.

A study performed by various scientists, including our Laboratory, indicated that inactivation of some subunits of various classes of SWI/SNF complexes i.e. central ATPase subunits, SWP73 non-core subunits, etc. leads to the occurrence of so called synthetic lethality. This phenomenon has been observed for the first time in 1922 in fruit fly where it was shown that the combination of two viable mutations leads to the lethality. Interestingly, it has been recently proven that synthetic lethality may be used successfully to treat some types of cancer providing an attractive possibility to exploit the results we have gathered.

In our study, we have shown that SWI/SNF CRCs play an important, evolutionarily conserved role in hormonal crosstalk by

Left to right: Robert Bińkowski, Szymon Kubala, Maryam Jozghorbani, Magdalena Zaborowska, Jakub Chalimoniuk, Tomasz Sarnowski, Paweł Ćwiek, Jakub Szurmak, Magdalena Wilga, Jakkapong Kluebsoongnoen.

the maintenance of fine tuning of hormone signalling pathways through precise control of expression of genes belonging to particular pathways. Moreover, we proposed that *Arabidopsis* lines carrying T-DNA insertional mutations in genes encoding SWI/SNF subunits may serve as a perfect model for studying evolutionarily conserved function of chromatin remodelling complexes in a direction 'from plants to human'. Of note, we also designed and patented an innovative platform (based on an *Arabidopsis* mutant line) for identification of new compounds with properties suitable for treatment of such human metabolic disorders as type 2 diabetes, etc. As our Laboratory is in possession of a unique collection consisting of about 90,000 *Arabidopsis* T-DNA insertional mutant lines, the study may be easily extended towards other evolutionarily conserved or diversified processes upon necessity.

Given the fact that more than 20% of human cancers are characterized by mutations in genes encoding subunits of SWI/SNF CRCs, we extensively studied the role of SWI/SNF impairment in various cancer types. We focused on systematic assessment of the role of SWI/SNF CRCs function alteration or inactivation in several cancer types including clear cell renal cell carcinoma, salivary advanced adenoid cystic carcinoma, bladder, prostate, breast and uterus cancers.

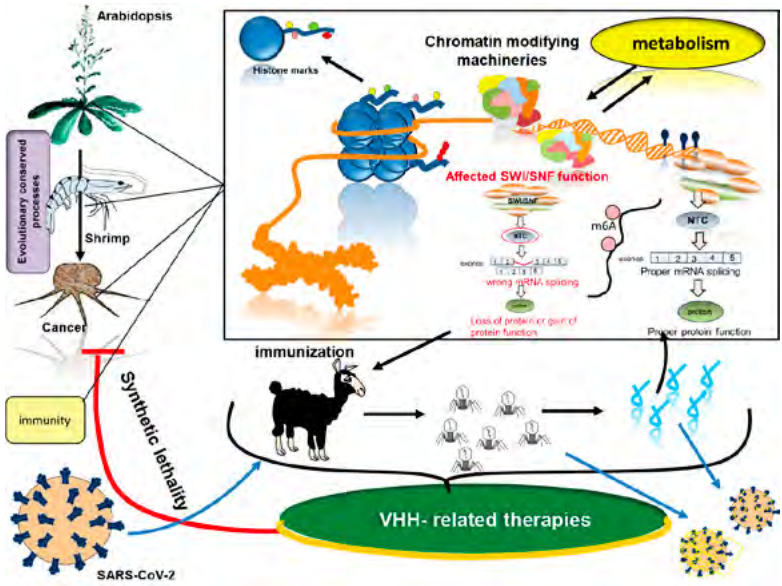
In the course of our study we found that SWI/SNF CRCs are involved directly in the control of various important regulatory pathways in the cell i.e. by interactions with their key elements. Basing on our results we conclude that SWI/SNF CRCs may play an important role in the control of metabolic processes via interaction with e.g. TOR and AMPK master regulators, but also are involved the regulation of alternative spli-

cing, alternative transcription start site (TSS) selection, RNA modification, DNA repair, ribonucleotide biosynthesis and protein modification. We found that SWI/SNF CRCs may play a vital role in the immune response, host-pathogen interactions and response to environmental cues. Last but not least, we identified a so far unrecognized mechanism of signal transduction involving membrane kinase receptors and SWI/SNF CRCs.

Despite the fact that relatively we know much about the function of SWI/SNF complexes, there is a complete lack of knowledge about that what happens just after the loss of SWI/SNF activity, because there are no good tools suitable for precise, inducible inactivation of their activity. In order to fill this gap, we are currently developing the technology based on VHH which will allow us to precisely target subunits of SWI/SNF CRCs in studied models with the aim to inactivate them. Furthermore, the VHHs developed by us may serve as precise tools for molecular biology.

At the beginning of 2020, when the SARS-CoV-2 outbreak appeared, we successfully employed the techniques and methodologies available in the laboratory, and in collaboration with Maria Sklodowska-Curie National Research Institute of Oncology we completed the collaborative project focused on the construction of VHH and M13 bacteriophage-based SARS-CoV-2-inactivating compound.

In summary, we uncovered the evolutionarily conserved roles of ATP-dependent SWI/SNF chromatin remodelling complexes in hormonal crosstalk in human and plants, and proposed utilization of *Arabidopsis* mutants with inactivated subunits of SWI/SNF CRCs



The graphical overview of past and ongoing studies carried out in the laboratory.

for studying evolutionarily conserved and evolutionarily diversified regulatory processes. We discovered the occurrence of synthetic lethality between various mutations inactivating subunits of SWI/SNF CRCs. We also recognized a dual function of SWI/SNF CRCs impairment in cancer development. We found that SWI/SNF CRCs are involved in an evolutionarily conserved manner in various regulatory processes including control of metabolism, DNA repair, RNA modifications, alternative splicing, alternative TSS selection, immune system modulation, host-pathogen interactions etc. We developed tools based on VHH and M13 bacteriophage which may be suitable for construction of compounds against SARS-CoV-2 and other existing and future pathogens with pandemic potential. We recognized intriguing, evolutionarily conserved interaction between membrane receptors and SWI/SNF CRCs and indicated a non-canonical function of membrane receptors. We additionally indicated the SWI/SNF CRCs and other epigenetics-related machineries as molecular targets in Hodgkin lymphoma.

Our future plans mainly focus on the further investigation of SWI/SNF CRCs role in the main regulatory processes in studied model organisms. We are interested in a better understanding of the role of SWI/SNF CRCs and other chromatin-related machineries in cancer development and progression, with special focus on exploitation of our results and knowledge to propose/design innovative methods of cancer treatment. Given the possession of a ready-to-use pipeline for design and identification of VHH and/or VHH-M13 bacteriophage based compounds against various pathogenic diseases, our additional long term future plan is to use this approach and skills upon necessity in case of future outbreaks appearance.

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Laboratory of Molecular Basis of Aging and Rejuvenation

Dr hab.
Ulrike Topf

Protein homeostasis is essential for normal cellular function in a healthy organism and loss of protein homeostasis leads to diseases. Our research focuses on identifying cellular mechanisms and pharmacological interventions that contribute to the maintenance of protein homeostasis during cellular stress and organismal ageing.

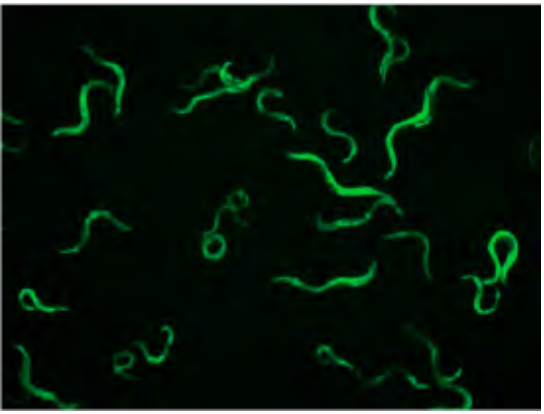
Age is the single greatest risk factor for the development of diseases such as cancer, diabetes, cardiovascular disease and neurodegenerative disorders. People are living longer, placing an increasing burden on society. Therefore, we need to develop novel interventions to keep the elderly population healthy for longer. How to address the arising problem if science is lagging in understanding the fundamentals of the aging process on the organismal, cellular and molecular level? We know that the balance between production of proteins and the removal of old proteins and damaged cellular components is a key characteristic of functional cells and health in organisms. This delicate homeostasis is lost during aging and is one of the main causes of the development of aging-related diseases. We hypothesize that we can exploit protective mechanisms in the cell to adjust protein homeostasis and be beneficial for the health of the aged organism. We are using the baker`s yeast, *Saccharomyces cerevisiae,* and the roundworm *Caenorhabditis elegans* to identify the reasons for protein homeostasis (proteostasis) collapse during aging. Both organisms are perfectly suited for research on the molecular and organismal level. These model organisms helped to reveal many fundamental mechanisms on how to extend lifespan. Our long-term goal is to understand better the complex crosstalk within the proteostasis network. This will allow us to develop novel interventions that alter or even ameliorate health deficits during aging.

Protein homeostasis is based on the well-adjusted cooperation between protein synthesis, protein folding and protein degradation. Multiple steps regulate each

Left to right: Katarzyna Jonak, Kamila Liput, Ramin Zadali, Hrudya Mohan, Spoorthy Gowda, Ulrike Topf, Monika Stasiak, Arun Kumar, Somayeh Shahmoradi Ghahe

of these fundamental cellular processes to adjust biogenesis of proteins to the current requirements of the cell. Under cellular stress conditions, the cell activates stress response mechanisms, which activate transcriptional, translational and post-translational processes that allow faster recovery and return to protein homeostasis. Chronic cellular stress, often manifested in pathologies, lead to a tipping point in proteostasis balance, where stress response mechanisms are no longer efficient enough to activate repair processes. Finally, failure of protein homeostasis is a hallmark of aging and aging-associated diseases. Our current understanding of mechanisms that are essential to maintain a correctly folded proteome is well detailed. However, little is known why these mechanisms fail during aging and how they can be modulated to delay aging and the accompanied decrease in health span.

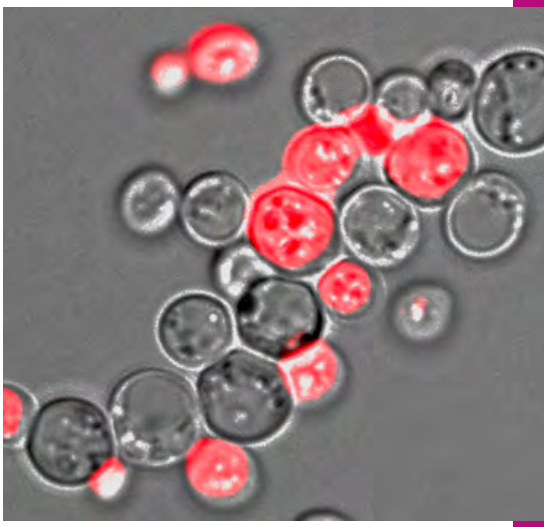
In many cases, molecular chaperones activate in response to cellular stress, assisting in the folding of newly synthesized proteins, but also play a role in the assembly of macromolecular complexes, disaggregation of misfolded proteins and in protein degradation. Thus, molecular chaperones are important mediators of proteostasis. In my laboratory, we have been studying the role of the co-chaperone prefoldin. Prefoldin is a protein complex consisting of six different subunits. Its main function is to help the folding of newly synthesized proteins.



C. elegans showing activation of the stress respons to heat shock

It is doing this by binding to polypeptides and handing it over to another chaperone that assists the protein to gain its functional conformation. The action of prefoldin does not require cellular energy, which makes it a perfect candidate to explore its role during cellular stress conditions characterized by decreased availability of energy. From our studies, we have learned that prefoldin can have versatile functions beyond its canonical role in the cell. Researchers in my laboratory have identified previously underappreciated functions of prefoldin during mitochondrial biogenesis and the assembly of the proteasome. Strengthening these processes would be beneficial for aged cells. Future research will need to focus on how to modulate the function of prefoldin for the benefit of old organisms.

The activation of many cellular stress response pathways leads to a down-regulation of cellular protein synthesis (translation) and changes in the synthesis of new proteins helping to adapt to the stress condition. Translation is a highly energy-consuming process, and cells try to conserve energy during stressful conditions. Protein synthesis declines with age. However, interventions that decrease protein synthesis early in life of model organisms can be beneficial for longevity. Thus, modulation of translation can serve as a protective mechanism for health benefits. There are many mechanisms to regulate translation, but we do not fully understand how it is controlled. Reactive oxygen species (ROS) could be important in controlling translation because their levels increase during ageing. Low levels of ROS can activate thiol-based redox switches, and adding ROS to the cell slows down protein synthesis. We applied a proteome-wide quantitative mass spectrometry approach to identify which proteins are sensitive to oxidative modification during aging and



Aged yeast cells. Dead cells are stain in red

identified many components of the protein synthesis machinery. Our findings during aging open a previously unknown possibility of regulating how many and what kind of proteins can be produced to adjust to a possibly harmful situation that would otherwise compromise cellular function and organismal health. My research team uses genetic and biochemical approaches to further investigate the changes in ribosomal protein composition within the ribosome complex and the ribosomal proteins post-translational modifications upon oxidative stress and during the aging process. We want to know how oxidative modifications affect the fate of single proteins involved in translation, how their oxidation influences protein synthesis, and how these changes in the translation apparatus affect organismal health.

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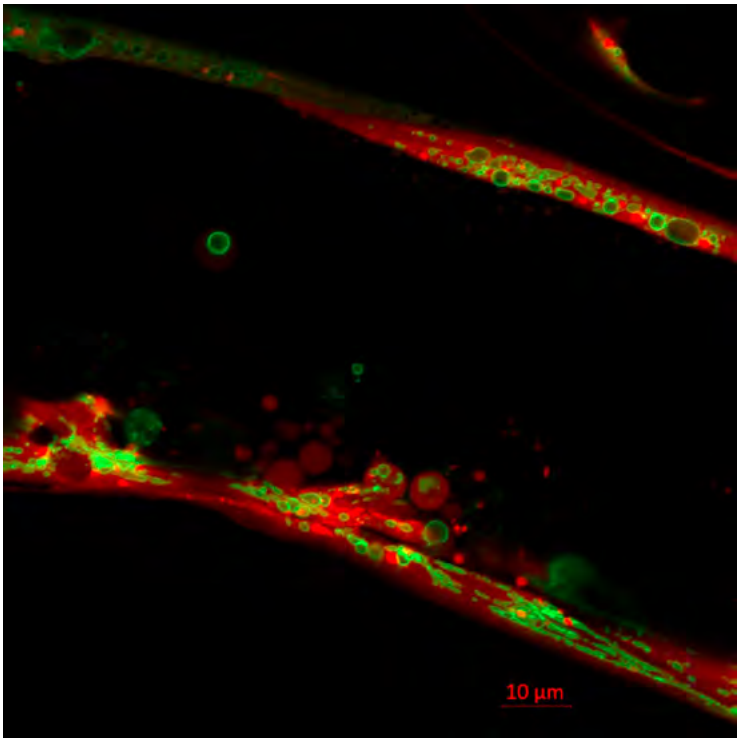
Laboratory of Animal Molecular Physiology

Dr
Michał Turek

The Laboratory of Animal Molecular Physiology investigates the functions and regulations of extracellular vesicles (EVs) across various biological contexts. The lab focuses on understanding how EVs are involved in removing cellular waste and facilitating intercellular communication. Utilizing *Caenorhabditis elegans* as a model organism, the research aims to uncover fundamental biological processes relevant to human health and disease.

Most living organisms consist of various cell types that constantly communicate with each other. During this communication, there may be an exchange of information in the form of nucleic acids as well as an exchange of building materials in the form of proteins. One of the carriers of this type of materials are the so-called extracellular vesicles (EVs). EVs are surrounded by a lipid membrane with biological material enclosed inside. After breaking away from the parent cell and often traveling considerable distances, they can be absorbed by another cell. In addition to the mentioned function in intercellular communication, vesicles can also be used to remove harmful, malfunctioning proteins as well as defective cell organelles. Despite decades of dedicated research, our understanding of EV regulation remains somewhat rudimentary. The majority of our knowledge has been gleaned from studying isolated EVs obtained from single cell types, neglecting the complex interactions within multicellular organisms. It is becoming increasingly evident that to fully comprehend EV regulation, we must shift our focus towards investigating them within the dynamic context of multicellular organisms, embracing a holistic perspective that accounts for the intricate interplay between different cell types and organ systems.

The Laboratory of Animal Molecular Physiology, headed by Dr Michał Turek, investigates EV function and their non-cell autonomous regulation extending across diverse biological contexts, encompassing developmental processes, dysregulated cell proliferation, immune responses, and the intricate dynamics of social interactions among organisms. Furthermore, its mem-



This image showcases *Caenorhabditis elegans* muscle exophers containing mitochondria. The muscles visible have released these specialized extracellular vesicles, highlighted with green fluorescence indicating the mitochondrial outer membrane (GFP) and red fluorescence marking the vesicle's protein components (RPN-5::wrmScarlet). A scale bar of 10 µm is included to provide a reference for the size of the structures observed.

bers aim to unravel the epigenetic mechanisms steering EV formation probing the roles of long non-coding RNAs and small RNAs in EV regulation and function. In their research they use *Caenorhabditis elegans* as a model organism, as this small, transparent nematode worm has become a powerful tool in scientific research. This organism is favoured by researchers for several key reasons: it has a simple structure, is easy to grow in large numbers, and has a short lifecycle, allowing for the observation of multiple generations in a short span of time. Remarkably, despite its simplicity, *C. elegans* shares many biological characteristics with humans, including essential cellular processes and gene functions. This makes it an excellent model for studying human diseases, genetics, and developmental biology. Additionally, its transparency allows direct observation of cellular activities and developmental stages, providing valuable insights into complex biological phenomena in a more controlled

Left to right: Ramakrishnan Ponath Sukumaran, Justyna Polaczyk, Satya Vadlamani, Michał Turek, Agata Szczepańska, Klaudia Kołodziejska

and observable environment than would be possible with more complex organisms.

Recent studies conducted by the Laboratory of Animal Molecular Physiology have illuminated remarkable aspects of cellular mechanisms relevant to aging and intercellular communication. In their study published in EMBO Reports, researchers discovered that muscle cells in *C. elegans* expel large EVs known as exophers, which are instrumental in promoting reproductive fitness by transporting essential yolk proteins to developing embryos. This process, driven by signals from developing embryos, optimizes the nutritional support for the offspring, enhancing their growth and development. In another article in Nature Communications, the laboratory explored the pheromone communication among *C. elegans* which influences the dynamics of somatic EV production. This study reveals a sophisticated system where cellular communication, mediated through EVs, is closely tied to the organism's environmental interactions and social behaviours.

These findings underscore the laboratory's innovative use of *C. elegans* to elucidate fundamental biological processes. Through

these concerted efforts, its members aim to advance our understanding of the complex interplay between non-cell autonomous and epigenetic regulation of EV formation in a metazoan model organism. By shedding light on these processes, their research endeavours hold the promise of offering valuable insights into the broader implications of EV regulation in human health and disease.

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* co-first author

† co-corresponding author

Laboratory of Intracellular Immunity

Dr
Michał Wandel

We are interested in understanding the interactions of pathogenic microorganisms with their human host. We explore the molecular details of how cell-intrinsic and cytokine-enhanced immunity protects the interior of the host cell against bacterial and fungal invasion, and also how pathogens evade the intracellular immune system.

The research group has been established at the IBB PAS in 2021 through generous funding from EMBO (Installation Grant), NAWA (Polish Returns) and NCN (OPUS, SONATA). The laboratory is led by Dr Michal Wandel, who returned to Warsaw from Cambridge in the UK, where he completed his PhD at the University of Cambridge and post-doc at the renowned MRC Laboratory of Molecular Biology. Presently the group consists of two PhD students and a post-doc. In our newly equipped and refurbished laboratory we endeavour to understand intracellular mechanisms of anti-microbial immunity.

The immune system identifies and eliminates microorganisms that breach the integrity of our tissues, but we are only beginning to uncover the intracellular mechanisms that engage invading pathogens to restrict infection. Not only specialised immune cells, but also regular cells (e.g. epithelial cells) have evolved complex autonomous mechanisms to detect and respond to infection. Exposure to cytokines, e.g. interferons, enhances cell-autonomous immunity for efficient control of diverse intracellular pathogens through the induction of target genes. On the other hand, pathogens devise various adaptation strategies to evade or overcome those defence mechanisms.

A prime example of the interferon-induced pro-inflammatory immunity factors are the GTPases from the guanylate-binding proteins (GBPs) family. GBPs sense cytosolic bacteria to form a signalling platform, which by recruiting and activating the cytosolic lipopolysaccharide receptor, Caspase-4, initiates pyroptotic death of infected cells and, via the pro-inflammatory cytokine IL-18, informs immune cells of the ongoing infection (Photo 4). However, despite recent advances, and in contrast to relatively well-studied anti-viral

Left to right: Ammarah Shabbir, Vartika Gurdaswani Khubchandani, Aleksandra Bajger, Michał Wandel

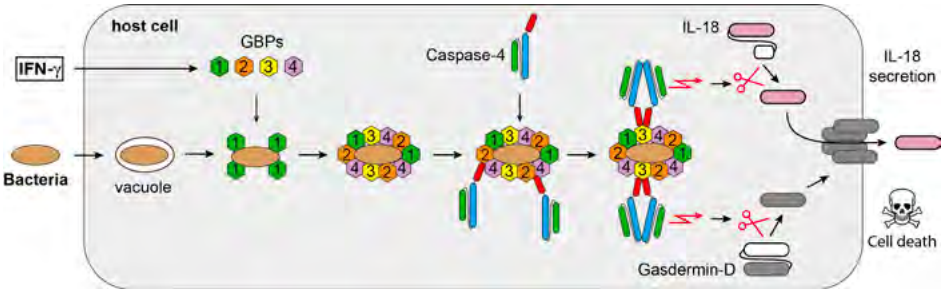
immunity, the molecular details of how cytokine-enhanced immunity protects the cell interior against bacterial, and especially fungal pathogens, is severely understudied.

Currently, at the Laboratory of Intracellular Immunity we investigate the cytokine-induced anti-pathogen state activated to resist bacterial infection. We are also initiating a research program to study the host-fungi interactions and anti-fungal cell-intrinsic and innate immune defences. At the molecular level, we research how our body's cells recognise microorganisms and to what immune response it leads – which signalling pathways are activated, what is the fate of the detected pathogen and the host cell, and why? Also, we are interested in the subversive strategies that pathogens have developed to evade the immune system. We employ large-scale screening approaches to identify novel host factors of the cell-autonomous and cytokine-inducible anti-microbial defence mechanisms of innate

Laboratory space

immunity. Moreover, we aim to determine the specific molecular mechanisms of action of newly identified immunity factors.

Using cutting-edge technologies, we are driving a deeper understanding of how core immunological processes are regulated in favour of the host, or the pathogen, and how these new mechanisms might be harnessed to treat diverse diseases. We employ state--of-the-art screening approaches to identify host restriction factors required for defence against pathogenic microorganisms. Using a combination of molecular biology, cell biology, genetics, proteomics, biochemistry and microbiology, we determine the specific mechanism of action of novel immunity factors, to understand their effector functions, downstream signalling and pro-inflammatory potential. More specifically, our primary experimental models are mammalian cells infected *in vitro* with pathogens in the safe environment of a modern BSL2 cell culture laboratory. We have extensive experience in cell manipulation (e.g., transfection, re-



Schematic illustration of the GBP-CASP4 signalling platform

troviral/lentiviral transduction, RNAi gene silencing, and CRISPR/Cas9 technology). Amongst many techniques, we routinely use standard techniques of molecular biology and biochemistry (e.g. cloning and mutagenesis of DNA, large scale recombinant protein expression and purification from bacterial systems, Western blotting), and cell biology of infection (e.g. fluorescent microscopy).

Our research through identification of host restriction factors may allow future development of novel therapeutics directed against pathogenic microorganisms, including difficult to treat multidrug resistant strains. Furthermore, since interferon plays an essential role in the development of systemic autoimmunity, understanding of the molecular mechanisms initiating pro-inflammatory processes may lead to better treatment of inflammatory diseases.

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Laboratory of Yeast Genetics and Molecular Biology*

Prof. dr hab.
Teresa Żołądek

The subject of our studies are the mechanisms of protein transport between subcellular compartments to understand better how defects of transport contribute to human diseases. We utilize yeast models of neurodegenerative diseases to discover ways to overcome defects that are caused by pathogenic mutations, learn more about the function of disease-related proteins, and discover new drugs and drug targets to treat selected diseases.

In the past, we studied processes that are dependent on ubiquitination involving homologous ubiquitin ligases, yeast Rsp5, and human NEDD4. We showed that ubiquitination is required for the functioning of the actin cytoskeleton and endocytosis, and we showed Rsp5 participation in actin polymerisation *in vitro*. Moreover, we show the contribution of Rsp5/Nedd4-dependent ubiquitination in lipid biosynthesis, lipid droplet formation, endosomal sorting, vesicular transport between Golgi apparatus and endoplasmic reticulum, nuclear-cytoplasmic transport of tRNA, heme biosynthesis and phosphatidylinositol-3-phosphate (PI3P) dependent formation of IPOD (Insoluble Protein Deposit), the structure which is important for proteostasis. We also analyzed various aspects of Rsp5 regulation, such as PKA kinase-dependent phosphorylation and its nuclear-cytoplasmic shuttling.

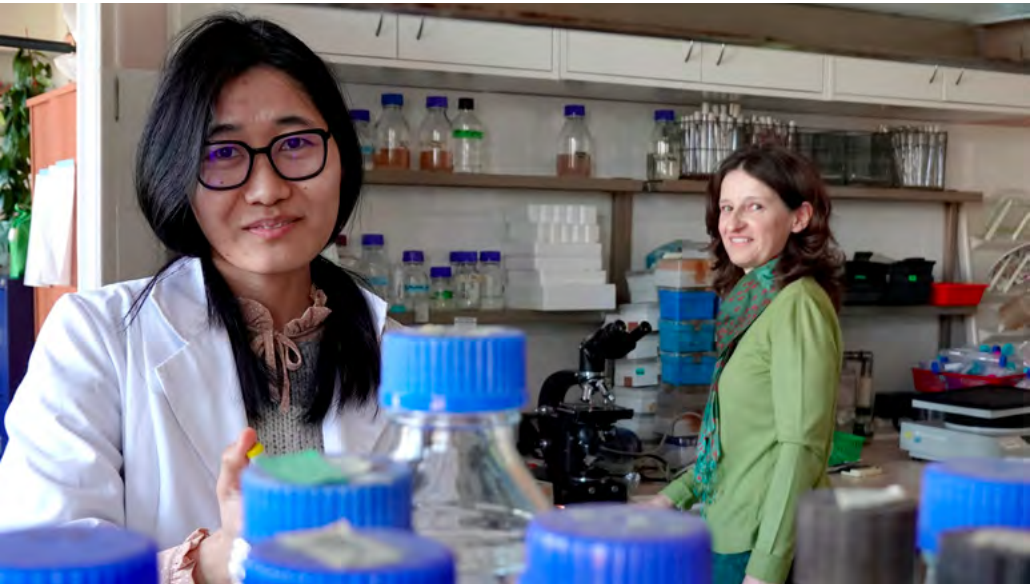
Logo of the Laboratory of Yeast Genetic and Molecular Biology

The genetics studies turned our attention to Atg2 and Vps13 family proteins, specially

* The laboratory was active until 30.06.2024

to human ATG2A and VPS13A, which were later shown to be bulk lipid transporters located in the membrane contract sites between various organelles. We used yeast to model a rare neurodegenerative disease, *VPS13A*-dependent chorea-acanthocytosis (ChAc) and found new phenotypes caused by point mutation *vps13-I2749R* corresponding to a *VPS13A-I2771R* mutation found in ChAc patients. We showed that amino acid substitution in the APT1 domain of Vps13 caused by this mutation disturbs its functioning by weakening PI3P binding, changing its lipid-binding specificity, and affects Vps13 localization. One of the phenotypes of *vps13-I2749R* and *vps13*Δ, hypersensitivity to SDS (sodium dodecyl sulphate, a detergent commonly found in cosmetics and household products), was successfully applied for isolation of multicopy suppressors, second site suppressors, and chemical suppressors. Thus, this yeast model constitutes a simple platform suitable for high throughput screens. Analysis of these suppressors showed increased activation of calcium signalling in *vps13* mutants and indicated calcium-dependent phosphatase, calcineurin, as a potential drug target for ChAc and other neurodegenerative diseases caused by mutations in human *VPS13A-D* genes. Finding the suppressor *RCN2* gene pointed to Rcn2 and other specific peptides and chemical inhibitors of calcineurin as potential treatments for these diseases. The other group of multicopy suppressors identified encode iron and copper transporters (Fet4, Ctr1), and we showed that the mechanism of their action relies on causing an

Left to right: Kierutheile Thou, Joanna Kamińska, Teresa Żołądek, Marek Skoneczny



Kierutheile Thou and Joanna Kamińska working in the laboratory

increase in intracellular iron content. This is in agreement with the fact that copper is required for the functioning of the high-affinity iron uptake system. Moreover, copper salts were found among chemical suppressors. This pointed to copper and iron dyshomeostasis in cells defective in Vps13, which was not known before, as a possible new target for ChAc treatment.

The localization of Vps13 in various membrane contact sites is dependent on interactions with specific proteins and lipids, characteristic for different subcellular compartments. Besides the lipid binding by the APT1 domain, we also identified Arf1, the GTPase, as a Vps13 and VPS13A binding protein. To find other proteins that determine the localization and regulation of Vps13, we purified Vps13-TAP from yeast cells by a pull-down method and identified interacting proteins by mass spectrometry. Among the identified proteins, we found the Rsp5 ubiquitin ligase of the NEDD4 family. The Vps13-Rsp5 interaction was confirmed by immunoprecipitation and western blot analysis. This prompted us to analyse the genetic interaction between *VPS13* and *RSP5* genes, and we found that additional copies of the *RSP5* introduced into *vps13*Δ mutant cells increase their sensitivity to SDS. We also observed a lower level of Rsp5 E3 ubiquitin ligase in *vps13*Δ compared to the wild-type cells. These and other results suggest that the Vps13-Rsp5 interaction may be important for the coordination of lipid synthesis with the lipid transport capacity of the yeast and human cells.

The phenotype of the hypersensitivity to SDS of *vps13*Δ cells was also used to screen the Prestwick library of repurposed drugs. Three groups of drugs that restored growth were found, among them copper ionophores and flavonoids, which bind copper and iron, respectively. These findings correlate well with suppressor studies. These newly identified potential repurpose drugs may be effective in alleviating defects caused by *VPS13A* gene mutations in humans, but this requires further study in other cell models. A human HeLa cell-based model with regulated silencing of the *VPS13A* gene is now established to continue the analysis. This is important since, to date, there are no specific treatments for *VPS13*-related diseases; only symptomatic treatment is used.

We also studied *S. cerevisiae* Hsp31, structurally similar to human DJ-1, a Parkinson's disease-related protein. It is a stress response and stress-protecting protein displaying the activity of glyoxalase III, decomposing toxic compound methylglyoxal. Hsp31 is also suggested to have a chaperone function. We recently discovered it has a periplasmic localization where it can serve as the first line of defense against environmental stresses. We identified amino acid residues within the Hsp31 polypeptide that are important for its secretion, and we demonstrated that non-secreted Hsp31 does not protect against these stresses. Since Hsp31 does not possess a recognizable secretion signal, it is likely secreted via one of unconventional protein secretion (UPS) routes. Therefore, we performed a genome-wide screening to identify proteins involved in UPS in *S. cerevisiae*. Our screen revealed Ssd1, a translational repressor that regulates the expression of a subset of genes encoding proteins involved in transactions on the cellular boundary. UPS is essential for the invasiveness of human pathogens, such as *Candida albicans*. Therefore, studying UPS is of great medical importance.

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Fluorescence Microscopy Facility

Dr
Anna Anielska-Mazur

The Fluorescence Microscopy Facility serves as a hub within IBB where both qualitative and quantitative research on biological specimens, employing light microscopy techniques, can be carried out. The facility is open to IBB research staff and also extends its services to external units. We actively engage in collaborative projects, offer training opportunities, and promote science and our institute.

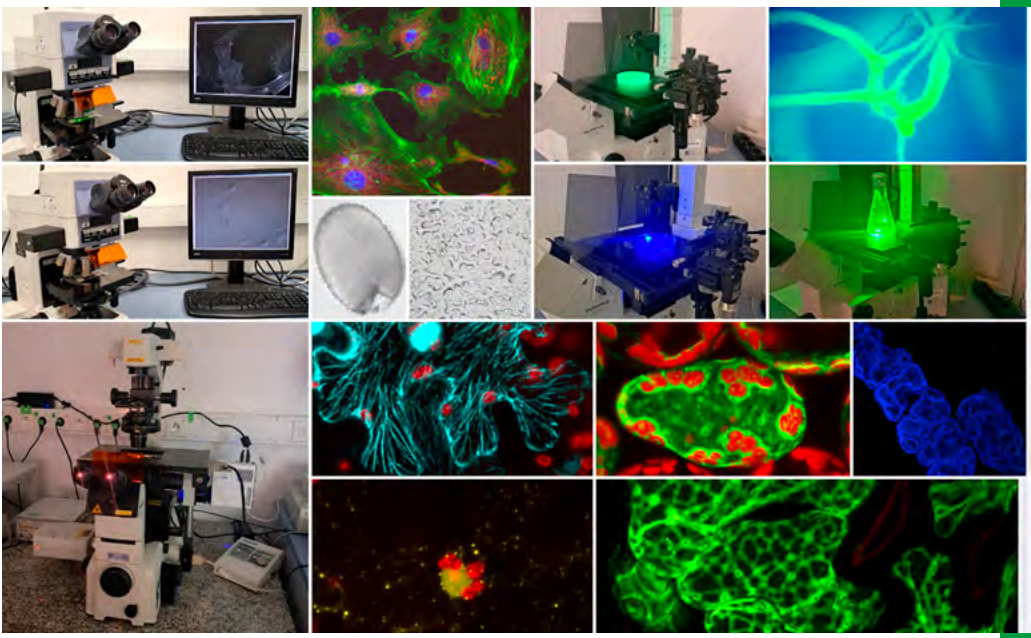
The Fluorescence Microscopy Facility functions as both a service and research unit, equipping researchers with the necessary tools and expertise for imaging cells, tissues, and organisms, and conducting qualitative and quantitative analyses. Crucially, the Facility's service also encompasses providing training to IBB researchers in specimen preparation techniques, imaging, and basic image analysis. Researchers affiliated with our institute, after receiving suitable training, have the flexibility to conduct independent experiments using the Facilities resources or seek help from our staff, including technical assistance, imaging support, and consultation. We also extend paid services to scientists from other institutions, subject to availability.

The Facility offers capabilities for capturing naturally contrasted and stained specimens using a color camera, as well as fluorescent specimens using a monochromatic camera or a confocal system. Moreover, we provide options for registering and observing images in transmitted light with Nomarski or phase contrast. We also offer light microscopes equipped with image recording systems and a rotary microtome (Leica RM2145). Specifically, our light microscopy instrumentation features two widefield fluorescence microscopes (Eclipse E800 Nikon and IX-70 Olympus) and the confocal microscope (Nikon EZC1 confocal laser scanning microscope). Our microscopy systems are optimized for imaging on glass slides or in chambered cover glass. Facility equipment is available to users through a reservation system for registered users.

The Facility's staff bring years of experience in imaging a variety of samples, including live and preserved plant cells and tissues, nematodes, fixed mammalian cells, fungi,

yeast, protozoa, and bacteria, to assist our research community of users in acquiring the best possible microscopy data to achieve their research goals. While our analyses span across organisms and cells from diverse taxonomic groups, our lab is particularly specialized and experienced in research on plant material. Our daily experience has been gained over 15 years. Over the past three years, our services have been utilized by more than 15 research laboratories within our institute, as well as departments at the University of Warsaw and Warsaw University of Life Sciences. The lab itself does not engage in independent scientific research projects; rather, it contributes to scientific projects conducted elsewhere as a collaborating entity. Our main collaborators are from the Laboratory of Plant Protein Phosphorylation, the Laboratory of Lipid Biochemistry and the Laboratory of Plant Pathogenesis, among many others. In general, we record signals from preparations following immunolocalization, histochemical staining, or binding to selective markers. We capture fluorescence from living samples where the protein of interest is fused with intrinsically fluorescent proteins (IFPs) such as cyan fluorescent protein (CFP) or green fluorescent protein (GFP) or others, in transiently or stably transformed cells, tissues, or organisms. Additionally, we provide support in the selection of transformants. Our commonly conducted analyses focus on identifying, localizing, and colocalizing molecules, macromolecules, organelles, and cellular compartments, as well as their behavior during environmental changes and physiological processes. We measure interactions between selected proteins using the bimolecular fluorescence complementation (BiFC) method, and we assess the dynamics and diffusion of IFP-tagged proteins through fluorescence recovery after photobleaching (FRAP). We also perform real-time analysis occurring in plant cells or tissues during development processes and stress, considering various plant mutants and the reversibility of changes (IFPs, FM 4-64), and monitor the formation and disaggregation of biomolecular condensates in the cytoplasm and

Left to right: Anna Anielska-Mazur and Małgorzata Lichocka



the nucleus. Captured data can be analyzed using various software (Lucia ORCA, EZ-C1 viewer and ImageJ/FIJI), and facility staff can provide support upon user demand through their extensive experience.

The obtained microscopy data complements other types of analyses performed by researcher/users, providing a broader picture of the issue under study, and is utilized in Master's theses, doctoral dissertations, conference materials, and scientific publications. In addition to provision of our services for research and providing individual training, the Facility's activity over the last few years has included coordinating a course on microscopy for the Doctoral School entitled 'Biological Imaging', participating in promoting science and the institute by conducting workshops for school students and lectures for students at the Faculty of Biology and Earth Sciences of the Jagiellonian University. We are open to scientific collaboration and other activities. We invite potential users and collaborators to visit the facility to discuss their microscopy needs with our staff.

Mass Spectrometry Facility

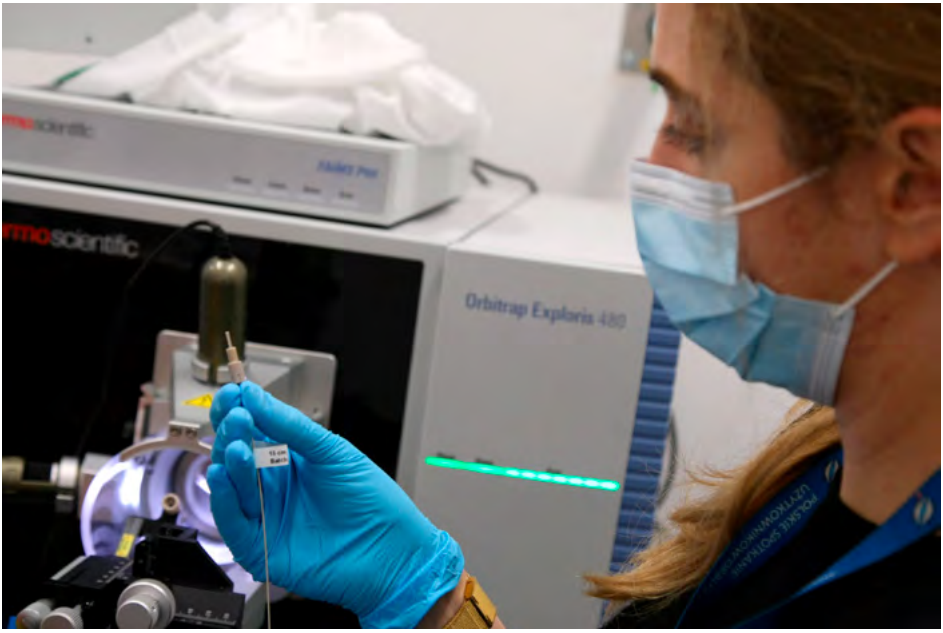
Prof. dr hab.
Michał Dadlez

The Facility offers proteomic, metabolomic, lipidomic, and structural analyses. Recognized for its expertise and advanced technology, it is a hub for scientific research and implementation of projects for biomedicine-oriented groups. The Facility provides essential diagnostic services to hospitals and conducts both small-molecule and proteomic analyses, using advanced techniques for protein identification and characterization.

The Mass Spectrometry Facility (MSF), led by prof. Michał Dadlez, has been operating since 2001 and specializes in proteomic, metabolomic, lipidomic, and structural analyses conducted on behalf of internal and external laboratories as well as companies, both domestic and international.

The Facility is continuously expanding its portfolio of instruments, which currently includes eleven LC-MS and one GC-MS system, with three new mass spectrometers introduced in 2021 and 2022. Two new Evosep One - Exploris 480 systems allowed the Facility to expand the scope of analytical methods and speed up proteomics measurements five-fold, when compared to previous capabilities. MSF also possess a range of other equipment, allowing for the preparation of virtually any type of material for analysis. This enables the Facility to conduct MS analyses for many scientific institutions and other entities outside academia. Given the scale of the Facility's operations, providing analyses for numerous external entities, and the concentration of state-of-the-art MS measurement technology, significant computational power, and a team of professionals with extensive experience in one place, the Facility is currently recognized as one of the largest and best-equipped MS laboratories, not only in Poland, but also in Europe.

The MSF collaborates in numerous scientific and implementation projects in the field of biomedicine, and also provides access to analyses as a scientific service. Currently, mass spectrometry-based analyses have become a required standard, and many research projects cannot be conducted at an appropriate level without continuous access to the infrastructure and expertise of a pro-



New Exploris 480 mass spectrometers are in good hands of Bianka Świderska.

teomic and metabolomic laboratory. The Facility performs several thousand proteomic and metabolomic determinations annually.

During the last three years, the Facility's researchers have expanded their knowledge and experience by cooperating with around 50 research institutions (including Warsaw University, Jagiellonian University, Warsaw Medical University, multiple Institutes of the Polish Academy of Sciences, among others) and 20 biotechnological and pharmaceutical companies (e.g. Adamed, FiLeClo, Captor Therapeutics, Sandoz Poland, Molecure, BGW). In addition, the laboratory provides diagnostic services for 15 hospitals, mainly therapeutic drug monitoring (immunosuppressants, antirheumatic drugs) and amino acids analyses. In 2010, as a part of the Mass Spectrometry Laboratory, a specialized workgroup was established for therapeutic drug monitoring, pharmacokinetic studies, and metabolomic profiling. It is listed in the national registry of diagnostic medical laboratories, held by the National Chamber of Laboratory Diagnosticians, under number 3089 as Laboratory of Drugs and Metabolites Research.

Small-molecule research in the MSF focuses on gut microbiome-metabolome interactions, and their influence on disease development, progression, and on potential treatment applications in different fields of medicine (oncology, cardiology, dermatology). The second area of our interest is searching how dietary, lifestyle or environmental conditions affect the nutritional values of

Many spectrometers available in the facility ensure uninterrupted work. The spectrometers shown provide quantitative analyses of small molecules (metabolomics), also serving polish patients with immunosuppressive drugs monitoring.

mothers' milk using global metabolomics and lipidomics studies, as well as targeted quantitative analyses.

The proteomics division of MSF undertakes projects spanning basic research as well as studies focusing on human diseases and parameters critical to the fisheries industry, pig farming, and equine breeding. The scope of analyses includes not only protein identification, determination of their interactors and post-translational modifications, but also complex large-scale differential analyses and development of protein diagnostic panels. One example of employing proteomics in medical research was the project conducted under the leadership of Prof. MD Piotr Laudański , focused on identifying protein markers for endometriosis. Through global proteomic analyses of plasma, peritoneal fluid and extracellular vesicles, the aim was to discover protein markers allowing for non-invasive diagnosis of endometriosis through patient blood testing. Based on the research results, a selection of 40 proteins has been identified for targeted testing in a larger patient group, a task scheduled for completion within this year.

For protein structure analysis we use the methodology of monitoring protein structural dynamics by measuring hydrogen--deuterium exchange along with developing new data analysis software.

1. New Exploris 480 mass spectrometers are in good hands of Bianka Świderska.
2. The miracle of transfer of molecules from solvent to the gas phase by electrospray happens here – the ion source is a critical element of a mass spectrometer.
3. A combination of two systems coupling liquid chromatography separation with mass spectrometry (LC-MS) provides enormous capacity of 24 h a day, 7 days a week automated sample analysis for proteomics, thanks to efficient autosamplers (orange block in front).
4. Many spectrometers available in the facility ensure uninterrupted work. The spectrometers shown provide quantitative analyses of small molecules (metabolomics), also serving polish patients with immunosuppressive drugs monitoring.
5. Also molecules from the gas phase, volatile compounds can be analyses in GC-MS instruments.

DNA Sequencing and Synthesis Facility

Dr
Robert Gromadka

The Facility is a leading-edge hub for genomic research. We offer a comprehensive suite of services that support a wide range of research endeavors. We perform sequencing based on classical Sanger chemistry and second and third generation sequencing, from sample preparation to data analysis. We offer synthesis of various standard and labeled primers. We help choose the right method, tailored to the planned experiments.

The Sequencing and Synthesis unit team has experience performing classical Sanger sequencing (since the 1990s) and next generation sequencing (NGS) since the advent of massive parallel sequencing strategies (454 sequencing). Currently we perform high throughput sequencing on Illumina (since 2012) and Oxford Nanopore (since 2014) platforms. Our facility also provides synthesis services of DNA/RNA oligonucleotides with fast turnaround time (typically 24-48 h), which are crucial in various molecular biology applications by enabling the amplification, sequencing, and analysis of specific DNA sequences.

The DNA Sequencing and Synthesis Facility has been involved in many national and international sequencing projects. Our experienced team has been engaged in multiple functional genomic projects, including sequencing of a fragment of the first eukaryotic genome of baker's yeast, *Saccharomyces cerevisiae*, in the 1990s, the first megabase-sized chromosome of *Paramecium spp*., the first genome of the important tuber crop potato, *Solanum tuberosum*, and the first reference genome of the human opportunistic pathogen, *Prototheca wickerhamii*. We have also taken part in many metagenomics and bacterial genome sequencing projects, with the results published in reputable international journals. We are a coexecutor of grants based on sequencing techniques.

Our facility is equipped with advanced infrastructure for DNA/RNA sample quality and quantity assessment such as Biorad PFGE apparatus, PicoDrop, Qubit and Agilent FemtoPulse. FemtoPulse is a powerful and automated pulsed-field capillary electrophoresis system ideal for long-read NGS

Left to right: Karolina Żuchniewicz, Piotr Dzierzbicki, Małgorzata Filipiak, Jarosław Cieśla, Ewa Kalińska, Robert Gromadka, Beata Babińska, Jan Gawor, Karolina Wylot

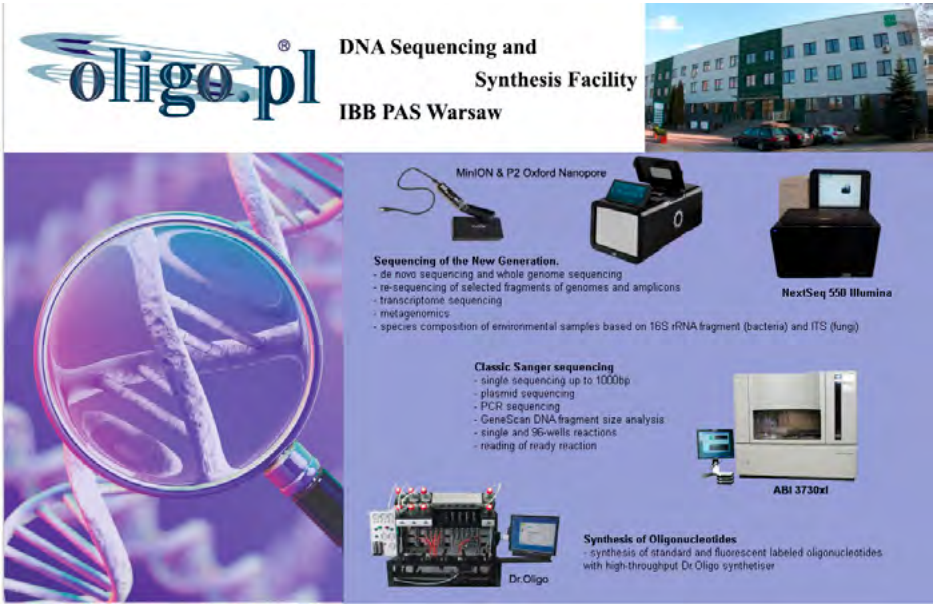
QC, gDNA, small RNA, or cfDNA analysis from low concentration samples. It has the ability to separate high molecular weight DNA up to 165 kb and detect nucleic acids down to 50 fg/μL input concentration. Also in our possession are high-throughput sequencers for classical sequencing, ABI3730 and ABI3730xl; next-generation sequencing instruments, MiSeq and NextSeq 550 sequencers (Illumina); and third-generation long-read sequencing, MinION. GridION and P2 Solo instruments. Through a collaboration with the Mother and Child Institute in Warsaw, we also have access to an Illumina NovaSeq 6000 ultra-deep sequencing system.

Depending on specific requirements, we can provide sequencing with a low or high throughput. We perform sample preparation and quality control assessments for each of our platforms. Each sequencing service includes data processing, quality control, and data delivery. We deliver high-quality, filtered sequence data and associated quality scores. We also provide comprehensive data analysis services based on customer demands. Our facility focuses on the sequencing of small genomes (bacteria and viruses) and medium-size genomes of eukaryotes (fungal and algal genomes). Based on strong expertise in long-read sequencing, the generation of complete genomes with high consensus accuracy is one of our great advantages. We also offer targeted amplicon sequencing for microbiome analysis, such as selected variable regions of 16S rRNA or fungal rDNA conserved regions of the internal transcribed spacer fragments. To measure changes in expression levels and discover

novel transcripts or single-nucleotide polymorphisms, we can conduct transcriptome and metatranscriptome RNA sequencing.

Recently, we participated in the implementation of IBB projects such as: sequencing and sequence analysis of genomes of *Streptococcus pyogenes* bacteria to selected groups of strains (serotypes), in which a non-random association with resistance to antibiotics was observed; sequencing the genomes of bacteriophages and their host genomes in search of the molecular basis of broad specificity and survival strategies in the host; sequencing and analysis of libraries of a *Streptococcus anginosus* mutant pool to identify virulence factors. We are co-contractors of projects in cooperation with other scientific institutes such as the National Medicines Institute (sequence analysis of genomes of *Streptococcus pneumoniae* bacteria to characterize invasive serotype 19A in Poland); the Department of Medical Microbiology of the University of Warsaw (sequence analysis of algae genomes of the genus Prototheca sp. in order to identify potential virulence genes); the University of Life Sciences in Lublin (sequencing and analysis of mitochondrial genomes of dogs to identify mutations responsible for cancer processes); the Institute of Genetics and Biotechnology of the University of Warsaw (sequencing of human mitochondrial genomes to identify point mutations). Additionally, we provide services to other laboratories at Ochota Campus and many other institutions in Poland. In recent years, we have provided services for over 100 scientific institutions and more than 20 companies every year.

For more information, please visit our website at www.oligo.pl.



Cell Culture and Protein Production Facility

Dr hab.
Ewa Szołajska

The Facility provides IBB researchers with access to laboratory space and equipment essential for mammalian and insect cell culture research. We also provide cell preparation and experimental support to groups whose research does not routinely involve *in vitro* animal cell culture. In addition, the Facility offers services for protein production using the baculovirus-insect cell expression system.

The Cell Culture and Protein Production Facility provides IBB researchers and PhD students with access to laboratory space and equipment essential for research involving mammalian and insect cell culture. Central to this function are our cell culture hoods, which provide an aseptic environment for cell manipulation, and carbon dioxide (CO_2) incubators, which provide the ideal conditions for the survival and growth of mammalian cells. In addition to standard CO_2 incubators, the HypoxyLab bench-top incubator and workstation (Oxford Optronix), which precisely controls the oxygen level, is also available. The CKX41 Olympus inverted microscope is used for regular cell observation, *while the Invitrogen Countess Automated Cell Counter provides accurate cell and viability counts.*

The Facility's flow cytometer, a BD FACSCalibur cell analyzer and sorter, is currently used for studies conducted in the yeast model system. Measurements include analyses of cell cycle, DNA content and replication, reactive oxygen species and apoptosis markers.

Members of several laboratories are regular users of the Facility's infrastructure, but we also provide cell preparation and experimental support to groups whose research does not routinely involve *in vitro* mammalian and insect cell culture. One such collaboration involved assessing the safety of the future use of a peptide inhibitor in the early phase of SARS-CoV-2 infection, developed by the group of Prof Piotr Zielenkiewicz. *In vitro* viability testing in a human lung cell model showed no cytotoxicity of the hexapeptide. This suggested that a peptide that effectively blocks the interactions between the viral spike protein and

Left to right: Ewa Szołajska, Małgorzata Podsiadło-Białoskórska

the human receptor, angiotensin-converting enzyme 2, is a good candidate for further drug development.

We also participated in the research conducted by Prof. Tomasz Sarnowski's group in collaboration with Mahidol University (Thailand). The study involved analyses of the activity of the promoter region of the gonad-inhibiting hormone (*GIH*) gene of the black tiger shrimp, *Penaeus monodon*, performed in SF9 insect cells. A possible role of the ecdysone receptor in modulating *GIH* gene expression was demonstrated. These results may have useful implications for improving shrimp production in the future.

For the research carried out in the laboratory of Prof. Wojciech Bal, we provided cancer cells to generate a zebrafish embryo melanoma model, which was used to develop the method of *in vivo* detection of different types of melanin radicals, applying electron paramagnetic resonance spectroscopy.

Currently, we are participating in research on the identification of host cell interactors of the Zika virus capsid protein conducted by the laboratory of Prof. Michał Dadlez. Our participation includes co-immunoprecipitation experiments performed in the neuroprogenitor cell model.

The Facility is also involved in services for drug discovery, offered by the IBB in the framework of the POL-OPENSCREEN (Polish Platform of Screening Infrastructure for Biological Chemistry) consortium. We carry out two *in vitro* tests: PAMPA (Parallel Artificial Membrane Permeability Assay) and Caco2 cell permeability assay. The data generated by both tests can be used to predict the *in vivo* intestinal absorption of tested compounds.

The Facility offers the production of recombinant proteins using a baculovirus expression system (BES), which allows for complex post-translational modifications. In previous years, components of the human SKI complex involved in the degradation of cytoplasmic mRNA, wild-type and mutant proteins were produced. Recently, in collaboration with Prof. Róża Kucharczyk's group, we obtained the wild-type and mutant variants of a *Saccharomyces cerevisiae* mitochondrial protein. An example of the successful production of large protein complexes is virus-like particle, adenoviral dodecahedron (Dd), which is built from

Cell culture room, Małgorzata Podsiadła Białoskórska

the virus capsid protein. Dd has been the subject of our studies for many years and its use as a vaccination platform is protected by a European patent. The potential of Dd as a carrier for non-immunogenic small molecules for the generation of specific antibodies is currently being investigated in a non-commercial collaboration with a French biotechnology company.

In addition to the production of proteins in the BES, the facility offered several in-house-produced reagents suitable for the purification of proteins and RNA and the preparation of DNA libraries.

HypoxyLab™ workstation. The purchase was funded by an OPUS NCN project led by dr hab. Justyna McIntyre, a member of the Laboratory of Mutagenesis and DNA Damage Tolerance.

BD FACSCalibur cell analyzer and sorter, equipment manager dr. hab. Michał Dmowski, member of the Laboratory of DNA Replication and Genomic Stability.

Laboratory of Biological NMR

Dr
Igor Żukow

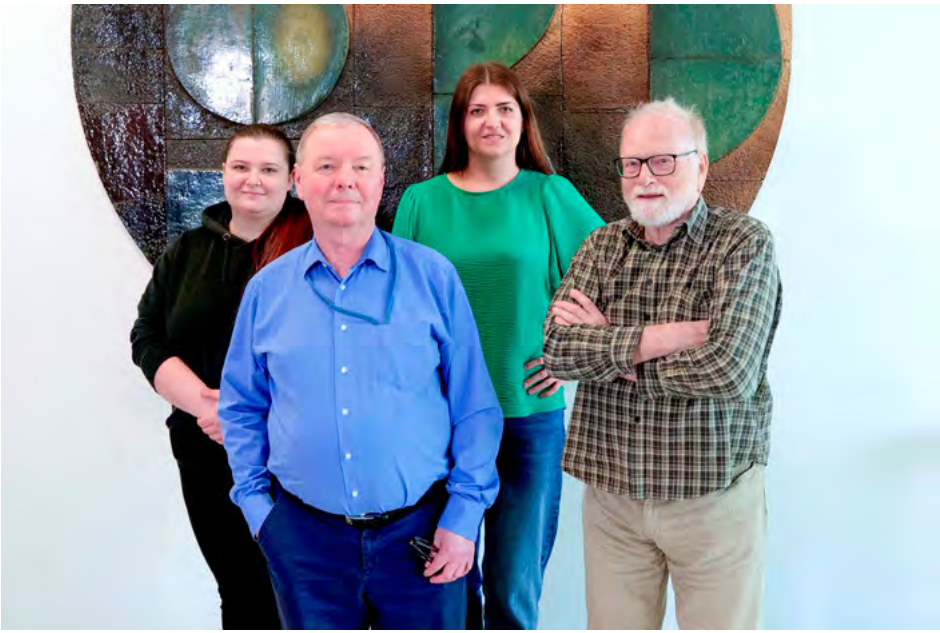
The Laboratory of Biological NMR studies protein-protein and protein-ligand interactions using heteronuclear multidimensional nuclear magnetic resonance (NMR) spectroscopy as a tool for the structural determination of biomolecules. Our research aims to understand molecular dynamic processes in the protein backbone based on ^{15}N relaxation measurements.

The Laboratory of Biological NMR has two installed Varian Inova NMR spectrometers, operating at magnetic fields of 9.6 T (^{1}H resonance frequency 400 MHz) and 11.7 T (^{1}H resonance frequency 500 MHz). NMR spectrometers are equipped with a triple $^{1}H/^{13}C/^{15}N$ resonance probehead, z-gradient, and temperature unit and are fully capable of recording multidimensional NMR data.

In the past, our facility has realized several research projects based on the evaluation of high-resolution 3D structures using NMR data. There are several 3D structures of the calcium-binding human protein S100A1, which were solved in apo (Ca free) and holo (Ca loaded) forms. Our studies explore the structural alterations observed in S100A1 on nitrosylation of the C-terminal cysteine. The other structures solved in our facility include parvulin from *Archaeum symbiosum*, the first catalytic half-domain from ubiquitin-activating enzyme E1, the Val57Gly mutant of human cystatin C, and several others.

Analysis of the molecular dynamics of the protein backbone based on ^{15}N relaxation experiments is another important aspect of laboratory studies. Experimental data of ^{15}N relaxation rates in longitudinal (R1) and transverse (R2) directions, together with ^{1}H-^{15}N NOE values, provide the possibility to analyse molecular dynamic processes with Model Free and/or spectral density mapping approaches in a residue-specific way. The presence of two installations in the Laboratory makes it possible to acquire experimental data in two different magnetic fields, which substantially increases the quality of the performed analysis.

Our research is impossible without developing protocols for the synthesis and



Left to right: Julia Podsiedlik, Igor Żukow, Lesia Kolomiiets, Andrzej Ejchart

purification of uniformly labeled ^{15}N- or ^{13}C,^{15}N-double labeled proteins suitable for heteronuclear multidimensional NMR experiments. The Laboratory of Biological NMR was the first laboratory in Poland to synthesize ^{13}C,^{15}N-double labeled protein in bacterial growth media. Recently, our experience and expertise have substantially expanded in the production of proteins containing fluorinated aromatic residues (^{19}F-Trp, ^{19}F-Tyr, and ^{19}F-Phe). This process introduces a paramagnetic probe into the protein structure, which makes it possible to extract additional long-distance constraints for the structural analysis of protein dynamics in solution.

At present, the Laboratory of Biological NMR conducts projects in collaboration with several laboratories at our institute. We have collaborated with the Laboratory of Biological Chemistry of Metal Ions (Head: Prof. W. Bal). Our joint studies include analysis of structural and dynamic properties on metal binding to the N-terminal fragment of the different Alzheimer-related peptides, Aβ(1-16) and Aβ(4-16). We explored the details of the oligomerization process in human glutathione under conditions of silver (Ag^+) ion saturation. In collaboration with the Laboratory of Molecular Basis of Biological Activity (Head: Prof. J. Poznański), we inspected the protein-ligand interactions of selected ligands with the CK2 kinase by utilizing ^{19}F NMR spectroscopy to obtain high-quality data.

The Laboratory also has strong collaborations with several research groups outside

of the institute. The joint grant with the Physical Department at Adam Mickiewicz University (Head: Prof. M. Kozak) is concerned with the analysis of structural changes in amylogenic peptides (Aβ) and proteins during interactions with metal nanoparticles.

Spektrometr NMR Varian 500

Strong contacts with the Chemical Department University of Gdańsk (Head: Prof. S. Rodziewicz-Motowidlo) include a joint grant focused on application of NMR spectroscopy to the evaluation of the high-resolution 3D structure of the herpes virus from several mammals in DPC micelles.

The Laboratory of Biological NMR is actively collaborating with various research groups, applying advanced NMR spectroscopy techniques to address challenges that have appeared during their studies. The laboratory offers extensive experience and expertise in the structural analysis of proteins in solution, exploration of molecular dynamics, and interactions between proteins-ligands or DNA-ligands, transmembrane proteins, and other areas. Our recent achievements in synthesizing fluorinated proteins have opened new opportunities for using ^{19}F NMR spectroscopy to acquire critical structural information about the binding of paramagnetic ions (such as Cu, Fe, or Mn) to proteins. This research is currently underway in our laboratory.

Laboratory of Microarray Analyses

Dr hab.
Marta Koblowska

Established in 2009, the Laboratory of Microarray Analyses is specialized in experiments related to genomics and transcriptomics for medicine and life sciences. The Laboratory conducts DNA microarrays (for gene expression, SNP, CNV, and genome-wide methylation analysis) and was involved in NGS sequencing (including RNA-seq, MNase-seq, and whole genome sequencing). Our team members actively participate in experimental design and data analysis.

Over the course of 15 years, the Laboratory of Microarray Analysis has utilized three dedicated systems for microarray analyses. These systems include two different Thermo Fisher platforms: the GeneChip® Scanner 3000 Targeted Genotyping System and the GeneAtlas® Personal Microarray System, as well as the Illumina HiScanSQ system. These platforms enabled transcriptomic profiling across various species, analyzing DNA methylation, single nucleotide polymorphisms (SNPs), and molecular karyotyping using cytogenetic microarrays. Additionally, the laboratory applied high-throughput DNA

Left to right: Marta Koblowska, Roksana Iwanicka-Nowicka

sequencing systems such as Illumina HiScanSQ, IonTorrent PGM, and IonTorrent Proton for numerous investigations. These included RNA-seq, whole human and *Arabidopsis* genome sequencing, determination of genomic locations of core histone modifications (ChIP-seq), and identification of nucleosome positions (MNase-seq) in *Arabidopsis*. The laboratory have also conducted metagenomic analyses, including 16S rRNA sequencing and full metagenome studies of microbial communities.

During this time, the Laboratory of Microarray Analysis performed over 5000 diverse analyses and collaborated with research groups from more than 20 scientific institutions, as well as commercial companies. Many collaborations involved project planning and research services utilizing the laboratory's equipment. The Laboratory's activities have also included providing training sessions for scientists from other research institutions in microarrays and next-generation sequencing technologies. These training programs covered cytogenetic microarrays, library preparation for ChIP-seq analysis, next generation sequencing (NGS) using Illumina sequencers, SNP microarray techniques, and courses for students and PhD students.

Currently, the Laboratory focuses on the application of DNA microarrays for transcriptomic analyses and the analysis of DNA methylation pattern. For simple and cost-effective gene expression profiling at the human, mouse, and rat transcriptome levels, we primarily use Thermo Fisher's Clariom S microarrays. These arrays allow the analysis of over 20,000 well-defined genes and enable rapid identification of expression biomarkers. Clariom S arrays perform well even with picogram RNA quantities and RNA isolated from FFPE samples. For more detailed transcriptomic analysis, we primarily use Clariom D microarrays, which allow the analysis of over 540,000 transcripts. Clariom D enables the analysis of splice variants in both protein-coding genes and lncRNAs. These arrays can be used with very small RNA quantities (as low as 100 pg). Clariom D provides an excellent alternative to RNA-

-seq analysis; they are easier to perform and significantly more cost-effective, and very importantly, the resulting data analysis is more straightforward than for RNA-seq data.

Over the last decade, abnormal DNA methylation patterns have been shown to contribute to gene expression dysregulation and are often the first indicators of changes associated with tumorigenesis. To identify different methylation patterns in tumor versus normal tissues, we use Human MethylationEPIC Arrays (Illumina). These arrays can lead to the identification of better disease biomarkers than those based on expression changes. The latest Infinium MethylationEPIC v2.0 BeadChip allows the assessment of methylation changes at over 930,000 individual sites across the entire human genome. It provides broad coverage of critical regions in the methylome, including CpG islands, enhancers, open chromatin regions, and other significant genomic areas.

The Laboratory of Microarray Analysis has made significant contributions to understanding gene expression and epigenetic regulation. The laboratory collaborated with the International Institute of Molecular and Cell Biology to identify pathways protecting cells from mitochondrial defects. This research led to a Nature publication in 2015. Additionally, the Laboratory contributed to a project with the IBB PAS and the University of Warsaw, revealing the role of a histone H1 variant in DNA methylation and *Arabidopsis* response to abiotic stresses. This work was published in Plant Physiology in 2015. More recently, the Laboratory has focused on the mechanisms controlling kidney cancer cells in collaboration with the Center of Postgraduate Medical Education, resulting in publications in journals such as Stem Cell Research & Therapy (2023), Cancer Cell International (2023), and Cancers (2019).

Piec do hybrydyzacji mikromacierzy DNA

Stacje płuczące do mikromacierzy DNA

Skaner do mikromacierzy DNA

PhD Training

PhD training in IBB PAS

IBB PAS offers graduate training for PhD Students within 'The Doctoral School of Molecular Biology and Biological Chemistry' and as a Partner in The Doctoral School of Translational Medicine 'Bench to Bedside – B 2 B 4 PhD", run by the Medical Centre for Postgraduate Studies. Our PhD Students are an essential part of the IBB PAS community and contribute significantly to its scientific endeavours.

Anna Muszewska- Head of the PhD School

PhD Students at IBB PAS are a heterogenous group that are currently trained within two legal systems, namely doctoral studies at the School of Molecular Biology, which will end at the end of 2024, and the Doctoral School of Molecular Biology and Biological Chemistry, which then become the single form of training. Some PhD Students enrolled at The Doctoral School of Molecular Biology and Biological Chemistry do their research projects at the Institute of Molecular Machines PAS, but most do their projects on-site within IBB PAS. On the other hand, among PhD students of the School of Molecular Biology, some realize their PhD projects at the International Institute of Molecular and Cell Biology and the National Medicines Institute. Together, the cohort of PhD students encompasses one hundred people from all around the world.

In the last few years, we have experienced an accelerated internationalisation of the applicant pool and the subsequent makeup of our PhD student community. Currently, there are 18/41 of non-polish speaking students at the Doctoral School of Molecular Biology and Biological Chemistry IBB, and 8/16 of first-year students came from other countries. Onboarding and integration of foreign students is facilitated by the close cooperation between the PhD School's administrative personnel and the Welcome Centre at IBB. The educational programme is offered in two disciplines: biological and chemical sciences. Those interested in translational medicine can pursue their degree with the 'Bench to Bedside – B 2 B 4 PhD', run by the Medical Centre for Postgraduate Studies, where IBB PAS is one of the partner institutions.

PhD Student Council, left to right: Mariia Kovalenko, Veena Manjunath (Deputy Chairwoman), Małgorzata Drabko (Chairwoman), Stephen Amankwah, Aleksander Kossakowski, Spoorthy Gowda (Secretary)

Students are offered biological and chemical training to gain advanced skills in medicinal chemistry, model organisms, protein structure and function, microscopic techniques or inorganic chemistry, to name just some of the latest courses. Being a scientist is not only the skills to perform scientific experiments, so we have built a portfolio of soft skills training in topics including the philosophy of science, scientific writing and communication, research ethics, grant proposal preparation, commercialization and intellectual property and other related topics. We offer additional courses in R and Python scripting languages to facilitate the processing of obtained results and to develop our students' digital skills. All courses are evaluated by the students through an anonymous survey. Student performance on the training programme is assessed by the Head of the PhD School with the PhD School Council. All PhD students attend weekly IBB Seminars and PhD Seminars. They also present their results to the whole scientific community at IBB once during the PhD Seminars and during the Scientific Symposium held every June.

The PhD students community have a representative on the IBB Scientific Council and their PhD Student Council. The latter organizes meetings for new PhD students, integrative and social activities, and takes

part in consultations and social aid assignments for those in need.

To enable participation in scientific exchange, IBB offers a travel budget of 12,000 PLN to each PhD candidate. PhD students apply for travel grants to participate in conferences, courses and workshops and report the travel output after completion. They also gain experience as project managers and primary investigators, writing mini-grant proposals for small 9-month-long projects with a budget of 20,000 PLN maximum. The projects are assessed by two external reviewers and only the ones with the highest marks are financed. The implementation of the project ends with a final report. Many students apply for National Science Centre Preludium grants, which offer them a greater budget and a small stipend. In the last 3 years there have been 18 Preludium applications from IBB students, and five of them were successful. Thanks to successful NAWA scholarship applications, several students have also completed internships at scientific institutions outside of Poland. In the last three years, two PhD Students obtained prestigious Awards of the Prime Minister for their outstanding PhD dissertations.

PhD students at IBB contribute to virtually all main research projects implemented at IBB PAS. Most of them publish an average of two scientific articles before the defence. Annually, IBB PhD Students publish 50 manuscripts, with an average Impact Factor of 5. Many PhD Students also engage in public outreach during the Science Festival, Scientific Picknick and the Night of Biologists.

Annually, 20-40 PhD degrees are granted at IBB PAS.

Doctoral Office, left to right: Anna Muszewska, Monika Wiczuk, Adrian Iwaniuk

Welcome to IBB Center

Welcome to IBB Center

The number of foreign researchers carrying out their scientific research at our Institute has been steadily increasing over the years. Along with their own individual scientific contributions, foreign researchers share their unique methodologies and diverse approaches, directly influencing the level of research conducted at IBB PAS. It also contributes to enriching the worldview of the IBB PAS community and building a diverse, intercultural scientific environment.

Aleksandra Kania – Welcome Center coordinator

In response to the growing interest among foreign researchers in the scientific opportunities at our Institute, the Welcome to IBB Center project was initiated at IBB PAS in June 2021. Since its inception, the center has played an important role in facilitating

Foreigners at IBB DAY – October 2023

Culinary workshop – May 2023

Intercultural integration workshop – April 2023

the integration and support of international researchers, doctoral candidates and interns joining our community. Notably, the number of foreign individuals within IBB has doubled since the establishment of the center (we currently host 80 foreigners from 27 different countries).

Funded by the National Academic Exchange Agency under the Welcome To Poland program, the Welcome Center serves as a vital hub for foreign scientists, providing comprehensive assistance with arrival logistics and legal formalities for their stay in Poland. Moreover, the center orchestrates networking, integration, and intercultural events, fostering a welcoming and inclusive environment for foreigners within both the IBB and Warsaw communities.

What have been the Welcome Center's achievements since its inception?

Organizational and informational activities:

- Implementation of an onboarding procedure, streamlining the admission process for foreign affiliates into IBB structures.
- Translation of internal IBB documents to ensure accessibility of English-language information.
- Development of the Welcome Center website and a guide outlining essential information for residing in Poland for new arrivals from overseas.
- Production of a promotional video highlighting IBB and the Welcome Center's initiatives.

Support for legal formalities:

- Assistance in obtaining visas and residence permits for foreign scientists.
- Ongoing support for foreign nationals in navigating other administrative procedures related to their stay in Poland.

Integration and intercultural activities:

- Organization of intercultural workshops aimed at fostering understanding of cultural differences and enhancing intercultural communication, and developing strong internal communities at IBB PAS.

Celebration of Divali – Indian Festival of Lights – November 2023

- Coordination of integration events such as the Easter Meeting, Divali Hindu Festival of Lights, and Nowruz Persian New Year Celebration, alongside smaller gatherings like musical evenings and sightseeing trips.

How has the Institute changed since the launch of the Welcome Center point?

Modern science is a highly internationalised pursuit, in which, to be competitive, a research institution must seek the brightest and best individuals worldwide and establish global collaborations with other leading international researchers and institutions. The establishment of the Welcome Center has marked a significant milestone in the internationalisation of our institute, bolstering its competitiveness among peer institutions. The establishment of the Center has made it easier to provide foreigners with the necessary conditions to engage in the Institute's community by alleviating administrative burdens, especially on their initial arrival in Warsaw. The Institute's scientific and administrative staff have also been supported in their cooperation with foreigners, including through the creation of transparent operating procedures in the area of recruitment and admission of foreign employees and students. The Welcome Center's activities both support the integration of communities and respond to the needs of working in an international and intercultural environment.

Henryk Arctowski Polish Antarctic Station

Stanisław RAKUSA-SUSZCZEWSKI

A short memoir of the founder of the Polish Antarctic Station

I returned in October 1974 from Antarctica, where I had spent two winters in the Russian and American expeditions, and had been to several stations on the continent, and at both poles; the geographical Amundsen-Scott station and the geomagnetic Vostok. I wrote to the Scientific Secretary of the Polish Academy of Sciences (PAS) and the Minister of Shipping, expressing the necessity of building a Polish Antarctic station in the South Shetland Islands. The station had the potential to serve our fishing fleet losing its fishing grounds to the north, under restrictions imposed by western countries. I further wrote that the station would be capable of providing weather forecasts for trawlers fishing in that area of the Antarctic, offering medical assistance to crews and holding fuel reserves for the fleet, as well as being a resting place for crew members in this remote part of the globe. This proved to be successful in the following years. The second argument was purely political. In 1974, only 12 countries were members of the Antarctic Treaty. Poland could obtain this status as the 13th country which, in the period of our isolation and the success of propaganda of the time, appeared convincing. However, it would have to make a financial and organisational effort related to maintaining a year-round research station. The third argument related to scientific objectives. Establishing such a station and joining the elite conducting research in the Antarctic, including, in particular, the identification of its biological resources, represented an attractive research topic. The nearby shelves of Argentina and the Falkland Islands, along with the Antarctic Peninsula, were rich in fish, unfamiliar to us. Before the station could be established, the possibility of fishing in the Antarctic had to be recognised and an ocean-biological and fishing expedition organised. This was the decision of the PAS authorities and the Ministry of Foreign Trade and Maritime Affairs (MHZGM). Financial

resources for this purpose were allocated by the Scientific Secretary of the PAS. The Maritime Fisheries Institute, owner of the research vessel r/v *ProfesorSiedlecki*, was the only partner with experience of two offshore fishing expeditions to the Argentine shelf and the sub-Antarctic fisheries of the Kerguelen Archipelago. For the purpose of examining fishing opportunities, a modern trawler t./ /Tazar from the Odra company participated in the expedition. Alongside fish, targeted identification of krill stocks, a key organism of the Antarctic ecosystem on which all major groups of Antarctic swimming and flying animals feed, was considered appropriate. As a scientific research object, krill is a little-known animal species with the largest biomass in the world. In the following years, it was the subject of an international BIOMASS programme, with Poland as a leader. This expedition's results were highly prospective, with fish and squid catches filling our food market which was insufficient at the time. The research results of the expedition were promptly published as monographs in the Polish Archives of Hydrobiology and in numerous international journals.

In May, 1976, after my return from a krill expedition, I began working towards a concept of an Antarctic station. In fact, the only real authority able to provide assistance to the Polish Academy of Sciences was the Ministry of Defence. Logistic preparations commenced, involving the purchase of 32 wooden containers from Stolbud, based in Bydgoszcz. This provided the opportunity to build a residential facility for 20 people, as well as meteorological and biological laboratories. Large halls were bought, including a steel hall to accommodate the workshop and power plant, as well as two wooden ones for storage, warehouse and garage. I created the plan for the main building myself, with the front of the building, the entrance placed centrally, the cloakroom, the living rooms arranged on the left and right, the dining room straight ahead, and the washroom in front of it. I ducted the main traffic in the centre of the building, while the wings, with tiny individual cabins, were supposed to be isolated. A T-shaped structure was created, which was later called 'a plane'. Operations at sea required separate equipment, which was obtained with the aid of the Navy. Fuel, oil, petrol and lubricants – a few thousand barrels – proved necessary. The station was established in 53 days at the site of my choice, i.e., Admiralty Bay on King George Island. Overall, 20 people decided to spend winter there, including a number of scientists. While in the country, I had my own workplace – the Polar Research Unit at the Institute of Ecology of the Polish Academy of Sciences (IE PAN). I undertook the managerial activity both in the country and in Antarctica. In 1992, the situation of the Institute of Ecology resulted in my becoming independent and leaving the IE PAN, under a decision made by Prof Leszek Kuźnicki, PhD, President of PAS. An independent Department of Antarctic Biology of the Polish Academy of Sciences (ZBA PAN) was established, together with the Arctowski

Station, for which I was made responsible. Initially, it was headquartered in a barrack in the Ochota District, and then in a villa bought with the funds I had received for my support in the construction of the Juan Carlos I Spanish Antarctic Station. I saved the funds allocated by the Committee for Scientific Research (KBN) and purchased a second house neighbouring the first one. The KBN allocated a considerable amount of money in 1998/1999 to upgrade the Arctowski Station, which I accomplished in one season by building new houses and laboratories, along with upgrading equipment. The Department of Antarctic Biology in Warsaw, coordinating all these activities, accommodated extensive library collections, archives and memorabilia from the 34 years of the operation of the Arctowski Station, and those earlier dating back to the 19th century of Polish Antarctic research. The operation of the Arctowski Station resulted in educating numerous scientists, not only biologists pursuing the programmes of their own centres established in almost all Polish universities and a few institutes of PAS. Some of them have reached world-class levels. My own output includes 13 doctoral theses which I have supervised, 14 reviews of doctoral theses and several habilitation theses, and four opinions for professorship. This is a reflection of the commitment to Antarctic research by the Department of Antarctic Biology and the opportunities it has created for other scientists and centres in the country, through the management of the Arctowski Station.

It has been 47 years, i.e., at least a generation, since the establishment of the Antarctic Station at Admiralty Bay on King George Island. National science has received numerous specialists in various specialities, including several biology majors. The choice of the subject matter is of utmost importance. Long gone are the days of describing the environment, producing maps, making inventories of flora and fauna species, often in the form of long-term monitoring. The basic processes of this specific geo-ecosystem and the trends of variation, including the effects of rising temperatures, have already been recognised. Modern science in biology is about genomics, biotechnology, genetics, and understanding the adaptations at the molecular level. Indeed, this was the main reason behind my decision to join the Department of Antarctic Biology of PAS to the Institute of Biochemistry and Biophysics of PAN. I believed that, due to the intellectual and equipment potential of the IBB PAS, the scientific staff of the ZBA PAS would be able to improve and modernise their scientific capabilities, while the IBB would gain access to an extensive collection of Antarctic microorganisms and the opportunity to undertake research into the metagenomics of Antarctic environments and the physiology of psychrophilic organisms involved in biotechnological processes. The IBB PAS has upheld, and will continue, the tradition of Polish research in the Antarctic by modernising the station and coordinating the research activities conducted there. Thanks to the tremendous concerted effort made by

individuals and the country's capabilities, we are part of a relatively small group of countries gathered in the Antarctic Treaty and its scientific organisations such as SCAR, COMNAP, and CCAMLR. Numerous individuals have had their lives transformed by the expeditions and stay at the Arctowski Station, and these have left a lasting mark on their memories. With the passage of time, this has been something I truly appreciate myself.

Henryk Arctowski Polish Antarctic Station Unit

Henryk Arctowski Polish Antarctic Station, situated on King George Island (South Shetland, Antarctica), amplifies research and cultural exchange at IBB PAS. Its remote location enables groundbreaking research in diverse fields ranging from glaciology and oceanography to biology and atmospheric science. Hosting international teams fosters collaboration, enriching both research output and global scientific cooperation.

The Polish Antarctic Station is located on King George Island in the South Shetland archipelago in Antarctica. It is 120 km away from the continent of Antarctica, and over 14,000 km away from Warsaw, where the entity managing it, the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, is located. The Polish Antarctic Station was named after the outstanding Polish researcher, Henryk Arctowski, who at the end of the 19th century was the scientific leader on board the ship Belgica during the first research expedition wintering in the Antarctic sea ice. On February 26, 1977, the activities of the Polish Station were officially launched, and since then it has been

Henryk Arctowski Polish Antarctic Station, main building "Samolot" ("Airplane") – author Marek Dąbrowski

The Antarctic, King George Island, Gentoo penguins – author Tomasz Kurczaba

used continuously as the Polish, year-round scientific and research facility in this part of the world. Its infrastructure is currently undergoing comprehensive modernization.

Left to right: Anna Kloc, Zofia Komorowska, Dariusz Puczko (Head), Przemysław Kapuściński

The Polish unit has become a center of innovative scientific research. It conducts research in the fields of biology, zoology, botany, microbiology, ecology, oceanography, hydrology, geology, geomorphology, glaciology, meteorology, among others. For many years, monitoring observations have been carried out continuously (ecological, geochemical, glaciological, hydrological and meteorological), the data from which are used by Polish and foreign scientists. In recent years, there have been more than 100 people from 36 institutions making a total of over 1,000 person-day stays at the Station each year.

The Polish Antarctic Station is operated by a 10-person winter group that works in Antarctica for over a year, and a summer group of several people that spends several months at the facility, the so-called Antarctic summer, as well as a team of people working in country to maintain the continuity of the Station's operation. The Station's infrastructure consists of various buildings and facilities that provide scientists with conditions for conducting research. The Station includes laboratories equipped with research equipment, living and social rooms for the crew, and warehouses and halls for storing equipment and supplies.

From 2020, comprehensive modernization works are being carried out at the Arctowski Polish Antarctic Station. These activities include the construction of a new main building, two warehouse halls and the revitalization of the existing infrastructure, including modernization of some technical

The Antarctic, King George Island, Adélie penguins – author Maciej Błaszkowski

The Antarctic, King George Island, southern elephant seals – author Łukasz Kreft

Henryk Arctowski Polish Antarctic Station, new main building – visualization

buildings, the energy and fuel systems, as well as the water supply network and the helicopter landing pad. These activities are particularly important in the context of the key role of the Arctowski Polish Antarctic Station as a special research device, as indicated in the "Strategy of Polish polar research" by the Polish Polar Consortium and the Polar Research Committee of the Polish Academy of Sciences. That report defined the directions of development of Polish research in the Arctic and Antarctic in terms of cognitive, economic and social utility and strengthening Poland's international position. A significant part of the budget allocated under the grant is dedicated to transport and logistics of this unique project.

Due to the extreme conditions in Antarctica, deliveries of materials and supplies are mainly by sea, and their scheduling depends on weather and seasonal conditions. A significant part of maintaining the infrastructure of the Henryk Arctowski Polish Antarctic Station are the costs of sea transport of people and supplies, fuel needed to produce energy, employment of staff, maintenance of machines and satellite communications, food, technical materials and specialist services.

The Polish Antarctic Station is not only a platform for conducting advanced scientific research, but also a place where cultural and educational exchanges take place. Through cooperation with Polish and foreign research and academic centers, the Station supports the development of international cooperation and promotes Polish science internationally. In its 70-year history, IBB PAS has constantly supported the development of Polish science and education, and the Arctowski Polish Antarctic Station remains one of the most important symbols of this commitment. In the future, the Station will continue its research mission, inspiring subsequent generations of scientists to discover the secrets of Antarctica and contributing to global progress in the field of science and environmental protection.

Administrative and non-scientific units

Administrative and non-scientific units

Jednostki administracyjne i pomocnicze

The Board of Directors

The entire activity of the Institute is managed by the Director, who also represents the Institute externally. The Director manages the Institute with the help of the Institute Management, which includes the Deputy Directors and the Chief Accountant.

Dyrekcja

Całokształtem działalności Instytutu zarządza Dyrektor, który także reprezentuje Instytut na zewnątrz. Dyrektor zarządza Instytutem przy pomocy Kierownictwa Instytutu, w skład którego wchodzą Zastępcy Dyrektora oraz Główna Księgowa.

Róża Kucharczyk (Director of General Affairs), Jarosław Poznański (Director), Agnieszka Sirko (Director of Science), Agnieszka Kruszewska (Director of Administration), Monika Kornacka (Chief Accountant)

Scientific Council

The Scientific Council exercises ongoing supervision over the Institute's activities, taking particular care of the high level of its scientific activity and the development of people starting and developing their scientific careers.

Rada Naukowa

Rada Naukowa sprawuje bieżący nadzór nad działalnością Instytutu, dbając zwłaszcza o wysoki poziom jego działalności naukowej i rozwój osób rozpoczynających oraz rozwijających karierę naukową.

Wojciech Bal
Chairman

Joanna Kruszewska
Vice Chairman

Agata Starosta
Vice Chairman

Aneta Bartosik
Secretary

Subordinate to the Director

Pion Dyrektora

Human Resources Unit

The scope of activities of the Human Resources Unit includes matters related to the employment relationship, civil law contracts, social and living matters.

Dział Kadr

Do zakresu działania Działu Kadr należą sprawy związane ze stosunkiem pracy umowami cywilnoprawnymi, sprawami socjalnymi i bytowymi.

Left to right:Katarzyna Frankowicz, Anna Czeczotko (Head), Aleksandra Kania (Manager of the Welcome to IBB Center*)

* More information on page 242

Subordinate to the Chief Accountant

Pion Głównej Księgowej

Finance and Accounting Unit

The scope of activities of the Finance and Accounting Unit includes matters related to the regulation and settlement of matters related to the records of business operations, control of these operations and settlements of business activities and settlements with employees. The Unit operates the Payroll Section and the Cash Register.

Dział Finansowo-Księgowy

Do zakresu działania Działu Finansowo-Księgowego należą sprawy dotyczące regulowania i załatwiania spraw związanych z ewidencją operacji gospodarczych, kontroli tych operacji oraz dokonywanie rozrachunków z tytułu prowadzonej działalności gospodarczej i rozliczeń z pracownikami. W ramach Działu funkcjonuje Sekcja Płac oraz Kasa.

Left to right: Agnieszka Błażejewska-Sedrakian (Head of Payroll Section), Karolina Szyszka, Agnieszka Kosowicz, Ewa Pietrak, Barbara Kresa, Anita Gibert, Małgorzata Boguta, Monika Kornacka (Chief Accountant), Grażyna Deba, Jolanta Kazubska, Agnieszka Jastrzębska

Subordinate to the Director of Science

Pion zastępcy Dyrektora ds. Naukowych

Research Management Unit

The task of the Research Management Unit is to provide administrative support for research conducted by the Institute independently and in cooperation with other domestic or foreign institutions.

Dział Badań i Projektów Naukowych

Zadaniem Działu Badań i Projektów Naukowych jest zapewnienie wsparcia administracyjnego badań prowadzonych przez Instytut samodzielnie i we współpracy z innymi pacówkami krajowymi lub zagranicznymi.

Left to right: Anna Płochocka-Youseff, Adrian Iwaniuk, Monika Wiczuk, Magdalena Karońska, Katarzyna Jagiełło-Wilgat (Head), Magdalena Mardowska, Elżbieta Kołodziej, Dariusz Plaskota, Monika Flis, Anna Muniak (not on the photo)

Doctoral Schools*

The Institute implements a program for educating PhD students and supporting their scientific development in the disciplines of biological sciences and chemical sciences.

Szkoły Doktorskie

W Instytucie realizowany jest program kształcenia Doktorantów i wspierania ich rozwoju naukowego w dyscyplinach: nauki biologiczne i nauki chemiczne.

Doctoral Office. Left to right: Anna Muszewska (Head of the PhD School), Monika Wiczuk, Adrian Iwaniuk

* More description on page 238

Subordinate to the Director of General Affairs

Pion Zastępcy Dyrektora ds. Ogólnych

Library

The scope of the Library's activities includes supporting the Institute's employees in the field of bibliography and collecting and documenting their achievements.

Biblioteka

Do zakresu działania Biblioteki należy wsparcie pracowników Instytutu w zakresie bibliografii oraz gromadzenie, dokumentowanie ich dorobku.

Małgorzta Pacuła (Head), nieobecna na zdjęciu –Anna Krzywonos

Phytotron-Greenhouse Complex and Culture Medium Center

Phytotron-Greenhouse Complex (PGC) and Culture Medium Center (CMC) are managed by the Head of both Units. The scope of PGC activities includes servicing research plants and laboratories in the field of plant breeding as well as programming and operation of technical devices constituting PGC. The scope of activity of the CMC includes servicing Research Units in the preparation of solid and liquid media, including dishes with solid media, solutions for preparing media and buffers.

Kompleks Fitotronowo-Szklarniowy i Pożywkarnia

Kompleks Fitotronowo-Szklarniowy (KFS) oraz Pożywkarnia zarządzane są przez Kierownika obu Jednostek. Do zakresu działania KFS należy obsługa Zakładów i Pracowni Naukowych w zakresie hodowli roślin oraz programowania i obsługi urządzeń technicznych stanowiących KFS. Do zakresu działania Pożywkarni należy obsługa Zakładów i Pracowni Naukowych w zakresie przygotowywania podłoży stałych i płynnych, w tym szalek z podłożami stałymi, roztworów do przygotowania podłoży oraz buforów.

Left to right: Krzysztof Kokoszka (Head), Agnieszka Safonow, Marcin Barański, Iwona Rosa, Bogdan Golczyk, Krzysztof Kokoszka, Bartosz Pac, Helena Karczewska

Isotope Laboratory and Radiological Protection Inspector

The scope of activities of the Isotope Laboratory includes, among others: providing support to research plants and laboratories in the field of work requiring the use of higher concentrations of isotopes. The Radiation Protection Inspector supervises and checks compliance with internal procedures and work instructions in the field of nuclear safety and radiological protection.

Pracownia Izotopowa i Inspektor Ochrony Radiologicznej

Do zakresu działania Pracowni Izotopowej należy m.in. obsługa Zakładów i Pracowni Naukowych w zakresie prac wymagających używania wyższych stężeń izotopów. Inspektor Ochrony Radiologicznej nadzoruje oraz kontroluje przestrzegania procedur wewnętrznych i instrukcji pracy w zakresie bezpieczeństwa jądrowego i ochrony radiologicznej.

Left to right: Jerzy Pawłowicz, Agnieszka Maciejewska (IOR), Marcin Barański

Subordinate to the Director of Administration

Pion Zastępcy Dyrektora ds. Ogólnych

Secretariate

The scope of the Secretariat's activities includes supporting the current functioning of the Director's Secretariat and supporting the Institute's management.

Sekretariat

Do zakresu działania Sekretariatu należy obsługa bieżącego funkcjonowania Sekretariatu Dyrektora oraz kierownictwa Instytutu.

Left to right: Karolina Krassowska, Patrycja Jabłońska

Administration and Technical Unit

The scope of activities of the Administration and Technical Unit includes matters related to administrative and technical services, maintenance and renovation of premises, and the implementation of recommendations arising from occupational health and safety and fire protection regulations.

Dział Administracyjno-Techniczny

Do zakresu działania Działu Administracyjno-Technicznego należą sprawy w zakresie obsługi administracyjnej i technicznej, konserwacja i remonty pomieszczeń, oraz realizacja zaleceń wynikających z przepisów bezpieczeństwa i higieny pracy i ochrony przeciwpożarowej.

Left to right: Tomasz Dobrzański (Head of Technical Section), Katarzyna Szostak, Mariusz Wójcik, Piotr Kula, Katarzyna Król (Head), Mariola Jabłońska, Dorota Krawczyk, Małgorzata Szulc, Krzysztof Komar, Robert Furmanek. Not on the photo: Barbara Gorowicz, Agnieszka Safonow, Zdzisława Sałańska, Elżbieta Strzelecka, Joanna Żochowska

Purchasing Unit and Public Procurement Unit

The scope of activity of the Purchasing Unit and Public Procurement Unit includes handling orders from all organizational units regarding equipment, devices and materials, reagents, running the office and organizing, conducting and monitoring the process of purchases carried out at the Institute based on internal regulations and the provisions of the Public Procurement Law.

Dział Zaopatrzenia i Zamówień Publicznych

Do zakresu działania Działu Zaopatrzenia i Zamówień Publicznych należy obsługa zamówień spływających ze wszystkich jednostek organizacyjnych dotyczących aparatury, urządzeń i materiałów, odczynników, prowadzenie Kancelarii oraz organizowanie, prowadzenie i monitorowanie procesu realizacji zakupów prowadzonych w Instytucie w oparciu o regulacje wewnętrzne oraz przepisy ustawy Prawo zamówień publicznych.

Left to right: Sebastian Patyk, Katarzyna Górska, Marcin Nowik (Head of Purchasing Unit), Maria Kałuża, Piotr Wójcik, Albert Mądry (Public Procurement Unit), Marta Kozłowska (not on the photo)

Henryk Arctowski Antarctic Station Unit*

Henryk Arctowski Antarctic Station Unit ensures the proper and uninterrupted functioning of the special research facility located on King George Island, South Shetland Islands.

Dział Stacja Antarktyczna im. Henryka Arctowskiego

Dział Stacja Antarktyczna im. Henryka Arctowskiego zapewnia prawidłowe i nieprzerwane funkcjonowanie specjalnego urządzenia badawczego znajdującego się na Wyspie Króla Jerzego, Szetlandy Południowe.

Left to right: Anna Kloc, Zofia Komorowska, Dariusz Puczko (Head), Przemysław Kapuściński

* More description on page 246

Occupational Health and Safety Inspector (OHS Inspector)

The scope of activities of the Occupational Health and Safety Inspector includes organizing projects aimed at preventing threats to the life and health of employees and improving occupational health and safety conditions.

Inspektor ds. Bezpieczeństwa i Higieny Pracy (Inspektor BHP)

W zakresie działania Inspektora BHP leży organizowanie przedsięwzięć mających na celu zapobieganie zagrożeniom życia i zdrowia pracowników oraz poprawę warunków bezpieczeństwa i higieny pracy.

Marzena Sieńko

IBB PAS Archive

The scope of activities of the IBB PAS Archive includes archiving documents created at the Institute, taking into account the provisions of the IBB PAS Office Instruction.

Archiwum IBB PAN

Do zakresu działań Archiwum IBB PAN należy prowadzenie archiwizacji dokumentów wytworzonych w Instytucie z uwzględnieniem zapisów Instrukcji Kancelaryjnej IBB PAN.

Mariola Jabłońska

IT Support Unit

The scope of activity of the IT Support Unit includes, among others: processing requests for support to employees and doctoral students as part of the current operation of the ICT infrastructure, maintaining the network infrastructure, computing infrastructure and data storage systems of the Institute, developing the technical level of the Institute's ICT infrastructure.

Dział Wsparcia Informatycznego

Do zakresu działania Działu Wsparcia Informatycznego należy m. in. realizowanie zgłoszeń o udzielanie wsparcia pracownikom i doktorantom w ramach bieżącej eksploatacji infrastruktury teleinformatycznej, utrzymania infrastruktury sieciowej, obliczeniowej i systemów przechowywania danych Instytutu, rozwój poziomu technicznego infrastruktury teleinformatycznej Instytutu.

Left to right: Hubert Twardowski, Łukasz Kniżewski (Head), Krzysztof Komar, Mateusz Jastrzębski, Tomasz Michalak (not on the photo), Jan Solak (not on the photo)

Management of IBB PAS (1954-2024)

Kierownictwo IBB PAN (1954-2024)

Directors/Dyrektorzy

- Prof. Józef Heller (1954-1966)
- Prof. Wacław Gajewski (1967-1972)
- Prof. Kazimierz Lech Wierzchowski (1972-1981)
- Prof. Andrzej Paszewski (1981-1984)
- Prof. Jerzy Buchowicz (1984-1987)
- Prof. Kazimierz Kleczkowski (1987-1990)
- Prof. Włodzimierz Zagórski-Ostoja (1990-2007)
- Prof. Piotr Zielenkiewicz (2007-2019)
- Prof. Jarosław Poznański (2019-present)

Chairmen of Scientic Council/ /Przewodniczący Rady Naukowej

- Prof. Janusz Supniewski (1954-1964)
- Prof. Józef Heller (1964-1981)
- Prof. Wacław Gajewski (1981-1992)
- Prof. Zofia Lassota (1993-1997)
- Prof. Andrzej Paszewski (1997-2011)
- Prof. Andrzej Jerzmanowski (2011-2018)
- Prof. Andrzej Dziembowski (2019)
- Prof. Wojciech Bal (2020-present)

Directors of Science*/Zastępcy Dyrektora ds. Naukowych

- Prof. Ignacy Reifer (1963-1966)
- Prof. Władysław Kunicki-Goldfinger (1967-1968)
- Prof. Przemysław Szafrański-Szeliga (1967-1974)
- Prof. Kazimierz Lech Wierzchowski (1969-1972)
- Prof. Zofia Lassota (1972-1975)
- Prof. Tadeusz Chojnacki (1975-1978)
- Prof. Michał Bagdasarian (1978)
- Prof. Andrzej Paszewski (1979-1981)
- Prof. Maria Monika Jeżewska (1981-1985)
- Prof. Celina Janion (1985-1990)
- Prof. Grażyna Muszyńska (1990-1996)
- Dr hab. Piotr Cegłowski (1996-2003)
- Prof. Piotr Zielenkiewicz (2003-2007)
- Prof. Piotr Jonczyk (2007-2019)
- Dr hab. Roman Szczęsny (2019-2023)
- Prof. Agnieszka Sirko (2024-present)

*According to the statute of IBB PAS in force from 1967 to 1972, two such deputy directors were appointed/ /Wg Statutu IBB PAN obowiązującego w latach 1967-1972 powoływano dwóch takich zastępców

Directors of General Affairs*/ /Zastępcy Dyrektora ds. Ogólnych

- Prof. Zofia Lassota (1970-1972)
- Prof. Andrzej Paszewski (1978-1979)
- Prof. Włodzimierz Zagórski-Ostoja (1981-1984)
- Doc. Bernard Wielgat (1984-2000)
- Prof. Piotr Zielenkiewicz (2000-2003)
- Prof. Piotr Jonczyk (2003-2007)
- Prof. Jacek Bardowski (2007-2015)
- Dr hab. Elżbieta Kraszewska (2016-2017)
- Prof. Jarosław Poznański (2018-2019)
- Prof. Róża Kucharczyk (2019-present)

Directors of Administration/ /Zastępcy Dyrektora ds. Administracyjnych

- Michał Kalisiak (1956-1967)
- Stanisław Ciszewski (1967-1973)
- Andrzej Lipiński (1973-1979)
- Janusz Staniusz (1979-1981)
- Gustaw Lang (1981-1982)
- Kazimierz Moes (1983-1985)
- Jerzy Podolak (1985-1986)
- Kazimierz Hawrot (1987)
- Ignacy Kosior (1987-2007)
- Lech Laskowski (2007-2011)
- Agnieszka Kruszewska (2011-present)

Chief Accountants/Główni Księgowi

- Czesław Filipowicz (1954-1963)
- Jadwiga Kapaon-Kacprzak (1964-1969)
- Ewa Kańska (1970-1987)
- Lucyna Andruszenko (1987-1993)
- Grażyna Deba (1993-2022)
- Monika Kornacka (2022-present)

Head of Research Management Unit/Kierownicy Działu Badań i Projektów Naukowych

- Dr Danuta Dębczyńska (1971-1995)
- Ałła Bulanda (1995-2005)
- Katarzyna Jagiełło-Wilgat (2005-present)

*In certain years, the position of director of general affairs was vacant/w pewnych latach stanowisko zastępcy dyrektora ds. ogólnych było nieobsadzone

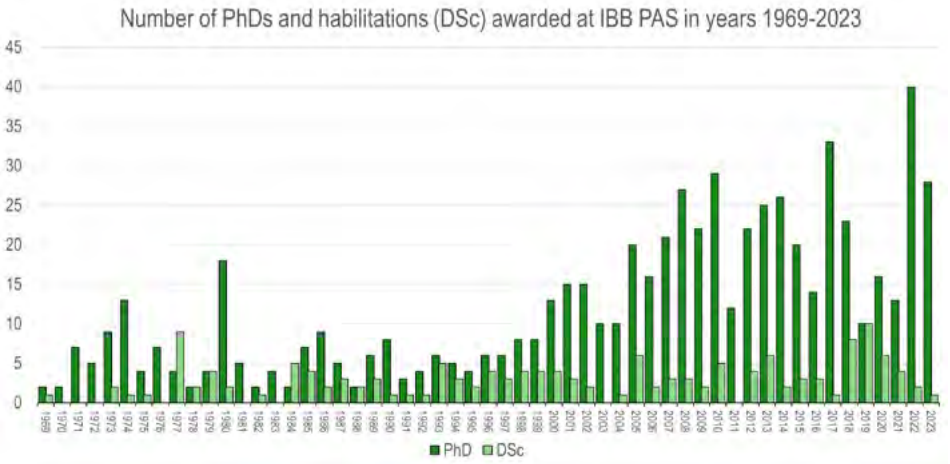
Numerical summary of the selected achievements

Numerical summary of the selected achievements in the last decade (2014-2023)

Publications in peer reviewed journals (total)	**1797**
Grants obtained (total)	**253**
National Science Centre (NCN)	203
The National Centre for Research and Development (NCBiR)	11
Foundation for Polish Science (FNP)	11
Polish National Agency for Academic Exchange (NAWA)	9
Ministry of Science and Higher Education (MNiSW)	7
Other	12
Patents obtained (total)	**98**
Polish	57
Foreign	41
Scientific degrees awarded by IBB PAS	
PhD	223
Habilitation (DSc)	40

Number of scientific degrees awarded at IBB PAS in years 1969-2023

Total numbers: over 650 PhD and over 150 Habilitations (DSc)



IBB PAS

The Institute of Biochemistry and Biophysics PAS is a member of the following scientific consortia and networks:

- Global Biodiversity Information Facility
- EU-OPENSCREEN
- Polish Polar Consortium
- Consortium Center for Pre-Clinical Research and Technology (CePT)
- European Plant Science Organisation
- Polish Union of Innovative Medical Biotechnology Companies BioInMed – Academic Platform
- Biocentrum Ochota
- Mazovian Hydrogen Valley cluster

GBIF eu-openscreen POLISH POLAR CONSORTIUM CePT Centrum Badań Przedklinicznych i Technologii epso bioinmed Biocentrum ochota

IBB PAS in the World

IBB PAS Community in October 2023

70 YEARS

INSTITUTE
OF BIOCHEMISTRY
AND BIOPHYSICS
POLISH ACADEMY
OF SCIENCES

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