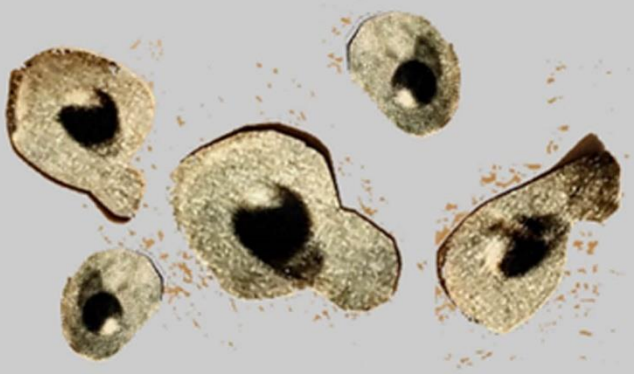
Doskonała Nauka



ABSTRACT BOOK

1st Polish Yeast Conference

Rzeszów
June 22-24, 2022

1st Polish Yeast Conference

Dear colleagues, the participants of the 1st Polish Yeast Conference,

With this conference, we started a new initiative, organizing the platform for meeting Polish yeast researchers from different cities and research centers between themselves and also with their foreign collaborators. Contemporary cell biology and biotechnology use yeast as one of the most important model organisms and as important producer of biofuels, low-molecular weight compounds, enzymes, recombinant proteins etc. Important research in these fields are carried out in Polish laboratories of wide geography: Warsaw, Cracow, Wrocław, Poznań, Gdańsk, Łódź, Lublin, Rzeszów and others. Planned conference will allow to meet with colleagues from other laboratories, to establish new contacts, to strengthen existing ones and to develop useful collaborations so important for science development. We also invite foreign colleagues which collaborate with Polish yeast researchers, so our conference will be attended by researchers from USA, Japan, UK, France, Sweden, Belgium, Slovakia and Ukraine. It means that our conference in fact will be simultaneously Polish and international one! In total, we have more than 100 registered participants with 52 oral presentations and tens of posters. Our conference was planned to be organized in 2020, however, COVID-19 pandemics postponed it for two years. Now, the conference is organized in hybrid mode (both in-site and on-line), however, only around 10 presentations will be delivered on-line while the rest will occur in-site.

Rzeszów yeast scientists are happy to host our respected colleagues from other Polish cities and abroad! Rzeszów is known as city of students as being of middle size (around 250,000 population) we have near 100,000 students! Yeast research in Rzeszów is mostly resides in the University of Rzeszów. Scientists are involved in studying yeast molecular genetics, cell biology and biotechnology. Of known topics, autophagy, yeast cell longevity, biofuel and vitamin production are developed. New directions of research involve production of anti-COVID-19 vaccine and bacterial antibiotics in yeast.

In addition to science, the program of our conference predicts social program including the concert of Subcarpathian Accordion Quintet "Ambitus V" and the conference dinner. Participants also could visit beautiful places of city downtown and vicinities like Łańcut museum palace and many others.

We feel that this conference is important for scientists. If we are right and if the conference will be technically well organized, we will be happy to have such conferences on the regular basis, say, once in two-three years.

Welcome to 1st Polish Yeast Conference in Rzeszów!

With deep respect,

Andriy Sibirny
The Chair of the Organizing Committee

Rzeszów, June 14, 2022

Scientific Committee

Chair, Prof. dr hab. Andriy Sibirny, University of Rzeszów
Vice-Chair, Prof. dr hab. Teresa Żołądek, Institute of Biochemistry and Biophysics
Prof. dr hab. Marek Tchórzewski, Maria Curie-Skłodowska University
Prof. dr hab. Ryszard Korona, Jagiellonian University
Dr Adrianna Skoneczna, Associate professor, Institute of Biochemistry and Biophysics
Dr Ewa Maciaszczyk-Dziubińska, Associate professor, University of Wroclaw
Dr Ewelina Celińska, Associate professor, Poznań University of Life Sciences
Dr Aleksandra Mirończuk, Associate professor, Wrocław University of Environmental and Life Sciences
Dr Maciej Wnuk, Associate professor, University of Rzeszów

Organizing Committee

Head, Prof. dr hab. Andriy Sibirny, University of Rzeszów
Vice-Head, Dr Maciej Wnuk, Associate professor, University of Rzeszów
Dr Anna Lewińska, Associate professor, University of Rzeszów
Dr Grzegorz Chrzanowski, Associate professor, University of Rzeszów
Dr Małgorzata Kus-Liśkiewicz, Associate professor, University of Rzeszów
Dr Renata Zadrąg-Tęcza, Associate professor, University of Rzeszów
Dr Justyna Ruchała, University of Rzeszów
Dr Leszek Potocki, University of Rzeszów

Sponsors:

Ministerstwo Edukacji i Nauki

Doskonała Nauka

Platinum Sponsors:

Federation of European Microbiological Societies

Gold Sponsors:

Luminex
complexity simplified.

ALCHEM
ALCHEM GRUPA Sp. z o.o.

Silver Sponsors:

LALLEMAND

Łańcut
BROWAR

Honorary Patronage:

Władysław Ortyl – Marshal of the Podkarpackie Voivodeship

Program

June 22-24, 2022 (Wednesday - Friday)

Day 1 (June 22)

9.00 – 16.00 Registration

13.00 – 13.15 Opening Ceremony

Andriy Sibirny – Chair of the Conference
Idalia Kasprzyk – Vice-Rector of the University of Rzeszów
Róża Kucharczyk – Deputy Director of General Affairs of Institute of Biochemistry and Biophysics, Polish Academy of Sciences
Hiroshi Takagi – Vice-Chair of International Commission on Yeasts
Terrance G. Cooper – Secretary of the Financial and Policy Committee of the International Yeast Research Community
Grzegorz Węgrzyn – Scientific Excellence Council

13.15 – 14.00 Keynote Lecture 1

Terrance G. Cooper, University of Tennessee Health Science Center, Memphis, Tennessee, USA
Multivariant global control of the major nitrogen-responsive transcription activator, Gln3

14.00 – 15.30 Session 1 Yeast cell biology and transport

Chairs:
Renata Zadrąg-Tęcza, University of Rzeszów, Rzeszów
Ewa Maciaszczyk-Dziubińska, University of Wrocław , Wrocław

14.00 – 14.15 **Michał Małecki**, University of Warsaw, Warsaw
Role of uridylation in cytoplasmic mRNA Decay

14.15 – 14.30 **Marek Skoneczny**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
New import pathway to peroxisomes: some answers, more questions

14.30 – 14.45 **Renata Zadrąg-Tęcza**, University of Rzeszów, Rzeszów
Cell size implications for the reproductive capacity of yeast cells

14.45 – 15.00 **Zbigniew Lazar**, Wrocław University of Environmental and Life Sciences, Wrocław
Characterization of sugar and glycerol metabolism in the yeast *Yarrowia lipolytica*

15.00 – 15.15 **Aneta Urbanek**, University of Wrocław, Wrocław
Interplay between *Candida albicans* transporters, plasma membrane and cell wall

15.15 – 15.30 **Marta Semkiv**, Institute of Cell Biology, NAS of Ukraine, Lviv
Autophagic degradation of cytosolic proteins in the methylotrophic yeast *Komagataealla phaffii*

15.30 – 16.00 Coffee break

16.00 – 17.30 Session 2 Sensing, signaling, and stress response

Chairs:
Maciej Wnuk, University of Rzeszów, Rzeszów
Marek Skoneczny, Institute of Biochemistry and Biophysics,

Polish Academy of Sciences, Warsaw

16.00 – 16.30 **Oleh Stasyk**, Institute of Cell Biology, NAS of Ukraine, Lviv
Glucose sensing and signaling in the methylotrophic yeast *Ogataea polymorpha*

16.30 – 16.45 **Jennifer Tate**, Tennessee Health Science Center, Memphis, Tennessee, USA
N-terminal Gln3 phosphorylation/dephosphorylation in the control of Gln3 localization

16.45 – 17.00 **Kamilla Grzywacz**, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań
Emerging functions of ribosome-associated noncoding RNAs during stress response in *Saccharomyces cerevisiae*

17.00 – 17.15 **Krzysztof Liberek**, University of Gdańsk, Gdańsk
Yeast chaperones in refolding of proteins from aggregates

17.15 – 17.30 **Małgorzata Adamczyk**, Warsaw University of Technology, Warsaw
New role of RNA polymerase III in shaping metabolic network activity and stress response in *Saccharomyces cerevisiae*

17.30 – 19.00 Session 3 Genetic Control of Cellular Processes

Chairs:

Marek Tchórzewski, Maria Curie-Skłodowska University in Lublin, Lublin

Kamilla Grzywacz, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań

17.30 – 17.50 **Marek Tchórzewski**, Maria Curie-Skłodowska University in Lublin, Lublin
The influence of ricin-mediated rRNA depurination on the translational machinery using *Saccharomyces cerevisiae* as experimental model

17.50 – 18.05 **Małgorzata Cieśla**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
RNA polymerase III transcription, novel layers of regulation

18.05 – 18.20 **Ulrike Topf**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Crosstalk between mitochondria and cytosolic translation machinery

18.20 – 18.35 **Paweł Golik**, Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw
Mitochondrial RNA degradation and stability in *Candida albicans* and the evolution of yeast nucleo-mitochondrial interactions

18.35 – 18.50 **Dorota Rzechonek**, Wrocław University of Environmental and Life Sciences, Wrocław
Regulation of erythritol utilisation in *Yarrowia lipolytica*

19.00 Subcarpathian Accordion Quintet "Ambitus V" Concert in the University of Rzeszów Senate Room

Day 2 (June 23)

9.00 – 10.30 Session 4 Genome maintenance

Chairs:

Adrianna Skoneczna, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw

Robert Wysocki, University of Wrocław, Wrocław

9:00 – 9:20 **Adrianna Skoneczna**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Post-translational regulation of Rad51 recombinase in yeast *S. cerevisiae*

9:20 – 9:40 **Dorota Dziadkowiec**, Faculty of Biotechnology, University of Wrocław, Wrocław
The role of yeast SWI2/SNF2 DNA dependent translocases in genome stability maintenance

9:40 – 10:00 **Michał Dmowski**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Contribution of non-catalytic subunits of the helicase-polymerase complex to the maintenance of genome stability in yeast

10:00 – 10:15 **Ireneusz Litwin**, Scientific Excellence Hub - Centre for DNA Repair and Replication, University of Wrocław, Wrocław
Identification of new cohesin interactors in yeast

10:15 – 10:30 **Karol Kramarz**, Scientific Excellence Hub - Centre for DNA Repair and Replication, University of Wrocław, Wrocław
Impact of SUMOylation at replication stress sites in fission yeast

10.30 – 11.00 Coffee break

11.00 – 12.30 Session 5 Yeast as a model of human diseases and drug testing
Chairs:
Teresa Żołądek, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Róża Kucharczyk, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw

11.00 – 11.30 **Sylvie Friant**, University of Strasbourg, Strasbourg, France
Humanization of yeast cells to study human proteins and patient mutations in rare diseases

11.30 – 11.45 **Róża Kucharczyk**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Mechanisms of ATP synthase defects due to mutations in mitochondrial *ATP6* gene - yeast studies

11.45 – 12.00 **Joanna Kamińska**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Helpful yeasts - how to find therapy for patients with Vps13 proteins deficit?

12.00 – 12.15 **Andrzej Kochański**, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw
Pathogenic effect of *GDAP1* mutations causative for CMT4A disease in a yeast model

12.15 – 12.30 **Monika Staniszewska**, Warsaw University of Technology, Warsaw
New trends in search for antifungal therapies

12.30 – 13.30 Lunch

13.30 – 15.00 Session 6 Yeast biodiversity and evolution
Chairs:
Ryszard Korona, Jagiellonian University, Cracow
Jarosław Marszałek, University of Gdańsk, Gdańsk

13.30 – 13.45 **Ľubomír Tomáška**, Comenius University in Bratislava, Slovakia
A runaway evolution of telomeres in ascomycetous yeasts

13.45 – 14.00 **Jarosław Marszałek**, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk
Evolutionary Biochemistry of yeast Hsp70/J-protein chaperones substrate binding cycle

14.00 – 14.15 **Szymon Kaczanowski**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
Yeast as a model of evolution of apoptosis

14.15 – 14.30 **Chiranjit Panja**, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
YOR020W-A (MCO10): characterizing the unknown "subunit L" of mitochondrial ATP synthase of *Saccharomyces cerevisiae*

14.30 – 14.45 **Monika Opałek**, Institute of Environmental Sciences, Jagiellonian University, Cracow
Fitness advantage of phenotypic heterogeneity in *Saccharomyces cerevisiae* populations

14.45 – 15.00 **Marcin Plech**, University of Edinburgh, Edinburgh, United Kingdom
Deep mutational scanning of human mendelian disease genes in yeast

15.30 – 18.00 Poster Session with coffee and cakes

18.00 – 23.00 Conference Dinner

Day 3 (June 24)

8.30 – 9.15 Keynote Lecture 2
Volkmar Passoth, Swedish University of Agricultural Sciences, Uppsala, Sweden
Oleaginous yeasts for biochemicals, feed and food from lignocellulose

9.15 – 10.45 Session 7 Yeast Biotechnology
Chairs:
Ewelina Celińska, Poznań University of Life Sciences, Poznań
Zbigniew Lazar, Wrocław University of Environmental and Life Sciences, Wrocław

9.15 – 9.45 **Hiroshi Takagi**, Nara Institute of Science and Technology, Japan
Proline new science and technology in yeast

9.45 – 10.00 **Olena Dmytruk**, University of Rzeszów, Rzeszów
Production of the bacterial antibiotics roseoflavin and aminoriboflavin by recombinant strains of the yeasts *Candida famata* and *Komagataella phaffii*

10.00 – 10.15 **Katarzyna Kosiorowska**, Wrocław University of Environmental and Life Sciences, Wrocław
Metabolic engineering of *Yarrowia lipolytica* yeast to poly(ethylene terephthalate) degradation

10.15 – 10.30 **Aksyniia Tsaruk**, Institute of Cell Biology, NAS of Ukraine, Lviv
The effect of carbon source, aeration and pH control on L-lactic acid production by methylotrophic yeast *Ogataea polymorpha*

10.30 – 10.45 **Andriy Sibirny**, University of Rzeszów, Rzeszów

Construction of the humanized strains of *Komagataella phaffii* producing intracellular, secreted and surface displayed SARS-CoV-2 antigens as potential vaccines against COVID-19

10.45 – 11.15 Coffee break

11.15 – 12.45 Session 7 Yeast Biotechnology (continued)

Chairs:
Aleksandra Mirończuk, Wrocław University of Environmental and Life Sciences, Wrocław
Justyna Ruchała, University of Rzeszów, Rzeszów

11.15 – 11.30 **Ewelina Celińska**, Poznań University of Life Sciences, Poznań
Omics-guided engineering of a secretory pathway for enhanced synthesis of secretory proteins in *Yarrowia lipolytica*

11.30 – 11.45 **Patrick Fickers**, Liege University, Liege, Belgium
Erythritol metabolism: from fundamental research to biotech application

11.45 – 12.00 **Milan Čertík**, Slovak University of Technology, Bratislava, Slovakia
Yarrowia lipolytica as a platform for production of tailor-made lipids

12.00 – 12.15 **Mateusz Szczepańczyk**, Wrocław University of Environmental and Life Sciences, Wrocław
Molecular mechanism of polyols assimilation by yeast *Yarrowia lipolytica*

12.15 – 12.25 **Justyna Ruchała**, University of Rzeszów, Rzeszów
Thermotolerant yeast *Ogataea polymorpha* as promising producer of the second generation ethanol

12.25 – 12.35 Maria Gorczyca, Poznań University of Life Sciences, Poznań
Co-expression of selected transcription factors modulates synthesis of heterologous proteins in *Yarrowia lipolytica* under stress conditions

12.35 – 12.45 **Marcin Sypka**, Łódź University of Technology, Łódź
Cold-adapted yeasts - the source of valuable biomolecules

12.45 – 13.45 Lunch

13.45 – 15.15 Session 8 Pathogenic and probiotic yeasts

Chairs:
Monika Staniszewska, Warsaw University of Technology, Warsaw
Maria Rąpała-Kozik, Jagiellonian University, Cracow

13.45 – 14.00 **Maria Rąpała-Kozik**, Jagiellonian University, Cracow
Living together - the role of *Candida albicans* in the formation of polymicrobial biofilm

14.00 – 14.15 **Justyna Karkowska-Kuleta**, Jagiellonian University, Cracow
The host put up against the pathogen's wall - the function of surface-exposed *Candida* molecules

14.15 – 14.25 **Marcin Zawrotniak**, Jagiellonian University, Cracow
Neutrophil responses to fungal infections

14.25 – 14.35 **Maciej Masłyk**, The John Paul II Catholic University of Lublin, Lublin
In search of effective anti-*Candida albicans* agents

14.35 – 14.45 **Monika Kordowska-Wiater**, University of Life Sciences, Lublin
Application of *Saccharomyces cerevisiae var. boulardii* in probiotic food - study on legume sprouts

14.45 – 15.00 **Małgorzata Cytryńska**, Maria Curie-Skłodowska University in Lublin, Lublin

Close encounters of *Candida albicans* with different antimicrobial peptides and proteins

15.00 – 15.15 **Dorota Kręgiel**, Łódź University of Technology, Łódź
Production and biological activity of pulcherrimin from *Metschnikowia pulcherrima* clade

15.15 – 15.45 Oral presentations of the best poster presenting authors (young scientists PhD/postdocs < 35yo.)

15.45 – 16.00 Best Poster Awards. Closing Ceremony
Andriy Sibirny – Chair of the Conference

PODKARPACKI KWINTET AKORDEONOWY
„AMBITUS V”
Kierownictwo artystyczne: Mirosław Dymon

afiliowany przy Uniwersytecie Rzeszowskim

Podkarpacki Kwintet Akordeonowy „AMBITUS V” powstał w 2005 r. z inicjatywy pracowników naukowo-dydaktycznych Instytutu Muzyki Uniwersytetu Rzeszowskiego, absolwentów Akademii Muzycznej w Krakowie i Uniwersytetu Jagiellońskiego: profesorów UR Mirosława Dymona i Pawła Palucha. Pełnią oni również rolę kierownictwa artystycznego zespołu, opracowując i aranżując utwory, które wykonują. W skład PKA wchodzą jeszcze wychowankowie klasy akordeonu (PP, MD) w Zespole Szkół Muzycznych nr 1 w Rzeszowie: Tomasz Blicharz (absolwent Akademii Muzycznej w Katowicach), Michał Stefanik oraz Mariusz Siuśta, będący absolwentami kierunku edukacja artystyczna w zakresie sztuki muzycznej Instytutu Muzyki UR. Repertuar zespołu jest rozległy i zróżnicowany. Zawiera zarówno utwory oryginalne, pisane na kwintet akordeonowy jak również transkrypcje dokonane przez członków zespołu, prof. Włodzimierza Lecha Puchnowskiego, prof. Ryszarda Sviaczkiewicziusa i in. Kwintet prowadzi działalność koncertową na terenie Polski Płd., nagrywał dla TVP3 oraz Radia Rzeszów. Wraz z innymi wykonawcami brał udział w nagraniu CD dla MTU Aero Engines pt. *Zakochani w Rzeszowie – Verlibt in Rzeszów* (2008) oraz autorskiej CD pt. *AMBITUS V* z utworami muzyki klasycznej (J. S. Bach, F. Farkaš, W. A. Mozart, L. Boëlmann, F. Mendelssohn – Bartholdy) i rozrywkowej (A. Chaczaturian, D. Ellington/ J. Tizol, A. Piazzolla) (2009). W latach 2015-2022 kilkakrotnie z wielkim powodzeniem koncertował w Niemczech (Bielefeld, Saarbrücken), na Węgrzech (Szeged) i Słowacji (Presov).

ACCORDION QUINTET "AMBITUS V"

Subcarpathian Accordion Quintet "AMBITUS V" was created in 2005 on the initiative of scientific and didactic staff from the Institute of Music at the University of Rzeszow, graduates of the Academy of Music in Krakow and the Jagiellonian University: professors UR Mirosław

Dymon and Paweł Paluch. They also act as artistic management of the band, developing and arranging the songs they perform. This quintet also includes pupils of the accordion class (PP, MD) in the Complex of Music Schools No. 1 in Rzeszow: Tomasz Blicharz (a graduate of the Academy of Music in Katowice), Michał Stefanik and Mariusz Siuśta, who graduated from the art education in the field of musical art in the Institute of Music at the University of Rzeszow. The band's repertoire is extensive and diverse. It contains both original works, written for the accordion quintet as well as transcripts made by the band members, Prof. Włodzimierz Lech Puchnowski, Prof. Ryszard Sviaczkiewiczius and others. The quintet conducts concert activities in the South of Poland, also they recorded for the channel TVP3 and Radio Rzeszow. Together with other performers, they participated in the recording of CD for MTU Aero Engines entitled Zakochani w Rzeszowie – Verlibt in Rzeszow (2008) and the author's CD entitled AMBITUS V with classical music (J. S. Bach, F. Farkaš, W. A. Mozart, L. Boëlmann, F. Mendelssohn – Bartholdy) and popular music (A. Khachaturian, D. Ellington/ J. Tizol, A. Piazzolla) (2009). In 2015-2022 they toured several times with great success in Germany (Bielefeld, Saarbrücken), Hungary (Szeged) and Slovakia (Presov).

Programme

1. Astor Piazzolla - Oblivion
2. Wolfgang Amadeusz Mozart – Divertimento B-dur KV 137
    1. Andante
    2. Allegro di molto
    3. Allegro assai
3. Tomaso Albinoni – Adagio g-moll
4. J. Strauss – Polka Tritsch – Tratsch - Polka, op. 214
5. Jacob Gade – El Choclo
6. Jacob Gade – *Jalousie (Zazdrość)*
7. Carlos Gardel _ Por una Cabeza
8. Astor Piazzolla – S.V.P. (*S'il vous plait*)
9. Astor Piazzolla – Adios Nonino
10. Astor Piazzolla – Libertango

KEYNOTES LECTURES

KL1 MULTIVARIANT GLOBAL CONTROL OF THE MAJOR NITROGEN-RESPONSIVE TRANSCRIPTION ACTIVATOR, GLN3

Terrance G. Cooper*

Microbiol., Immunol. & Biochem., Univ. of TN, Memphis, Tennessee U.S.A.
*tcooper@uthsc.edu

GATA-family Gln3 and Gat1 are Nitrogen Catabolite Repression-sensitive transcription activators. In nitrogen replete conditions, they sequester in the cytoplasm as Ure2-Gln3 and Ure2-Gat1 complexes. As a result, transcription of genes associated with poor nitrogen source transport and metabolism is minimal. As nitrogen availability declines, Gln3 and Gat1 enter the nucleus and ramp up NCR-sensitive transcription permitting cells to scavenge poor environmental nitrogen sources. GATA-family Dal80 and Gzf3 are transcriptional repressors that compete with Gln3 and Gat1 for promoter binding. Although the 4 GATA factors bind to similar sequences, they are not redundant. They differ markedly in regulation and function. The transcription of all but Gln3, is NCR-sensitive, Gln3-dependent, as well as autogenously and cross-regulated. Nitrogen-responsive Gln3 regulation is achieved via the action of 2 global nitrogen-responsive protein kinases, TorC1 and Gcn2. In replete intracellular amino acids, TorC1 phosphorylates Gln3 and Tap42 and inhibits Gcn2. TorC1-bound, phosphorylated Tap42 binds the Sit4 and PP2A phosphatases inactivating them. In sparse nitrogen, the opposite occurs. TorC1 activity decreases, Tap42-Sit4/PP2A complexes release from TorC1 and dephosphorylate Gln3 which dissociates from Ure2 and enters the nucleus. Additionally, Gcn2, required for nuclear Gln3 entry, becomes active and inhibits TorC1. In short, TorC1 and Gcn2 cross-regulate one another's activities and oppositely regulate Gln3 nuclear localization. However, further observations pointed to additional layers of regulation. Nuclear Gln3 is far more phosphorylated than when it is in the cytoplasm. This posits that Gln3 must be dephosphorylated to enter the nucleus and then becomes phosphorylated within it. Whether or not Gln3 must bind to its target promoters once it is in the nucleus is determined specifically by glutamine levels. When glutamine is low, Gln3 must bind to its target promoters before it can exit from the nucleus, whereas in high glutamine Gln3 can exit the nucleus without binding to its target promoters. Upon nuclear exit of the highly phosphorylated Gln3, in replete nitrogen conditions, it is dephosphorylated in a Sit4/PP2A-dependent manner. In other words, both free Sit4/PP2A present in nitrogen excess, and Tap42-Sit4/PP2A present when nitrogen is scarce dephosphorylate cytoplasmic Gln3. Sit4, PP2A and Ure2 are all required to maintain cytoplasmic Gln3 in a dephosphorylated form. Paradoxically, both rapamycin and glutamine analogue, methionine sulfoximine (Msx), treatment elicit nuclear Gln3 localization. However, only rapamycin elicits Gln3 dephosphorylation. To better understand this paradox, we parsed and characterized Gln3's regulatory domains. Since Tor1 phosphorylates Gln3, it was not surprising to find that Tor1 interacts with a Gln3 C-terminal a-helix. This a-helix is required for cytoplasmic Gln3 sequestration and, for rapamycin-induced, Sit4-dependent, nuclear Gln3 localization. Abolishing this a-helix permits nuclear Gln3 localization in the absence of both rapamycin treatment and Sit4 activity. Paradoxically, however, Sit4 continues to dephosphorylate Gln3. Searching for the site of Sit4-dependent dephosphorylation, we located an N-terminal, Tor1-interacting a-helix whose integrity is required for rapamycin-induced nuclear Gln3 localization. It is the ser/thr residues in this site that are

dephosphorylated in response to rapamycin treatment. Assembling these observations, we concluded that the C-terminal Tor1-Gln3 site functions negatively to sequester Gln3 in the cytoplasm when nitrogen is plentiful, whereas the N-terminal Tor1-Gln3 site functions positively supporting nuclear Gln3 localization. The 2 sites function autonomously and collaboratively in Gln3 control. This may also explain why functional Gtr-Ego components, that activate the TorC1 complex, are required for nuclear Gln3 localization. The most recent observations point to yet further, but yet to be understood, fine-tuning of nitrogen-responsive Gln3 regulation. The Whi2-Psr1/2 complex suppresses TorC1 activity following a shift from high- to low-amino acid medium, but that suppression has little influence on NCR-sensitive protein production beyond those proteins associated with major amino acid interconversions that occur following the shift. Yet the loss of Whi2 dramatically changes the levels of 58 proteins that are largely unrelated beyond those associated with carbohydrate metabolism and oxidative stress.

NIH GM35642-27, Van Vleet Chair, LTAUSA18162, RVO 61388971.

KL2 OLEAGINOUS YEASTS FOR BIOCHEMICALS, FEED AND FOOD FROM LIGNOCELLULOSE

Volkmar Passoth*, Jule Brandenburg, Mikołaj Chmielarz, Giselle Martín-Hernandez, Yashaswini Nagaray, Bettina Müller, Johanna Blomqvist

Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-75007 Uppsala, Sweden
*volkmar.passoth@slu.se

Microbial lipids produced from lignocellulose and crude glycerol can serve as sustainable alternatives to vegetable oils, which production is in many cases accompanied with monocultures, land use changes, or rain forest clearings. Our projects aim to understand the physiology of microbial lipid production, optimise the production and establish novel applications of microbial lipid compounds.

We have established methods for fermentation and intracellular lipid quantification. Following the kinetics of lipid accumulation in different strains, we found high variability in lipid formation even between very closely related oleaginous yeast strains on both, wheat straw hydrolysate and crude glycerol. For example, on complete wheat straw hydrolysate we saw that one *Rhodotorula glutinis* strain, when starting assimilating xylose also assimilated the accumulated lipids, while a *Rhodotorula babjevae* strain could accumulate lipids on xylose.

Two strains (*Rhodotorula toruloides* CBS14 and *R. glutinis* CBS3044) were found to be best out of 27 tested to accumulate lipids on crude glycerol. Interestingly, presence of hemicellulose hydrolysate stimulated glycerol assimilation in both strains.

Apart from microbial oil, *R. toruloides* also produces carotenoids. First attempts of extraction using the classical acetone- based method showed that β-carotene is the major carotenoid. However, there are indications that there are also substantial amounts of torulene and torularhodine, which have a very high potential as antioxidants.

Session 1

Yeast cell biology and transport

Oral presentations

S1_O1 ROLE OF URIDYLATION IN CYTOPLASMIC MRNA DECAY

Michał Małecki*, Maciej Grochowski, Lidia Lipińska-Zubrycka

Institute of Genetics and Biotechnology, Department of Biology, University of Warsaw, Poland
*m.malecki@igib.uw.edu.pl

Messenger RNA (mRNA) degradation is an essential step in gene expression. Due to the existence of several redundant pathways mRNA degradation is difficult to study and many fundamental questions still remain unsolved. Bulk mRNA decay is initiated by poly(A) tail shortening followed by removal of 5' cap which leaves mRNA molecule accessible for cytoplasmic 5'-3' exonuclease Xrn1. It is believed that in eukaryotes 5'-3' directed degradation is a dominant pathway, however, mRNA can be degraded from 3'-end by the exosome complex supported by the SKI complex activity. A less understood factor implicated in bulk mRNA decay is a pervasive cytoplasmic uridylation of mRNA 3'-ends. Uridylation is catalysed by cytoplasmic uridyltransferases (TUTases) (Rissland el al., 2009), while 3'-5' exonuclease Dis3l2 preferentially targets uridylated RNAs (Malecki et al., 2013). In higher eukaryotes uridylation of mRNAs with shortened poly(A) tails facilitates LSM complex binding which in turn accelerates decapping and 5-3' decay of those transcripts. Intriguingly elimination of uridylation has no significant impact on cell physiology and only minor molecular consequences has been reported (Scheer et al., 2021). Therefore, contribution and significance of uridylation for bulk mRNA decay are not clear.

We used fission yeast to establish the role of uridylation in mRNA decay. Fission yeast offers all advantages of a simple unicellular model while their mRNA is pervasively uridylated similarly to situation in higher eukaryotes. Using yeast genetics and both genome-wide and gene focused 3'-RACE we discovered that uridylation participates in mRNA turnover in two independent ways. Main fission yeast TUTase Cid1 uridylates adenylated mRNAs. Such uridylation induces LSM complex binding that accelerates decapping and 5-3' decay while at the same time protects 3'-end from further shortening. Messengers that escape this main pathway can be completely deadenylated, we found that such molecules can be oligouridylated by the second fission yeast TUTase Cid16. According to our data mRNA oligouridylation triggers its decay by U-specific 3-5' exonuclease Dis3l2. Thus we established that oligo(A) tails uridylation by Cid1 protects mRNAs 3'-ends and shifts decay balance towards 5-3' direction, while oligouridylation of deadenylated mRNAs by Cid16 triggers their removal by 3-5' decay pathway.

Interestingly our results strongly suggest that redirecting mRNA decay to 3-5' pathway leads to clashes with ongoing translation. Therefore, we propose that the main role of pervasive uridylation of poly(A) tails is to route mRNA decay to 5-3' pathway which helps to avoid interference between mRNA translation and decay.

Rissland O. S. & Norbury C. J. 2009 *Nat Struct Mol Biol* 16: 616–623.
Malecki M. et al. 2013 *EMBO J* 32: 1842–1854.
Scheer H. et al. 2021 *Nat Commun 121* 12: 1–17.

S1_O2 NEW IMPORT PATHWAY TO PEROXISOMES: SOME ANSWERS, MORE QUESTIONS

Marek Skoneczny*

Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warszawa, Poland
kicia@ibb.waw.pl

Peroxisomes are ubiquitous organelles that perform specific tasks within eukaryotic cells and contain a limited number of enzymes that function in specific metabolic pathways to accomplish them. The import mechanisms by which most of these proteins enter the peroxisomal lumen are well established and are based on the typical signal-receptor principle. Two such signals are known: C-terminal peroxisomal targeting signal 1 (PTS1), recognized by the TPR domain of the Pex5p receptor, and N-terminal PTS2, recognized by the WD-40 domain of the Pex7p receptor. However, some proteins in *Saccharomyces cerevisiae* are imported into peroxisomes via an alternative, poorly characterized route that engages the Pex5p receptor but does not rely on the interaction between the PTS1 and TPR domain. Instead, their import mechanism involves binding hitherto unknown signal sequences within the cargo proteins to the Pex5p receptor N-terminal region. We called this hypothetical signal PTS3.
In the course of our studies, we revealed that four proteins, acyl-CoA oxidase (AOx), carnitine acetyltransferase (Cat2p), a multifunctional enzyme of the β-oxidation pathway (Fox2p), and catalase A (Cta1p) are imported, partially or exclusively via this alternative, PTS1-independent route. Furthermore, aiming to determine the structure of the PTS3 targeting signal, we identified amino acid residues within AOx polypeptide that are important for its import into peroxisomes, presumably comprising this signal. These residues are scattered across AOx polypeptide but located adjacent to each other on the deduced 3D structure of this protein. Such type of signal is called a Signal patch. Our findings expand the knowledge of the mechanisms of translocation of proteins across the peroxisomal membrane and indicate the need to reevaluate the peroxisomal import paradigm.
At the same time, our findings raise several questions: Are those proteins the only ones imported via the PTS3 route in *S. cerevisiae*? What does the import signal in these proteins look like? What is the exact nature of the domain in Pex5p, recognizing this signal? Peroxisomal proteins having neither PTS1 nor PTS2 signals do exist in other species. Does the PTS3-dependent peroxisomal import mechanism operate in those organisms? Is it present in other yeast species, other fungi, perhaps also in plants and animals, or only in *S. cerevisiae*?

S1_O3 CELL SIZE IMPLICATIONS FOR THE REPRODUCTIVE CAPACITY OF YEAST CELLS

Renata Zadrąg-Tęcza[*], Magdalena Kwolek-Mirek, Roman Maślanka
Department of Biology, Institute Biology and Biotechnology, University of Rzeszów, Rzeszów, Poland
[*]*retecza@ur.edu.pl*

Cell size is the most fundamental feature, which sets the scale of all cell biological processes. The cell size determines both the spatial organization of the intracellular structures and the adjustment of the scale of biosynthetic processes ensuring appropriate level of macromolecules such as proteins, lipids and nucleic acids. The dynamic balance to adjust the level of biosynthetic process to the changes in cell size ensures the maintenance of the physiological efficiency of cells (Schmoller and Skotheim, 2015). Cell size changes may have significant impact on cell physiology, inter alia by affecting intracellular distances, surface-to-volume ratio or DNA-to-cytoplasm ratio. Hence, in the case of many cell types, physiological efficiency reaches its maximum within the medium-size cell range, which suggest existence of a certain optimum size range for cell physiological efficiency. However, how cell size impacts on different cellular processes and how cells control their size is still open question.
The studies conducted by our team with using the *Saccharomyces cerevisiae* yeast cells have shown the causal relationship between the cell size and the reproductive capacity (expressed as the number of buds produced by the mother cell during its life). The gradual increase in cell size, results from the mechanism of cytokinesis, leads to the achievement of an excessive cell size - hypertrophy state - that prevents it from entering into the subsequent cell cycle (Biliński et al. 2012). Our experiments showed that the cell size increase rate during a single cycle as the important factor that has an impact on reproductive capacity of the cell. Searching the factors which can modulate the rate of cell size increase we have focused on the type and availability of nutrients as they determine the energy and biosynthetic metabolism of the cell, thus affecting the physiological and structural changes accompanying the increase in cell size. Using an experimental approach based on conditions differing under the level of glucose available to cells, we established that cell size is directly connected with biosynthetic processes. Glucose, as the predominant carbon source for most organisms, plays the main role in many metabolic pathways thus strongly influencing biosynthetic processes and the growth rate of cell size. Hence, the metabolic alterations decreasing the biosynthetic capabilities and the related decrease in cell size may lead to slower achievement of the hypertrophy state and result in extension of the proliferation capacity (Maślanka and Zadrąg-Tęcza, 2020). Our results suggest that the mechanism of cell size regulation is associated with the strategies of optimization allocation cellular resources.

Biliński T. et al. FEMS Yeast Res. 2012, 12:97-101.
Maślanka R. and Zadrąg-Tęcza R. Int J Mol Sci. 2020, 21.
Schmoller K. M. and Skotheim J. M. Trends in Cell Biol. 2015, 25:793-802.

S1_O4 CHARACTERIZATION OF SUGAR AND GLYCEROL METABOLISM IN THE YEAST *YARROWIA LIPOLYTICA*

Piotr Hapeta[1,2], Patrycja Szczepańska[1], Eduard Kerkhoven[3], Anne-Marie Crutz-Le Coq[4], Cécile Neuvéglise[5], Jean-Marc Nicaud[4], **Zbigniew Lazar**[1*]

[1]*Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland*
[2]*Department of Bioengineering and Imperial College Centre for Synthetic Biology, Imperial College London, London SW7 2AZ, UK*
[3]*Department of Biology and Biological Engineering, Division of Systems and Synthetic Biology, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden*
[4]*Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78352 Jouy-en-Josas, France*
[5]*SPO, INRAE, Montpellier SupAgro, Univ Montpellier, 34060, Montpellier, France*
[*]*zbigniew.lazar@upwr.edu.pl*

The yeast *Yarrowia lipolytica* are microorganisms with high industrial potential, especially valued for their predisposition to the production of chemical compounds used in food, pharmaceutical as well as fuel and energy industries. For growth and biosynthesis of the desired products, *Y. lipolytica* use a wide range of carbon sources, including hydrophobic (lipids, hydrocarbons) and hydrophilic (monosaccharides, glycerol) substrates, which can also be obtained as waste or by-products from other industries. Despite the enormous amount of research related to these microorganisms, the knowledge on the functioning of their central carbon metabolism still needs to be completed, in particular, in order to implement efficient biotechnological processes on an industrial scale.

The preferential use of glycerol over glucose when these substrates are mixed is an interesting and unique characteristic of *Y. lipolytica*. We have attempted to explain this phenomenon, as well as the influence of individual carbon sources and nitrogen availability on global gene expression in this yeast. The whole transcriptome sequencing of the cells grown under chemostat conditions indicated that gene expression in *Y. lipolytica* is more dependent on nitrogen concentration than the type of carbon source used. In particular, expression of hexokinase (*YlHXK1*) proved to be extremely susceptible to changes in nitrogen availability. Moreover, high expression of *YlHXK1* in media with low nitrogen concentration appeared to influence the expression of other genes, including those related to the Krebs cycle and erythritol biosynthesis. We also characterized the hexokinase from *Y. lipolytica* and determined the influence of its unique spatial structure on metabolism and its regulation. Overexpression of hexokinase reversed the preferential use of glycerol in favor of glucose.

Furthermore, overexpression of hexose transporters and hexokinases (native and fructophilic from *Schizosaccharomyces pombe*) proved that the preferential use of glucose over fructose in cultures with a mixture of these sugars depends primarily on the affinity of the native hexokinase for glucose. Interestingly, the combination of overexpression of any of the tested hexokinases with hexose transporters led to the production of significant amounts of polyols (31.5 g/L) from glucose and fructose, which was previously possible with the use of glycerol as a carbon source. In addition, overexpression of fructophilic hexokinase and hexose transporters allowed to shorten the process time and resulted in a 23% improvement in the

polyol biosynthesis from glucose and fructose compared to the processes with glycerol. In the future research, the fine-tuning of the pentose phosphate pathway will be of decisive importance in the NADPH generation, indispensable in lipid biosynthesis.
On the other hand, the biosynthesis of a specific class of lipids, i.e., phospholipids, performed better in glucose-based media. Through a series of genetic modifications of the phospholipid biosynthesis pathway, we showed that glycerol (including raw glycerol) can also be a good substrate for production of these compounds by *Y. lipolytica*.

S1_O5 INTERPLAY BETWEEN *CANDIDA ALBICANS* TRANSPORTERS, PLASMA MEMBRANE AND CELL WALL

Aneta Urbanek[*], Daria Derkacz, J. Suchodolski, J. Muraszko, Anna Krasowska[*]

Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, 50-383 Wroclaw, Poland
[*]*anna.krasowska@uwr.edu.pl*

Candida albicans is an opportunistic fungal pathogen of humans that is responsible for causing life-threatening systemic infections. *C. albicans* can gain several mechanisms of resistance to commonly used antifungals such as azoles. The main reason for *C. albicans* drug resistance is the presence of ABC transporters in the plasma membrane, mainly the Cdr1 efflux pump, which is responsible for decreasing the intracellular concentration of drugs. The primary target for azole antifungal agents is lanosterol 14a-demethylase (Erg11p) encoded by the *ERG11* gene. This enzyme is involved in the biosynthesis of ergosterol, a crucial component of the plasma membrane (PM). Our results show that targeted gene disruption of *ERG11* can result in the resistance to drugs targeting ergosterol such as azoles and amphotericin B. The altered PM structure of the *C. albicans erg11Δ/Δ* strain contributes to the delocalisation of the Cdr1p from the PM to vacuoles and causes its activity decreases (Suchodolski et al. 2019).
It should be highlighted that the lack of ergosterol in PM induces changes in the composition and structure of the cell wall of *C. albicans*. For instance, in the presence of fluconazole , we observed the local accumulation of chitin and a higher level of unmasked chitin in the WT strain, whereas those effects were visible in the *erg11*Δ/Δ mutant without fluconazole treatment. In addition, the lack of ergosterol in the *erg11Δ/Δ* mutant led to the exposure of β-glucan on the fungal cell surface (Suchodolski et al. 2020). Immune evasion of the host is supported by masking immune-stimulating cell wall components, such as chitin and β-glucan. Cell wall remodelling caused by the lack of ergosterol in PM, may expose these components and lead to the recognition of *C. albicans* by the host's immune system.
At the same time, when the fungal cell wall is altered and remodelled by known antifungals, it could be also made susceptible to other compounds, such as lipopeptides, e.g. surfactin (SU). Discovering new and efficient antifungal compounds is relatively difficult, thus one prominent drug development strategy is to look for a synergistic combination of existing antifungals with other active compounds.

Suchodolski J. et al. Microorganisms. 2019, 7, 378.
Suchodolski J. et al. Pharmaceutics. 2020, 12, 314.

S1_O6 AUTOPHAGIC DEGRADATION OF CYTOSOLIC PROTEINS IN THE METHYLOTROPHIC YEAST *KOMAGATAEALLA PHAFFII*

Marta Semkiv[1*], Anastasiya Zazulya[1], Olena Dmytruk[1,2], Grzegorz Węgrzyn[3], Andriy Sibirny[1,2]

[1]*Department of Molecular Genetics and Biotechnology, Institute of Cell Biology NAS of Ukraine, Drahomanov St, 14/16, Lviv, 79005, Ukraine*
[2]*Department of Microbiology and Molecular Genetics, University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland*
[3]*Department of Molecular Biology, University of Gdansk, Bażyńskiego 8, 80-309 Gdansk, Poland*
[*]*smarta0309@gmail.com*

Cellular homeostasis relies on the precise balance of the synthesis and degradation of the cellular components. Usually, the degradation of proteins with short lifespan occurs in the proteosomes, whereas proteins with long life span and whole organelles are degraded by autophagy. Some mechanisms of autophagy have been investigated using yeast *Pichia pastoris* (*Komagataella phaffii*). This yeast is considered to be one of the most efficient producers of recombinant proteins, in particular insulin, hepatitis B virus surface antigen, interferons, alcohol oxidase enzyme, nitrilase, etc. The important prerequisite of the creation of protein-producing strains is the maximum reduction in the level of degradation of the protein in the cytosol. However, the mechanisms of degradation of their own cytosolic proteins as well as recombinant proteins of biotechnological significance with cytosolic localization in *P. pastoris* remain unclear.

A selection system has been developed for identification of the strains of yeast *P. pastoris* with deletion of the genes associated with autophagic degradation of cytosolic proteins. For this purpose, the *LAC4* gene of *Kluyveromyces lactis* encoding β-galactosidase, fused to the fluorescent label GFP, was expressed under the control of a methanol-induced promoter of the formaldehyde dehydrogenase gene in strains *P. pastoris* GS200 (*arg4 his4*) and SMD1163 (*his4 pep4 prb1*), in which the process of autophagy is disrupted. The obtained recombinant strains produced β-galactosidase during growth on medium with methanol, and after transfer of cells to medium with glucose, degradation of this cytosolic protein occurred. Because derivatives of strain SMD1163 showed a slower decrease in β-galactosidase activity than derivatives of strain GS200, it can be argued that the degradation of recombinant protein occurs in part by autophagy. Based on the engineered strain GS200/LAC4, insertion mutants were obtained in which the process of β-galactosidase degradation was prolonged after transfer to glucose medium compared to the original strain. In one of the obtained mutants, the integration site of the insertion cassette was identified – the integration occurred in the region of the open reading frame of the gene encoding β-1,6-N-acetylglucosaminyltransferase. Insertional and deletion mutants with disruption of this gene revealed impaired process of degradation of heterologous enzyme β-galactosidase, and also native enzymes formaldehyde dehydrogenase and formate dehydrogenase.

This work was supported by grant of The Polish National Agency for Academic Exchange and Ministry of Education and Science of Ukraine for the joint Ukraine-Poland R&D projects.

Poster presentations

S1_P1 THE ROLE OF ERGOSTEROL AND SPHINGOLIPIDS IN THE LOCALIZATION OF *CANDIDA ALBICANS* Cdr1p EFFLUX PUMP IN DETERGENT-RESISTANT MICRODOMAINS

Jakub Muraszko[1], Aneta Urbanek[1], **Daria Derkacz**[1*], Marcin Łukaszewicz[1], Przemysław Bernat[2], Anna Krasowska[1]

[1]Faculty of Biotechnology, University of Wrocław, 50-383 Wrocław, Poland
[2]Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, University of Łódź*, Banacha 12/16, 90-237* Łódź*, Poland*
[*]daria.derkacz@uwr.edu.pl

The human opportunistic pathogen *Candida albicans* is a part of the normal microbiota of healthy individuals. In immunocompromised patients causes a life-threatening disease named candidiasis. A limited number of antifungal therapies, multiple virulence factors and various resistance strategies make *C. albicans* infections challenging to treat.

One of the targets for antifungal therapeutics (e.g., azoles or polyenes) is ergosterol – a lipid crucial for maintaining the proper structure and functioning of *Candida* plasma membrane (PM). Inhibition of ergosterol biosynthesis caused by azoles induces multiple resistance mechanisms. This includes increased expression of genes encoding proteins involved in ergosterol biosynthesis (e.g., *ERG11*) or overproduction of *C. albicans* transporters (e.g., Cdr1p – *Candida* drug resistance protein 1) responsible for pumping out drugs from fungal cells [1].

Additionally, apart from ergosterol, sphingolipids (SLs) play a key role in multi-drug resistance. The primary role of SLs is the segregation of PM components and the formation of PM detergent-resistant microdomains (DRMs). Interestingly, *Saccharomyces cerevisiae* with deletion of genes involved in ergosterol biosynthesis shows increased SLs synthesis with smaller polar head groups (mannose-inositol-P-ceramide and inositol-P-ceramide (MIPC and IPC)) and decreased SLs synthesis with the larger polar head groups (mannose-(inositol-P)2-ceramide ($M(IP)_2C$)) [2]. Furthermore, $M(IP)_2C$ biosynthesis inhibition corresponds to a lowered level of Cdr1p and an elevated susceptibility to azoles [3]. This suggests that the presence of SLs is essential for developing drug resistance mechanisms that involve drug transporters. Thus, further investigating correlations between ergosterol, SLs, and drug transporters are relevant.

We prove that Cdr1p localizes in DRMs in both *C. albicans* WT and *erg11Δ/Δ* strains using the novel spot variation fluorescence correlation spectroscopy (svFCS) technique. Additionally, we performed a microscopic study of Cdr1p-GFP localization in both *C. albicans* strains, and we compared it with the localization of Pma1p-GFP, which is considered to concentrate in DRMs naturally. In order to investigate whether the decrease of the total amount of SLs alters Cdr1p-GFP localization, we treated *C. albicans* WT and *erg11Δ/Δ* strains with the inhibitor of SLs biosynthesis - aureobasidin A (AbA). We demonstrate that treatment with AbA leads to a diminished total SLs content in both *C. albicans* strains, which correlates with delocalization of both Pma1p-GFP and Cdr1p-GFP.

This suggests that proper localization of Cdr1p depends on the presence of both ergosterol and SLs in *C. albicans* PM. Substantial changes in PM lipid composition can lead to alterations in the localization and activity of proteins residing in fungal PM. Considering the limited number of clinically employed antifungal therapies, it is worth further investigating the interplay between SLs and Cdr1p.

1. Hargrove T. Y., et al. Structural analyses of *Candida albicans* sterol 14α-demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol biosynthesis. *J Biol Chem*. **2017**; 292(16), 6728-6743.
2. Valachovic M., et al. Cumulative mutations affecting sterol biosynthesis in the yeast *Saccharomyces cerevisiae* result in synthetic lethality that is suppressed by alterations in sphingolipid profiles. *Genetics*. **2006**; 173(4), 1893-908.
3. Gao J., et al. *Candida albicans* gains azole resistance by altering sphingolipid composition. *Nat Commun*. **2018**; 9(1), 4495.

S1_P2 THE MECHANISMS OF DEGRADATION OF METHANOL CATABOLISM ENZYMES OF FORMALDEHYDE DEHYDROGENASE AND FORMATE DEHYDROGENASE IN METHYLOTROPHIC YEAST *KOMAGATAELLA PHAFFII*

Dmytruk O[1,2*], Dmytruk K[1], Sibirny A[1,2]

[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, 79005 Ukraine
[2]Department of Biotechnology and Microbiology, University of Rzeszów, Rzeszów, 35-601 Poland
[*]verbaolena@gmail.com

The investigation of the mechanisms of cytosolic protein degradation is of great fundamental and applied importance. The decrease in the specific activity of formaldehyde dehydrogenase (Fld1) and formate dehydrogenase (Fdh1) in the wild type strain GS200, the strain with the deletion of the *GSS1* hexose sensor gene, and the strain that is defective in autophagy pathway SMD1163 of *Komagataella phaffii* during short-term and long-term induction with methanol, with or without the addition of the MG132 (proteasome degradation inhibitor), was investigated. It was shown that the duration of cell incubation on methanol had no particular effect on the inactivation of enzymes. The effect of the proteasome inhibitor MG132 was insignificant. Catabolic inactivation of cytosolic and peroxisomal enzymes was damaged in the *gss1* mutant since glucose signaling was impaired. Fld1 and Fdh1 are degraded via the vacuolar pathway regardless of the duration of methanol induction. Such a conclusion was confirmed by western blot analysis and fluorescence microscopy studies.

S1_P3 THE YNL320w – THE UNCHARACTERIZED PROTEIN INVOLVED IN GLUATHIONE HOMEOSTASIS IN *S. CEREVISIAE*

Kasabuła Marta[1*], Chiranjit Panja[1], Róża Kucharczyk[1]

[1]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland*
[*]*kasabulam@ibb.waw.pl*

Although *S. cerevisiae* is one of the longest-used model organisms in scientific research, the function of many of its genes and their protein products is still unknown. One such gene is YNL320w. It belongs to the serine hydrolase family, of which the catalytic triad: serine, histidine, aspartic acid and their environment are highly conserved. It retained more than 35% sequence similarity across all species categories, with more than 70% similarity in fungi. The Ynl320w is highly similar to human ABHD17 and ABHD13 hydrolases. ABHD17 has been shown to have depalmitoylase activity, like another member of this family: ABHD10. Deletion of the YNL320w gene allows cells with GSH1 gene deletion (*gsh1Δ*, lacking gamma glutamylocysteine synthase and thus defective in glutathione (GSH) synthesis) to tolerate the lack of GSH and improves their growth on media without supplementation of this tripeptide (Yadav S. et al. G3, 2020). Similar genetic interaction was found by the authors between *gsh1Δ* and *fmp40Δ*, which encodes a mitochondrial protein having ampylase activity, shown by us to be involved in regulation of global protein glutathionylation and redox homeostasis (Sreelatha A. et al. Cell, 2018). We found that Ynl320w-GFP fusion protein co-localizes with mitochondria. The *gsh1Δ* cells grow slower on respiratory medium. This phenotype is recovered by deletion of YNL320w gene. The recovery of the respiration in *gsh1Δ* depends on the activity of the three mitochondrial redoxins Prx1, Grx2 and Trx3. These results indicate that Ynl320w is another protein that regulates redox homeostasis in the cell and more over important in regulating cellular respiration in the function of redox homeostasis.

Yadav S. et al. G3 (Bethesda). 2020 10(1):371-378
Sreelatha A. et al. Cell. 2018 175(3):809-821.

Research funded by–UMO-2018-31-B-N23-01117 and the Doctoral School of Molecular Biology and Biological Chemistry of IBB PAS

S1_P4 A NEW MECHANISM OF ATP SYNTHASE SUBUNITS 6 (ATP6) AND 9 (ATP9) TRANSLATION REGULATION BY THEIR ASSEMBLY IN YEAST MITOCHONDRIA

Kabala A.M.[1, 2], Binko K.[1, 2], Godard F.[1], Dautant A.[1], Baranowska E.[2], Skoczen N.[1,2], Gombeau K.[1], Bouhier M.[1], Becker H.D.[3], Ackerman S.H.[4], Steinmetz L.M.[5], Tribouillard-Tanvier D.[1], **Kucharczyk R**.[2,*], and di Rago J.-P. [1]

[1]Univ. Bordeaux, CNRS, IBGC, UMR 5095, F-33000 Bordeaux, France
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[3]Université de Strasbourg, CNRS, Institut de Biologie Moléculaire et Cellulaire, F-67084 Strasbourg Cedex, France
[4]Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan, USA
[5]EMBL, Genome Biology Unit, 69117 Heidelberg, Germany. Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA
[*]roza@ibb.waw.pl

The yeast mitochondrial ATP synthase is composed of 17 subunits, assembled in different stoichiometry, of which 3 (subunits *6*, *8*, and *9*) are encoded by mitochondrial genes while the 14 others have a nuclear genetic origin. Within the membrane domain (FO) of this enzyme, the subunit *6* and a ring of 10 identical subunits *9* transport protons across the mitochondrial inner membrane coupled to ATP synthesis in the extra-membrane structure (F1) of ATP synthase. As a result of their dual genetic origin, the ATP synthase subunits are synthesized in the cytosol and inside the mitochondrion. The mechanisms that prevent unbalanced production of ATP synthase subunits are as-yet poorly understood. We show that the rate of translation of the subunits *9* and *6* is enhanced in strains with mutations leading to specific defects in the assembly of these proteins. We show that the translational regulation of subunit *6* involves several components of ATP synthase, at least the F1 and subunit *9*, the chaperoning activities of two proteins (Atp10 and Atp23) that are required to assemble subunit *6*, and the 5'UTR of *ATP6* mRNA, as a means to couple the synthesis of this protein to its incorporation into ATP synthase. Subunit *9* of ATP synthase is also subject to such a mechanism. The subunit *9* translation is correlated with its oligomerization into the ring. Three proteins (Aep1, Aep2 and a fragment of Atp25) required to express subunit *9*, the F1, and a protein (Phb1) are involved in the stabilization of newly synthesized subunit *9*. These assembly-dependent feedback loops are presumably important to limit the accumulation of harmful assembly intermediates of ATP synthase that have the potential to dissipate the mitochondrial membrane electrical potential and the main source of chemical energy of the cell.

The work was supported from the NSC funding to RK: UMO-2011/01/B/NZ1/03492.

S1_P5 INVESTIGATING THE INTERACTION LANDSCAPE OF UBIQUITINATION ENZYMES USING A NANOLUC-BASED PROTEIN COMPLEMENTATION ASSAY

Natalia Łazarewicz[1,2*], Gaëlle Le Dez[2], Ewa Blaszczak[1], Robert Wysocki[1], Gwenaël Rabut[2]

[1]Department of Genetics and Cell Physiology, Faculty of Biological Sciences, University of Wrocław, Kanonia 6/8, Wrocław, 50-328, Poland
[2]Institut de Génétique et Développement de Rennes, CNRS UMR 6290, Université de Rennes 1, 35000 Rennes, France
[*]natalia.lazarewicz@uwr.edu.pl

Protein-protein interactions (PPIs) create a complex network that determines proper functioning of eukaryotic cells. Among the PPIs, protein ubiquitination is one of several crucial enzymatic cascades in the cell. It is necessary for the regulation of many cellular processes such as cell growth, proliferation and signaling. This process involves three classes of enzymes, E1, E2, and E3. Ubiquitin ligases (E3s) are the most abounded and determine the diversity of ubiquitination processes. As these enzymes recognize the modified substrate proteins and provide ubiquitination specificity, they became attractive targets for treatments against diseases caused by aberrations in protein ubiquitination. For instance, overexpression of certain ubiquitin ligases is linked to various types of cancer and neurological diseases in humans. Therefore, understanding the molecular action of ubiquitin ligases is essential to develop processes-specific drugs.

In recent years, many techniques have been developed to study the networks of PPIs. Among these methods, protein complementation assays (PCA) facilitate research on PPIs in living cells, in conditions closest to physiological. Here, we present the studies on ubiquitin ligases, which are involved in diverse cellular processes such as DNA damage response or cell cycle control in the yeast *Saccharomyces cerevisiae*. We investigate the mechanism of action, interacting partners, and substrates' recognition mechanisms to decipher their cellular consequences. For this purpose, we use a modern protein-fragment complementation assay (the NanoBiT® technique).

Our preliminary results showed that among the high confidence results we found the interactors previously described in the literature as well as novel putative interactors. Using luciferase-based technique, we were able to show proteasomally degraded substrates. In addition, we optimized a high-throughput cell growth and measurements, which can be applied in research of different enzymatic pathways. This may also contribute to the development of novel therapies for various diseases in the future.

[1] Błaszczak E, Biochem Soc Trans. 49(3). (2021)
[2] Dixon AS, ACS Chem Bio. 11(2). (2016)

This research was funded in part by the National Science Centre, Poland grant number: 2021/41/N/NZ2/00551 (NCN, Poland)

S1_P6 LESS IS MORE AND MORE IS LESS, BUT THE TYPE IS ALSO IMPORTANT IMPLICATIONS OF GLUCOSE AND FRUCTOSE METABOLISM ON THE REPRODUCTIVE POTENTIAL OF THE *SACCHAROMYCES CEREVISIAE* YEAST

Roman Maślanka[*], Renata Zadrąg-Tęcza

Department of Biology, Institute of Biology and Biotechnology, College of Natural Sciences, University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland
[*]*rmaslanka@ur.edu.pl*

Yeast cells have two general ways to obtain energy, fermentation or aerobic respiration, and molecular shift between them depending on the availability of carbon sources in the environment. Carbohydrates, which are the primary group of carbon sources, are used in cellular processes not only as a source of energy but also to provide a carbon skeleton for biosynthetic processes. Hence, their availability connected with the activity of the cAMP/PKA pathway may influence on several physiological parameters of the cells, *inter alia* on biosynthetic capacity, growth rate, and size of the yeast cells. Our previous results provide evidence that high glucose concentration (calorie excess – CE) and connected with it changes in cell metabolic fluxes increase the biosynthetic capabilities of yeast cells and their cell size, but simultaneously decrease the reproductive potential of the cells (Maślanka and Zadrąg-Tęcza, 2019). Besides glucose, also fructose is the preferred and easily fermented carbon source for yeast cells. Although the metabolic pathway of fructose fermentation is very similar to that of glucose, there are some small discrepancies between glucose and fructose fermentation. The precise molecular cause of this phenomenon is unclear, but there are assumptions that the discrepancy in glucose/fructose fermentation might be connected with the kinetic characteristics of transporters and phosphorylating enzymes of the fermentation pathway or/and differences in catabolite repression caused by fructose compared to glucose (Berthels et al., 2004). The aim of the study was to analyze the effect of fructose and different glucose concentration in the medium on the reproductive capacity and parameters which assess the metabolic status of the yeast cells. The studies were conducted using yeast cells of WT (BY4741) and mutant strains: Δ*hxk2* (with impairment in the glycolytic pathway) and Δ*aim14* (devoid of a gene not directly connected with carbon metabolism). The obtained results show that cells of used strains significantly differ in reproductive capacity. Both mutant strains have greater reproductive potential than the wild-type strain in all of the analyzed conditions. However, the reproductive potential of the Δ*hxk2* strain did not change between conditions with different glucose concentrations, whereas the reproductive potential of the Δ*aim14* strain decreased with the increased glucose concentration (the same as was noted for the WT strain). In the case of a medium with fructose, all of the analyzed strains have slightly increased reproductive potential in comparison to a medium with glucose of the same concentration. The differences in reproductive potential between media with glucose and fructose were not associated with significant changes in the cell metabolic parameters. However, we observed increased ATP content in the cells cultured on the medium with fructose. These results suggest that there are some differences between the fermentation metabolism of glucose and fructose, which may have long-term consequences, among others related to the reproductive potential of cells.

Maślanka and Zadrąg-Tęcza. J Cell Physiol. 2019; 234: 17622– 17638.

Berthels et al. FEMS Yeast Research. 2004; 4: 683-689.
This research was supported by grant no 2021/05/X/NZ3/00162 National Science Centre

S1_P7 BIOLOGICAL ACTIVITY OF NEWLY SYNTHETIZED QUATERNARY AMMONIUM SALTS AGAINST MICROORGANISMS

Ewa Obłąk[1], **Edyta Mazurkiewicz[1*]**, Łukasz Lamch[2], Kazimiera A. Wilk[2]

[1]Department of Physicochemistry of Microorganisms, Faculty of Biological Sciences, University of Wrocław, ul. S. Przybyszewskiego 63, 51-148 Wrocław, Poland
[2]Department of Organic and Pharmaceutical Technology, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[*]ewa.oblak@uwr.edu.pl

Quaternary ammonium salts (QAS) are widely used in industry (preservatives, detergents), agriculture (fungicides, biocides) and medicine (disinfectants, gene or drug carriers). Their widespread use causes resistance to these compounds among pathogenic microorganisms. Therefore, it is still a need to design and synthesize new QAS chemical structures with bactericidal, fungicidal, anti-adhesive and anti-biofilm properties.
The biological activity of two groups of gemini quaternary ammonium salts, bromides and methyl carbonates with different lengths of hydrophobic chains (C12, C16) against yeast and yeast-like fungi was investigated. The best inhibitory and fungicidal activity against the tested strains *Saccharomyces cerevisiae* Σ1278b, *Candida albicans* ATCC 10231 and *Rhodotorula mucilaginosa* ATCC 4056 was shown by bromide with 12-carbon alkyl chains. Moreover, the tested surfactants had the ability to eradicate the biofilm produced by *C. albicans* ATCC 10231. Compounds with 16 - carbon atoms in the alkyl chains showed a stronger anti-biofilm effect than C12. Moreover, it was found that the deposition of gemini QAS on the surfaces of stainless steel, glass and silicone reduced adhesion of *C. albicans* ATCC 10231 to these surfaces. The best anti-adhesive effect was shown by bromide with 12-carbon hydrophobic chains. This surfactant also inhibited the filamentation of *C. albicans*.
In order for these compounds to be used for application, they were tested for cytotoxicity, mutagenicity and haemolysis. It was shown that C12 methyl carbonate was not haemolytic. Moreover, the tested gemini surfactants were not cytotoxic to *S. cerevisiae* Σ1278b cell or mutagenic (at concentrations equal to ¼ MIC).

S1_P8 CONSTRUCTION AND APPLICATION OF THE Bi-GENOMIC MITOCHONDRIAL-SPLIT-GFP STRAIN FOR SCREENING THE MITOCHONDRIAL MATRIX ECHOFORMS OF DUALY LOCALISED PROTEINS

Gaetan Bader[1], Ludovic Enkler[1], Yuhei Araiso[1], Marine Hemmerle[1], Krystyna Bińko[2], Emilia Baranowska[2], **Aneta Więsyk[2]***, Johan-Owen De Craene[1], Julie Ruer-Laventie[3], Jean Pieters[1], Deborah Tribouillard-Tanvier[4], Bruno Senger[1], Jean-Paul di Rago[4], Sylvie Friant[1], Hubert Dominique Becker[1], Róża Kucharczyk[2]

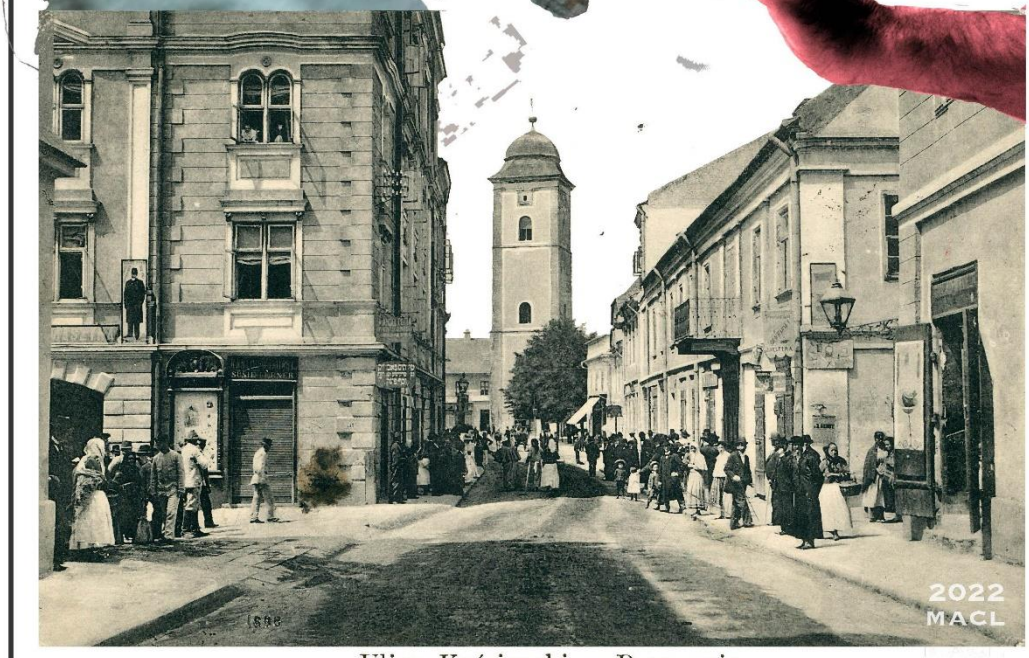
[1]Universite de Strasbourg, CNRS UMR7156, Genetique Moleculaire, Genomique, Microbiologie, Strasbourg, France
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[3]Biozentrum, University of Basel, Basel, Switzerland
[4]Institut de Biochimie et Genetique Cellulaires, CNRS UMR5095, Universite de Bordeaux, Bordeaux, France
[*]anetaw@ibb.waw.pl

A single nuclear gene can be translated into a protein that distributes in many cellular compartments. Accumulating evidences show that mitoproteomes contain lots of proteins having also another cellular localization, termed echoforms. Unraveling the existence of mitochondrial echoforms using current GFP (Green Fluorescent Protein) fusion microscopy approaches is extremely difficult, especially for the cytosolic proteins, because the GFP signal of the cytosolic echoform will almost inevitably mask that of the mitochondrial one. We therefore engineered a yeast strain expressing a new type of Split-GFP that we termed Bi-Genomic Mitochondrial-Split-GFP (BiG Mito-Split-GFP). In this strain the sequence encoding the non-fluorescent fragment of $GFP_{\beta 1\text{-}10}$ (first ten beta sheets) was integrated into the mitochondrial genome and is therefore translated from the mitochondrial machinery while the other ($GFP_{\beta 11}$) is fused to the nuclear-encoded protein of interest translated in the cytosol. The self-reassembly of this Bi-Genomic-encoded Split-GFP is confined to mitochondria and only when the protein of interest will be present in the matrix. We could authenticate the mitochondrial importability of any protein or echoform from yeast, but also from other organisms such as the human Argonaute 2 mitochondrial echoform.

The work is supported from the NSC funding to RK: UMO-2018-31-B-NZ3-01117.

Session 2

Sensing, signalling, and stress response



Ulica Kościuszki w Rzeszowie.

Oral presentations

S2_O1 GLUCOSE SENSING AND SIGNALING IN THE METHYLOTROPHIC YEAST *OGATAEA POLYMORPHA*

Olena G. Stasyk[1,2], **Oleh V. Stasyk[2]***

[1]*Ivan Franko Lviv National University, Hrushevky Str. 4, Lviv, 79005, Ukraine*
[2]*Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine*
**stasyk@cellbiol.lviv.ua*

The molecular mechanisms of glucose sensing and signaling for catabolite regulation were most extensively studied on the model of bakers' yeast *Saccharomyces cerevisiae*. However, contrary to this species evolutionary adapted to aerobic sugar fermentation, other so-called Crabtree-negative yeasts exhibit distinct from *S. cerevisiae* mechanisms of glucose signaling.

We identified in the methylotrophic yeast *Ogataea polymorpha* two putative glucose sensors homologous to hexose transporters. Hxs1 (hexose sensor) appeared to belong to the group of classical yeast non-transporting glucose receptors like *S. cerevisiae* Snf3 and Rgt2. Hxs1 is required for glucose-regulated expression of a functional *O. polymorpha* hexose transporter Hxt1, but is not directly involved in catabolite repression or inactivation pathways in *O. polymorpha*. Truncation of Hxs1 C-terminal fragment abrogated its signaling, whereas conserved substitution $Hxs1^{R203K}$ transformed the protein into a constitutively signaling form. Another *O. polymorpha* hexose transporter-like protein Gcr1 (glucose catabolite repression) has been identified as a "tale-less" sensor required for glucose repression and uptake. Similarly to non-transporting sensors, substitution $Gcr1^{R165K}$ converted the protein into an aberrantly signaling form that profoundly impaired utilization of various carbon substrates. Simultaneously, expression of $Gcr1^{R165K}$ in *gcr1Δ* deletion mutant did not restore glucose transport or repression pathway. Gcr1 apparently has a sensing function in the absence of glucose, participating in the transcriptional induction of peroxisomal alcohol oxidase and normal methylotrophic growth. Gcr1 overexpression led to the increased sensitivity to extracellular 2-deoxyglucose, supporting its functionality as a glucose carrier. Gcr1 may have originated from horizontal gene transfer to yeasts from *Eurotiales* fungi because its orthologue is absent in the genomes of most yeasts, with exception of a few closely related to *O. polymorpha* species. We proposed that Gcr1 represents a previously unknown type of yeast glucose sensor – a transceptor.

Stasyk O.V. et al. J. Biol. Chem. 2004, 279(9):8116-25.
Stasyk O.G. et al. Eukar. Cell. 2008, 7(4):735-46.
Stasyk O.G. et al. Int J Biochem Cell Biol. 2018, 103:25-34.

S2_O2 N-TERMINAL GLN3 PHOSPHORYLATION/DEPHOSPHORYLATION IN THE CONTROL OF GLN3 LOCALIZATION

Jennifer J. Tate*

Department of Microbiology, Immunology & Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee 38163, U.S.A.
*jtate@uthsc.edu

The normal home of *Saccharomyces cerevisiae* ranges from rotting fruit and vegetables in the sun to water-soaked soil in the rain, i.e., from nitrogen replete to starvation environments. Its finely tuned nutrient sensing and responding systems permit it to thrive in good times and survive in bad ones. The principal regulators evolved to cope with these changing environments consist of two nitrogen catabolite repression- (NCR-) sensitive transcription activators, Gln3/Gat1, and control of their localization by two global nitrogen-responsive protein kinases, Target of Rapamycin Complex 1 (TorC1) and Gcn2. When available intracellular amino acid levels are high, active TorC1 inhibits Gcn2 and phosphorylates Gln3, thereby preventing its nuclear entry. As intracellular amino acids dwindle, TorC1 activity decreases and Gcn2 activity, required nuclear Gln3 and Gat1 entry, increases. In other words, TorC1 and Gcn2 oppositely regulate Gln3's localization as well reciprocally regulating one another's activities. Early experiments demonstrated that Tor1 phosphorylates Gln3. We subsequently demonstrated that Tor1 physically interacts with an a-helix$_{656\text{-}666}$ in its C terminus. This C-terminal a-helix is required for wild type, rapamycin-induced, Sit4 phosphatase-dependent nuclear Gln3 localization, but not for rapamycin-induced Gln3 dephosphorylation. Searching for the location of rapamycin-induced dephosphorylation, we found that Gln3 lacking residues 384-730 could enter the nucleus in the absence of Sit4 in both repressive and derepressive growth conditions. Surprisingly, however, Sit4 retained its ability to dephosphorylate Gln3$_{1\text{-}384}$. Further experiments demonstrated that nuclear entry of Gln3$_{1\text{-}384}$ could only occur if an N-terminal **U**re2 **R**elief **S**equence (Gln3$_{URS,\ 247\text{-}282}$) remained intact. It turned out that this Gln3$_{URS}$ contained a Tor1-interacting a-helix (Gln3$_{275\text{-}289}$) situated within an unstructured coiled-coil region (Gln3$_{249\text{-}289}$). It was this N-terminal Gln3$_{URS}$, Tor1-interacting sequence whose dephosphorylation rapamycin induced. Rapamycin treatment resulted in 8 of the 13 Gln3$_{URS}$ serine/threonine residues being dephosphorylated 3–15-fold with 3 of them by 10–15-fold. Importantly, phosphomimetic aspartate substitutions of the Gln3$_{URS}$ serine/threonine residues abolished the N-terminal Gln3–Tor1 interaction, rapamycin-elicited nuclear Gln3 localization, and 1/2 of the derepressed levels of nuclear Gln3 localization. Cytoplasmic sequestration of Gln3 with defects in its URS, however, remained intact in repressive conditions due to the presence of the C-terminal Gln3-Tor1 interaction site. Together, our experiments demonstrated that the N- and C-terminal Gln3–Tor1 interaction sites function both autonomously and collaboratively to regulate NCR-sensitive and rapamycin-induced Gln3 localization and phosphorylation/dephosphorylation.

1. Tate, J.J., Rai, R., De Virgilio, C., and Cooper, T.G. 2021. N- and C-terminal Gln3-Tor1 interaction sites: one acting negatively and the other positively to regulate nuclear Gln3 localization. Genetics. 217(4):iyab017. doi: 10.1093/genetics/iyab017. PMID: 33857304.

2. Tate, J.J., Tolley, E.A., and Cooper, T.G. 2019. Sit4 and PP2A Dephosphorylate Nitrogen Catabolite Repression-Sensitive Gln3 When TorC1 Is Up- as Well as Downregulated. Genetics. 212(4):1205-1225. doi: 10.1534/genetics.119.302371. PMID: 31213504.
3. Tate, J.J., Rai, R., and Cooper, T.G. 2018. More than One Way in: Three Gln3 Sequences Required To Relieve Negative Ure2 Regulation and Support Nuclear Gln3 Import in *Saccharomyces cerevisiae*. Genetics. 208(1):207-227. doi: 10.1534/genetics.117.300457. PMID: 29113979.
Supported by NIH GM35642-27, Harriet S. Van Vleet Chair of Excellence, LTAUSA18162, RVO 61388971.

S2_O3 EMERGING FUNCTIONS OF RIBOSOME-ASSOCIATED NONCODING RNAS DURING STRESS RESPONSE IN *SACCHAROMYCES CEREVISIAE*

Kamilla Grzywacz*

Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland
*kamilla.grzywacz@ibch.poznan.pl

In the past years, it became evident that small non-protein-coding RNAs (ncRNAs) play key roles in gene expression regulation, especially at transcription and translation levels. Recently, we have described a novel class of ncRNAs, namely ribosome-associated noncoding RNAs (rancRNAs), which directly bind and regulate the ribosome function in yeast *Saccharomyces cerevisiae*. It was the first report presenting the possibility of regulation of the ribosome function by direct interactions with small noncoding RNAs. We described several types of rancRNAs in *Saccharomyces cerevisiae*, derived from many cellular RNAs, including mRNAs, rRNAs, tRNAs and snoRNAs. We further demonstrated that the mRNA-derived rancRNA_18 is needed for rapid shutdown of global translation and efficient growth resumption under hyperosmotic conditions. We have shown that small RNAs derived from tRNAs (tRFs, tRNA-derived fragments) are widespread in yeast and are capable of specific stress-dependent interactions with the ribosomes which, as a consequence, lead to protein biosynthesis regulation during non-optimal growth conditions. Furthermore, we discovered that the levels of snoRNA-derived small RNAs (sdRNAs) in the cytoplasm and their association with the ribosomes are dependent upon stress conditions but independent from snoRNA expression. Moreover, both sdRNAs and snoRNAs interact with translating ribosomes in a stress-dependent manner. Likely consequential to their ribosome association and protein synthesis suppression features, yeast sdRNAs may exert inhibitory activity on translation.

This work was supported by the National Science Center grant no. DEC-2017/27/B/NZ1/01416.

S2_O4 YEAST CHAPERONES IN REFOLDING OF PROTEINS FROM AGGREGATES

Hubert Wyszkowski, Wiktoria Sztangierska, Tomasz Chamera, Agnieszka Kłosowska,
Krzysztof Liberek*

Intercollegiate Faculty of Biotechnology, University of Gdańsk, 80-307 Gdańsk, Abrahama 58
**krzysztof.liberek@ug.edu.pl*

Native proteins often possess a low margin of stability. Changes in environmental conditions challenge conformation of proteins and lead to their aggregation. This process is controlled and prevented by a group of proteins called molecular chaperones. Efficient disaggregation and refolding of proteins trapped in aggregates is crucial for thermotolerance in yeast and depends on the cooperation between the Hsp104 disaggregase and the Hsp70 chaperone. Multifunctional Hsp70 chaperones are harnessed to specific roles by J-domain proteins (JDPs, also known as Hsp40s). Interaction with the J-domain of these cochaperones stimulates ATP hydrolysis in Hsp70, which stabilizes substrate binding. In yeasts, two classes of JDPs, Class A (Ydj1) and Class B (Sis1), engage Hsp70 in the reactivation of aggregated proteins. Although intensely studied, many mechanistic details of how the two JDP classes regulate protein disaggregation are still unknown.

By using reconstituted yeast chaperone system we investigate individual steps of protein disaggregation in the context of functional differences between the yeast Ydj1 and Sis1 JDPs. With real-time biochemical tools, we show that Ydj1 alone is superior to Sis1 in aggregate binding, yet it is Sis1 that recruits more Hsp70 (Ssa1) molecules to the substrate. The distinctions of Sis1 strictly depend on the interaction between the CTDI domain of the JDP and the EEVD motif in Ssa1, a quality specific to most of Class B JDPs. This interaction conditions more Ssa1 crowding on aggregates potentiating entropic pulling of the chaperone-bound polypeptides. This, what we refer to as aggregate remodeling by the Hsp70 system, results in enhanced Hsp104-dependent protein recovery. Our data indicate a mechanism by which the Class A and B JDPs contribute to the disaggregation efficacy in a divergent manner.

S2_O5 NEW ROLE OF RNA POLYMERASE III IN SHAPING METABOLIC NETWORK ACTIVITY AND STRESS RESPONSE IN *SACCHAROMYCES CEREVISIAE*- WHAT'S GOING ON?

[1]Szatkowska R., [1]Stempak K.,[1]Furmanek E., [2]Ludwig Ch., **[1*]Adamczyk M**

[1]Laboratory of Systems and Synthetic Biology, Chair of Drugs and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland
[2]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK
[*]madamczyk@ch.pw.edu.pl

Stress conditions, including heat shock, oxidative stress, osmotic stress, and toxic metals, are deleterious to normal cellular function. Oxidative stress and oxidative damage have been strongly correlated with aging and several devastating diseases including Parkinson's disease, Lou Gehrig's disease (amyotrophic lateral sclerosis ALS), rheumatoid arthritis, and cancer. Cells employ a number of defense mechanisms to sense and respond appropriately to oxidative stress. Enzymes such as superoxide dismutases and catalases play critical roles in oxidative stress protection through catalyzing the conversion of ROS to less harmful products (Beyer 1991). Free radical scavenging activities are also exacerbated by small antioxidant molecules, including glutathione, thioredoxin, and ascorbic acid (Halliwell 2012). Yeast cells lacking functional systems for combating oxidative stress are highly sensitive to dioxygen and redox-cycling drugs, fail to grow on respiratory carbon sources (Gralla and Valentine 1991). Maf1 protein is a general repressor of RNA Polymerase III (RNAP III) (Boguta 1997). High activity of RNAP III is considered a hallmark of human cell tumourigenesis. A variety biochemical, phenotypical and metabolic changes were observed in *maf1Δ* mutant (Cieśla 2008, Adamczyk and Szatkowska 2017, Szatkowska 2019). For example, *maf1Δ* is unable to grow on glycerol at non-permissive temperature, additionally the mutant strain shows oxidative stress response under favourable growth conditions (Szatkowska 2019). Several discoveries have been made on the transcriptional and post-transcriptional level revealing new insights into the regulatory mechanism of Maf1 in the nucleus. The main goal of the study was to obtain sufficient biochemical and metabolic data to understand the system-level metabolic perturbations in Maf1 deficient cells causing it lethality. ^{13}C flux analysis using [1,2-^{13}C] glucose and [U-^{13}C] glycerol tracers, combined with *in silico* modelling served in this study as an approach to tackle the role of tRNA synthesis in metabolism modulation of *S. cerevisiae*. In this talk, I will show that changes in RNAP III activity (in *maf1Δ* and in its suppressor *rpc128-1007*) is an important layer of regulation of TCA cycle activity in yeast, leading to death or survival.

This work was supported by National Science Centre Poland (SONATA BIS 2012/05/E/NZ2/00583 to M.A.), by Warsaw University of Technology (Nchem2 504/04632/1020/43.120010 to M.A.) within the Excellence Initiative: Research University (ID-UB) programme BIOTECHMED-3 Advanced (1820/334/Z01/POB4/2021 to M.A.) and EMBO Short-term Fellowship to R.Sz.

1.Beyer *et al.* 1991 *Prog Nucleic Acid res Mol Biol*
2.Halliwell 1994, 2012 *Nutrition Rev.*
3.Gralla and Valentine 1991 *J Bacteriol*
4.Boguta *et. al.* 1997 *Gene*
5.Cieśla *et. al.* 2007 *Mol Cell Biol*
6.Adamczyk and Szatkowska 2017 *PlosONE*
7.Szatkowska *et.al.* 2019 *Biochemical Journal*

Poster presentations

S2_P1 FMP40 - THE ONLY KNOWN AMPYLASE IN *SACCHAROMYCES CEREVISIAE* - ROLE IN REDOX HOMEOSTASIS

Suchismita Masanta*, Chiranjit Panja, Róża Kucharczyk

Laboratory of Bioenergetics and Mechanisms of Mitochondrial Diseases, Institute of Biochemistry and Biophysics PAS, Pawińskiego 5A, Warsaw, Poland
**masantas@ibb.waw.pl*

AMPylation (adenylation) is one of the post-translational protein modifications (PTM) leading to the diversification of protein functions and activity, in which the AMP is attached through its α phosphate to the serine, threonine or tyrosine residues via a phosphodiester linkage. Recently with our collaborators, we discovered that the intermembrane space-localized yeast Fmp40 protein of unknown function, the homologue of human SelO, has the ampylase activity, conserved in SelO proteins of human, yeast and bacteria. We have shown that yeast SelO protein is involved in response of cells to hydrogen peroxide and menadione treatment: cells lacking the Fmp40 ampylase died faster than the wild type cells upon H_2O_2 and menadione treatment. E. coli SelO ampylates glutaredoxin GrxA, and the glutathionylation level of proteins is reduced in bacterial and yeast cells lacking SelO (1).
Here we show that *fmp40Δ* cells, like the cells lacking the mitochondrial redoxins Prx1, Trx3 or Grx2, are resistant upon exposure to high concentrations of the hydrogen peroxide - phenotype dependent on the presence of the Grx2. The growth phenotype of double mutants *prx1Δfmp40Δ* and *trx3Δfmp40Δ* are more resistant compared to a single one upon oxidative stress. We found the slow growth of the double mutant *sod1Δ fmp40Δ* in the respiratory medium which indicates that Fmp40 may have an impact on oxidative phosphorylation activity of yeast mitochondria - the matter is under investigation. Sod1 is a superoxide dismutase, neutralizing the superoxide anion (O2•−) (2). Fmp40 ampylates Prx1, Trx3 and Grx2 *in vitro* and it is active under both reducing and non-reducing conditions and forms dimer *in vitro*. A higher accumulation level of Prx1 protein has been found in *fmp40Δ* cells. Furthermore, we observed that Fmp40 has a matrix-localized echo form whose pool is increased upon higher oxidative stress.

1. Sreelatha A. et al. (2018), Cell 175(3):809-821
2. Mühlenhoff et al. (2020), Biol. Chem. 401(12):1407-1428

Acknowledgement (Funding): Doctoral School of Molecular Biology and Biological Chemistry Institute of Biochemistry and Biophysics, Polish Academy of Science

S2_P2 HETEROLOGOUS EXPRESSION OF LIVERWORT *ACR3* GENE IN BUDDING YEAST REVEALED A NOVEL FEATURE OF PLANT ARSENITE TRANSPORTERS

Katarzyna Mizio[*], Jacek Staszewski, Kacper Zbieralski, Robert Wysocki, Donata Wawrzycka, Ewa Maciaszczyk-Dziubińska

Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8 Wrocław, Poland

[*]*katarzyna.mizio@uwr.edu.pl*

ACR3 is the ubiquitous family of membrane transporters that confer high-level resistance to arsenicals in bacteria and fungi. The *S. cerevisiae* Acr3 (ScAcr3) is a plasma membrane proton gradient driven arsenite and antimonite transporter and the best studied member of the ACR3 family. Acr3 homologues have been identified in algae and land plants with the exception of flowering plants, however, their function remains largely uncharacterized. Recently, we cloned and expressed the liverwort *Marchantia polymorpha* orthologue (*MpACR3*) in budding yeast cells. We found that MpAcr3 is able to fully complement arsenite sensitivity of the yeast *acr3*Δ strain. Similar to ScAcr3, MpAcr3 displays an As(III)/H^+ antiporter activity that requires highly conserved cysteine and glutamate residues located in TM4 and TM9 transmembrane regions. Interestingly, we discovered that unusually long N-terminal region of MpAcr3 acts as a metalloid sensor and regulates subcellular localization of the transporter, which seems to be a unique feature of plant members of the ACR3 family. Our research proves that *S. cerevisiae* serves as an excellent model to study function, specificity and trafficking of plant membrane transporters.

This work was supported by the National Science Centre, Poland, grant No. 2019/35/B/NZ3/00379.

S2_P3 SNORNA-DERIVED SMALL RNAS AS POSSIBLE REGULATORS OF TRANSLATION

Anna M. Mleczko, Piotr Machtel, Mateusz Walkowiak, Anna Wasilewska, **Piotr J. Pietras***, Kamilla Bąkowska-Żywicka

Department of Functional Transcriptomics, Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Noskowskiego 12/14 61-704 Poznań, Poland
ppietras@ibch.poznan.pl

In recent years, a number of small RNA molecules derived from snoRNAs have been observed. This relatively new topic is currently the object of intense research. It has been observed, that snoRNA-derived small RNAs (sdRNAs) in cells are mainly involved in microRNA pathway. However, similar molecules have been observed in *Saccharomyces cerevisiae*, which is an organism lacking miRNA machinery. Both sdRNAs and snoRNAs may directly interact with translating ribosomes in a stress-dependent manner. Likely consequential to their ribosome association and protein synthesis suppression features, yeast sdRNAs may exert inhibitory activity on translation. To clarify this, we set up an *in vitro* translation system for *S. cerevisiae* grown under optimal conditions. When the assay was performed in the presence of synthetic sdR67, sdR83 or sdR128 we observed a reproducible inhibitory effect on translation. To investigate whether *in vitro* effects have a physiological significance in yeast we used electroporation to introduce synthetic sdRNAs into *S. cerevisiae* cells. With this assay, we proved that yeast sdRNAs can decrease translational efficiency in vivo. Because both snoRNAs and ribosomes are universally conserved, we tested if sdRNA-mediated repression of translation is functionally conserved in other eukaryotic species as well. To test this possibility, we examined three cell-free *in vitro* translation systems using wheat germ extracts, rabbit reticulocyte lysates, and HeLa cell lysates. The addition of S. cerevisiae sdRNAs reproducibly inhibited *in vitro* protein biosynthesis in the wheat germ system. *In vitro* translation was very mildly inhibited by yeast sdR67 in the rabbit reticulocyte but not by sdR83 nor sdR128. No inhibition was observed in human systems. These data suggest that *S. cerevisiae* sdRNAs might potentially inhibit translation systems in selected eukaryotes.

Mleczko, A.M., Machtel, P., Walkowiak, M., Wasilewska, A., **Pietras, P.J**., Bąkowska-Żywicka, K. Levels of sdRNAs in cytoplasm and their association with ribosomes are dependent upon stress conditions but independent from snoRNA expression. Sci Rep 9, 18397 (2019). https://doi.org/10.1038/s41598-019-54924-2

This work was supported by the National Science Center grant no. DEC-2017/27/B/NZ1/01416.

S2_P4 CHANGES IN THE RIBOSOME-ASSOCIATED SMALL NONCODING RNAS IN *SACCHAROMYCES CEREVISIAE* AS A RESPONSE TO ENVIRONMENTAL STRESS

Anna Wasilewska-Burczyk[1*], Piotr J. Pietras[1], Weronika Pawlik[1], Jan G. Kosiński [2], Marek Żywicki [2], Kamilla Grzywacz [1]

[1]*Department of Functional Transcriptomics, Institute of Bioorganic Chemistry Polish Academy of Sciences, Zygmunta Noskowskiego 12/14, 61-704 Poznań, Poland*
[2] *Department of Computational Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland*
[*]*awasilewska@ibch.poznan.pl*

It is now well established that the proper ribosome functioning is crucial to the health of the cell and that its function is tightly regulated with ribosome-associated factors, which provide means to prepare the translational program of stressed cells for recovery. The scientific interest of our group is to identify and characterise the ribosome-associated noncoding RNAs (rancRNAs) in *S. cerevisiae*. So far we have shown that small RNAs derived from tRNAs (tRFs, tRNA-derived fragments) and snoRNAs (sdRNAs) are widespread in yeast (16 + publi SciRep) and are capable of specific stress-dependent interactions with the ribosomes which, as a consequence, lead to protein biosynthesis regulation during non-optimal growth conditions (1). However, the precise molecular function as well as the trigger for the origin of stress-induced rancRNAs in yeast needs further examination.

Our aim was to characterise rancRNAs interacting with different subpopulations of the yeast ribosomes during environmental stress. Due to the significant changes in cell metabolism, we have subjected yeast cells to 3 different environmental conditions (normal growth, lack of sugar, osomotic), based on our previous work on stress-induced rancRNAs. To gain a detailed insight of rancRNA interactions with the components of the ribosomes, we have combined the polysome profiling with the rancRNA-seq. Therefore, we have generated cDNA libraries from small RNAs (sized 20–50 nt) that co-purify with ribosomal particles under different environmental conditions and sequenced separately small RNAs associated with the polysomes, monosomes, small and large ribosomal subunits as well as the cytosolic RNAs.

After sequencing, we have obtained a pool of 35 840 reads mapped to the reference yeast genome. Most of the rancRNAs were the processing products derived predominantly from rRNAs (especially in the monosomes), tRNAs and snoRNAs (both types associated mostly with the polysomes and the ribosomal subunits). Within rancRNAs we have identified tRFs derived from all 41 yeast tRNA isoforms as well as sdRNAs derived from all yeast snoRNAs. Moreover, the composition of the libraries from the ribosomal subpopulations differed between the environmental conditions. The difference in the types of rancRNAs between ribosomal samples was also manifested with a different length distribution. The following tRFs were the most abundant: 5'-tRF^{Ala}(AGC)L, 3'-tRF^{tAsp}(GUC)G1, 5'-tRF^{tTyr}(GUA)F2, 3'-tRF^{tAla}(AGC)K2, and 5'-tRF$^{tA(}$AGC)L. We were able to distinguish clear differences in their association with the ribosomal particles between different growth conditions. For example, 5'-tRF^{Ala}(AGC)L association with the polysomes was 2.6 times higher in sugar starvation than in normal growth conditions and 1.5 higher than in osmotic stress. The most abundant sdRNAs are: sdRNA70, sdRNA128 and sdRNA10. sdRNA70 association with the polysomes was nearly 1.8 times higher in sugar starvation than in osmotic stress and 2.6 times higher than in optimal growth

conditions. Bąkowska-Żywicka K, et al. (2016) FEMS Yeast Res, 16(6). pii: fow077. *This work was supported by the National Science Center grant no. DEC-2017/27/B/NZ1/01416.*

S2_P5 α-ARRESTIN Art10 PLAYS A ROLE IN ARSENIC TOLERANCE IN BUDDING YEAST

Kacper Zbieralski*, Renata Broda, Olga Szostek, Robert Wysocki, Donata Wawrzycka

Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8, Wrocław, Poland

**kacper.zbieralski2@uwr.edu.pl*

Art10 is a poorly characterized protein of the α-arrestin family, whose members are known to regulate ubiquitination of plasma membrane proteins in cooperation with the Rsp5 ubiquitin ligase. Takahashi *et. al.* (2010) demonstrated that in the yeast *Saccharomyces cerevisiae* overexpression of the Art10 protein confers high-level resistance to sodium arsenite. Nevertheless, the function of Art10 remains elusive. In order to further investigate the role of Art10 in the metalloid resistance mechanisms in yeast, we cloned and expressed *ART10* in *S. cerevisiae* under control of its native promoter and the *MET17* promoter. We confirmed that overexpression of *ART10* enhances yeast tolerance to trivalent arsenic [As(III)] and antimony [Sb(III)], but not to pentavalent arsenic [As(V)]. Interestingly, overexpression of Art10 increased sensitivity of yeast cells to cadmium [Cd(II)]. However, *ART10* deletion did not influence tolerance to these compounds. We showed that the Art10-GFP fusion protein localized in the cytoplasm; however, additional plasma membrane localization was observed when overexpressed. Considering the function of α-arrestins in the membrane protein proteostasis, we investigated a role of Art10 in the stability regulation of the plasma membrane Acr3 transporter, a low affinity $As(III)/H^+$ and $Sb(III)/H^+$ antiporter.

Takahashi T, Yano T, Zhu J, Hwang GW, Naganuma A. Overexpression of FAP7, MIG3, TMA19, or YLR392c confers resistance to arsenite on Saccharomyces cerevisiae. J Toxicol Sci. 2010 Dec;35(6):945-6. doi: 10.2131/jts.35.945. PMID: 21139346.

This work was supported by the University of Wrocław Initiative of Excellence-Research University IDUB grant BPIDUB.4610.13.2021.KP.A

S2_P6 *SACCHAROMYCES CEREVISIAE* AS A HETEROLOGOUS MODEL SYSTEM TO STUDY PROPERTIES OF PLANT ARSENIC TRANSPORTERS

Kacper Zbieralski*, Jacek Staszewski, Anna Janiczek, Inga Bona, Katarzyna Mizio, Robert Wysocki, Ewa Maciaszczyk-Dziubińska, Donata Wawrzycka

Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8, Wrocław, Poland

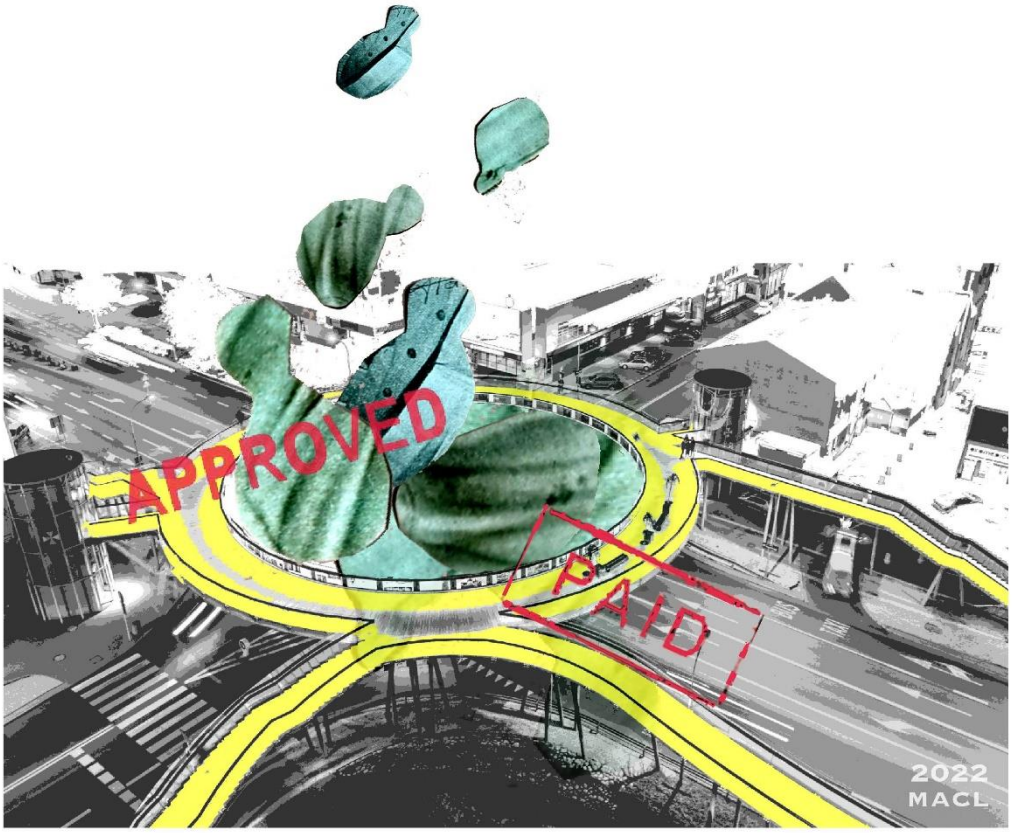
*kacper.zbieralski2@uwr.edu.pl

Efflux of arsenicals is the most efficient way of conferring high-level resistance to these toxic compounds. The most studied arsenic transporters are members of the ACR3 family, which is widespread among bacteria and fungi. A multitude of putative Acr3 orthologues have been recently identified in green algae and lower plants, although their function in these organisms remains elusive. In order to further investigate the properties of plant Acr3 transporters, we cloned and heterologously expressed several ACR3 orthologues of green microalgae *Coccomyxa subellipsoidea* (CsACR3), *Raphidocelis subcapitata* (RsACR3), *Chlamydomonas eustigma* (CeACR3), moss *Physcomitrella patens* (PpACR3), fern *Pteris vitatta* (PvACR3) and gymnosperm *Picea sitchensis* (PsACR3) in *Saccharomyces cerevisiae.* We found that these proteins confer distinct levels of resistance to arsenite, arsenate and antimonite in the yeast *acr3* mutant. Most notably, the expression of RsACR3, PpACR3 and PvACR3 accounts for exceptional tolerance of yeast cells to arsenate. Moreover, we observed that the plant ACR3 transporters display differences in the subcellular localization. Strikingly, we demonstrated that the localization of CeACR3-GFP and PpACR3-GFP is regulated in response to the metalloid stress. Our results provide insight in the properties of poorly characterized plant members of the ACR3 family using budding yeast as a heterologous model system.

This work was supported by the National Science Centre, Poland, grant No. 2019/35/B/NZ3/00379.

Session 3

Genetic control of cellular processes



Oral presentations

S3_O1 THE INFLUENCE OF RICIN-MEDIATED rRNA DEPURINATION ON THE TRANSLATIONAL MACHINERY USING *SACCHAROMYCES CEREVISIAE* AS EXPERIMENTAL MODEL

Monika Szajwaj[1], Leszek Wawiórka[1], Eliza Molestak[1], Barbara Michalec-Wawiórka[1], Mateusz Mołoń[2], Iwona Wojda[3], **Marek Tchórzewski[1*]**

[1]*Department of Molecular Biology, Maria Curie-Skłodowska University, Lublin, Poland*
[2]*Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszów, Poland*
[3]*Department of Immunobiology, Maria Curie-Skłodowska University, Lublin, Poland*
[*]*marek.tchorzewski@mail.umcs.pl*

The plant toxin ricin belongs to the group of type-II ribosome inactivating proteins (RIPs) and is classified as N-glycosidase, which exclusively recognizes a universally conserved stem-loop on a large ribosomal subunit known as the sarcin-ricin loop (SRL). Ricin removes specific adenine base at the SRL in large rRNA, which constitutes an rRNA component of the GTPase associated center, which together with the ribosomal P-stalk is responsible for binding and stimulation of GTPase activity of translational GTPases. The generally accepted model of ricin action assumes that direct inactivation of ribosomes by depurination of a specific adenine residue within the SRL on the 60S ribosomal subunit is a major source of its toxicity; the current model proposes that SRL depurination leads to protein synthesis inhibition, evoking ribotoxic stress with concomitant induction of numerous metabolic pathways, which lead to cell death. However, the direct relationship between the depurination and its impact on the translational machinery *in vivo* has never been satisfactorily explained. We approached a long-standing question about the influence of SRL depurination on the functioning of the translational machinery. We have shown that a small subset of depurinated ribosomes exert an effect on cell metabolism, indicating that minute modification within the ribosomal pool is sufficient to elicit a toxic effect and ricin driven depurination does not affect notably any particular step of translation. The SRL depurination observed in a small fraction of ribosomes blocks cell cycle progression with no effect on cell viability in yeast cells. Thus, we propose that ribosomes with depurinated SRL represent a small imprinted ribosomal pool, which generates a specific signal for the cell to halt the cell cycle.

S3_O2 RNA POLYMERASE III TRANSCRIPTION, NOVEL LAYERS OF REGULATION

Małgorzata Cieśla*

Laboratory of tRNA Transcription, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland
*goges@ibb.waw.pl

Three highly related RNA polymerases (Pols) are multi-subunit complexes responsible for transcription in eukaryotic cells. Each of these enzymes is involved in transcription of a specific sets of genes. The largest polymerase, Pol III, synthesizes tRNA, 5S rRNA and other small, non-coding RNAs. Earlier studies on biogenesis of eukaryotic Pols focused in particular on Pol II. Relatively less is known about the assembly and nuclear import of the Pol III complex. We are the first to describe the function of yeast Rbs1 protein in Pol III biogenesis. Our research has revealed that Rbs1 is an assembly factor of Pol III that binds to the Pol III complex, facilitates its translocation to the nucleus, and returns back to the cytoplasm with the participation of the Crm1 exportin. Further on, we showed that Rbs1 binds to the 3' untranslated regions (3'UTR) of selected mRNAs and controls their post-transcriptional expression. The Rbs1 sequence within the R3H domain was found to be necessary for mRNA interaction. We revealed a regulatory link between Rbs1 and Rpb10, a common subunit to all three Pols. Overexpression of Rbs1 increased the abundance of *RPB10* mRNA which correlated with suppression of Pol III assembly defects. We propose a co-translational model of the Pol III complex assembly where Rbs1 binds to mRNAs encoding Rpb10 and Rpc19, the subunits initiating the Pol III assembly process. Identification of Rbs1 homologues in other higher organisms indicates that the molecular regulatory mechanism of Rbs1 may be conserved in evolution.

This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012].

S3_O3 CROSSTALK BETWEEN MITOCHONDRIA AND CYTOSOLIC TRANSLATION MACHINERY

Karolina Gościńska, Somayeh Shahmoradi Ghahe, **Ulrike Topf***

Laboratory of Molecular Basis of Aging and Rejuvenation, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
utopf@ibb.waw.pl

Mitochondria are essential organelles for most eukaryotic cells. Despite they possess their own genome, mitochondrial biogenesis vastly depends on nuclear genes that encode mitochondrial proteins. The translation machinery located outside of mitochondria in the cytosol is necessary to produce these proteins. Thus, mitochondria need to possess mechanisms to signal their need for newly synthesized proteins. Sophisticated import machinery translocate proteins destined to mitochondria into the organelle. Consequently, we found that slowdown of protein import into mitochondria leads to accumulation of mitochondrial precursor proteins in the cytosol, which causes proteotoxic stress. In order to maintain cellular protein homeostasis, cells respond to such mitochondrion-derived stress by decreasing global cellular protein synthesis. While translation attenuation is a common response to various stress conditions signals from mitochondria and mechanistic consequences for the translation machinery remain poorly understood. The overproduction of reactive oxygen species could serve as a stress signal to regulate non-mitochondrial events. Using a global quantitative proteomics approach, we demonstrate that translation machinery components including ribosomes are sensitive to oxidative modifications, which are reversible (Topf *et al*, Nat Commun., 2018). Our findings propose a previously underappreciated concept of translation regulation that involves redox switches on the ribosomes. However, consequences of such redox switches for the modulation of translation remain to be characterised. We propose that the oxidation of thiol residues of ribosomal proteins creates a sub-pool of ribosomes that are necessary for the production of proteins under cellular stress conditions.

Part of this work is supported by the National Science Centre grant 2019/34/E/NZ1/00367.

S3_O4 MITOCHONDRIAL RNA DEGRADATION AND STABILITY IN *CANDIDA ALBICANS* AND THE EVOLUTION OF YEAST NUCLEO-MITOCHONDRIAL INTERACTION

Paweł Golik[1,2*]

[1]*Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawińskiego 5A, 02-106 Warsaw, Poland*
[2]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland*
[*]*p.golik@uw.edu.pl*

Yeasts show remarkable variation in the organization of their mitochondrial genomes, yet there is little experimental data on organellar gene expression outside few model species. *Candida albicans* is interesting as a human opportunistic pathogen, and as a representative of a clade that is distant from the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Unlike them, it encodes seven Complex I subunits in its mtDNA. In *C. albicans* mitochondria the 14 protein-coding genes, two ribosomal RNA genes, and 24 tRNA genes are expressed as eight primary polycistronic transcription units (Kolondra et al., 2015).

The main evolutionary force shaping the mitochondrial genomes of yeasts is frequent recombination, constantly breaking apart and joining genes into novel primary transcription units which influences the features of gene expression, such as the presence of secondary promoter sites that are inactive, or act as "booster" promoters, simplified transcriptional regulation and reliance on posttranscriptional mechanisms. Evolutionary divergence of the mitochondrial genome between distant yeast species entailed to significant changes in the repertoire and function of nuclear-encoded genes involved in the expression of organellar genes, including specific factors from the pentatricopeptide (PPR) repeat family, as well as mitochondrial ribonucleases. RNA degradation by a two-component 3'-5' exoribonuclease mtEXO complex is essential for shaping the mitochondrial transcriptome, and it is required to maintain the functional demarcation between transcription units and non-coding genome segments (Łabędzka-Dmoch et al., 2021). Another RNase, Pet127p, is involved in intron degradation, but is not essential for the respiratory activity (Łabędzka-Dmoch et al., 2021).

Kolondra A. et al. BMC Genomics 2015, 16:827.
Łabędzka-Dmoch K. et al., RNA Biol. 2021, 18:303–317.
Łabędzka-Dmoch K. et al., RNA 2022, 28(5):711-728.

S3_O5 REGULATION OF ERYTHRITOL UTILIZATION IN *YARROWIA LIPOLYTICA*

Dorota Rzechonek[*], Aleksandra Mirończuk

Department of Biotechnology and Food Microbiology, Wrocław University of Environmental Life and Sciences, Wrocław, Poland
[*]*dorota.rzechonek@upwr.edu.pl*

Erythritol is a four-carbon polyalcohol, produced by yeast *Yarrowia lipolytica* in response to hyperosmotic stress. Subsequently, erythritol can be re-assimilated and this process is regulated by transcriptional factor EUF1. Until now, four genes encoding enzymes of erythritol utilization pathway were the only known targets of this transcriptional factor. The RNA-seq analysis, performed in this study, revealed that EUF1 up-regulates numerous other genes involved in carbon metabolism, DNA repair and membrane transport. Among them, there are elements of two important metabolic pathways: pentose phosphate pathway (PPP) and glyoxal cycle.

High concentrations of erythritol in the medium significantly affects the gene expression of the PPP enzymes, which confirms earlier assumptions, that erythritol derivatives are further transformed by this pathway. On the other hand, in presence of erythritol, EUF1 up-regulates most of the genes of glyoxal cycle – important for catabolism of fatty acids or acetate. Thus, this unexpected association sheds a new light on the role of erythritol in metabolism.

Poster presentations

S3_P1 INACTIVATION OF MAF1, THE GENE ENCODING THE NEGATIVE REGULATOR OF RNA POLYMERASE III, HAS MULTIPLE EFFECTS ON TRANSLATION

Alicja Armatowska*, Jan Słupski, Magdalena Boguta and Małgorzata Cieśla

Laboratory of tRNA Transcription, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland
*a.armatowska@ibb.waw.pl

In all eukaryotes, tRNA synthesis is carried out by RNA polymerase III (Pol III). Maf1, as a master regulator of Pol III transcription, represses the synthesis of tRNAs under conditions that are detrimental to yeast growth. In *maf1Δ* cells improper Pol III regulation leads to increased transcription of tRNA precursors (pre-tRNA), resulting in impaired pre-tRNA maturation and saturation of the tRNA export machinery (Karkusiewicz et al., 2011). Furthermore, levels of modification enzymes are insufficient to modify all of the synthesized pre-tRNAs (Arimbasseri 2018). Inactivation of the *MAF1* gene in yeast leads to a decrease in *SUP11* activity and increased translation fidelity (Kwapisz et al., 2002). It is possible that the tRNA accumulated in *maf1Δ* cells does not reach the translation machinery or is not fully functional in reading mRNA codons, making the process slower and more accurate.

We showed that overexpression of the *TEF1/TEF2* genes, encoding the translation elongation factor eEF-1α, suppresses the anti-suppressor phenotype and respiratory deficient phenotype of the *maf1Δ* strain. Next, we characterized an additional, previously unknown phenotype of *maf1Δ* cells on minimal, glucose medium – a delayed lag-phase. Overproduction of the translation elongation factor eEF-1α, encoded by *TEF1/TEF2* genes also, resulted in suppression of this growth defect and a shorter delay in restarting proliferation. We connected the observed growth defect with the efficiency of translation in *maf1Δ* cells. Our data indicate that the level of protein synthesis is decreased in *maf1Δ* cells and can be restored by overproduction of the translation elongation factor eEF-1α. Moreover, polysomes profiling assay revealed reproducible changes in the *maf1* deletion mutant which may indicate a potential defect in the biosynthesis of the ribosomal subunits. We also observed specific changes in mitochondrial translation in *maf1Δ* cells. *de novo* levels of mitochondrial encoded proteins are changed while mRNA levels of mitochondrial genes did not differ significantly between the mutant and wild-type cells.

The obtained results support our hypothesis that *MAF1* deletion has multiple effects on translation. We suggest that changes in the pool of mature, functional tRNAs can affect growth and both – cytoplasmic and mitochondrial translation in *maf1Δ* cells. However, the mechanism explaining how excess pre-tRNA synthesis in *maf1Δ* directly affects translation remains unknown.

Karkusiewicz I. et al. (2011) *J Biol Chem*, 286, 39478–39488.
Arimbasseri G.A. (2018) *Biochim Biophys Acta*, 1861, 354–360.
Kwapisz M. et al. (2002) *Current Genetics*, 42, 147–152.

This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012].

S3_P2 ANALYSIS OF EVOLUTIONARILY CONSERVED OXIDATION CHANGES OF PROTEINS DURING EUKARYOTIC AGEING

Katarzyna Jonak*, Ulrike Topf

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland
*k.jonak@ibb.waw.pl

Age-related diseases are a growing medical challenge. Many health conditions associated with ageing, such as cancer or Alzheimer's disease, are linked to changes in the redox status of aged cells. In an older cell, the level of oxidation increases and global protein production slows down, which correlates with its damage, destruction, and eventually death. However, the exact link between oxidation, protein synthesis, and age-dependent decay remains unknown. Hitherto, research focused on oxidation as a damaging agent that leads to the deterioration of a cell, tissue or whole organism during later stages of ageing. Interestingly, a new hypothesis has been recently proposed based on changes in oxidation in younger cells of yeast *S. cerevisiae*. It has been observed that defects in protein production caused by elevated oxidation can be reversed as soon as the physiological conditions are restored (Topf et al., 2018). This fact indicates the existence of a mechanism, called a translational redox switch, that rapidly responds to changes to adjust protein synthesis rates in cells of various eukaryotes. We hypothesize that elevated oxidation may serve as a messenger to modulate stress defence at the early stage of ageing until concentrations of reactive oxygen species (ROS) become too high in the aged cell leading to its death. Such modulation of protein translation may safeguard the protein homeostasis and health of the cell during ageing. Thus, the aim of the presented research is the exploration of the evolutionary conservation of age-dependent early redox changes focusing on the proteins involved in global protein production.

To address the aim of the project, we are developing the first cross-species database of age-dependent oxidation of highly sensitive cysteine residues. The database explores the changes in the oxidation of specific cysteine sites across different species using as the main base the dataset from chronologically aged yeast *S. cerevisiae*, along with datasets from adult worms *C. elegans* and mice *M. musculus*. By comparing the mass spectrometry data of globally oxidised proteome, we observed a high number of proteins significantly oxidised during the early stages of ageing, among them well-conserved ribosomal proteins. The database currently in development is a fundamental step for the exploration of the novel concept of the oxidation-dependent regulation of protein synthesis in aged organisms rather than oxidation-dependent damage.

Topf U. et al. (2018) *Nature Communications*, 9(1):1-17.

S3_P3 RETROGRADE REGULATION IN YEAST *CANDIDA ALBICANS*

Diu Hoang Thi Bui[1], Wiktoria Kabza[1], **Karolina Łabędzka-Dmoch[1*]**, Jakub Piątkowski[1], Paweł Golik[1,2]

[1]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawińskiego 5a, 02-106 Warsaw, Poland
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland,
[*]karolina.labedzka.dmoch@igib.uw.edu.pl

Retrograde signaling (RTG) is a pathway that responds to various mitochondrial dysfunctions and facilitates the adaptation of the cell to stress. We investigate the RTG pathway in *Candida albicans*, a potentially pathogenic yeast that prefers respiratory metabolism over fermentation, is Crabtree negative, and contains subunits of complex I encoded in the mitochondrial genome (opposite to *S. cerevisiae*). We aimed to describe the retrograde response in respiratory-deficient strains of *C. albicans*, in order to identify the mechanisms and effectors that are different from those already known in *S. cerevisiae*.

The main factor involved in the RTG pathway in yeast is the heterodimeric basic helix-loop-helix/leucine zipper transcription factor Rtg1-Rtg3. In *S. cerevisiae* upon mitochondrial dysfunction, the Rtg1p/Rtg3p heterodimer translocates from the cytoplasm to the nucleus, where it binds to the R-box (5'-GTCAC-3') in the promoters of RTG effectors, where it activates their transcription.

We show that the *C. albicans* orthologs, CaRtg1p and CaRtg3p interact with each other *in vivo* and *in vitro*. Both transcription factors are localized to the nucleus independently of the respiratory state of the cell. CaRtg3p is essential for nuclear localization of the dimer, it is a phosphoprotein, but phosphorylation does not influent its localization.

Among possible effectors of the RTG pathway, we identified the alternative oxidase (AOX). It is present in *C. albicans* in addition to the classical respiratory chain, but is absent in *S. cerevisiae*. AOX is constitutively expressed at a low level in WT, and its main function is to reduce the ROS stress in mitochondria. We show no expression of AOX in the strains lacking CaRtg3p or CaRtg1p (by RNA seq and northern blot). We demonstrate binding of CaRtg1/3p dimer to the promoter of CaAOX *in vitro* (EMSA). CaAOX expression may be regulated by retrograde response in *C. albicans*.

S3_P4 TRANSCRIPTION EFFICIENCY OF tRNA GENES DEPENDS ON THE RSP5 LIGASE

Aleksandra Łopusińska*, Magdalena Boguta and Małgorzata Cieśla

Laboratory of tRNA Transcription, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland
*o.lopusinska@ibb.waw.pl

Transfer RNAs (tRNAs) are essential molecules that convert information contained in mRNA into amino acid sequence. tRNA synthesis in eukaryotes is catalyzed by RNA polymerase III (Pol III). Transcription of tRNA genes is tightly regulated by the nutrient and energy status of the cells and by the post-translational modifications of the components of Pol III machinery. Large-scale proteomic studies report that components of the Pol III machinery are modified by ubiquitination and are potential substrates for the ubiquitin ligase, Rsp5. The aim of the study was to elucidate the role of Rsp5 in the control of tRNA transcription. First, we analyzed the effect of the *rsp5* mutations on tRNA levels. A mutation in the catalytic HECT domain (*rsp5-1*) and a mutation in the WW domain that affects the interaction with the substrates (*rsp5-19*) of the Rsp5 ligase markedly increased the levels of individual tRNAs. We speculated that Rsp5 regulates the transcription efficiency of tRNA genes. Therefore, the effect of *rsp5* mutations on Pol III occupancy at tRNA genes was examined by the chromatin immunoprecipitation approach. We observed that the *rsp5-1* mutation by changing the Rsp5 catalytic activity increased Pol III occupancy at tRNA genes. One potential substrate for ubiquitination by Rsp5 is Rpb10, the common subunit of all RNA Pols, involved in their assembly. Substitution of the potential ubiquitination site, Lys 59 in Rpb10 results in altered interactions between subunits of both Pol III and Pol I. Examination of the role of Rsp5 ligase in ubiquitination of Rpb10 and the effect on assembly of Pols and transcription efficiency require further studies.
This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012].

S3_P5 THE TRANSCRIPTIONAL COUPLING OF FUNCTIONALLY-RELATED GENES CAN BE COORDINATED BY ADJACENT GENE COREGULATION

Dmitry Pokushalov[1, 2], D. Tyler Boone[1, 3], **Michael A. McAlear[1*]**

[1]Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT, 06457; dpokushalov@wesleyan.edu (D.P.); dboone@wesleyan.edu (D.T.B)
[2]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[3]Molecular and Cellular Biology Graduate Program, Guarini School for Graduate and Advanced Studies at Dartmouth College, Hanover, NH 03755
[*]mmcalear@wesleyan.edu

The genes required for ribosomal biogenesis are amongst the most highly regulated genes, and they encode for proteins that drive a large fraction of the total cellular economy in organisms like yeast. To produce a ribosome, yeast cells need to co-express multiple rRNAs, ~ 80 ribosomal proteins (RPs), and a large set of extra-ribosomal rRNA and ribosome biogenesis genes (known as the RRB or ribi regulon). Significantly, a large fraction of the genes from the RP and RRB regulons are organized as adjacent gene pairs. A genetic analysis of the adjacent *MPP10-MRX12* RRB gene pair has revealed that the proper coregulation of both *MPP10* and *MRX12* depends on the *cis*-elements from within *pMPP10*. To investigate the modularity of adjacent gene coregulation (AGC), we relocated *MRX12* to a different chromosome; it continued to be properly coregulated even when the *cis*-elements in *pMPP10* were mutated. We defined the distance limit at which *pMPP10* can regulate *MRX12* by separating the adjacent pair with transcriptionally inactive DNA, showing that *MRX12* is capable of autonomous regulation when separated by at least another 3 kbp from *MPP10*. We analyzed the phylogeny of the RP regulon in varied yeast species and found that new RP pairs have been formed and split throughout evolution. However, changes in gene adjacency did not correlate with changes in transcriptional coregulation. Within the RP regulon the cytosolic and mitochondrial RPs are distinctly regulated with varying tightness depending on both species and experimental conditions. Lastly, we performed a whole-genome synteny analysis to show that the dynamics of pair formation and loss does not differ between functionally-related and unrelated genes. In summary, our results suggest that adjacent gene coregulation is an ancient, dynamic, and modular form of transcriptional control that can link the expression patterns of immediate gene neighbors.

S3_P6 YOR020W-A (MCO10): CHARACTERIZING THE UNKNOWN "SUBUNIT L" OF MITOCHONDRIAL ATP SYNTHASE OF *SACCHAROMYCES CEREVISIAE*

Chiranjit Panja*, Aneta Wiesyk, Katarzyna Niedźwiecka, Emilia Baranowska, Róża Kucharczyk
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland;
**chiranjit@ibb.waw.pl*

In *S. cerevisiae*, the uncharacterized protein Yor020W-A (**m**itochondrial **c**lass **o**ne protein of **10** kDa, Mco10), was previously found to be associated with mitochondrial ATP synthase [1] and also referred to as a new 'subunit L' in a recent study of fungal ATP synthases [2]. However, recent cryo-EM structures of *S. cerevisiae* ATP synthase failed to ascertain Mco10 as a bona fide subunit of the complex. Also, nothing is known yet of possible role Mco10 plays in the structure and function of ATP synthase. Surprisingly, the N-terminal fragment of Mco10 is very similar to Atp19 (subunit *k*) of ATP synthase. Atp19, along with the subunits Atp20 and Atp21 plays a major role in formation and stabilizing the dimers of ATP synthase. Biochemical analysis revealed in spite of similarity in sequence and evolutionary lineage, Mco10 and Atp19 differ significantly in function. This is the first work to establish Mco10 being a unique candidate for a 'monomer specific' subunit having role in calcium homeostasis and permeability transition pore.

References:

[1] Morgenstern M, Stiller SB, Lübbert P, et al. Definition of a High-Confidence Mitochondrial Proteome at Quantitative Scale. **Cell Rep**. 2017;19(13):2836-2852. doi: 10.1016/j.celrep.2017.06.014

[2] Liu S, Charlesworth TJ, Bason JV, et al. The purification and characterization of ATP synthase complexes from the mitochondria of four fungal species. **Biochem J**. 2015;468(1):167-175. doi: 10.1042/BJ20150197

Funding: *Grant Number NCN 2018/31/B/NZ3/0111 of National Science Centre, Poland.*

S3_P7 THE FUNCTION OF THE MOLECULAR CO-CHAPERONE PREFOLDIN DURING CELLULAR HOMEOSTASIS

Ismail Tahmaz*, Ulrike Topf

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
*itahmaz@ibb.waw.pl

Protein homeostasis is maintained by correct protein folding and the elimination of misfolded proteins to avoid protein aggregation which can be hazardous to the cell. Newly synthesized proteins can be detected by chaperones in order to ensure proper folding. Protein misfolding and aggregation can occur when the cooperation of chaperones and their supporting co-chaperones is disrupted. As a result, proteostasis can be disrupted, leading to the emergence of numerous diseases.

Prefoldin is a co-chaperone, a heterohexameric protein complex, made up of two type of subunits; α (Pfd3 and Pfd5) and β (Pfd1, Pfd2, Pfd4 and Pfd6) in eukaryotes and archea. The prefoldin complex is responsible for transferring cytoskeletal proteins such as actin and tubulin to the TRiC/CCT complex as canonical function. However, few studies also showed that prefoldin is also involved in prevention of protein aggregation. Mitochondria function depend on the production of proteins in the cytosol and import of proteins into the organelle. Mitochondrial dysfunction can result in mistargeting mitochondrial proteins to the cytosol causing a burden for cellular homeostasis. Whereas the import mechanisms of mitochondrial proteins are well described, less is understood about the fate of mitochondria targeted proteins in the cytosol under normal growth conditions and cellular stress. According to our findings, prefoldin is required to withstand a range of exogenous stressors including antimycin A, an inhibitor of mitochondrial respiratory chain activity. We investigated prefoldin's role in mitochondrial biogenesis in terms of physiological activity. We observed that although all yeast cells depleted of individual prefoldin subunits showed respiratory function, some of them, such as cells lacking *PFD2*, showed a distinct mitochondrial phenotype. We are interested in revealing how prefoldin preserves cellular and/or mitochondrial protein homeostasis and what cellular factors are necessary to recover from the stress conditions.

This work is supported by the National Science Centre grant 2018/31/B/NZ1/02401.

S3_P8 IDENTIFICATION OF CONSEQUENCES OF RIBOSOME HETEROGENEITY FOR CELLULAR PROTEIN HOMEOSTASIS

Ramin Zadalimomen*, Ulrike Topf

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
*rzadalimomen@ibb.waw.pl, utopf@ibb.waw.pl

Ribosome is a complex machine that translates the mRNA-encoded genetic information into proteins. It consists of ribosomal proteins, ribosomal RNA, and ribosomal-associated proteins (RAPs). Initially, scientists believed that the ribosome was a machine with unalterable protein and RNA subunits; in this regard, there were various hypotheses between scientists and most of them thought ribosomes are passive machines with no regulatory function. However, subsequent discoveries and evidence suggest that ribosomes may be more heterogeneous than previously thought. Studies analyzing the ribosome's structural heterogeneity as the result of changes in ribosomal protein composition are emerging.
Despite the discovery of ribosome heterogeneity, a causal link between different cellular stress including mitochondrial dysfunction, and translational output is largely unknown. In this regard, studies on the inherent mechanisms of protein synthesis activated during stress are of great value and can also provide insight into the role of ribosomal heterogeneity in ribosomopathy. We use yeast Saccharomyces cerevisiae as a model to study how ribosome heterogeneity influences the identity of newly synthesized proteins upon mitochondrial stress and analyse the impact of selected ribosomal proteins on ribosome function and translation under various stress conditions.
This work is supported by the National Science Centre grant 2019/34/E/NZ1/00367.

Session 4

Genome maintenance

Oral presentations

S4_O1 POST-TRANSLATIONAL REGULATION OF RAD51 RECOMBINASE IN YEAST *S. CEREVISIAE*

Justyna Antoniuk Majchrzak[1], Anna Długajczyk[1], Tuguldur Enkhbaatar[1], Joanna Kamińska[1], Marek Skoneczny[1], Daniel Klionsky[2], **Adrianna Skoneczna**[1*]

[1]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland*
[2]*Life Sciences Institute and Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA*
[*]*ada@ibb.waw.pl*

DNA double-strand breaks (DSBs) are particularly harmful lesions, especially when an error-free DNA repair pathway is not available. In such circumstances, cells are forced to use error-prone recombination pathways to repair the DNA breaks just to be able to resume the cell cycle and continue growth. However, using the error-prone recombination pathways leads to genome rearrangements, loss of chromosomal fragments, or even whole chromosomes. One of the major factors involved in the recombinational repair of DNA damage is recombinase Rad51. The Rad51 binds ssDNA and forms nucleofilaments responsible for presynaptic complex formation. We previously noticed that the level of Rad51 is highly increased when illegitimate recombination is engaged in repair. Because we expect that a high level of Rad51 may lead to genome instability and the regulation of the Rad51 protein turnover is not known yet, we focused on this issue. We showed that level of Rad51 is regulated by proteolysis via SUMO- and ubiquitin-dependent manner. The SUMOylation of Rad51 depends on Mms21 SUMO ligase. The ubiquitination of Rad51 depends on multiple E3 enzymes, including SUMO-targeted ubiquitin ligases. We also demonstrate that post-translational modifications of Rad51 may lead to opposite effects. Ubiquitin-dependent degradation of Rad51 depends on Rad6, Rad18, Slx8, Dia2, and APC complex. Rsp5-dependent ubiquitination leads to Rad51 stabilization.

This work was supported by the National Science Center grant 2016/21/B/NZ3/03641.

S4_O2 THE ROLE OF YEAST SWI2/SNF2 DNA DEPENDENT TRANSLOCASES IN GENOME STABILITY MAINTENANCE

Jakub Muraszko[1], Karol Kramarz[2], Gabriela Baranowska[1], Anna Barg-Wojas[1], **Dorota Dziadkowiec[1*]**

[1]Faculty of Biotechnology, University of Wrocław, Poland
[2]Academic Excellence Hub - Research Centre for DNA Repair and Replication, University of Wrocław, Poland
[*]dorota.dziadkowiec@uwr.edu.pl

The regulation of DNA metabolic processes at vulnerable regions of the genome, such as centromeres, is essential for maintaining genome integrity. The consequences of deleting genes encoding proteins that fine-tune these processes can often go undetected because the global DNA damage or replication stress responses can counteract the lack of specific activities. However, dysregulation of these genes' activity through over-expression can perturb the system more dramatically.
Schizosaccharomyces pombe Rrp1 and Rrp2 belong to a unique class of Rad5/16-like SWI2/SNF2 enzymes, including human HLTF and SHPRH as well as *Saccharomyces cerevisiae* Uls1, that have both translocase and E3 ubiquitin ligase activities. We demonstrate that both Rrp1 activities may contribute to the regulation of Rad51 recombinase at the centromeres. Additionally we show that overproduction of Rrp1 leads to the decrease in global histone levels and spreading of the centromeric histone variant Cnp1 (CENP-A) away from the central core of the centromere. This perturbs centromere function and causes the accumulation of abnormal repair intermediates, chromosome instability and consequent viability loss.
Some of Rrp1 functions are shared by Rrp2 and Uls1 indicating that these SWI2/SNF2 enzymes contribute to genome stability maintenance through the regulation of nucleosome dynamics and crucial repair factors at specific chromosomal regions.

Muraszko J, *et al.* Nucleic Acids Res. (2021) 49(12):6832-6848. doi: 10.1093/nar/gkab511.
Barg-Wojas A, *et al.* J Cell Sci. (2020) 10;133(3):jcs230193. doi: 10.1242/jcs.230193.

S4_O3 CONTRIBUTION OF NON-CATALYTIC SUBUNITS OF THE HELICASE-POLYMERASE COMPLEX TO THE MAINTENANCE OF GENOME STABILITY IN YEAST

Michał Dmowski*

Laboratory of DNA Replication and Genome Stability, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
*mdmowski@ibb.waw.pl

High fidelity DNA replication is required for faithful transmission of genetic material to daughter cells. Various exogenous and endogenous factors may impede the functioning of the replication machinery resulting in fork stalling, formation of unstable single-stranded DNA (ssDNA) regions, or double-strand brakes (DSB) and thus threaten genome stability with deleterious effect on cell survival.

To achieve faithful DNA duplication, multiprotein replisome structures are engaged at replication origins. The lagging DNA strand is replicated mainly by DNA polymerase delta (Pol δ), while the majority of leading strand replication is performed by DNA polymerase epsilon (Pol ε). In eukaryotic organisms, Pol ε together with the CMG helicase form the CMGE complex, an essential element of the replisome. In *Saccharomyces cerevisiae* CMG is composed of Cdc45, Mcm2-7 (helicase subunits), and GINS (Psf1-3 and Sld5 subunits). Pol ε is composed of Pol2 (the catalytic subunit) and Dpb2-4 accessory subunits.

It is evident, that the contribution of catalytic subunits of the replisome to DNA replication is essential. However, growing pieces of evidence now demonstrate the importance of non-catalytic subunits in this process. Our results demonstrate that mutations in genes encoding the non-catalytic Dpb2 subunit of Polε or Psf1 subunit of GINS, which affect interactions between replisome components, result in genomic instability pronounced by abnormal cell morphology, increased mutation rates, increased contribution of error-prone Pol ζ, impaired progression through the S phase, defective activation of replication checkpoint (Dmowski et al. 2017), and increased DNA repeat tracts instability (Jedrychowska et al. 2019). Additionally, we show that impaired functioning of the replisome in cells with defective GINS or Polε results in perturbations of CMGE helicase-polymerase functioning, severely affecting DNA replication what increases contribution of Pol δ to leading strand replication (Dmowski et al. 2022) and requires rescue by DNA repair mechanisms related to homologous recombination (Jedrychowska et al. 2019). These results gain importance given, that various disorders or cancer are caused by changes in protein levels or mutations in human POLE2 or GINS1 genes (homologs of yeast DPB2 and PSF1, respectively).

Dmowski M. et al. DNA Repair (Amst). 2022, 110:103272.
Dmowski M. et al. PLoS Genet. 2017, 13:1–24.
Jedrychowska M. et al. PLoS Genet. 2019, 15:e1008494.

This research was funded by the Foundation for Polish Science" [grant TEAM/2011-8/1] and the National Science Centre, Poland [grant No 2015/17/B/NZ1/00850 and 2017/26/M/NZ3/01044]

S4_O4 IDENTIFICATION OF NEW COHESIN INTERACTORS IN YEAST

Ireneusz Litwin[1*], Małgorzata Nowicka[2], Ewa Maciaszczyk-Dziubińska[2], Katarzyna Markowska[1], Robert Wysocki[2], Karol Kramarz[1]

[1]*Academic Excellence Incubator - Research Centre for DNA Repair and Replication, University of Wrocław, Kanonia 6/8, Wrocław, Poland*
[2]*Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8, Wrocław, Poland*
[*]*ireneusz.litwin@uwr.edu.pl*

Cohesin is a highly conserved, multiprotein complex whose canonical function is to hold sister chromatids together to ensure accurate chromosome segregation. Cohesin loading onto DNA depends on the Scc2-Scc4 cohesin loading complex that enables cohesin ring opening and topological entrapment of sister DNA molecules. In attempt to better understand how sister chromatid cohesion process is regulated, we performed a proteomic screen that identified the Isw1 chromatin remodeler as a cohesin binding partner. We revealed that the ISW1a complex modulates cohesin loading specifically at centromeres. We found that in the absence of Isw1 more Scc2 associates with centromeres resulting in excess cohesin loading at centromeres and pericentromeric regions. Surprisingly, Isw1-cohesin interaction seems to be non-essential for regulation of centromeric cohesion and probably mediates other cohesin functions. Finally, our results showed that the concomitant absence of ISW1a, Chd1 and Isw2 chromatin remodelers leads to centromeric sister chromatid cohesion defect. We suggest that these proteins restrict the activity of RSC chromatin remodeler at centromeres and propose that centromeric cohesin loading is regulated by positioning of canonical nucleosomes in the vicinity of centromeric nucleosome.

S4_O5 IMPACT OF SUMOYLATION AT REPLICATION STRESS SITES IN FISSION YEAST

Katarzyna Markowska, Ireneusz Litwin, **Karol Kramarz***

Academic Excellence Hub - Research Centre for DNA Repair and Replication, Faculty of Biological Sciences, University of Wrocław, Poland
*karol.kramarz@uwr.edu.pl

DNA replication can be altered by multiple endogenous and exogenous factors, which jointly refer to as replication stress (RS). To maintain genome stability, cells have evolved multiple salvage pathways, named as replication stress response (RSR). RSR coordinates the repair and restart of arrested replication forks (RFs) to complete DNA replication. RSR is regulated by various post-translational modifications (PTMs) and SUMOylation at arrested RFs is among the less understood PTMs. SUMO (small ubiquitin-like modifier) is covalently attached to target proteins as a monomer that can be further extended into chains. Recently, it was shown that SUMO chains promote re-localization of arrested RFs within nucleus for activation of relevant repair pathway at nuclear periphery. On the other hand, SUMO chains impede the DNA synthesis mediated by homologous recombination (HR) at RFs and must be removed to carry on DNA replication (Kramarz et al., 2020). In general, it is thought that SUMO chains signal for proteasomal degradation of modified proteins by recruitment of SUMO-targeted ubiquitin ligases and/or SUMO proteases (Schirmeisen et al., 2021). Here, I present the data that shed a light on SUMO chains as an integral part of RSR, fine tuning the dynamics of progression through S-phase and contributing to the resolution of replication intermediates arising at blocked RFs.

Kramarz K. et al. Nat Commun. 2020, 6;11(1):5643.
Schirmeisen K. et al. Genes (Basel) 2021, 12(12):2010.

Poster presentations

S4_P1 RECRUITMENT OF RAD51 RECOMBINASE TO THE REPAIR FOCI DEPENDS ON SUMOYLATION

Tuguldur Enkhbaatar[1*], Dorota Malicka[2], Marek Skoneczny[1], Adrianna Skoneczna[1]

[1] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland

[2] Warsaw University, Faculty of Biology, Miecznikowa 1, 02-096 Warszawa, Poland

[*]tuqdo@ibb.waw.pl

Homologous recombination (HR) is one of two known repair pathways that remove DNA double-strand breaks (DSBs). DSBs are hazardous types of lesions because, when left unrepaired lead to cell death, and when repaired incorrectly, they result in chromosomal rearrangements. Rad51 recombinase is engaged in DSB repair contributing to genome maintenance. At the DSB sites, after the resection of DNA ends, Rad51 binds to ssDNA, thus creating presynaptic filament, which then invades the homologous DNA duplex. Rad51 filament is also the interaction platform with other proteins involved in homologous recombination repair. Posttranslational modifications of Rad51 modify its activity, e.g., in *Saccharomyces cerevisiae*, phosphorylation of Ser125 and Ser375 by Cdc28 kinase promotes the DNA binding affinity of Rad51, phosphorylation of Ser192 affects ATPaes activity of Rad51, phosphorylation of Ser2 and Ser10 influences The Rad51 stability. We asked whether the other posttranslational modifications also influence Rad51 recombinase. Our data showed that Rad51 has different SUMOylation and ubiquitination profiles in cells overproducing different E3 ligases. For example, in the cells overproducing Mms21 SUMO ligase (subunit of Smc5-Smc6 complex), Rad51 was mono-SUMOylated. In cells overproducing Rsp5, a NEDD family ubiquitin ligase, Rad51 was modified with SUMO and ubiquitin and displayed a complicated pattern of higher molecular weight Rad51 forms. When Slx8, a subunit of SUMO Targeted Ubiquitin Ligase Slx5-Slx8 complex, was overproduced, Rad51 seemed to have an intermediary SUMOylation profile between mono- and poly-SUMOylation but was intensively poly-ubiquitinated. We asked how different SUMOylation profiles would influence the formation of Rad51 repair foci in cells challenged with zeocin. Using fluorescent microscopy and YFP-Rad51 fusion protein, we showed that Rsp5-dependent poly-SUMOylation of Rad51 resulted in increased repair foci number both in control and zeocin-treated cells. Overproduction of Slx8 led to decreased number of Rad51 repair foci after zeocin treatment. Overproduction of Mms21 did not affect Rad51 foci number in zeocin-treated cells but showed a higher number of Rad51 foci in control cells. In conclusion, we formulated a hypothesis where Mms21 is responsible for mono-SUMOylation of Rad51; Rsp5 further modifies Rad51 by adding more SUMO to this protein or stimulating poly-SUMO chain formation, which enables Rad51 recruitment to the DNA lesion. At the same time, Slx8 promotes degradation of Rad51 SUMOylated forms, likely contributing to its release from DNA.

S4_P2 MMS2 INDUCED POLYMERASE SWITCH IN *SACCHAROMYCES CEREVISIAE*

Hałas Agnieszka[1*], Krawczyk Michał[2], Śledziewska-Gójska Ewa[1]

[1]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland*
[2]*Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary*
[*]*halas.agnieszka@ibb.waw.pl*

Yeast Mms2 is an ubiquitin E2 variant (UEV) protein which is involved in synthesis of ubiquitin chains bound *via* lysine 63, together with E2 enzyme Ubc13. The best documented function of this protein complex is connected with polyubiquitination in concert with Rad5 ubiquitin ligase of monoubiquitinated replication processivity factor PCNA and activation of DNA damage tolerance pathway leading to damage avoidance in a process called template switch. This pathway functions in an error-free manner and deletion of *MMS2* results in mutator phenotype.

Interestingly, we show that overproduction of Mms2 from heterologous *MET25* promoter in yeast causes 5-7-fold increase in frequency of spontaneous mutations in yeast cells. Moreover, unlike known Mms2 functions, this new promutagenic function of Mms2 depends entirely on presence of polymerase zeta activity as our results show that the mutator effect is totally abolished by the deletion of *REV3* gene encoding catalytic subunit of this TLS polymerase zeta. Additionally this mutagenesis depends on *POL32* encoding accessory subunit shared by polymerase zeta and delta, pointing to the role of four subunit polymerase zeta in the mutagenesis.

The new promutagenic activity of Mms2 does not depend on Ubc13 ubiquitin conjugating enzyme and Rad5 ligase but Ubc4 conjugating enzyme and Rsp5 and Not4 ligases suggesting activity of alternative ubiquitination process.

Additionally mutagenesis induced by MMS2 overexpression requires Def1which had been shown to be involved in polymerases switch induced by DNA damage. We also demonstrate that the cellular level of Pol3 , the catalytic subunit of replicative polymerase delta can be affected by both absence of Mms2 and the increased level of this protein.

Altogether the results suggest that Mms2 overproduction causes decrease of cellular level of Pol3 which facilitates the replacement of polymerase delta with polymerase zeta in replication fork, which in turn leads to increased mutagenesis.

This work was supported by National Science Centre, Poland, Preludium Grant 2016/23/N/NZ3/01550.

S4_P3 TRANSLESION SYNTHESIS POLYMERASES IN MITOCHONDRIA OF *SACCHAROMYCES CEREVISIAE*

Katarzyna Bagińska[1,2], Martyna Wiśniewska[1], Renata Kuberska[1], Ewa Śledziewska-Gójska[1] and **Aneta Kaniak-Golik[1*]**

[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland
[2]University of Warsaw, Warsaw, Poland;
[*]anetak@ibb.waw.pl

Mitochondria, essential organelles in eukaryotic cells, possess their own streamlined multicopy genomes that have to be maintained in the functional state (so called *rho*+ genomes) to support the main mitochondrial activity: ATP production by oxidative phosphorylation. The ability of mitochondria to respire is, in turn, necessary for the proper functioning of the whole compartment and, in consequence, for the whole cell. The chief system for maintaining and safeguarding mtDNA is the conserved replicative mitochondrial DNA polymerase (Pol γ), belonging to the A-family of polymerases. In the budding yeast, it is a one-subunit highly processive polymerase called also Mip1. However, Pol γ is not the only polymerase in mitochondria. There are several accessory polymerases which are, in contrast to Polγ, localized to both the nucleus and mitochondria. Those accessory polymerases are less faithful than replicative polymerases and are capable of replicating damaged templates in a process called the translesion synthesis (TLS). There are three of them in mitochondria: 1) polymerase ζ (Polζ), from B-family of polymerases, that is composed minimally of 2 subunits, Rev3 and Rev7, 2) Rev1, and 3) polymerase η (Polη), encoded by the *RAD30* gene. The latter two belong to the family of Y-polymerases. TLS polymerases, especially Pol ζ and Rev1, are responsible for spontaneous and induced mutagenesis of the nuclear genome - they act there as mutators. In contrast, in mitochondria the three TLS polymerases do not influence significantly spontaneous mutagenesis of mtDNA, but prevent the accumulation of UV-induced point mutations in mtDNA - they all act as anti-mutators, which differs from the effects of Pol ζ's and Rev1's activities in the nucleus upon UV damage. On the other hand, upon UV damage Rev3 and Rev1 promote recombination processes that destabilize *rho*+ genomes, whereas Rad30 acts in a pathway stabilizing the functional genome under these conditions. We will present the results of our study on the regulation of TLS polymerase activities in the mitochondrion upon the genotoxic damage, in particular in relation to known mechanisms of their regulations in the nucleus.

S4_P4 ROLE OF URIDYLATION IN BULK MRNA DECAY

Lipińska-Zubrycka Lidia[1*], Grochowski Maciej[1], Małecki Michał[1]

[1]*Institute of Genetics and Biotechnology, University of Warsaw, Pawińskiego 5a, 02-106 Warsaw, Poland*
[*]*llipinska@biol.uw.edu.pl*

Messenger RNA (mRNA) degradation is an essential step in gene expression. Every mRNA produced in the nucleus meets its end in the cytoplasm to close the expression cycle. Due to the existence of several redundant pathways, RNA degradation is difficult to study and many fundamental questions remain unsolved. Bulk mRNA degradation is initiated by poly(A) tail shortening followed by removal of 5' cap which leaves mRNA molecule accessible for cytoplasmic 5'-3' exonuclease Xrn1. In eukaryotes, 5'-3' directed degradation is the main degradation pathway, however, mRNA can be degraded from 3'-end by the exosome complex supported by the SKI complex activity. A less understood factor implicated in bulk mRNA is a pervasive cytoplasmic uridylation of mRNAs 3'-ends. Uridylation is catalysed by cytoplasmic uridyltransferases, while 3'-5' exonuclease Dis3L2 preferentially targets uridylated RNAs. In higher eukaryotes, the uridylation of mRNAs with shortened poly(A) tails facilitates LSM complex binding, which accelerates decapping and 5-3' decay of those transcripts. Intriguingly elimination of uridylation has no impact on cell physiology and only minor molecular consequences have been reported. Therefore, the contribution and significance of uridylation for bulk mRNA decay are not clear.

We used the fission yeast model to establish the role of uridylation in mRNA decay. Fission yeast offers all advantages of a simple unicellular model while their mRNA is pervasively uridylated similarly to the situation in higher eukaryotes. Using yeast genetics and both genome-wide and gene-focused 3'-RACE we discovered that uridylation participates in mRNA turnover in two independent ways. Main fission yeast uridylyltransferase Cid1 uridylates adenylated mRNAs, this induces LSM complex binding which serves two purposes – it accelerates decapping and subsequent 5-3' decay while at the same time it protects 3'-end from more extensive shortening. Messengers that did not undergo this main pathway can be completely deadenylated, such molecules are either the target of SKI complex associated with exosome or are oligouridylated by second fission yeast uridylyltransferase Cid16 which triggers their decay by U-specific 3-5' exonuclease Dis3L2. Thus we established that oligo(A) tails uridylation by Cid1 protects mRNAs 3'-ends and shifts decay balance towards 5-3' direction, while oligouridylation of deadenylated mRNAs by Cid16 triggers their removal by 3-5' decay pathway.

Our results strongly suggest that 3-5' mRNA decay results in interference with ongoing translation. Therefore, the main role of pervasive uridylation of poly(A) tails is to redirect mRNA decay to the 5-3' pathway before transcripts will be completely deadenylated and marked to decay by 3-5' exonucleases.

S4_P5 UFD4 INTERACTS WITH COHESIN AND REGULATES COHESIN FUNCTIONS

Małgorzata Nowicka[1*], Ewa Maciaszczyk-Dziubińska [1], Katarzyna Markowska [2], Robert Wysocki [1], Ireneusz Litwin [1,2]

[1]*Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8, Wrocław, Poland*

[2]*Academic Excellence Incubator - Research Centre for DNA Repair and Replication, University of Wrocław, Kanonia 6/8, Wrocław, Poland*

[*]*malgorzata.nowicka@uwr.edu.pl*

Cohesin is a multiprotein, ring-shaped complex that holds sister chromatids together during the cell cycle from the beginning of S-phase until the onset of anaphase. Moreover, cohesin is central to chromosome metabolism, DNA repair and gene transcription. Thus, regulation of cohesin activity is crucial for cell viability. In order to identify novel regulators of cohesins, we performed a proteomic screen that identified Ufd4 as a potential cohesin interactor. Ufd4 is an ubiquitin-protein ligase (E3), which interactome and function in the cell is poorly understood. There is evidence that Ufd4 is responsible for chromosomal proteins ubiquitination and takes part in the global chromatin response to DNA damage.

In an effort to understand Ufd4 function in cohesin regulation, we confirmed cohesin-Ufd4 physical interaction with proximity ligation assay (PLA) and coimmunoprecipitation (CoIP). We showed that Ufd4 binds to cohesin throughout the cell cycle, however, is not involved in degradation of cohesin and its known regulators. In order to determinate whether Ufd4 affects cohesin function and binding to chromatin, we performed chromatin immunoprecipitation (ChIP), condensation and cohesion assays. We found that lack of *UFD4* leads to a mildly reduced cohesin levels at centromeres as well as chromosome arms and rDNA region. Furthermore, we observed moderate premature sister chromatid separation in the *ufd4* mutant but no defect in chromosome condensation.

Our results indicate that Ufd4 may be an auxiliary factor responsible for cohesin binding to chromatin and thus promoting sister chromatid cohesion.

S4_P6 COMPLEX GENOME REARRANGEMENTS IN THE MODEL EUKARYOTIC ORGANISM *SACCHAROMYCES CEREVISIAE*

Adrian Piróg*, Hanna Tutaj, Katarzyna Tomala, Ryszard Korona

Institute of Environmental Science, Jagiellonian University, 7 Gronostajowa Street, 30-387 Kraków, Poland
*adrian.pirog@doctoral.uj.edu.pl

Extensive changes in the genome are usually harmful. Research conducted in recent years has shown that genome instability can be the source of new traits and the adaptation of microorganisms to new or unfavorable environmental conditions (Berman 2016; Duffy et al. 2016). This is related to the increased rate of mutation, which favors the formation of a wide range of new phenotypes, which may contain sets of genes beneficial in a specific situation (Peter et al. 2018). We created a collection of 400 strains of yeast *Saccharomyces cerevisiae* and performed extensive tests for loss of heterozygosity (LOH). These strains were diploid and contained homozygous deletions of single genes involved in genome stabilization and one or two heterozygous markers (on different chromosomes) that allowed to track either single or multiple (at least double) events of LOH. We detected several dozen gene deletions causing a pronounced (10-100x) increase in the rate of LOH. Next, we checked whether the destabilization events were largely random or formed discernible patterns. Depending on the studied gene deletion, we observed characteristic changes in ploidy, either an average reduction or increase in the amount of DNA. Subsequently, strains exhibiting the most radical deviations from diploidy were subjected to long-term evolution to determine the changes that aneuploid cell undergo. We found that the initially highly aneuploid strains tended to return to the euploid state and improve their growth rate, often up to that of the wild strain. We also detected mutants that may contribute to the formation of adaptations that increase chronological lifespan. In conclusion, we identified dozens of gene deletions associated with greatly increased rates of multi-point gene destabilization. Such events can be considered one of the mechanisms of adaptive evolution by which cells can very quickly achieve highly altered genomic configurations during drastic episodes of genetic instability.

Berman J. Ploidy plasticity: a rapid and reversible strategy for adaptation to stress. *FEMS Yeast Res*. 2016;16(3):fow020

Duffy S, et al. Overexpression screens identify conserved dosage chromosome instability genes in yeast and human cancer. *Proc Natl Acad Sci USA*. 2016;113(36):9967-9976

Peter J, et al. Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates. *Nature*. 2018;556(7701):339-344

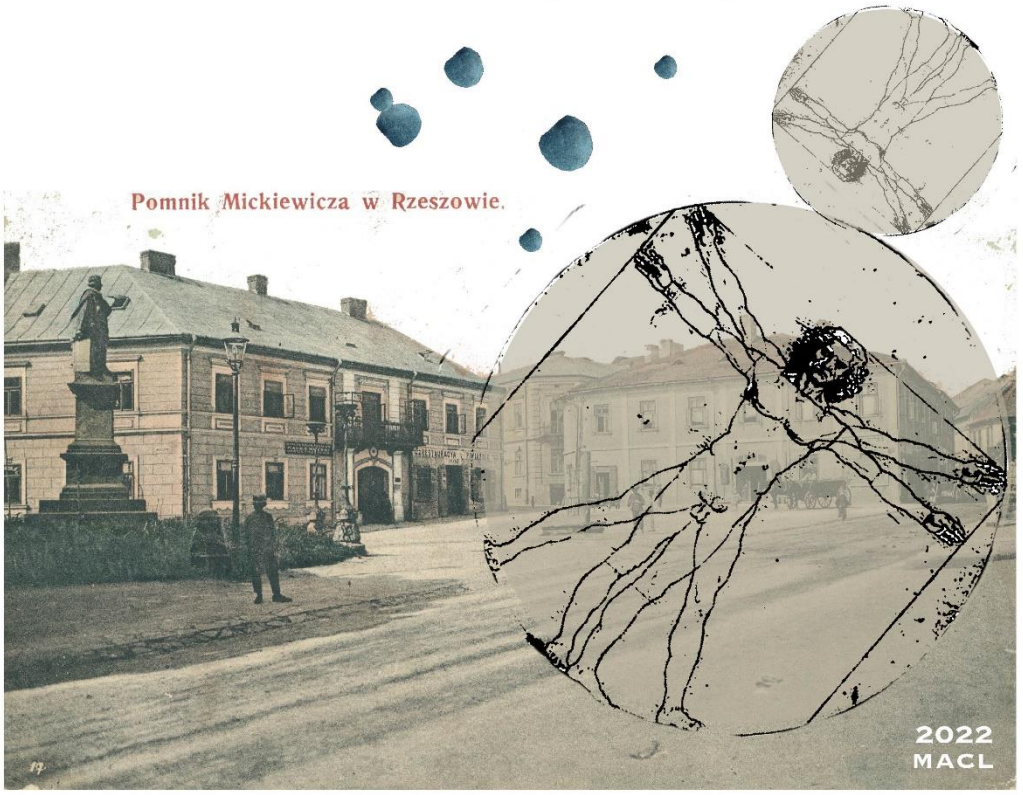
S4_P7 GENOME-SCALE PATTERNS IN THE LOSS OF HETEROZYGOSITY INCIDENCE IN *SACCHAROMYCES CEREVISIAE*

Hanna Tutaj[*], Adrian Piróg, Katarzyna Tomala, Ryszard Korona

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland
[*]*hanna.tutaj@doctoral.uj.edu.pl*

Mitotic recombination, and resulting from it loss of heterozygosity (LOH), is one of the key drivers of evolution in unicellular eukaryotes, such as the budding yeast *Saccharomyces cerevisiae*, and in metazoan tumors. However, it still remains unclear if LOH events happen randomly in the genome or if they follow any discernible patterns. To address this issue we created a set of selectable markers for both arms of all yeast chromosomes which were then introduced into several genetic backgrounds and tested in multiple environments. We provided meaningful new insights into the occurrence of the phenomena of loss of heterozygosity. Firstly, the rate of LOH decreases with the increase in the number of SNPs between homologous chromosomes so that divergence as small as half percent is enough to significantly reduce it. Secondly, the density of loss of heterozygosity events is higher on shorter chromosome arms, or more generally, in regions closer to telomeres, while pericentromeric regions are less affected by LOH. The observed uneven distribution of loss of heterozygosity events could be a result of uneven density of DNA damages but also of the changing mode of DNA repair and the resulting fitness effect. Additionally, the existence of some repetitive elements, such as ARS and LTR retrotransposons can alter the LOH density, not only locally but also at a whole chromosome arm scale. Finally, conditions preventing growth, such as under prolonged starvation, decrease the overall rate of loss of heterozygosity by about twenty five times. In conclusion, our study uncovered the existence of genome-wide patterns in the incidence of loss of heterozygosity in yeast.

Session 5
Yeast as a model of human diseases and drug testing



Oral presentations

S5_O1 HUMANIZATION OF YEAST CELLS TO STUDY HUMAN PROTEINS AND PATIENT MUTATIONS IN RARE DISEASES

Sylvie Friant*

University of Strasbourg, France
*s.friant@unistra.fr

We use heterologous expression of the cDNA of human genes, either wild-type or bearing patient mutations, in the yeast *Saccharomyces cerevisiae* to determine or to compare the *in vivo* activity of the human proteins. Yeast is a good model to study genetic diseases. Indeed 30% of human genes related to diseases have a yeast homologue and more than 50% of yeast genes can be successfully humanized (Kachroo et al., 2015). By using this approach, we analyse the cellular functions of the wild type versus the mutant version of new variants of unknown significance (VUS) or of new genes identified in patients suffering from rare diseases. Indeed, high-throughput sequencing allows the identification of new potentially pathological mutations for many patients with rare diseases. Functional validation is an essential step to validate these new mutations. The data obtained by the yeast humanization assays are completed by additional cell biology experiments (expression, intracellular localization, interactomics) on cells or tissues from unaffected control/patient variants.

S5_O2 MECHANISMS OF ATP SYNTHASE DEFECTS DUE TO MUTATIONS IN MITOCHONDRIAL *ATP6* GENE – YEAST STUDIES

Róża Kucharczyk*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
*roza@ibb.waw.pl

Mitochondrial diseases are a broad group of neuromuscular and metabolic diseases issued from defects in oxidative phosphorylation (OXPHOS), a process that provides cells with the energy rich ATP molecule. Despite its small coding capacity and high number of copies (several thousands in a single cell), mutations of the mitochondrial genome have been quite frequently associated to mitochondrial diseases (15% of the cases), and with the advent of novel genomic sequencing methods their list is rapidly expanding. Evaluating the pathogenicity of these mutations is difficult as they are found often in single cases or families and in heteroplasmic state (mutant and wild type mitochondrial DNA (mtDNA) molecules coexisting in one cell). Another difficulty is the absence of methods for genetically transforming human mitochondria. The baker yeast *Saccharomyces cerevisiae* is an invaluable model to evaluate the consequences of mtDNA mutations found in patients as it is possible to modify the yeast mtDNA in a well-defined nuclear genetic background and without the co-existence in cells of mutated and wild type mtDNA. Furthermore, owing to its good fermenting capacity yeast can survive mutations that inactivate OXPHOS. We took advantage of these attributes to investigate in yeast the functional consequences of more than eleven mutations of the mitochondrial *ATP6* gene found in patients with NARP, MILS, MLASA, BSCL and other diseases. The protein encoded by this gene (called the subunit *a*) is involved in the transfer of protons through the membrane domain of ATP synthase (FO) coupled to ATP synthesis. In light of recently described high resolution structures of ATP synthase, the consequences of subunit *a* substitutions were defined and pathogenic mechanisms at molecular level were proposed. This analysis provides also important information on the role of specific amino acid residues in the structural organization and functioning of ATP synthase proton channel.

The work was supported from the NSC funding to RK: 2016/23/B/NZ3/02098.

S5_O3 HELPFUL YEASTS - HOW TO FIND THERAPY FOR PATIENTS WITH VPS13 PROTEINS DEFICIT?

Joanna Kamińska*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
*kaminska@ibb.waw.pl

Neurodegenerative diseases are a growing problem in the aging society. Besides major neurodegenerative diseases there are numerous rare neurodegenerative disorders, such as those caused by mutations in human *VPS13* genes. There are four humans *VPS13* genes (*VPS13A*-*D*) associated with rare but severe neurodegenerative disorders, such as chorea-acanthocytosis (*VPS13A*), early-onset Parkinsonism (*VPS13C*), childhood-onset movement disorder (*VPS13D*) and neurodevelopmental disease - Cohen's syndrome (*VPS13B*). There are no therapies for these diseases and patients are treated only symptomatically. *VPS13* genes are evolutionary conserved and recent studies suggest that the Vps13 proteins are lipid transfer proteins located at membrane contact sites. We use yeast *Saccharomyces cerevisiae*, containing one nonessential gene coding for Vps13 protein, as a model organism both to study the effect of *VPS13* gene mutations on molecular level and to help develop novel therapeutic strategies for patients. For this end we searched for both multicopy and chemical suppressors of the growth defect of *vps13Δ* cells on medium with sodium dodecyl sulphate (SDS). These strategies resulted in identification of genes and substances which point to two molecular targets for potential treatment. The first is calcineurin, a calcium dependent protein phosphatase, which activity is higher in the *vps13Δ* mutant. We identified the way to specifically limit its activity genetically by titration of the calmodulin, a calcineurin activator, or by increasing the level of the Rcn2 protein, the negative regulator of calcineurin. The administration of ethylene glycol tetraacetic acid (EGTA), which sequesters calcium, or FK506, the known calcineurin inhibitor was also effective. The second identified target is iron homeostasis. We found that the iron level in *vps13Δ* is lower than in wild-type strain and showed that both the administration of iron salts and the increase in the level of iron transporter Fet4 help the *vps13Δ* mutant. Since the iron uptake system is copper-dependent, similar effect results from addition of copper salts or copper ionophores or by increasing the cellular level of copper transporters.
These our findings open area for further investigation of molecular pathogenesis of diseases caused by mutations in *VPS13* genes using higher eukaryotic models and developing new therapies.

S5_O4 PATHOGENIC EFFECT OF *GDAP1* MUTATIONS CAUSATIVE FOR CHARCOT-MARIE-TOOTH 4A DISEASE IN A YEAST MODEL

Andrzej Kochański*

Neuromuscular Unit, Mossakowski Medical Research Institute, Polish Academy of Sciences

*akochanski@imdik.pan.pl

Charcot-Marie-Tooth type 4A disease (CMT4A) belongs to an extremely heterogenous group of hereditary motor sensory neuropathies (HMSN), in which over 100 genes have been shown to be mutated and involved in the molecular pathogenesis. The patients affected with CMT4A disease suffer from slowly progressive wasting and weakness of distal muscles of lower and upper limbs, which is accompanied by sensory disturbances. Some of the CMT4A patients are wheelchair-bound from early infancy. CMT4A disease is caused by the mutations located in the *GDAP1* gene. Some of them are recurrent, while others have been reported only in single patients. Similarly to other neurodegenerative disorders also in CMT4A disease there is a question whether a newly detected sequence variant (SNP) is pathogenic for disease and how its causative –pathogenic effect may be tested. In this study we proposed a new yeast-based model to assess the pathogenic effect of sequence variants located within *GDAP1* gene. We expressed in yeast the wild-type and known pathogenic variants of *GDAP1* gene. Human GDAP1 protein was produced in yeast cells. We also assessed mitochondrial morphology and function of *GDAP1*-expressing strains. GDAP1 protein localization was analyzed in yeast. The various *GDAP1* sequence variants expressed in the yeast resulted in specific effect in the tests, we performed. We have shown that pathogenic effect of *GDAP1* sequence variants may be validated in the yeast-based model.

S5_O5 NEW TRENDS IN THE SEARCH FOR ANTIFUNGAL THERAPIES

Małgorzata Gizińska, Anna Staniszewska, Michalina Kazek, Mirosława Koronkiewicz, Łukasz Kuryk, Małgorzata Milner-Krawczyk, Joanna Baran, Paweł Borowiecki, **Monika Staniszewska***

Centre for Advanced Materials and Technologies Warsaw University of Technology
*monika.staniszewska@pw.edu.pl

Candida albicans CNB1 plays a role in the response *in vitro* and *in vivo* to stress generated by PB-WUT-01, namely 1,3-dimethyl-7-(2-((1-(3-(perbromo-2H-benzo[d][1,2,3]triazol-2-yl)propyl)-1H-1,2,3-triazol-4-yl)methoxy)propyl)-1H-purine-2,6(3H,7H)-dione. The antifungal mechanism involved the calcineurin pathway-regulated genes *SAP9-10*. *Galleria mellonella* treated with PB-WUT-01 (at 0.64 μg/mg) showed limited candidiasis and remained within the highest survival rates. The histochemical inspection of the larvae in the control group (CAI4 + PBS) revealed the dissemination of yeasts, while the larvae under the PB-WUT-01 treatment (0.64 μg/mg) showed limited infection and remained within the highest survival rates. These data suggested a potential application of PB-WUT-01 in treating the *C. albicans* infection *in vivo.* Using flow cytometry, we assessed the chitin content of the PB-WUT-01-treated CAI4 and *cnb1Δ/CNB1* following the staining with calcofluor white (CFW) of 2.5 μg mL^{-1}. The treatment of CAI4 with PB-WUT-01 led to a decrease in the chitin content (independent of conc. value) vs untreated counterpart. The *cnb1Δ/CNB1* mutant showed a decrease in the staining index (SI) after 18-hour treatment at 20 μM. Contrariwise, the elevated SI value after 18-h treatment at sub-MIC = 5 μM compared to the untreated counterpart was noted. The confocal microscopy analysis detected changes in chitin levels of *cnb1Δ* in comparison with the *cnb1Δ/CNB1* and CAI4 cells.

It is assumed that *CNB1* plays an important role in the cell wall changes associated with apoptotic death. The morphogenesis process was dependent on *CNB1*, whereas *cnb1Δ/CNB1* and *cnb1Δ* were not at all filamentous. Moreover, we established the *C. albicans* filamentation reduced in the biofilm under osmolarity generated by NaCl (125 μM). It was evident that when salt was added to RPMI, it caused an increase in the HO342 nuclei accessibility in the upper layer of the biofilm. Contrariwise, since the wild type *C. albicans* biofilm's response to PB-WUT-01 differed from that of NaCl, it is assumed that PB-WUT-01 does not act *via* the induction of the osmotic stress pathway in *C. albicans*. Furthermore, the signaling pathway responsible for apoptosis and the hyphae formation coincided because the morphological switching was inhibited and apoptosis was induced in the mutant treated with PB-WUT-01.

The molecular mode of action of PB-WUT-01 was rationalized by *in silico* docking studies toward both human and *C. albicans* calcineurin A (CNA) and calcineurin B (CNB) complexes, respectively. PB-WUT-01 acting as a calcineurin inhibitor in the *C. albicans* cells enhances the cells' susceptibility. Therefore it could be a suitable alternative treatment in patients with candidiasis. Our results strengthen the PB-WUT-01's potential as a promising inhibitor of calcineurin, which warrants a possibly novel antifungal approach for further investigation. In addition, the docking studies of PB-WUT-01 and *C. albicans* 6TZ6 calcineurin revealed dominant and subtle interactions in the formed complex, which plays a fundamental role in a very high potency of the studied inhibitor. It was established that the origin of such high affinity of PB-WUT-01 to 6TZ6 is due to the formation of unique multiple polar interactions

between inhibitor's xanthine-1,2,3-triazole-TBBt core structures and neighboring amino acid residues (i.e. CnC-Y30, CnC-I60, CnC-Y97, CnA-E408, and CnA-W401) present in the LxVP binding groove of the 6TZ6 protein. This discovery is expected to inspire the development of 6TZ6 inhibitors in the future.

Gizińska et al. Bioorg Med Chem Lett. 2020 Dec 1;30(23):127545. doi: 10.1016/j.bmcl.2020.127545. Epub 2020 Sep 12. PMID: 32931913.

Poster presentations

S5_P1 TETRAZOLE DERIVATIVES BEARING BENZODIAZEPINE MOIETY ACTION MODE AGAINST VIRULENCE OF *CANDIDA ALBICANS*

Marta Rogalska*

Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

*marta.rogalska2.stud@pw.edu.pl

Candidiasis, an opportunistic fungal disease, is a critical worldwide problem causing about 700 000 deaths per year (Hossain S. et al. 2021). Due to the emergence of new forms of *Candida* resistance and the toxicity of current drugs, which also interfere with other drugs patients are taking; it is essential to search for new antifungal therapeutic agents that are not toxic to the patient. Especially today, when COVID-19 patients are at an increased risk of developing fungal infections, particularly after having been treated with immune-suppressing drugs specific antifungals are necessary to increase survival.

Derivatives of tetrazole and benzodiazepine show remarkable pharmaceutical potential- indicate many types of activities incl. against fungal, bacteria, viruses, cancer, antihypertensive, antidiabetic, anticonvulsant. Thereupon, a series of novel tetrazole derivatives bearing benzodiazepine moiety were synthesized and studied for search potential antifungal activity. Based on screening tests on the model strain of *Candida albicans* ATCC SC5314, determining the degree of inhibition of fungal cell growth and the MFC factor (minimal antifungal concentration), the two most effective compounds were selected: 5-(2-bromophenyl)-7-fluoro-1-[3-(5-(4-chlorophenyl)-2H-tetrazol-2-yl)propyl]-1,3-dihydro-2H-1,4-benzodiazepin-2-one hereinafter referred to as 6c and 5-(2-bromophenyl)-7-fluoro-1-[3-(5-(2-chlorophenyl)-2H-tetrazol-2-yl)propyl]-1,3-dihydro-2H-1,4 benzodiazepin-2-one as 6d. Moreover, their *in vitro* cytotoxicity against the VeroE6 cells was low. *In vivo* toxicity to the model organism of *Galleria mellonella* larvae (survival, mobility, coconization, melanization and health score) was also determined. The tetrazole derivatives altered the hyphal morphology, chitin deposition and membrane permeation in planktonic and sessile cells. 6c or 6d caused PS translocation and the membrane permeation in planktonic and accidental dependent permeabilization (ADP). Moreover, using flow cytometry analyses there was determined *Candida* response to stress associated with compounds treatment 6c or 6d (non- and protoplast cells), and changes in the cell cycle. The higher content (%) of reactive oxygen species in C. albicans wt. cells treated was noted. 6d showed CNB1- dependent action in candidiasis. The HSP90 levels were increased with the cnb1Δ / cnb1Δ and cnb1Δ / CNB1 mutants with Ca2+ supplementation in medium (calcineurin stress pathway CNB1). The compounds affected the *Candida* biofilm's viability. Summing up, the selected tetrazole derivatives bearing benzodiazepine moiety may be potential therapeutics with a strategy aimed at *Candida albicans* virulence factors causing accidental passive cell death without toxicity against eucaryotic cells.

M. Staniszewska T. Zdrojewski, M. Gizinska, M. Rogalska, Ł. Kuryk, A. Kowalkowska, E. Łukowska-Chojnacka (2022). Tetrazole derivatives bearing benzodiazepine moiety- synthesis and action mode against virulence of *Candida albicans*. European Journal of Medicinal Chemistry 230; 114060

S. Hossain, E. Lash, A. O. Veri, L. E. Cowen (2021). Functional connections between cell cycle

and proteostasis in the regulation of *Candida albicans* morphogenesis. Cell Reports 34; 8; 109791

S5_P2 THE GTPase Arf1 IS A DETERMINANT OF YEAST Vps13 LOCALIZATION TO THE GOLGI APPARATUS

Kołakowski Damian[1], Rzepnikowska Weronika[2], Kaniak-Golik Aneta[1], **Żołądek Teresa[1*]**, Kamińska Joanna[1]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland; damian.kolakowski@ibb.waw.pl (D.K.); anetak@ibb.waw.pl (A.K.-G.); [*]teresa@ibb.waw.pl (T.Z.); kaminska@ibb.waw.pl (J.K.)

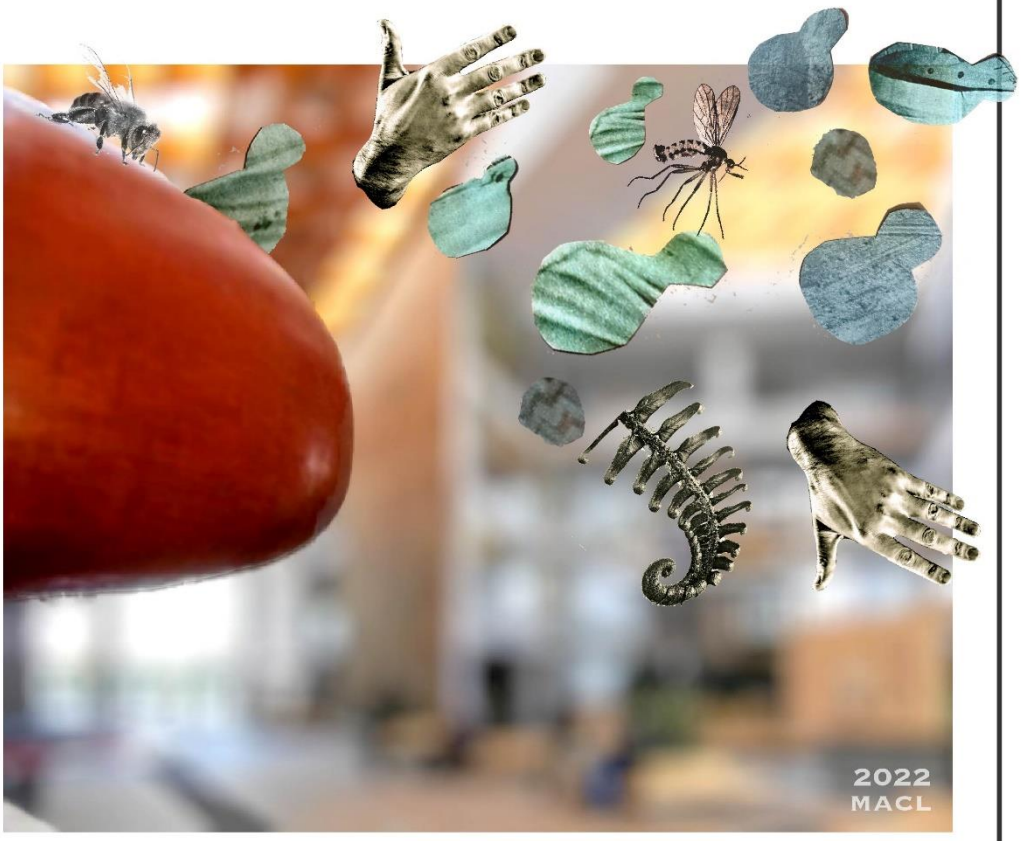
[2]Neuromuscular Unit, Mossakowski Medical Research Institute, Polish Academy of Sciences, Pawińskiego 5, 02-106 Warsaw, Poland; wrzepnikowska@imdik.pan.pl (W.R.)

VPS13 proteins are evolutionarily conserved. Mutations in the four human genes (*VPS13A-D*) encoding VPS13A-D proteins are linked to developmental or neurodegenerative diseases. The relationship between the specific localization of individual VPS13 proteins, their molecular functions, and the pathology of these diseases is unknown. Here we used a yeast model to establish the determinants of Vps13 interaction with the membranes of Golgi apparatus. We analyzed the different phenotypes of the *arf1-3 arf2*Δ *vps13*Δ strain, with reduced activity of the Arf1 GTPase, the master regulator of Golgi function and entirely devoid of Vps13. Our analysis led us to propose that Vps13 and Arf1 proteins cooperate at the Golgi apparatus. We showed that Vps13 binds to the Arf1 GTPase through its C-terminal Pleckstrin homology (PH)-like domain. This domain also interacts with phosphoinositol 4,5-bisphosphate as it was bound to liposomes enriched with this lipid. The homologous domain of VPS13A exhibited the same behavior. Furthermore, a fusion of the PH-like domain of Vps13 to green fluorescent protein was localized to Golgi structures in an Arf1-dependent manner. These results suggest that the PH-like domains and Arf1 are determinants of the localization of VPS13 proteins to the Golgi apparatus in yeast and humans.

Kolakowski et al., Int. J. Mol. Sci. 2021, 22, 12274, https://doi.org/10.3390/ijms222212274.

Session 6

Yeast biodiversity and evolution



Oral presentations

S6_O1 A RUNAWAY EVOLUTION OF TELOMERES IN ASCOMYCETOUS YEASTS

Ľubomír Tomáška[1*], Regina Sepšiová[1], Filip Červenák[1], Zuzana Brzáčová[1], Jozef Nosek[2]

Departments of [1]Genetics and [2]Biochemistry, Comenius University in Bratislava, Faculty of Natural Sciences, Ilkovičova 6, Mlynská dolina B-1, 842 15 Bratislava, Slovakia
[*]lubomir.tomaska@uniba.sk

The ends of linear DNA chromosomes (telomeres) are essential loci whose main function is to protect genome integrity of the cell. Although numerous mechanisms of telomere maintenance and their involvement in physiological as well as pathological cellular states have been described, several important questions remain unanswered. These include: How does a cell deal with a crisis encountered during the first stages of the telomeric sequences loss? What are the means of co-evolution of telomeric sequences with DNA-binding proteins mediating their functions? What are the structural features of telomeric sequences that make them suitable for maintenance of chromosomal ends? Terminal sequences of nuclear chromosomes are usually composed of an array of short G-rich repeats that are similar in organisms as diverse as protozoa, plants, and mammals. With a few, most eukaryotes have only minor variations of the sequence 5'-TTAGGG-3'. In contrast, ascomycetous yeasts exhibit a great variability in both length and sequence of their telomeric repeats. Such variations challenged other factors (e.g., telomerase, telomere-binding proteins (TBPs)) that must have co-evolved to maintain their functions at telomeres. Taking advantage of biodiversity of ascomycetous yeasts and an unprecedented variability of telomeres in this taxonomic group combined with a wealth of genomic data and available experimental tools, we address the above questions to (1) elucidate novel mechanisms involved in cellular adaptations to the loss of telomeric sequences and (2) explore the diversity of yeast telomeric repeat sequences to identify their common sequence and structural characteristics and means of their co-evolution with telomere-binding proteins. To this end we are testing a hypothesis that telomeric repeats, in spite of their sequence heterogeneity, exhibit distinct structural features that are important for telomere maintenance. Furthermore, using a combination of bioinformatic and biochemical approaches we found that double-stranded (ds) TBPs exhibit various degrees of flexibility of binding to their cognate DNA substrates. Although our research is aimed at telomeres, its results is instrumental in addressing of general questions such as description of cellular mechanisms and genomic responses involved in dealing with intracellular stress, principles of fine-tuning of interactions between DNA and specific DNA-binding proteins, understanding of physiological roles of secondary structures of DNA, and evolution of eukaryotic genomes.

Tomáška Ľ. & Nosek J. (2020). J. Mol. Evol. 88(4):293-318.
Juríková K. et al. (2020). J. Biol. Chem. 295(27):8958-8971.
Červenák F. et al. (2021). Genome Biol. Evol. 13(2):evaa268.

This work is supported by the grants APVV-19-0068 and VEGA (1/0061/20)

S6_O2 EVOLUTIONARY BIOCHEMISTRY OF YEAST HSP70/J-PROTEIN CHAPERONES SUBSTRATE BINDING CYCLE

Jarosław Marszałek*

Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland
*jaroslaw.marszalek@ug.edu.pl

Systems consisting of Hsp70 chaperone, and its obligatory J-protein co-chaperone are the most versatile molecular chaperone machines. Present in all domains of life, they are key to many aspects of proteostasis via their ability to cyclically interact with a myriad of substrate polypeptides. Typically, a limited number of Hsp70s interact with a larger number of J-proteins that recruit their partner Hsp70 to interact with substrates. The fundamentals of the cyclical interactions between Hsp70/J-protein and substrates are well established. Much less is known about: how the specificity of these interactions for various cellular functions is determined; how systems in a cellular compartment can be insulated from one another; how such systems have evolved over time. I will focus on the Hsp70/J-protein system involved in the biogenesis of iron-sulfur (FeS) clusters-cofactors in yeast because it provides a window into how specificity of substrate-Hsp70 interactions can change. In this system the interaction between substrate protein, the scaffold on which clusters are built, and its J-protein (Hsc20) have stayed constant. However, the system's Hsp70 has changed during evolution, in some cases interacting only with the scaffold in others having many substrate proteins. Interestingly, although the scaffold's interactions with Hsc20 and Hsp70 are critical, they do not explain the specialized system's functional insulation from other Hsp70/J-protein systems. Rather it is J-protein Hsc20's interaction with Hsp70 that is responsible for the system functional insulation.

S6_O3 YEAST AS A MODEL OF EVOLUTION OF APOPTOSIS

Szymon Kaczanowski*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw
*szymon@ibb.waw.pl

Eukaryotic apoptotic cell death is a central mechanism that regulates multicellular development. However, mitochondrial apoptotic-like cell death - a form of primordial apoptosis – also occurs in unicellular organisms such as yeast or unicellular protists. The origin of mitochondrial apoptosis is a fundamental question of biology. The 'endosymbiotic hypothesis' of its origin was proposed for the first time by Guido Kroemer [1] who observed that the release of apoptotic factors from the mitochondria initiated apoptosis in the so-called permeability transition.

We aim to experimentally verify this hypothesis and show that the apoptotic machinery is a primeval adaptation acquired during mitochondrial domestication. *Saccharomyces* Genome Database as well as literature search suggested role of four apoptotic proteins i.e. protease metacaspase MCA1, nuclease NUC1, and apoptosis-inducing factor NDI1 and HTRA/Omi protease NMA111 in core yeast apoptotic machinery. These genes were used for the study as their deletion decreases apoptotic activity while overexpression induces apoptosis. We plan to test the retention of ancient functions of these gene orthologs across the various kingdoms i.e. bacteria, protists, plants, and animals etc. in yeast by their ability to replace their yeast orthologs. The codon-optimized orthologs were commercially synthesized and were cloned in front of CPS1 terminator of self-constructed common vector. Yeast mutants containing ortholog replacements were created using homologous recombination strategy. Furthermore, GFP tagged version of these orthologs were constructed and used to verify the expression and localization of these foreign genes in the yeast. Fluorescence microscopy analysis shows that the fusion proteins for NDI1, NUC1, MCA1 as well as NMA111 localized to respective compartments which some leakages. The proper expression and size of GFP tagged proteins was confirmed by western blot. The recombination mutants were further tested to check functional complementation using drop tests as well as Annexin-V/PI co-staining assay after apoptotic induction with 200 mM acetic acid. At least six out of ten selected orthologs of yeast apoptotic genes were able to significantly revert the sensitivity to apoptotic induction in contrast to deletion borne resistance.

He hypothesized that apoptosis originated from the ancestral mechanism of killing eukaryotic cells and this ancient mechanism evolved into current apoptotic mechanisms. Various phylogenetic studies, including ours [2-4], confirmed that apoptosis-initiating factors usually are of bacterial origin, similarly as is the case with mitochondria. In the presented study, we tested the hypothesis that the function of apoptotic factors is in principle evolutionary conserved since mitochondrial domestication. According to this hypothesis mechanism of apoptosis of all eukaryotes evolved together during mitochondrial domestication. Following the hypothesis of antagonistic pleiotropy, ancient evolutionary conserved non-apoptotic functions established selection constraints favoring preservation of mitochondrial programmed cell death. This hypothesis implies that heterologous expression of apoptotic factors from remotely related organisms complements gene deletion mutations of these factors. We tested this hypothesis using yeast *S. cerevisiae* as a model.

References:

1.Kroemer G: **Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution**. *Cell Death Differ* 1997, **4**(6):443-456.

2.Kaczanowski S, Klim J, Zielenkiewicz U: **An Apoptotic and Endosymbiotic Explanation of the Warburg and the Inverse Warburg Hypotheses**. *Int J Mol Sci* 2018, **19**(10).

3.Koonin EV, Aravind L: **Origin and evolution of eukaryotic apoptosis: the bacterial connection**. *Cell Death Differ* 2002, **9**(4):394-404.

4.Kaczanowski S, Sajid M, Reece SE: **Evolution of apoptosis-like programmed cell death in unicellular protozoan parasites.** *Parasit Vectors* 2011, **4**:44.

S6_O4 YOR020W-A (MCO10): CHARACTERIZING THE UNKNOWN "SUBUNIT L" OF MITOCHONDRIAL ATP SYNTHASE OF *SACCHAROMYCES CEREVISIAE*

Chiranjit Panja*, Aneta Więsyk, Katarzyna Niedźwiecka, Emilia Baranowska, Róża Kucharczyk

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
*chiranjit@ibb.waw.pl

In *S. cerevisiae*, the uncharacterized protein Yor020W-A (**m**itochondrial **c**lass **o**ne protein of **10** kDa, Mco10), was previously found to be associated with mitochondrial ATP synthase [1] and also referred to as a new 'subunit L' in a recent study of fungal ATP synthases [2]. However, recent cryo-EM structures of *S. cerevisiae* ATP synthase failed to ascertain Mco10 as a bona fide subunit of the complex. Also, nothing is known yet of possible role Mco10 plays in the structure and function of ATP synthase. Surprisingly, the N-terminal fragment of Mco10 is very similar to Atp19 (subunit *k*) of ATP synthase. Atp19, along with the subunits Atp20 and Atp21 plays a major role in formation and stabilizing the dimers of ATP synthase. Biochemical analysis revealed in spite of similarity in sequence and evolutionary lineage, Mco10 and Atp19 differ significantly in function. This is the first work to establish Mco10 being a unique candidate for a 'monomer specific' subunit having role in calcium homeostasis and permeability transition pore.

References:

[1] Morgenstern M, Stiller SB, Lübbert P, et al. Definition of a High-Confidence Mitochondrial Proteome at Quantitative Scale. **Cell Rep**. 2017;19(13):2836-2852. doi: 10.1016/j.celrep.2017.06.014

[2] Liu S, Charlesworth TJ, Bason JV, et al. The purification and characterization of ATP synthase complexes from the mitochondria of four fungal species. **Biochem J**. 2015;468(1):167-175. doi: 10.1042/BJ20150197

Funding: *Grant Number NCN 2018/31/B/NZ3/0111 of National Science Centre, Poland.*

S6_O5 FITNESS ADVANTAGE OF PHENOTYPIC HETEROGENEITY IN *SACCHAROMYCES CEREVISIAE* POPULATIONS

Monika Opałek[1*], Bogna Smug[2], Dominika Włoch-Salamon[1]

[1]*Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University*
[2]*Małopolska Centre of Biotechnology, Jagiellonian University*
[*]*monika.opalek@doctoral.uj.edu.pl*

Environmentally driven phenotypic heterogeneity is widespread among clonal microbial populations, as it provides fitness advantage via various social strategies, e.g. bet-hedging, cross-feeding, division of labour. Clonal populations of *Saccharomyces cerevisiae* laboratory stains can differentiate into at least two phenotypically distinct cell types when exposed to starvation. A fraction of cells actively cease growth, exit the mitotic cell cycle and enter a reversible growth-arrested state - quiescence (Q cells). The other cells (non-quiescent, NQ) do not undergo directional changes and stop at various stages of the cell cycle when nutrients are depleted. Q cells are resistant to multiple kinds of stress (long-term starvation, heat shock, antifungals) and are considered as ones that enable population's survival. However, we recently showed that the coexistence of Q and NQ cells can provide a fitness advantage, since Q cells survive long starvation better, while NQ cells start to proliferate faster if the starvation period is short (Opalek, Smug, Doebeli, & Wloch-Salamon, 2022). Currently, we are running an experimental evolution aiming to explore adaptative value of Q:NQ ratio in variety of ecological scenarios and starvation regimes.

Keywords: phenotypic heterogeneity, experimental evolution, quiescence, *Saccharomyces cerevisiae*, fitness

References:

Opalek, M., Smug, B., Doebeli, M., & Wloch-Salamon, D. (2022). On the Ecological Significance of Phenotypic Heterogeneity in Microbial Populations Undergoing Starvation. *Microbiology Spectrum*. https://doi.org/10.1128/spectrum.00450-21

Acknowledgements:

The project is supported by National Science Centre (OPUS project to DWS, No.2017/25/B/NZ8/01035), Jagiellonian University (Minigrant POB BioS project to BS, PSP U1U/P03/NO/03.32, UJ), Faculty of Biology JU (projects N18/DBS/000003 and MNW/000013 to MO and DWS)

S6_O6 DMS OF hGCH1: CHARACTERIZATION OF MOLECULAR MECHANISMS OF HUMAN DISEASE IN YEAST

Marcin Plech*, Hasan Cubuk, Greg Kudla, Joseph Marsh

Institute of Genetics and Cancer, MRC HGU, University of Edinburgh, United Kingdom
*marcin.plech@ed.ac.uk

Quick diagnosis and detailed understanding of molecular mechanisms of rare genetic disorders will improve quality of life of patients and their families. Our team focuses on investigating damaging protein variants of human genes in yeast competition experiments. This has been facilitated by the advent of Deep Mutational Scanning (DMS) which combines Next Generation Sequencing technologies with high-throughput screening assays.

We have performed DMS of the human GTP cyclohydrolase 1 (hGCH1), which has been implicated in DOPA-responsive dystonia, a neurological movement disorder. Our results revealed clustering of deleterious mutations around substrate binding sites, ligand pockets and across interfaces within the native GCH1 homodecamer. Crucially fitness of individual mutants in yeast is predictive of pathogenicity in humans. Our empirical measurements outperform popular computational Variant Effect Predictors (including supervised and unsupervised VEPs).

Most studies to date have aimed only at identification of loss of function (LOF) mutations as this was the sole perceived source of genetic disorders. However, pathogenic mutations can exert a wide range of effects on proteins, including gain of completely new functions (GOF) and dominant negative effects (DN) which denotes poisoning of whole protein complexes by incorporation of defective subunits. In our experiments we have utilised a set of vectors allowing variable expression of hGCH1. We have also assessed our libraries during co-expression with the WT hGCH1 (introgressed into the yeast gDNA). These treatments are designed to enable better characterization of disease mechanisms including correct annotation of mode of inheritance of individual mutations (dominant / recessive) and distinction between LOF, DN, or GOF status. Our ultimate goal is to build inference models that combine the mode of inheritance with other factors to predict pathogenicity of mutants and accurately assess the risk of individual missense variants.

Poster presentations

S6_P1 MULTIPLE REGION HIGH RESOLUTION MELTING-BASED TECHNIQUE FOR DIFFERENTIATION OF FOOD-DERIVED YEASTS AT SPECIES LEVEL

Monika Borkowska*, Ewelina Celińska

Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland
*monika.borkowska@up.poznan.pl

Yeasts comprise a large and divergent group of microorganisms playing fundamental role in numerous foods production. To monitor the production processes, validate authenticity and safety of foods, reliable methods of the microbes identification and differentiation are needed. Nowadays, an improved fluorescent-detection system and next-generation software programmed for high resolution melting (HRM) analysis currently allow the differentiation of DNA fragments obtained in qPCR even in single base differences. In the present study, we explored the potential of three region-based qPCR-based HRM analysis for differentiation of food-derived yeast isolates. The three targeted fragments (26S rDNA, 18S rDNA, ITS) were carefully analyzed to dock the primers at inter-species conservative regions, flanking polymorphic regions of ~ 200 bp. Thirty eight yeast species, including own isolates and reference, type strains, were used as a training material. The collection of yeast strains spanned *Pichia*, *Clavispora*, *Candida*, *Yarrowia*, *Kluyveromyces*, *Saccharomyces*, and *Wickerhamomyces*. To reliably assess the HRM technique potential, we cross-validated the identification results by conventional Sanger sequencing and mass spectrometry of total proteins of the yeast isolates. Conducted studies demonstrated that considering only a single qPCR-HRM region renders misleading clustering and taxonomic classification. Combined analysis of the three proposed here regions resulted in adequate clustering at species level resolution.

S6_P2 APOPTOSIS AND MITOCHONDRIAL DOMESTICATION: CO-EVOLUTION?

Vandana Kaushal*, Joanna Klim, Urszula Zielenkiewicz, Anna Kurlandzka, Szymon Kaczanowski

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland
*vandana@ibb.waw.pl

Apoptosis, a common type of programmed cell death, is a tool that allows cells to self-destruct when stimulated by the appropriate trigger. In addition to higher eukaryotes, it's also reported in unicellular eukaryotes as well as prokaryotes. We aim to experimentally verify that the apoptotic machinery is a primeval adaptation acquired during mitochondrial domestication. *Saccharomyces* Genome Database as well as literature search suggested role of four apoptotic proteins i.e., protease metacaspase MCA1, nuclease NUC1, and apoptosis-inducing factor NDI1 and HTRA/Omi protease NMA111 in core yeast apoptotic machinery. These genes were used for the study as their deletion decreases apoptotic activity while overexpression induces apoptosis. We plan to test the retention of ancient functions of these gene orthologs across the various kingdoms i.e., bacteria, protists, plants, and animals etc. in yeast by their ability to replace their yeast orthologs. The codon-optimized orthologs were commercially synthesized and were cloned in front of CPS1 terminator of self-constructed common vector. Yeast mutants containing ortholog replacements were created using homologous recombination strategy. Furthermore, GFP tagged version of these orthologs were constructed and were used to verify the expression and localisation of foreign genes in the yeast. Fluorescence microscopy analysis shows that the fusion proteins for NDI1, NUC1, MCA1 as well as NMA111 localized to respective compartments which some leakages. The proper expression and size of GFP tagged proteins was confirmed by western blot. The recombination mutants were further tested to check functional complementation using drop tests as well as Annexin-V/PI co-staining assay after apoptotic induction with acetic acid. All the selected orthologs of *NDI1* were able to significantly revert the sensitivity to apoptotic induction while for rest of genes, at least one of the ortholog had complementation effect.

S6_P3 LIMITED IMPACT OF GENETIC INTERACTION NETWORK ON THE EVOLUTIONARY TRAJECTORIES IN YEAST *S. CEREVISIAE*

Joanna Klim*, Urszula Zielenkiewicz, Anna Kurlandzka, Szymon Kaczanowski

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland
*klim@ibb.waw.pl

The impact of genetic interaction networks on evolution is a key issue. Previous studies have demonstrated that functionally related genes frequently interact with one another, and they establish functional modules, i.e., groups of genes involved in the same biological process. We experimentally tested the hypothesis that compensatory evolutionary modifications, such as mutations and transcriptional changes, occur frequently in genes from perturbed modules of interacting genes using *S. cerevisiae* as a model. We investigated modules lacking *COG7* or *NUP133*. Strains lacking each of these genes were subjected to experimental evolution in continuous cultures and the evolved populations were examined at the genomic and transcriptomic levels. It was found that for both functional modules: genetic interactions, the modular structure of genetic networks, and the adaptive landscape described by the genetic interaction network did not have a significant impact on the process of evolution of yeast populations after gene deletion (Klim *et al*., 2021). In fact, most of the gene inactivations were predicted to be neutral. Similarly, transcriptome changes mostly signified adaptation to growth conditions rather than compensation for the absence of the tested genes. Our findings show that modular structure of the network and the genetic interactions described by others have very limited effects on the evolutionary trajectory following gene deletion of module elements in tested experimental conditions and have no significant impact on short-term compensatory evolution. Interestingly, we identified a few genes that were mutated more than once across all yeast populations. Mutations in these genes may be beneficial in our experimental conditions, regardless of genetic background. The issue of potential driver mutations is subjected to further research.

Klim J. *et al.* BMC Ecol Evo. 2021, 21, 99

The work was supported by the Polish National Science Center: 2014/13/B/NZ8/04719, 2018/29/N/NZ2/00902.

S6_P4 THE LAG PHASE LENGTH AS A MEASURE OF POPULATION FITNESS

Monika Opałek[1*], Bogna Smug[2], Dominika Włoch-Salamon[1]

[1]*Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University*
[2]*Małopolska Centre of Biotechnology, Jagiellonian University*
[*]*monika.opalek@doctoral.uj.edu.pl*

The lag phase is the first one of four typically recognized phases of the unicellular microbial growth curve. The growth kinetic measured as a change in population density in time, can be represented by a sigmoid curve with the following phases: (1) the lag phase, when cells adjust to a new environment; (2) the exponentially growing phase (or log/logarithmically), when cells divide with a maximum rate and their density doubles at each doubling time, (3) the stationary phase, when cells cease divisions due to nutrients depletion, and if the measurements are conducted long enough, the fourth, (4) decline/death phase can be observed, where population's density drops due to the cell death.

Lag phase length not only can be used as a fitness parameter for basic science but is also an important parameter in industrial microbiology. However, calculating lags may be challenging as it is method and parameter-dependent. The most frequently used methods to determine lag duration are: (1) based on max growth acceleration, where a point of the growth curve where the second derivative is maximal is identified; (2) exponential method, where the intersection of the initial density line and the tangent line to the growth curve at the maximal uptake rate is identified; (3) based on biomass increase, where an increase in biomass (or absorbance) of a certain value is identified.

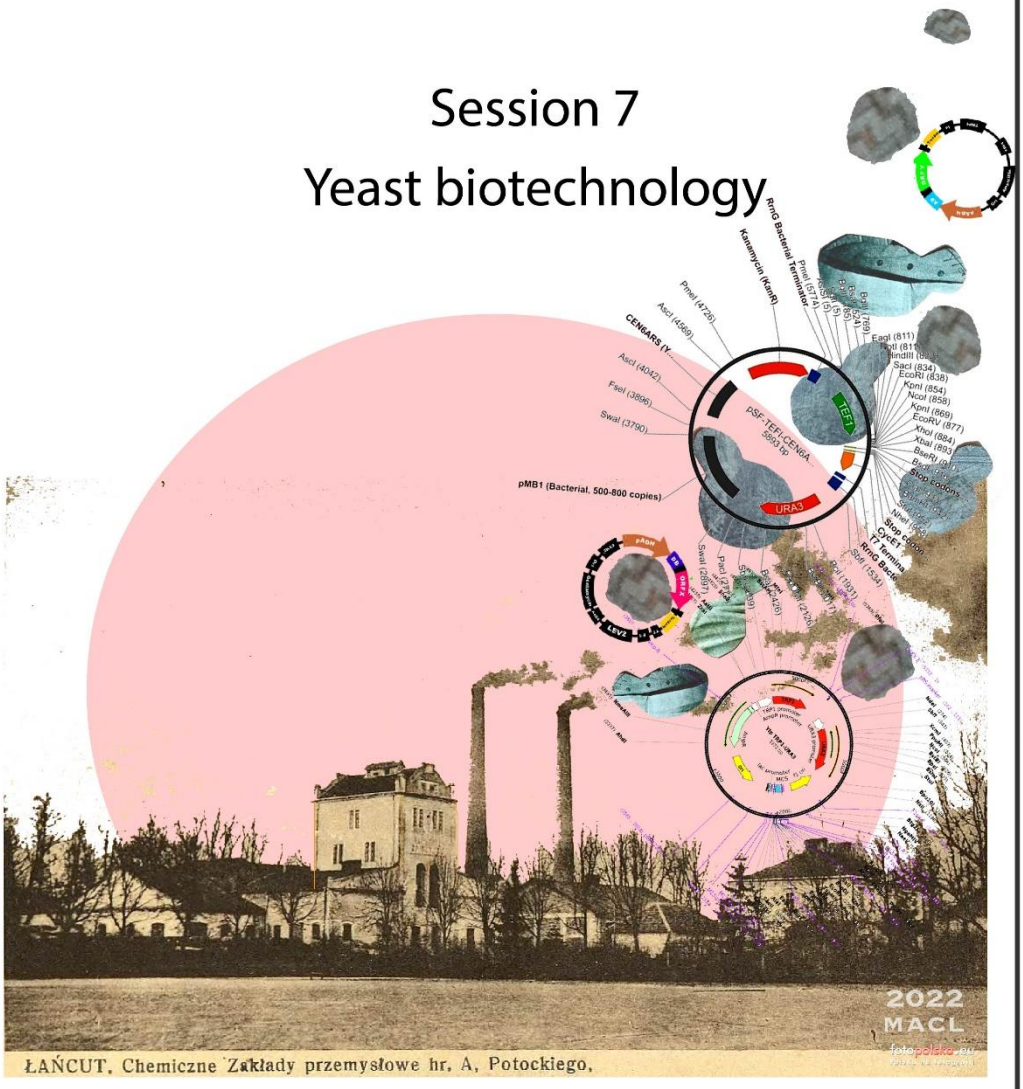
During the conference, I will present a web server that we developed to help experimental biologists to calculate lags and visualize possible discrepancies between methods.

The microbial lag phase length calculator is freely accessible under the following address: https://microbialgrowth.shinyapps.io/lag_calulator/

Acknowledgements:
The project is supported by National Science Centre (OPUS project to DWS, No.2017/25/B/NZ8/01035), Jagiellonian University (Minigrant POB BioS project to BS, PSP U1U/P03/NO/03.32, UJ), Faculty of Biology JU (projects N18/DBS/000003 and MNW/000013 to MO and DWS) and by the programme "Excellence Initiative–Research University" at the Jagiellonian University in Kraków, Poland."

Session 7

Yeast biotechnology



Oral presentations

S7_O1 PROLINE NEW SCIENCE AND TECHNOLOGY IN YEAST

Hiroshi Takagi*

Division of Biological Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan
*hiro@bs.naist.jp

In the yeast *Saccharomyces cerevisiae*, proline (Pro) is a pivotal and multifunctional amino acid that is used not only as a nitrogen source but also as a stress protectant and energy source. Therefore, Pro metabolism is known to be important in maintaining cellular homeostasis. The metabolic regulation of Pro, including its biosynthesis, cellular localization, transport, and degradation, is thus of great interest. We recently observed that intracellular Pro regulates the replicative lifespan of *S. cerevisiae*. The deletion of *PUT1*, which encodes the Pro oxidase Put1, and the expression of *PRO1*^{I150T}, which encodes the Ile150Thr variant of γ-glutamyl kinase, resulted in the accumulation of Pro and extended the lifespan of yeast cells. Inversely, we discovered that Put1-catalyzed Pro oxidation controls the chronological lifespan of *S. cerevisiae*. Pro metabolism may thus have a physiological role in maintaining the lifespan of yeast cells.

Pro is a predominant amino acid in grape must, but it is poorly utilized by *S. cerevisiae* in wine-making processes. This sometimes leads to a nitrogen deficiency during fermentation and Pro accumulation in wine. Although the presence of other nitrogen sources under fermentation conditions is likely to interfere with Pro utilization, the inhibitory mechanisms of Pro utilization remain unclear. We first constructed a Pro auxotrophic yeast strain and identified an inhibitory factor by observing the growth of cells when Pro was present as a sole nitrogen source. Intriguingly, we found that Arginine (Arg), and not ammonium ion, clearly inhibited the growth of Pro auxotrophic cells. In addition, Arg prevented the Pro consumption of wild-type and Pro auxotrophic cells, indicating that Arg is an inhibitory factor of Pro utilization in yeast. Next, quantitative PCR analysis showed that Arg partially repressed the expression of genes involved in Pro degradation and uptake. We then observed that Arg induced the endocytosis of the Pro transporters Put4 and Gap1, whereas ammonium induced the endocytosis of only Gap1. Furthermore, we propose a possible mechanism mediated by the ubiquitin ligase Rsp5 and its adaptor protein, Art3. We found that the ubiquitination activity of Rsp5 was essential for the Arg-induced endocytosis of Put4. Because Put4 contains no Rsp5-binding motif, we next screened an adaptor protein that plays a role in the Arg-induced endocytosis of Put4. Our genetic and biochemical analyses clearly revealed that the *ART3* gene-disrupted cells were defective in Put4 endocytosis, indicating that Art3 is a key regulator for Put4 endocytosis. More importantly, we discovered that deletion of *ART3* remarkably canceled the inhibitory effects of Arg on Pro utilization.

Hence, our results may involve an important mechanism for Arg-mediated inhibition of Pro utilization in yeast. The breeding of wine yeast strains that can efficiently assimilate the abundant Pro in grape must could be promising for the improvement of wine quality.

A. Nishimura *et al*., ***Microorganisms***, **9**, 1650 (2021).
A. Nishimura *et al*., ***Yeast***, **37**, 531-540 (2020).
A. Nishimura *et al*., ***Biochem. Biophys. Res. Commun.***, **531**, 416-421 (2020).

I am grateful to Drs. Akira Nishimura and Ryoya Tanahashi in my lab. This research was in part supported by grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (Research program on development of innovative technology).

S7_O2 PRODUCTION OF THE BACTERIAL ANTIBIOTICS ROSEOFLAVIN AND AMINORIBOFLAVIN BY RECOMBINANT STRAINS OF THE YEASTS *CANDIDA FAMATA* AND *KOMAGATAELLA PHAFFII*

Dmytruk K.V.[1], Ruchala J.[2], **Dmytruk O.V.[1,2]***, Tsyrulnyk A.O.[1], Andreieva Y.A.[1], Fedorovych D.V.[1], Chrzanowski G.[2], Motyka O.I.[3], Fayura L.R.[1], Mattanovich D.[4], Marx H.[4], Sibirny A.A.[1,2]

[1]*Institute of Cell Biology National Academy of Sciences of Ukraine, Drahomanov St, 14/16, Lviv, 79005, Ukraine*
[2]*University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland*
[3]*Research Institute of Epidemiology and Hygiene of the Danylo Halytsky Lviv National Medical University, Zelena St, 12, 79005, Lviv, Ukraine*
[4]*BOKU University of Natural Resources and Life Sciences, Muthgasse 18, 1190, Vienna, Austria*
*dmytruk77@gmail.com

Soil actinomycetes *Streptomyces davaonensis* and *Streptomyces cinnabarinus* synthesize antibiotic roseoflavin (RoF), the natural analog of riboflavin. RoF synthesis starts from flavin mononucleotide and proceeds through immediate precursor aminoriboflavin (AF), which also possesses antibiotic properties. Both flavin antibiotics strongly inhibit growth of many Gram-positive bacteria, including pathogenic ones. RoF accumulation by the natural producers is rather low while AF accumulation is negligible. Yeasts have many advantages as biotechnological producers relative to bacteria, however, no recombinant producers of bacterial antibiotics in yeasts are known. We possess riboflavin and flavin mononucleotide overproducers of the flavinogenic yeast *Candida famata* and riboflavin overproducers of *Komagataella phaffii (Pichia pastoris)* which were used as hosts for construction of the producers of flavin antibiotics. Synthetic genes *rosB, rosC* and *rosA* with adapted codons for both yeast species, which encode enzymes of RoF synthesis, have been expressed in *C. famata* and *K. phaffii*. Additionally, in riboflavin overproducers, yeast gene *FMN1* coding for riboflavin kinase, was overexpressed. Resulted both *C. famata* and *K. phaffii* yeast transformants accumulated AF (overexpression of *FMN1* and *rosB*) or RoF (overexpression of *FMN1, rosB, rosC* and *rosA*). The structure of accumulated antibiotics was confirmed by ultra-HPLC and mass spectrometry. AF isolated from cultural liquid of *C. famata* transformant inhibited growth of *Staphylococcus aureus* (including MRSA) and *Listeria monocytogenes*. Accumulation of RoF in *K. phaffii* in bioreactor reached 129 mg/L which exceeded published data on accumulation of this antibiotic by the recombinant strain of the native producer, *S. davaonensis*.

This study was supported by National Research Foundation of Ukraine grant 2020.01/0090

S7_O3 METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* YEAST TO POLY(ETHYLENE TEREPHTHALATE) DEGRADATION

Katarzyna Ewa Kosiorowska*, Aleksandra Maria Mirończuk

Wrocław University of Environmental and Life Sciences, Department of Biotechnology and Food Microbiology, Chełmońskiego 37, 51-630, Wrocław, Poland
*katarzyna.kosiorowska@upwr.edu.pl

Poly(ethylene terephthalate) (PET) is one of the world's most widely used plastic material with wide range of usage eg. packaging, construction and automobile industry. PET is classified as a non-biodegradable polymer, whose large accumulation in the environment cause harmful problem towards our ecosystems. To the date, the enzymes from hydrolase class such as cutinases, lipases and PETase were classified as capable to hydrolyse ester bonds present in PET. In this work we have used *Yarrowia lipolytica* yeast strain as a host organism to extracellular production of cutinase from *Fusarium solani* pisi and PETase from *Ideonella sakaiensis*. Due to unordinary capabilities of this unconventional yeast to assimilate atypical carbon sources such as alkane, can grow in the high pH range, and large production of native lipases we have performed plastic degradation studies directly in the shake flask cultures with the modified strains. In our work we investigated the capability to PET degradation during cultivation, furthermore, we have checked supplementation effect on the degradation efficiency with the use of various salts and olive oil at different concentrations. PET plastic material used in this study were not prior pre-treated. We have determined the plastic degradation capacity by the amount of released PET hydrolysis products such as terephthalic acid (TPA), mono-(2-hydroxyethyl) terephthalic acid (MHET) and with the use of ultraperformance liquid chromatography (UPLC). In the present study, we also tested the ability to assimilate terminal PET degradation products by *Y. lipolytica* and compared the ability to hydrolyze MHET by modified strains producing cutinase and PETase. In addition, we have verified changes in the structure of the PET films incubated for 28 days in the cultures by using scanning electron microscopy (SEM). Analysis of the PET surface structure revealed numerous cracks and pits.

This work was financially supported by the National Science Centre, Poland, project UMO-2017/27/B/NZ9/02218.

S7_O4 THE EFFECT OF CARBON SOURCE, AERATION AND PH CONTROL ON L-LACTIC ACID PRODUCTION BY METHYLOTROPHIC YEAST *OGATAEA POLYMORPHA*

Aksyniia Tsaruk[1*], Roksolana Vasylyshyn [1], Andriy Sibirny[1,2]

[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology of National Academy of Sciences of Ukraine, Drahomanova St 14/16, Lviv, 79005, Ukraine
[2]Department of Microbiology and Biotechnology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601, Poland
[*]aksyniiatsaruk@gmail.com

Global demand for L-lactic acid rapidly increases activating research in this field. L-lactic acid is an industrially important chemical compound with numerous applications in food, medical, pharmaceutical industry and also is used for production of renewable biodegradable biopolymer poly-lactic acid as an alternative to petroleum-based plastics. Current industrial production of lactate is mainly carried out by lactic acid bacteria, however implementing genetically modified yeast as lactic acid producers has several important advantages. The methylotrophic yeast *Ogataea polymorpha* is generally considered as a robust microorganism due to its tolerance to high temperatures and low pH. This yeast is also able to efficiently metabolize two main lignocellulosic sugars, glucose and xylose. Lignocellulose is a low-cost, abundant and renewable material for production of high-value compounds that could also be successfully utilized for lactate biosynthesis. *O. polymorpha* naturally does not possess the lactate dehydrogenase (LDH) that catalyzes the reduction of pyruvate to L-lactic acid but it could be introduced from other organisms (bacteria, fungi, protists and mammals).
In this study, the alcohol fermentation pathway of wild type strain of the yeast *O. polymorpha* was successfully altered by introduction of lactate dehydrogenase gene. For this purpose, *LDH* gene derived from filamentous fungus *Rhizopus oryzae* was selected. It was placed under the control of the strong constitutive promotor of glyceraldehyde-3-phosphate dehydrogenase gene. *O. polymorpha* strain was transformed by electroporation procedure, and the transformants were selected on YPD medium containing nourseothricin. The target gene integration in transformants was confirmed by PCR. Selection of strains for fermentation process was carried out on YDP medium containing 0.002% of bromophenol blue (BPB) which is a pH indicator that changes color from yellow at pH 3 to purple at pH 4.6. Plates were inoculated and incubated at 37^{o}C for 24 hours. *O. polymorpha* transformants that developed distinct yellow color around its colony on YPD with BPB medium due to biosynthesis of lactic acid was selected for further investigation. The effect of carbon source, aeration and presence of neutralizing agent on production of L-lactic acid were investigated. Engineered *O. polymorpha* strain demonstrated higher level of lactate production on minimal medium with 10% glucose compared to minimal medium with 10% xylose. It has been suggested that lactate production requires a lot of ATP because it is consumed largely to energize the efflux of lactic acid out of the cell. Our data has shown that cell aeration has a positive impact on lactic acid production. Lactic acid accumulation causes cellular toxicity making neutralization of the medium during fermentation necessary for efficient L-lactic acid production. The $CaCO_3$ was

used as a neutralizing agent and it was shown to effectively stabilize the pH and increase lactic acid production.

S7_O5 CONSTRUCTION OF THE HUMANIZED STRAINS OF *KOMAGATAELLA PHAFFII* PRODUCING INTRACELLULAR, SECRETED AND SURFACE DISPLAYED SARS-COV-2 ANTIGENS AS POTENTIAL VACCINES AGAINST COVID-19

[1,2*]Sibirny A.A., [2]Semkiv M.V., [2]Dmytruk O.V., [2]Vasylyshyn R.V., [2]Fayura L.R., [2]Zazulia A.Z., [3]Gaffke L., [3]Podlacha M, [3]Cyske Z, [3]Pierzynowska K, [1]Ruchała J., [3]Węgrzyn G., [2]Dmytruk K.V.
[1]*University of Rzeszów, Poland*
[2]*Institute of Cell Biology, NAS of Ukraine, Lviv*
[3]*Gdansk University, Poland*
[*]*sibirny@cellbiol.lviv.ua*

Currently, many vaccines against coronavirus SARS-CoV-2 have been developed or are in the development. One of the promising vaccines could be the inactivated microbial cells with surface displayed viral antigens. Thus, it was proposed to use genome reduced *Escherichia coli* with surface-expressed SARS-CoV-2 fusion peptide and attenuated *Francisella tularensis* which expressed coronavirus M and N proteins as vaccines (Maeda et al., 2021; Jia et al. 2021). In similar way, experiments have been started on the yeast *Saccharomyces cerevisiae* with surface displayed RBD domain of S protein (Gao et al., 2021). Such vaccines could be used orally or as spray. However, bacteria do not process glycosylated proteins whereas *S. cerevisiae* is characterized by fungal processing resulted in hyperglycosylation. We work with humanized strains of the methylotrophic yeasts *Komagataella phaffii* (*Pichia pastoris*) and *Ogataea polymorpha* with human type of protein glycosylation. In the first round of experiments, we constructed *K. phaffii* and *O. polymorpha* strains producing intracellular and secreted versions of RBD under control of alcohol oxidase promoter *AOX1*p induced by methanol. Besides, yeast strains producing fusion peptide and two heptad repeats of the spike protein as well as strains producing putative virus-like particles have been constructed. The aim of the current study was to construct *K. phaffii* strains with surface displayed RBD fragment of SARS-CoV-2 spike protein. DNA sequence of RBD protein with *K. phaffii* adapted codons have been designed and synthesized chemically. Then it was expressed under control of *AOX1* promoter. Binding of RBD to outer surface of yeast cell was achieved using signaling sequence of α-factor of *S. cerevisiae* at the 5'-terminus whereas sequence of *S. cerevisiae SAG1* gene was localized at the 3'terminus of the RBD protein. Induction of RBD protein synthesis was achieved by incubation of glycerol-grown cells in the medium with methanol. Surface displayed RBD localization in the methanol-induced cells was proved using double immunolabeling with anti-RBD and secondary fluorescent antibodies and by western blotting. In similar way, surface displayed RBD protein was obtained for probiotic yeast *Saccharomyces boulardii*. In this case, expression of RBD was achieved using constitutive *TEF1* promoter of *S. cerevisiae*. Intraperitoneal administration to mice of the purified intracellular or secreted RBD protein, fusion peptide and two heptad repeats fragment, putative virus-like particles were highly immunogenic while produced serum exerted efficient neutralization of spike protein binding with ACE2 receptor. The same results on high immunogenicity and neutralization activity were obtained after oral administration of *K. phaffii* cells with surface displayed RBD which were inactivated by 10 min heating at 60^{o}C. Future prospects of current device for development of the efficient anti-COVID vaccine will be discussed.

Gao et al., Microb Cell Fact. 2021 May 5;20(1):95. Jia et al., NPJ Vaccines. 2021 Mar 30;6(1):47. Maeda et al., Proc Natl Acad Sci U S A. 2021 May 4;118(18):e2025622118.

This study was partially supported by National Research Foundation of Ukraine, grant 2020.01/0080.

S7_O6 OMICS-GUIDED ENGINEERING OF SECRETORY PATHWAY FOR ENHANCED SYNTHESIS OF SECRETORY PROTEINS IN *YARROWIA LIPOLYTICA*

Paulina Korpys-Woźniak, **Ewelina Celińska***

Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, 60-637 Poznań, Poland
*ewelina.celinska@up.poznan.pl

Synthesis and secretion of proteins is a complex biological process, involving multiple molecular functions and spanning many cellular compartments. Due to this complexity, fishing out limiting steps (which differ depending on the target protein and synthesis rate) is very challenging. Comprehensive description of molecular events taking place in a cell, as provided by omics studies, greatly assists such search.

In the present study, we used global transcriptomics data, describing molecular processes taking place in *Yarrowia lipolytica* cells under over-synthesis of biochemically different proteins, as guidelines for designing a secretory pathway engineering strategy. Previously identified differentially-expressed-genes (DEGs) were used here as "secretory helpers" (SHs), co-over-expressed with the target recombinant proteins (r-Prots). Twelve SHs, functionally spanning the whole translation-translocation-folding-maturation-excretion pipeline, were analyzed. The genes were co-transformed with an easy-to-track reporter, and tested under two temperatures.

Our results indicated clear distinction in the effects triggered by the SHs natively operating in SYNTHESIS or TRANSPORTATION of the polypeptides. The former group (*i.a. RPL3, SSA5, SSA8*) significantly enhanced synthesis of the protein irrespective of the culturing temperature, however, for efficient secretion the secretory pathway's capacity must have been released by applying decreased temperature (25◦C). Exploitation of the genes involved in protein trafficking (like *SSO1, CWP11*) as the SHs, did not give such spectacular results in terms of the amounts of the target polypeptide, however, their overexpression allowed to assist the secretory pathway in maintaining its capacity under not favorable thermal conditions. Presumably, what underlies such relationship between the secretory pathway's capacity and the temperature, is either increased or decreased rate of transcription; which, if excessive - induces stress in the secretory pathway, and causes loss in the final product; and if balanced - gives sufficient capacity to the folding machinery to correctly process the nascent polypeptides. Our results suggest that under decreased temperature, the biological processes of transcription and translation are balanced, as the amounts of transcripts and protein products were then highly correlated.

This study provides generalizable guidelines to be applied in cloning/culturing strategies aiming at enhancement of heterologous protein secretion in *Y. lipolytica*.

Funding: *Ministry of Education and Science, DI 2017 000 947*

S7_O7 ERYTHRITOL METABOLISM: FROM FUNDAMENTAL RESEARCH TO BIOTECH APPLICATIONS

Patrick Fickers*

Microbial Processes and Interactions, TERRA Teaching and Research Centre, University of Liège - Gembloux Agro-Bio Tech, Belgium
*pfickers@uliege.be

Y. lipolytica is a non-conventional yeast, well-known for its unusual metabolic properties. Based on its ability to secrete high amounts of proteins and metabolites of biotechnological interest, *Y. lipolytica* has several industrial applications, including heterologous protein synthesis or organic acid production. Under osmotic stress, the yeast produces erythritol, a four-carbon sugar alcohol. As this metabolite was found embed with sweetening properties, applications as food additive have been developed. Here, a metabolic strategy yielding to strains with increased erythritol production will be detailed together with a bioconversion process leading to erythrulose, the first intermediate of erythritol catabolism. From erythritol metabolism, we developed new efficient promoters and recipient strains that could be used for recombinant protein production and metabolic engineering. As a case study, the lipase CalB from *Candida antartica* was cloned under the control of the best-developed promoter. The lipase titer obtained in bioreactor was compared to those obtained in *P. pastoris*, another well know cell factory.

S7_O8 *YARROWIA LIPOLYTICA* AS A PLATFORM FOR PRODUCTION OF TAILOR-MADE LIPIDS.

Milan Čertík*, Peter Gajdoš, Jaroslav Hambalko, Veronika Urbaníková

Institute of Biotechnology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic
milan.certik@stuba.sk

Yarrowia lipolytica is a dimorphic oleaginous yeast and is considered as an attractive host for aimed re-design of lipid metabolic pathway. Apart from its natural ability to accumulate a high amount of lipid in cells during utilization of cheap carbon substrates, several metabolic engineering strategies have been employed for not only increasing the lipid content but also for production of various lipid structures. Genes coding biosynthesis of unusual lipid structures in plants and insects have been characterized and codon-usage optimized for *Y. lipolytica*. Overexpression of *FAE1* gene coding for fatty acid elongation involved in biosynthesis of very long chain fatty acids (VLCFA) from field pennycress (*Thlaspi arvense*) in *Y. lipolytica* possessing *fad2* deletion led to accumulation of erucic acid (22:1ω9, EA) and VLCFAs in the yeast lipids, exclusively in triacylglycerols (Gajdoš et al, 2020a). Construction of strain with strong promoter and mutation in beta-oxidation resulted in 7.6 times improved VLCFA biosynthesis in *Y. lipolytica*. Introduction of *EeDAcT* gene from *Euonymus europaeus* (coding for diacylglycerol acetyltransferase catalyzing formation of 3-acetyl 1,2-diacylglycerol - acDAG) in *Y. lipolytica* caused formation of acDAG in the yeast intracellular lipids (Gajdoš et al, 2020b). Interestingly, acDAG was accumulated in all strains regardless to their possibility to either form or not form storage lipid particles. Expression of *MsexD2* and *MsexD3* genes from tobacco hornworm moth (*Manduca sexta*) which encode two fatty acid desaturases, resulted in biosynthesis of mainly C16:1Δ^{11} fatty acid (11-14% of total fatty acids) in *Y. lipolytica*. Production of other new fatty acids C14:1Δ^{11} and C18:1Δ^{13} were also observed in trace amounts. Media supplementation with precursors C16:1Δ^{11} and C16:2$\Delta^{10,12}$ led not only to formation of C16:2$\Delta^{10,12}$ and C16:3$\Delta^{10,12,14}$, but also new C18 fatty acids with two and three double bonds were detected. Accumulation of long chain alcohols in *Y. lipolytica* expressing *BlucFar1* and *BlapFar4* bumblebee fatty acid reductase genes was also confirmed (Hambalko J. et al., 2021). However, the strain with *BlucFar1* gene formed C18, C20, C22, C24 fatty alcohols, while C16 alcohols were observed in strain with *BlapFar4* gene. *Y. lipolytica* was able to biosynthesize 4% of conjugated punicic acid (PuA) in intracellular lipids when *FADX* gene from pomegranate (*Punica granatum*) coding for biosynthesis of PuA was introduced to the yeast. Thus, oleaginous yeast *Y. lipolytica* with rational metabolic engineered strategies showed a great potential for production of various tailor-made lipid structures with their several applications in pharmaceutical, nutritional and industrial fields.

Gajdoš P. et al. FEMS Microbiol. Letters, 2020a, 367(6): fnaa042
Gajdoš P. et al. Yeast 2020b, 37(1): 141-147
Hambalko J. et al. Frontiers in Bioeng. Biotechnol., 2021, 8(1): article No. 593419

The work was supported by grants APVV-17-0262, APVV-20-0166 and VEGA 2-0012-20.

S7_O9 MOLECULAR MECHANISM OF POLYOLS ASSIMILATION BY YEAST *YARROWIA LIPOLYTICA*

Mateusz Szczepańczyk[*], Aleksandra M. Mirończuk

Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland
[*]*mateusz.szczepanczyk@upwr.edu.pl*

Yarrowia lipolytica is an oleaginous yeast commonly used in lipid and polyol production. Despite the years of research of the molecular pathways resulting in polyol synthesis, there are still gaps of knowledge. Most of the enzymes involved in this process are well described, while some are still under investigation. One of the main issues regarding the molecular mechanism of polyol assimilation is the lack of characterized polyol transporters. The aim of this study was to identify the potential polyol transporters based on the analysis of changes in gene expression and further genetic engineering of the modified strains. The transporters selected for further investigations were chosen based on the *in silico* and literature analysis. Strains used in the experimental phase (wild type, modified strains and strain after adaptive laboratory evolution (ALE)) were grown on media with different polyols as a sole carbon source for further analysis. The results indicate the intricate interplay between the polyol synthesis pathways and the compound transport.

S7_O10 THERMOTOLERANT YEAST *OGATAEA POLYMORPHA* AS PROMISING PRODUCER OF THE SECOND GENERATION ETHANOL

Justyna Ruchała[1*], Olena Kurylenko[2], Roksolana Vasylyshyn[2], Marta Semkiv[2], Olena Dmytruk[1], Aksyniia Tsaruk[2], Anastasiya Zazulia[2] , Kostyantyn Dmytruk[2], Andriy Sibirny[1,2]

[1]Instititute of Biology and Biotechnology, University of Rzeszów, Pigonia 1, 24-310 Rzeszów, Poland

[2]Institute of Cell Biology, National Academy of Science of Ukraine, Lviv 79005, Ukraine

[*]jruchala@ur.edu.pl

Development of the feasible process for biofuel production from lignocellulose is very important task of modern microbiology and biotechnology. Thermotolerant yeast *Ogataea polymorpha* is the promising organism for further development as it robustly grows and ferments glucose and xylose at 45-50^{o}C which could be applicable for simultaneous saccharification and fermentation.

Xylose is the main pentose sugar released from the lignocellulosic biomass, which has a high potential as a renewable feedstock for bioethanol production. The thermotolerant yeast *Ogataea (Hansenula) polymorpha*, in contrast to *Saccharomyces cerevisiae*, is able to metabolize and ferment not only glucose but also xylose. Using combination of the methods of metabolic engineering (deletion of *CAT8* gene, overexpression of *XYL1*, *XYL2*, *XYL3*, *DAS1* and *TAL2* genes) and classical selection, mutant strains of *O. polymorpha* have been isolated which accumulated near 16 g/L of ethanol form xylose at 45 ^{o}C (Kurylenko et al., 2014; 2018; Ruchala et al., 2017). Increase in ethanol production from xylose was achieved due to deletion of *HAP4-A* and *TUP1* genes whereas deletion of both *MIG1* and *MIG2* reduced the amount of ethanol produced from both glucose and xylose (Kurylenko et al. 2021). To overcome glucose inhibition of xylose utilization, native Hxt1 transporter of *O. polymorpha* has been engineered and overexpressed (Vasylyshyn et al., 2020).

Here we showed the important role of transcription factor Azf1 and sugar sensor Hxs1 in xylose alcoholic fermentation. Deletion of both *AZF1* and *HXS1* genes strongly reduced ethanol production from xylose whereas overexpression of both genes activated alcoholic fermentation of this pentose. Glucose fermentation in *hxs1Δ* deletion mutant also was strongly reduced. Accumulated data for the first time proved the role of sugar sensor in xylose fermentation in the natural xylose-metabolizing yeasts. Biotechnological potential of these findings will be discussed.

S7_O11 CO-EXPRESSION OF SELECTED TRANSCRIPTION FACTORS MODULATES SYNTHESIS OF HETEROLOGOUS PROTEINS IN *YARROWIA LIPOLYTICA* UNDER STRESS CONDITIONS

Maria Gorczyca[1*], Wojciech Białas[1], Jean-Marc Nicaud[2], Ewelina Celińska[1]

[1]*Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, 60-637 Poznań, Poland*
[2]*INRAE, AgroParisTech, Micalis Institute, Université Paris-Saclay, 78350 Jouy-en-Josas, France*
[*]*maria.gorczyca@up.poznan.pl*

Heterologous protein synthesis and stress response are both complex and inter-connected biological processes. Due to their complexity, effective fine-tuning of these processes can be executed only by modifying multiple targets. This can be done by manipulation with master regulators of biological processes – transcription factors (TFs). While *Yarrowia lipolytica* is a widely recognized yeast protein production platform, TFs implicated in stress response or recombinant protein (r-Prot) synthesis are still not well described.

The aim of this study was to examine potential implications of selected putative TFs in stress resistance and/or r-Prot synthesis in this yeast. The adopted strategy relied on co-over-expression (co-OE) of one of the TFs and a reporter r-Prot followed by phenotype studies under different environmental conditions. It was presumed that such an approach will further manipulate the activation status of the TF. The second goal was to evaluate this approach as a rationale engineering strategy to enhance stress resistance and/or r-Prot synthesis in *Y. lipolytica*.

Four TFs were selected for these experiments: two of unknown function, and two already annotated to biological processes. The co-OE'ing strains and a control were cultured under a combination of four environmental conditions at several levels: pH, oxygen availability (OA), temperature and osmotic stress. Growth and fluorescence from the reporter protein (expressed as total and specific FL) were read in time-intervals. Data analysis was assisted by mathematical modelling according to Response Surface Methodology (RSM). In addition, the strains' resistance to oxidative stress was analyzed in drop-tests, by growing them on plates with different oxidative agents.

The results demonstrated that, depending on the tested TFs, the total r-Prot yield could be increased by more than 2-fold. Furthermore, we noted specific implications of different TFs in alleviating growth retardation induced under specific conditions. Surprisingly, sensitivity towards oxidative stress was significantly decreased upon synthesis of any r-Prot. One of the tested TFs showed potential implication in acquiring resistance to oxidative stress. Finally, we conclude that co-OE of a carefully selected TF can be used as a strategy to enhance r-Prot production in *Y. lipolytica*.

Funding: *NCN OPUS: 2021/41/B/NZ9/00086*

S7_O12 COLD-ADAPTED YEASTS – THE SOURCE OF VALUABLE BIOMOLECULES

Marcin Sypka*, Aneta M. Białkowska

Institute of Molecular and Industrial Biotechnology, Łódź University of Technology, Stefanowskiego 2/22, 90-537, Łódź, Poland
*marcin.sypka@dokt.p.lodz.pl

The Earth's cold natural and anthropogenic environments are regarded as vast and mostly untapped source of extremophilic microbial life, with over 80% of our planet's ecosystems exhibiting temperatures below 5°C (Buzzini and Margesin 2014). Microorganisms that maintain structural integrity and normal functioning of a cell in low temperatures are known as psychrophilic or psychrotolerant - depending on their growth optima. Most commonly, psychrophiles exhibit an optimum growth temperature between 0°C and 20°C, while psychrotolerant microorganisms, though able to grow and function in such temperatures, have growth optima in the range from 20°C to 30°C (Buzzini and Margesin 2014). One particularly interesting group of extremophiles, due to their numerous biotechnological applications, is cold-adapted yeasts. As the result of an environmental pressure, these microorganisms developed many strategies to live and thrive in challenging conditions, often leading to the production of valuable biomolecules of industrial importance (Buzzini and Margesin 2014; Tsuji and Kudoh 2020). They are a potential source of enzymes, called psychrozymes, which offer novel opportunities for biotechnological exploitation based on their high catalytic activity at low temperatures and relatively low thermostability. Furthermore, they are able to synthesize antifreeze proteins (AFPs), cryoprotectant agents, exopolysaccharides, biosurfactants, pigments and other secondary metabolites (Tsuji and Kudoh 2020). During the course of the presentation, several groups of valuable biomolecules produced by cold-adapted yeast and their industrial significance will be discussed. Moreover, psychrophilic yeasts collection of the Institute of Molecular and Industrial Biotechnology, including species of *Cryptococcus*, *Rhodotorula, Gofeauzyma* and *Debaryomyces* genera and their representatives' biochemical profiles (Białkowska et al. 2017) will be presented.

Białkowska A.M. et. al. Polar Biol. 2017, 40(9):1787-1803
Buzzini P., Margesin R. (Eds.), Cold-adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance 2014, 1-549
Tsuji M., Kudoh S. Sustainability, 2020, 12(11):4518

Poster presentations

S7_P1 CELL ENGINEERING OF THE *KOMAGATAELLA PHAFFII* FOR CO-SUBSTRATE METABOLISM

Cristina Bustos[1,2*], Julio Berrios[1], Patrick Fickers[2]

[1]*School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085,Valparaíso 2362803, Chile*
[2]*Microbial Processes and Interactions, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Av. de la Faculté 2B, 5030 Gembloux, Belgium*
[*]*cv.bustos@student.uliege.be*

Komagataella phaffii (formerly known as *Pichia pastoris*) has become an increasingly important microorganism for recombinant protein production. This yeast species has gained high interest for the production of recombinant proteins. This has been facilitated principally by the use of a strong and tightly methanol-regulated promoter from the alcohol oxidase 1 (AOX1) gene. Even though the drawbacks associated with methanol utilization are related to its toxic cell effects, induction of cellular oxidative stress and its use arise high oxygen demand for catabolism. In operating systems based on the *pAOX1* expression, co-substrates such as sorbitol can be used to reserve methanol for the expression system's inducer. This non-conventional carbon source has no repressing effect on *pAOX1*, unlike glycerol or glucose. Sorbitol dehydrogenase (SDH) which is a NAD-dependent enzyme, oxidizes D-sorbitol into fructose that is subsequently converted into phospho-sugars. With the aim to improve sorbitol metabolism, SDH encoding gene was constitutively overexpressed in *K. phaffii* strain GS115 and the effect on both growth rate and biomass yield was investigated.

Acknowledge:
Agencia Nacional de Investigación y Desarrollo. Ministerio de Ciencia Tecnología e Innovación. Gobierno de Chile. Becas Doctorado Nacional grant number No. 21211138

S7_P2 THE QUEST OF LIGNINOLYTIC ENZYMES IN THE BIODIVERSITY AS NOVEL TOOLS FOR XENOBIOTIC DEGRADATION

Rémi Chausse[1*], Patrick Fickers[2], Vincent Phalip[3]

[1]*UMRT BioEcoAgro, University of Lille, University of Liege - Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Lille, France*
[2]*UMRT BioEcoAgro, University of Liege - Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Liege, Belgium*
[3]*UMRT BioEcoAgro, University of Lille, Lille, France*
[*]*remi.chausse@univ-lille.fr*

Xenobiotics generated by human activities are one of the major causes of environmental pollutions. In the context of the growing awareness on the environmental issue and the societal demand for greener and renewable processes, there is a need to find ways to stop the released of harmful compounds in nature. Like lignin these organics pollutants are very recalcitrant to degradation and are persistent in the environment causing threat to human health and wildlife (Arregui et al. 2019). The available processes of water treatment are not sufficient to get rid of these molecules and new methods need to be found. Ligninolytic enzymes has gathered a lot of attention during the past three decades. Three main types of enzymes are currently known to be ligninolytic: laccase, lignin peroxidases (LIP) and manganese peroxidases (MnP) (Wong 2009). These three groups display low substrate specificity which means they can operate on a wide array of different substrates. They initially target lignin components, but they can also degrade a wide panel of aromatic compounds such as xenobiotic, dyes and other pollutants (Dao et al. 2019) (Merino-Restrepo et al. 2020). That is the reason why research on the screening and detection of ligninolytic enzymes have generated much attraction, because of their tremendous applications in the valorisation of lignin and the bioremediation of polluted soils and waters. Enzymes represent thus one of the most promising fields of investigation for organic pollutants degradation. Their ability to operate under soft reactional conditions at moderate temperatures, pH and without the use of harmful solvents, garners the growing interest of researchers and industrials.

This present work aimed to find new enzymes from the biodiversity able to degrade xenobiotics such as bisphenol A (BPA) which is a well-known endocrine-disrupting compounds (EDCs) (Vos et al. 2000). Fungal stains were isolated from specific ecological niches and screened for the principal ligninolytic activities including laccase, Lip and MnP. Several environments were with different patterns of anthropic modifications were chosen to maximize the variety of isolated strains. The screening was carried out using different media with specific inductions to reveal the potential of each fungus. An Isolate belonging to the the complex species *Pseudallescheria boydii / Scedosporium apiospermum* was found with interesting laccase activity. Genome mining led to the identification of 9 putative laccase encoding genes.

One of them was cloned and the corresponding protein was produced with success in the dysmorphic yeast *Yarrowia lipolytica*. The recombinant protein was produced and semi-purified by ultrafiltration and its capacity to degrade bisphenol A was assed.

S7_P3 WHEY PERMEATE MANAGEMENT IN THE MICROBIOLOGICAL BIOSYNTHESIS OF VALUABLE COMPOUNDS COUPLED WITH FORMULATION OF PREBIOTICS CONTAINING YEAST BIOMASS

Karolina Drężek[*], Joanna Kozłowska, Paula Bardadyn, Joanna Żylińska-Urban, Jolanta Mierzejewska

Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology,00-664 Warsaw, Poland;
[*]*karolina.drezek@pw.edu.pl*

The dairy industry generates considerable waste with high COD (Chemical Oxygen Demand) and BOD (Biochemical Oxygen Demand) ratios, making them hazardous to the environment. The main is whey permeate (WP), rich in organic compounds, such as lactose and proteins. WP is generated during the production of whey protein concentrate after ultrafiltration. Its composition makes it a harmful waste to dispose of; however, it could be converted to a value-added product with an adequately selected microorganism (Belem & Lee, 1998). An example is the unconventional yeast species *Kluyveromyces marxianus*, which is capable of assimilating lactose, the main carbohydrate present in whey permeate.

The scientific objective of this work was an indication of possible management pathways for WP. Two independent approaches were tested. In the first, batch and continuous systems for natural 2-phenylethanol (2-PE) were developed. Under optimized conditions 8.29 ± 0.82 g of 2-PE was obtained (Drężek et al., 2021). The results also proved that the proposed idea of WP utilization makes it less harmful to the environment and significantly reduces its COD load. In the second approach, WP provided a substrate for bioethanol production. Herein, among other things, we examined the feasibility of using yeast biomass from the first bioprocess (2-PE biotransformation). We optimize ethanol synthesis at a maximum concentration of 54 g/L ethanol after 24 h of incubation at 30°C.

During the development of mentioned technologies, the aim was also to manage most of the process streams. As end products were isolated from culture broth, the biomass was a technical waste. Therefore, in this work, a solution that implies using inactive *K. marxianus* yeast as a culture medium component for lactic acid bacteria (LAB) was proposed. We succeeded in improving bacterial growth, confirming the validity of such an approach. Based on positive results, it may be suspected that most likely a synbiotic preparation consisting of both yeasts and bacteria can be introduced in the future. It could support the current probiotics used e.g. in fish farming (Kazuń et al., 2020).

Belem, M.A.; Lee, B.H. Crit. Rev. Food Sci. Nutr. 1998, 38(7): 565-98.
Drężek, K. et al. Molecules. 2021, 26, 7388. https://doi.org/10.3390/molecules26237388.
Kazuń, B. et al. Pol J Vet Sci. 2020, 23(4): 611-618. DOI 10.24425/pjvs.2020.135808

S7_P4 ENGINEERING *YARROWIA LIPOLYTICA* FOR C22 FATTY ACIDS PRODUCTION

Peter Gajdoš[*], Veronika Urbaníková, Mária Vicenová, Milan Čertík

Institute of Biotechnology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, Bratislava 81237, Slovak Republic
[*]*peter_gajdos@stuba.sk*

Yarrowia lipolytica is one of the most studied oleaginous yeasts. Its genome is fully sequenced and many tools for its manipulation are available. It has been used for production of many different compounds: proteins, organic acids, lipids and lipophilic molecules. Very long chain fatty acids (VLCFA) are one group of industrially interesting lipid molecules. Behenic acid (C22:0), erucic acid (C22:1Δ^{13}) and their derivatives are compounds with industrial application in cosmetics, lubricants, and fuels. The common source of VLCFA are plants from family Brassicaceae. These plants are source of genes responsible for synthesis of VLCFA as well. Microbial production of oils enriched with VLCFA is interesting alternative to their conventional production by plants. It was shown that *Y. lipolytica* has potential for production of such fatty acids, when *FAE1* coding β-ketoacyl-CoA synthase in *Thlaspi arvense* was expressed in strain Po1d (*MATA ura*3-302 *leu*2-270 *xpr*2-322) (Gajdoš et al., 2020). Since overall yield of VLCFA on Po1d was low, further manipulation of metabolic pathways in *Y. lipolytica* was performed with aim of increasing VLCFA accumulation. Biosynthesis of storage lipid was changed by expression of single diacylglycerol acyltransferase Dga1p, *FAE1* was overexpressed under strong constitutive promoter and β-oxidation was abolished by deletion of *MFE1.* In this way strain YL53 (*Δmfe*, *pTEF-DGA1*, *8UAS-pTEF-FAE1*) was constructed which produced almost 120 μg of VLCFA per g of produced biomass, which accounted for 34% of total fatty acids in biomass. The major very long chain fatty acid was C22:1Δ^{13} (13%) followed by C22:0 (10%) and C24:0 (8%). Minor VLCFA were C22:1Δ^{15}, C24:1Δ^{15}, and C24:1Δ^{17}. To conclude, redesign of lipid metabolism pathways had positive effect on production of VLCFA by recombinant *Y. lipolytica*.

Gajdoš et al. FEMS Microbiol Lett 2020, 367(6):fnaa042.

This work was supported by grant APVV-17-0262.

S7_P5 YEAST RIBOFLAVIN AS A NATURAL COMPONENT OF APATITE COMPOSITE FOR BONE REGENERATION

Justyna Gaweł[1], Justyna Milan[1], Daniel Broda[1], Dariusz Płoch[2], Jacek Żebrowski[1], **Małgorzata Kus-Liśkiewicz[1*]**

[1]Institute of Biology and Biotechnology, University of Rzeszów, Rzeszów 35-310, Pigonia 1, Poland
[2]Institute of Engineering Materials, University of Rzeszów, Rzeszów 35-310, Pigonia 1, Poland
[*]mkus@ur.edu.pl

Bone is one of the most commonly transplanted tissues worldwide (Shegarfi & Reikeras, 2009). Native bones have good natural healing potential, but if defects are too large, it is not able to properly repair itself. Biodegradable and biocompatible composites can be a potential solution to conventionally used bone implants. (Thompson i in., 2015).

In this study, composites of hydroxyapatite, chitosan, and riboflavin naturally synthesized by yeast *Candida famata* were prepared for possible use in regenerative medicine. Hydroxyapatite is a versatile platform for the development of bioceramics with multifaceted biological properties, such as antibacterial effects, osteoinduction, and angiogenesis regulation. Chitosan was chosen as a polymer matrix to manufacture the composite, because of biodegradable, biocompatible and non-toxic. Riboflavin is considered a significant nutritional and growth factor in humans and is recognized as an adjuvants to improve bone metabolism. To assess the level of riboflavin production, the effect of time and supplementation with glycine were performed. Determination and the assessment of the extracellular riboflavin was performed by HPLC or UV-Vis and fluorescence scans, respectively. Antioxidant properties and FT-IR spectroscopy was also done. Modulation of the proliferation and survival of the cells by riboflavin was performed with cytotoxic and cell migration assay. The results show higher ratio of cells proliferation of NIH3T3, and augmented *in vitro* wound-healing activity. Hence, this study concluded that extracellular riboflavin is biocompatible that would be used for the development of wound dressing and implantable material. Moreover, the protocol to obtain chitosan/hydroxyapatite/riboflavin scaffold, which could be suitable for bone filling, was optimized and visualized by SEM technique.

Shegarfi, H., & Reikeras, O. J Orthop Surg, 2009, 17(2), 206–211. https://doi.org/10.1177/230949900901700218

Thompson, E. M., Matsiko, A., Farrell, E., Kelly, D. J., & O'Brien, F. J. Tissue Eng Regen Med, 2015, 9(8), 889–902. https://doi.org/10.1002/term.1918

Chaves Neto AH, Yano CL, Paredes-Gamero EJ, Machado D, Justo GZ, Peppelenbosch MP, Ferreira CV. Toxicol In Vitro. 2010 Oct;24(7):1911-9. doi: 10.1016/j.tiv.2010.07.026. Epub 2010 Aug 3. PMID: 20688149.

S7_P6 FROM WASTE TO VALUABLE PRODUCT - OPTIMIZATION OF RIBOFLAVIN PRODUCTION FROM WHEY

Alicja Najdecka[1*], Daria Fedorovych[2], Justyna Ruchała[1], Andriy Sibirnyy[1,2]

[1]Department of Biology, University of Rzeszów, Ćwiklińskiej 2D, 35-601 Rzeszów, Poland
[2]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Lviv, Drahomanov St 14/16, Lviv 79005, Ukraine
[*]awojtun@ur.edu.pl

Whey is a waste by-product of dairy industry which comes from cheese and casein manufacture. Due to problem with disposal of huge amounts of whey it became an environmental problem. Throughout years, scientists developed different methods of processing whey waste from dairies. However, many of them does not ensure microbial conversion of lactose. *Candida famata* belongs to flavinogenic yeast which overproduce riboflavin (vitamin B2) and secrete it to culture media. Riboflavin is a precursor of flavin coenzymes FMN and FAD which are used in oxidative metabolism and other processes.
Our research is focused on genetic engineered strains of *Candida famata* which are able to grow on whey (around 5% of lactose content) with addition of ammonium sulfate only. Best producer so far appeared to be the strain with overexpression of genes *SEF1* (transcription activator), *RIB1* (GTP-cyclohydrolase II), *RIB7* (riboflavin synthase) and *RIB6* (3,4-dihydroxy-2-butanone-4-phosphate synthase) (Petrovska et al 2022).
Cultivation conditions are now optimized in bioreactor (benchtop bioreactor BioFlo 115, New Brunswick). Constant parameters such as temperature 28^{o}C, aeration 1 L/min, pH 5,5±0,5 and content of ammonium sulfate 3 g/l have been established. Further investigations are now focused on dissolved oxygen (DO) content. DO goes together with agitation which compensate oxygen demand during cultivation. Because *C. famata* might be sensitive to shear forces, a perfect solution between DO level and agitation have to be found.
It was found that under optimal conditions, the mentioned strain accumulated 2,5 g/l of riboflavin in batch culture on whey. These results are very promising. Further experiments including fed-batch cultivation are planned in near future.
Keywords: non-conventional yeast, riboflavin, milk whey, bioreactor

S7_P7 THE INFLUENCE OF CARBON SOURCE AND TEMPERATURE ON THE BIOSYNTHESIS OF ENZYMES BY PSYCHROPHILIC YEAST

Paweł Moroz, **Kacper Szymański***, Zbigniew Lazar, Anna Kancelista

Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław
*anna.kancelista@upwr.edu.pl

Many species of yeast occurring in extreme environments can be an excellent source of valuable enzymes and metabolites useful in the development of modern biotechnology processes. Most of the already characterized enzymes originate from mesophilic organisms. However, the current market is looking for ecological, and most importantly, economically viable, technologies. To answer this challenge, obtaining enzymes, e.g. lipases, highly active at low temperatures. In existing technologies, to obtain such enzymes, vegetable oils are used to induce their production, however, waste materials, e.g. oil cakes, can be also used. Psychrophilic microorganisms as well as products of their metabolism, can be used in processes where mild conditions are required (dairy industry – where low temperature is needed) or where the product itself is used at low temperature (e.g. detergents with psychrozymes). There are many advantages of using psychrophiles and their bioproducts, including: energy saving, elimination of thermal destruction of thermolabile components of the product and thermolability of the enzyme itself allowing for their easy and effective inactivation.

The aim of this work was to determine the influence of carbon source and temperature on the biosynthesis of selected hydrolytic enzymes. Yeast isolates were grown in media containing vegetable oils (rapeseed, sunflower and linseed) and oil cakes obtained after pressing of oils seeds (rapeseed, sunflower and linseed) at two temperatures: 16°C and 22°C. Among the tested isolates (10 strains), the highest lipase biosynthesis was found for three strains: D9, D14 and D19 identified as *Vishniacozyma tephrensis*, *Aureobasidium pullulans* and *Vishniacozyma victoriae*. The analyzed strains produced enzymes with different efficiencies, and their biosynthesis was carbon source and temperature depended. The highest activity was obtained for D14 strain grown on sunflower cake at 16°C. In contrast, lipase biosynthesis on linseed cake was most efficient at 22°C. In general, waste oil cakes were as efficient for lipase biosynthesis as the corresponding vegetable oils. The obtained results indicate the possible efficient waste management. Furthermore, using oil cakes for lipase production allowed also for efficient cellulases and xylanases biosynthesis. The accompanying enzymes were synthesized mostly at 22°C by all tested strains.

The new yeast isolates and their enzymes are very valuable for research and potential industrial application. However, to fully characterize their industrial potential as well as to optimize the biosynthesis processes, further studies are required.

Acknowledgements

The project was financed by the Minister of Education and Science, Poland as part of the programme "Student Scientific Circles Create Innovations" No. SKN/SP/496550/2021.

Session 8

Pathogenic and probiotic yeasts

Oral presentations

S8_O1 LIVING TOGETHER: THE ROLE OF *CANDIDA ALBICANS* IN THE FORMATION OF POLYMICROBIAL BIOFILM

Maria Rąpała-Kozik[1*], Miriam Gonzalez-Gonzalez[1,2], Magdalena Surowiec[1], Magdalena Smolarz[1], Ewelina Wronowska[1], Marcin Zawrotniak[1], Justyna Karkowska-Kuleta[1]

[1]*Department of Comparative Biochemistry and Bioanalytics Faculty of Biochemistry, Biophysics and Biotechnology*
[2]*Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Krakow, Gronostajowa 7-9, 30-387 Kraków, Poland*
[*]*maria.rapala-kozik@uj.edu.pl*

Candida albicans is the part of the microbiome that colonizes the oropharyngeal or vaginal mucosa and gastrointestinal tract of humans. Under certain conditions (immunosuppression or host antibiotic therapy), this commensal fungus can transform into an invasive pathogen that adheres to medical or dental devices, as well as mucous membranes, causing several mucosal infections. The transformation is correlated with the changes in the morphology of cells, from yeast to filamentous hyphae. The last one favors fungal coexistence with bacteria and multi-species biofilm formation, which often presents increasing thickness, virulence, and antimicrobial resistance.

One of the bacteria that *C. albicans* can accompany is *Porphyromonas gingivalis*, which mainly inhabits gingival pockets, where, growing under anaerobic conditions, it can cause periodontal diseases. As presented in our studies, the filamentous growth of *C. albicans*, often supported by the presence of human proteins in the medium, protects *P. gingivalis* cells from the uncomfortable condition by increasing oxygen consumption and favoring the growth of bacteria within the mixed biofilm. Such collaboration of microorganisms is supported by the tight interaction of proteins located on the surface of both pathogens, including the main fungal adhesin Als3 and the main bacterial virulence factors, gingipains, the cysteine proteases. These interactions are also assisted by the modification of fungal surface protein (citrullination) by a bacterial enzyme, peptidylarginine deiminase (PPAD), which enhanced mutual adhesion. The collaboration also involves an increased exposition of fungal moonlighting proteins within the mutual biofilm, which is correlated with a progression in host tissue adhesion and colonization.

Moreover, the biofilm matrix gives additional power to *P. gingivalis* cells, hiding them from the recognition by the host defense cells and increasing their resistance to antibiotics. But further development of the co-infection is likely to depend on the conditions prevailing at the place of biofilm formation, where increased bacterial growth may result in gradual elimination of the yeast and independent colonization by *P. gingivalis* the new, unexpected niche.

The National Science Center of Poland (grant No. 2019/33/B/NZ6/02284 awarded to MR-K) financially supported this work.

S8_O2 THE HOST PUT UP AGAINST THE PATHOGEN'S WALL - THE FUNCTION OF SURFACE-EXPOSED *CANDIDA* MOLECULES

Justyna Karkowska-Kuleta[1*], Dorota Satala[1], Kamila Kulig[2], Grażyna Bras[1], Ewelina Wronowska[1], Andrzej Kozik[2], Maria Rąpała-Kozik[1]

[1]Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

[2]Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387, Kraków, Poland

[*]justyna.karkowska@uj.edu.pl

The cell wall of pathogenic fungi from genus *Candida* – the most widespread fungal pathogens to humans – is a dynamic structure providing cells with mechanical stability, giving protection from adverse conditions and the ability to adapt and dynamically respond to changing environments. Importantly, the fungal cell wall remains in active and constant contact with host proteins and cells during pathogen invasion, also altering the host immunological response and facilitating the further development of infection.

The key components of the *Candida* cell wall are proteins that could be covalently bound to the polysaccharide scaffold of the cell wall or to other embedded proteins, as well as proteins that are more loosely attached to the cell wall. The first group contains typical fungal adhesins with agglutinin-like proteins from the Als protein family. The latter group includes proteins often referred to as moonlighting proteins, that are displayed at the fungal cell surface, although they predominantly occur inside the cell being involved in basic cellular metabolism. These proteins are devoid of a signal peptide that directs them to the classical secretory pathway, and their transport route to the cell surface and depositing mechanisms are still insufficiently understood. One hypothesis indicates the possible transport of intracellular proteins outside the cell as a cargo of fungal extracellular vesicles (EVs). EVs are spherical nanometer-sized structures surrounded by a lipid bilayer, carrying a diversified and plentiful load, and their secretion may have a significant impact on other microbial cells in the same infectious niche and on the host organism during the infection. In our studies, we obtained EVs from different *Candida* species, including the most common *C. albicans*, as well as emerging non-*albicans Candida* species – *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, indicating the presence of many atypical cell wall proteins inside these structures.

Protein moonlighting is a phenomenon of particular importance in the pathogenesis of infections caused by *Candida* fungi. Several enzymes with essential metabolic functions in the fundamental intracellular processes, including a glycolytic metalloenzyme enolase, perform entirely new, noncatalytic activity related to fungal virulence. Moonlighting proteins, acting in concert with the important typical adhesins of the fungal cell wall, are involved in the binding of human extracellular matrix proteins, including fibronectin and vitronectin, or key plasma proteins, like plasminogen or kininogen, to fungal cells. This phenomenon consequently may greatly facilitate fungal invasion of the host cells and further development of infection.

Acknowledgements:

This work was supported in part by the National Science Centre of Poland (grant no. 2019/33/B/NZ6/02284 to MR-K).

S8_O3 NEUTROPHIL RESPONSES TO FUNGAL INFECTIONS

Zawrotniak Marcin*, Smolarz Magda, Rąpała-Kozik Maria

Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
marcin.zawrotniak@uj.edu.pl

Candida albicans lives on the skin and mucous membranes as an opportunistic pathogen. In immunocompromised patients, yeasts cause difficult to treat infections called candidiasis*. C. albicans* uses several virulence factors to facilitate growth and avoid immune response, such as changing the morphology from blastospores to filamentous forms or the ability to form a dense biofilm. The biofilm reduces the penetration of biocidal agents and masks pathogen cells with glucans and mannans. In addition, *Candida* secretes aspartyl protases (Saps), which are responsible for the degradation of host proteins and the development of biofilm. The host immune system is equipped with cells specialized to defend against different pathogens. The first line of defense is neutrophils, cells capable of migration to the site of infection. The numerous surface and intracellular receptors of neutrophils allow them to recognize pathogenic microorganisms that invade the host. Neutrophils are equipped with several mechanisms of response to infection, such as phagocytosis and the release of neutrophil extracellular traps (NETs), called netosis. Phagocytosis provides intracellular inactivation of pathogen cells, which significantly reduces the resulting inflammation, as well as the spread of microorganisms; however, it is ineffective for large cell sizes or developed infection. Unlike phagocytosis, the netosis mechanism acts in the extracellular space. Structures made of DNA decorated with biocidal granular proteins are released outside of cells, forming extracellular traps. NETs binding pathogens at the site of infection, limiting their spread, and biocidal agents are responsible for their elimination. Breakdown of basal protective barriers by *C. albicans* induces a rapid neutrophil response and the formation of local inflammation. The classical neutrophil response pathway involves the phagocytosis of yeast cells, but this mechanism is only effective in the initial phase of infection. As we have shown, with the progress of infection and the change of the morphological form of *C. albicans,* the neutrophil response switches to netosis. Activation of the netosis mechanism occurs in response to selected factors of *C. albicans* exposed to the extracellular environment. Neutrophils can recognize the glucans and mannans present in the yeast cell wall, via dectin-1, Mac-1, and partially TLR2 and TLR4, resulting in the release of NETs. Similarly, some of the Saps released by the yeast are potent activators of the netosis mechanism; however, depending on the type of protease, an ROS-dependent or -independent netosis signaling pathway can be activated. On the other hand, *C. albicans* does not remain defenseless. Our studies have identified Sap6 protease as a factor inactivating immune cells. Sap6 is endocytosed after binding to the neutrophil surface and then, using a Trojan horse mechanism, protease accumulates in neutrophils, leading to proteolytic inactivation of NADPH oxidase, a key component of the netosis signaling pathway. As a result, damage to NADPH oxidase leads to activation of the apoptosis mechanism and Sap6-induced cell death. Neutrophils can also "eavesdrop" on intercellular communication of *C. albicans.* This pathogen releases quorum-sensing molecules, such as farnesol, farnesoic acid, and tyrosol. Analyzes by our team showed that farnesol through the Mac-1 and TLR2 receptors is an activator of the

ROS-dependent netosis mechanism. Our study showed that the neutrophil response mechanism to infection with *C. albicans* differs depending on the progression of the infection.
This work was supported by the Polish National Science Center (grant no. 2019/33/B/NZ6/02284 to MR-K).

S8_O4 IN SEARCH OF EFFECTIVE ANTI-*CANDIDA ALBICANS* AGENTS

Maciej Masłyk[1*], Monika Janeczko[1], Konrad Kubiński[1], Tomasz Goszczyński[2], Oleh Demchuk[3], Aleksandra Martyna[1], Anna Boguszewska-Czubara[4]

[1]*Department of Molecular Biology, The John Paul II Catholic University of Lublin, ul. Konstantynów 1i, 20-708 Lublin, Poland*
[2]*Laboratory of Biomedical Chemistry, Hirszfeld Institute of Immunology and Experimental Therapy,*
Polish Academy of Sciences, ul. Rudolfa Weigla 12, 53-114 Wrocław, Poland
[3]*Department of Chemistry, The John Paul II Catholic University of Lublin, ul. Konstantynów 1j, 20-708 Lublin, Poland*
[4]*Department of Medical Chemistry, Medical University of Lublin, ul. Chodźki 4A, 20-093 Lublin, Poland*
[*]*maciej.maslyk@kul.pl*

A small limited number of antifungal substances available necessitates the development of new selective antifungal therapies. This is of particular importance in the treatment of candidiasis caused by drug-resistant strains, which is a growing problem worldwide. Invasive candidiasis is a particular type of infection, which occurs very often among hospitalized patients. That is why, the development of new effective drug candidates is highly desirable.

Recently, we have developed two types of molecules with specific antifungal properties. One of them are based on the quinone oxime system and the other are boron clusters. The compounds show very good anti-*Candida* activities. Moreover, significant activity was observed against clinical strains, including those showing resistance to systemic antifungal drugs. The activities of developed substances start at 0.6 μg/ml. At the same time, they show no toxic effect on human cells or zebrafish embryonic development at the effective doses. This makes them promising candidates for the new potential antifungal substances.

S8_O5 APPLICATION OF *SACCHAROMYCES CEREVISIAE* VAR. *BOULARDII* IN PROBIOTIC FOOD – STUDY ON LEGUME SPROUTS

Monika Kordowska-Wiater[1*], Michał Świeca[2], Adam Staniszewski[1]

[1]*Department of Biotechnology, Microbiology and Human Nutrition, University of Life Sciences in Lublin, Skromna 8, Lublin, Poland*
[2]*Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Skromna 8, Lublin, Poland*
[*]*monika.kordowska-wiater@up.lublin.pl*

Probiotics according to WHO definition are "live microorganisms which when administered in adequate amounts confer a health benefit on the host".The taxonomic position of *S. cerevisiae* var. *boulardii* is debatable, but current literature and Index Fungorum claim it is a variety of *S. cerevisiae*, closely related to the wine strains. This probiotic yeast meets the functional features such as: ability to survive delivery to the target organ, interaction with host systems, antipathogenic properties and safety. Yeast *Saccharomyces cerevisiae* var. *boulardii* is successfully employed in the prevention and relieving symptoms of antibiotic-associated diarrhea, traveller's diarrhea, Crohn's and inflammatory bowel diseases. The positive effect of this yeast is associated with the abilities to release *in vivo* the substances that inhibit certain bacterial toxins and⁄ or their pathogenic effects, antisecretory activity and immunostimulatory effects on the intestinal mucosa. During illness, probiotics are usually delivered to human body as drugs or dietary supplements. *S. cerevisiae* var. *boulardii* may be also used in production of functional food: e.g. low-alcohol and alcohol-free beer and synbiotic yoghurts. In this study for the first time legumes sprouts were developed as carriers for probiotic yeast. Lentil and adzuki bean are an excellent source of nutrients and compounds with well-documented pro-health properties e.g. phenolics or vitamins. Most importantly, legumes contain high amounts of resistant starch and galacto-oligosaccharides being effectively metabolized by gut microbiota and probiotic organisms. Probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* was isolated from a commercial probiotic preparation. It was confirmed genetically. Lentil and adzuki bean seeds were soaked in probiotic water suspension (1×10^6 cfu/ g), imbibed, and dark-germinated in a growth chamber with spraying with water or probiotic water suspension on the 1st day of cultivation. Sprouting was run at 25 °C, 30 °C, and 35 °C for 4 days. The part of sprouts obtained was stored at 4 °C for 7 days. The highest count of yeast was found in the sprouts obtained from seeds soaked in inoculum and further cultivated at 30 °C–1.03 - 2.5×10^7 cfu/g. The growth of yeast was affected by both availability of nutrients and temperature of sprouting. After storage the population of *S. cerevisiae* var. *boulardii* was reduced by about 50% and 41% in the adzuki bean and lentil sprouts, respectively. After digestion *in vitro* of fresh adzuki bean and lentil sprouts count of yeast decreased by 22% and 28%, respectively. In the case of stored sprouts at 4 °C the reduction of *S. cerevisiae* var. *boulardii* was about 20% in the lentil sprouts. In stored adzuki bean sprouts yeast population was constant during digestion but in lentil sprouts reduction of yeast was on the level 25%. *S. boulardii* significantly improved microbiological quality of final products, because they lowered mold counts (by about 99%) and coliform counts (by about 71- 92%) compared with control. Co-cultures did not affect negatively on phenolics content and antioxidant capacity of synbiotics; however, diversify content of nutrients and their

digestibility. Legumes sprouts enriched with *S. cerevisiae* var. *boulardii* are a new functional product, which is characterized by a safety and desirable nutritional and prohealth quality.

S8_O6 CLOSE ENCOUNTERS OF CANDIDA ALBICANS WITH DIFFERENT ANTIMICROBIAL PEPTIDES AND PROTEINS

Sylwia Stączek[1], Aneta Sowa-Jasiłek[1], Agnieszka Zdybicka-Barabas[1], Monika Koziej[1], Paweł Mak[2], **Małgorzata Cytryńska[1*]**

[1]Department of Immunobiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[2]Department of Analytical Biochemistry, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland
[*]malgorzata.cytrynska@mail.umcs.pl

An opportunistic pathogenic fungus *Candida albicans* is a common inhabitant of human gastrointestinal and reproductive tracts. Antimicrobial peptides and proteins, e.g. histatin 5, LL-37, defensins, and lysozyme, are important humoral factors involved in control and elimination of *Candida* cells from human body.

In the search for molecules effective against *Candida*, researchers turned their attention to antimicrobial peptides found in various organisms. At the same time, in the study of pathogenicity as well as in the study of the effectiveness of potential antimicrobial molecules research began to use alternative model hosts, e.g. the greater wax moth *Galleria mellonella*. This insect species also produces a number of antimicrobial peptides and proteins with different properties. Among them anionic peptide 2, lysozyme and apolipophorin III exhibit anti-*Candida* activity. Apolipophorin III (apoLp-III) is an abundant hemolymph protein involved in lipid transport and immune response in *G. mellonella*. ApoLp-III binding to *C. albicans* cell surface resulted in changes in cell surface topology (AFM imaging) and transition from yeast-like to hyphal growth with formation of true hyphae (Zdybicka-Barabas et al., 2012). *G. mellonella* lysozyme (Gm lysozyme), similarly to its human counterpart, is a member of the c-type family of lysozymes. Our study provided evidence that Gm lysozyme can bind to protoplasts as well as to the intact *C. albicans* cells leading to decreased survival rate which was also correlated with reduced metabolic activity (LIVE/DEAD staining). Of note, Gm lysozyme did not hydrolyze typical chitinase and β-glucanase substrates, indicating that enzymatic activity towards cell wall components is not necessary for anti-*Candida* activity. Interestingly, *C. albicans* cells pre-treated with a potassium channel blocker were protected against Gm lysozyme action. Gm lysozyme-treated *C. albicans* cells exhibited typical apoptotic features: loss of mitochondrial membrane potential, externalization of phosphatidylserine, chromatin condensation and DNA fragmentation (Sowa-Jasiłek et al., 2016). *G. mellonella* anionic peptide 2 (AP2) is the unique 7 kDa peptide (pI 4.79) constitutively present in hemolymph in the concentration of 10–12 µM. The exposure (1-3h) to AP2 decreased the metabolic activity and survival rate of intact *C. albicans* cells, however the viability of protoplasts was not affected, suggesting an important role of the fungal cell wall in the peptide action. FTIR spectroscopy suggested AP2 interactions with the cell surface proteins and destabilization of protein secondary structures. Interestingly, the presence of amphipathic α-helices with exposed positively charged lysine residues was predicted in anionic AP2 by bioinformatic analysis (Sowa-Jasiłek et al., 2020).

Sowa-Jasiłek A. et al. Int. J. Mol. Sci. 2020, 21(6):1912.

Sowa-Jasiłek A. et al. Microbiol. Res. 2016, 193:121-131.
Zdybicka-Barabas A. et al. J. Insect Physiol. 2012, 58(1):164-177.

S8_O7 PRODUCTION AND BIOLOGICAL ACTIVITY OF PULCHERRIMIN FROM THE *METSCHNIKOWIA PULCHERRIMA* CLADE

Dorota Kręgiel[1*], Ewelina Pawlikowska[1], Beata Kolesińska[2], Maria Nowacka[3], Anna Rygala[1], Renata Vadkertiova[4]

[1]*Department of Environmental Biotechnology,* Łódź *University of Technology, Wólczańska 171/173, 90-530* Łódź*, Poland*
[2]*Institute of Organic Chemistry, Faculty of Chemistry,* Łódź *University of Technology, Żeromskiego 116, 90-924* Łódź*, Poland*
[3]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363* Łódź*, Poland*
[4]*Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská Cesta 9, 845 38 Bratislava, Slovakia*
[*]*dorota.kregiel@p.lodz.pl*

Pulcherrimin is a secondary metabolite of yeasts belonging to the *Metschnikowia pulcherrima* clade, and pulcherrimin formation is responsible for the antimicrobial action of its producers. In addition, understanding the environmental function of this metabolite can provide insight into various microbial interactions and enables the efficient development of new effective bioproducts and methods. Fifteen yeast strains of the genus *Metschnikowia* were characterized based on their production of pulcherrimin. This red pigment was isolated and its purity assessed using ^{1}H nuclear magnetic resonance spectroscopy. Under experimental conditions, pulcherrimin formation varied depending on both the tested strains and culture media. The best producers formed up to 240 mg/L of pulcherrimin in minimal medium with glucose as the carbon source, supplemented with 0.05% $FeCl_3$ and 0.1% Tween 80. The antimicrobial and antiadhesive action of pulcherrimin, as well as its protective properties under selected stressful conditions were also determined. Classical microbiological methods, microscopy, and physico-chemical testing were used. The results show that pure pulcherrimin does not have antimicrobial properties, but its unique hydrophilic nature may hinder the adhesion of hydrophilic bacterial cells to abiotic surfaces. Pulcherrimin also proved to be a good cell protectant against UV radiation and temperature stress.

Pawlikowska E. et al. Fermentation 2020, 6(4), 114.
Kręgiel D. et al. Molecules 2022, 27(6), 1855.

Poster presentations

S8_P1 QUINALIZARIN AS A POTENTIAL GROWTH INHIBITOR OF *CANDIDA ALBICANS* WITH ANTI-HYPHAE AND ANTI-BIOFILM PROPERTIES

Monika Janeczko[*] and Kamila Górka

[1]*Department of Molecular Biology, Faculty of Science and Health, The John Paul II Catholic University of Lublin, Konstantynów 1i, 20-708 Lublin, Poland*
[*]*monika.janeczko@kul.pl*

Quinalizarin (1,2,5,8-tetrahydroxyanthraquinone) is one of the main components of many herbal medicines and has good anti-tumor activity. It is effective in different types of tumor cells including breast cancer, prostate cancer, leukemia T cells and angiogenesis. Moreover, quinalizarin has been suggested as a promising drug prototype against human cytomegalovirus and HIV (Cozza G. et al., 2015).
The aim of this work was evaluation of the antifungal activity of quinalizarin against *Candida albicans*. *C. albicans* is the most common human fungal pathogen causing mucosal infections as well as systemic diseases. As a commensal, *C. albicans* asymptomatically colonizes mucosal surfaces. However, by any disturbance in the host's environment or due to dysfunction of the immune system, *C. albicans* can attack virtually any site in the host organism. The ability of this fungal species to transition from commensal to pathogen is related to a number of virulence factors. In particular, the ability to switch morphology and form biofilms are key properties in the pathogenesis of *C. albicans* (Tsui C. et al., 2016).
The antifungal effects of quinalizarin were investigated using reference *C. albicans* ATCC 10231 strain. The quinalizarin minimum inhibitory concentration (MIC) was 8 μg/ml. To investigate biofilm inhibition and degradation effect, MTT assay was used. Our results showed that quinalizarin at the concentrations 80 μg/ml and 160 μg/ml significantly inhibited the biofilm formation of *C. albicans,* reducing its viability to 39 % and 29.7 %, respectively. In turn, the mature biofilm was reduced up to 70 % and 62 % at the concentrations 80 μg/ml and 160 μg/ml, respectively. In addition, the specific mechanisms of this anti-biofilm effect of quinalizarin were tested by evaluating hyphae formation and extracellular polymeric substances (EPS) production rate (extracellular DNA, extracellular proteins, and carbohydrates). The effect of quinalizarin as a control on the hyphal growth of *C. albicans* cells was evaluated using both liquid and solid RPMI 1640 medium containing 10 % fetal bovine serum (FBS). Quinalizarin at 8 μg/ml reduced the amount of hyphae and promoted the growth of *C. albicans* in the yeast form. The contents of EPS of biofilm matrix under treatment of quinalizarin at 8 μg/ml, 40 μg/ml, 80 μg/ml and 160 μg/ml were not changed.
Our research evidences the effectiveness of quinalizarin as potential anti-*Candida* agents.

Cozza G. et al. Biomed Res Int. 2015, 2015:734127
Tsui C. et al. Pathog Dis. 2016, 74(4):ftw018

S8_P2 EXTRACELLULAR VESICLES PRODUCED BY PROBIOTIC YEAST – BASIC CHARACTERISTICS AND THEIR INFLUENCE ON HUMAN INTESTINAL CELLS

Patrycja Kowalska[1, 2], Małgorzata Milner-Krawczyk[1], Klaudia Marlicka[1], Kornelia Dobrzyńska-Kobylec[1], Dominika Wiśniewska[1], Maciej Trzaskowski[3], Agata Głuchowska[4], Grażyna Mosieniak[4], Karolina Drężek[1], **Jolanta Mierzejewska[1*]**

[1]Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland
[2]Doctoral School No. 1, Warsaw University of Technology, Warsaw, Poland
[3]Center for Advanced Materials and Technology CEZAMAT, Warsaw University of Technology, Warsaw, Poland
[4]Laboratory of Molecular Basis of Aging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[*]jolanta.mierzejewska@pw.edu.pl

Extracellular vesicles, shortly called EVs, are nanoparticles produced by various types of living cells, such as mammalian cells, protozoa, bacteria and fungi. Technically describing, these structures are encapsulated by a lipid bilayer and each type of cell produces EVs with a different bioactive cargo, e.g. proteins, RNAs, lipids.

The first such structures were identified for pathogenic yeasts, and we have little knowledge of EVs of other microscopic fungi. Therefore, our goal was to characterize EVs produced by probiotic *Saccharomyces boulardii* CNCM 1-745 (Enterol®, BIOCODEX) and verify whether they have a visible effect on the metabolism of human intestinal cells.

As a result of our research, the size and concentration of EVs in the liquid culture of *S. boulardii* were estimated using the nanoparticle tracking analysis (NTA). We noticed that in YPD medium at 37°C the yeast was releasing vesicles of various size, in the range of 20-300 nm. The EVs were also visualized using STEM and fluorescence microscopy. Since probiotic yeast can inhabit the human colon for at least a few days after their application, we tested the cytotoxicity of EVs on model cell lines isolated from colorectal tissues – HT29 and HCT116. Various EVs to cells ratios were tested, and we observed neither a significant cytotoxicity effect nor an enhanced induction of ROS production. Besides, we performed EVs` loading tests with NileRed (lipophilic fluorescence dye) and doxorubicin hydrochloride (hydrophilic fluorescence cytostatic drug). After incubating the loaded EVs with HT29 and HCT116 lines, both cargo reagents were transferred into cells, which we could monitor by fluorescence microscopy. Moreover, we confirmed that doxorubicin loading and transfer inside EVs did not inactivate the compound.

In conclusion, our research has shown that EVs of probiotic yeast are not cytotoxic and can transfer their cargo to human intestinal cells. This opens up space for further research into whether we can use these EVs as carriers for biologically active substances.

Studies presented herein were financed by BIOTECHMED-2 Advanced (POB Biotechnology and Biomedical Engineering) project granted by Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) programme.

S8_P3 THE EFFECT OF PESTICIDES ON CELL HETEROGENEITY IN *CANDIDA* SPP.

Potocki L.[1*], Baran A.[1], Oklejewicz B.[1], Szpyrka E.[1], Podbielska M.[1], Schwarzbacherová V.[2], Wnuk M.[1]

[1]*Department of Biotechnology, College of Natural Sciences, University of Rzeszów, Pigonia 1, 35-310 Rzeszów, Poland*
[2]*Department of Biology and Genetics, Institute of Genetics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia*
[*]*lpotocki@ur.edu.pl*

Yeasts of the genus *Candida* are commensal and opportunistic human pathogens, characterized by the ability of intercellular variability, so-called heterogeneity, which plays an essential role in adaptation to stress factors. Colonizing the human body, they come into contact with many xenobiotics, which in addition to drugs, heavy metals, and food compounds include pesticides used in modern agriculture to protect crops from pests.
This study was aimed to evaluate the impact of commonly used pesticides- triazole fungicide Tango® (epoxiconazole) and two neonicotinoid insecticide formulations Mospilan® (acetamiprid) and Calypso® (thiacloprid) as an underestimated and neglected source of cellular variability of microorganisms, including yeast of the *Candida* genus, which can lead to clonal heterogeneity with altered morphological, physiological and genetic profiles. The tested pesticides caused differences between cells of the same species in the studied populations in response to ROS accumulation, the level of DNA damage, changes in fatty acids (FAs) and phospholipid profiles, and changes in the percentage of unsaturated to saturated FAs, or the ability to form biofilms. Furthermore, for the first time, the effect of tested neonicotinoid insecticides on the change in the metabolic profile of colony cells during aging was demonstrated. Our data suggest that widely used pesticides, including insecticides, can increase cellular diversity in the population of *Candida* species known as clonal heterogeneity, which plays an important role in acquiring resistance to antifungal agents.

Keywords: *Candida*, stress, pesticide, intercellular variability, heterogeneity, resistance

S8_P4 YEASTS DECREASE MYCOTOXIN CONTAMINATION OF WINTER DURUM WHEAT GRAIN

Urszula Wachowska[1*], Michael Sulyok[2], Marian Wiwart[3], Elżbieta Suchowilska[3], Dariusz Gontarz[4], Wolfgang Kandler[2]

[1]*University of Warmia and Mazury, Department of Entomology, Phytopathology and Molecular Diagnostics, 10-720 Olsztyn, Prawocheńskiego 17, Poland*
[2]*Institute of Bioanalytics and Agro-Metabolomics Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad Lorenz Strasse 20, 3430 Tulln a.d. Donau, Austria*
[3]*University of Warmia and Mazury in Olsztyn, Plac Łódzki 3, Department of Plant Breeding and Seed Production, 10-724 Olsztyn, Poland*
[4]*PZZ Lubella GMW Sp. z o.o. Sp.k., Wrotkowska 1, 20-469 Lublin, Poland*
[*]*urszula.wachowska@uwm.edu.pl*

Fungi of the genus *Fusarium,* causing *Fusarium* head blight (FHB) in durum wheat, produce numerous mycotoxins, thus decreasing grain quality. Trichothecenes, including deoxynivalenol (DON) and nivalenol (NIV), are the most prevalent and toxicologically important mycotoxins. They exert both acute and chronic toxic effects in animals. Culmorin (CUL) and moniliformin (MON) have been less extensively researched, and their in vivo toxicity is low. The aim of this study was to determine the content of selected mycotoxins (DON, NIV, CUL and MON) produced by *Fusarium* fungi in the grain of several cultivars of winter durum wheat, which was grown in two different locations in Poland and was biologically protected with a strain of *Debaryomyces hansenii* yeasts. The severity of FHB and grain colonization by yeasts and *Fusarium* fungi were also evaluated. A total of 40 grain samples were analyzed.
DON was detected in 36 grain samples (16-1282 mg/kg), NIV in 19 samples (19-377 mg/kg), CUL in 21 samples (21-2877 mg/kg) and MON in 22 samples (10-1003 mg/kg). Biological treatment reduced the content of DON, NIV and CUL in most grain samples from northern Poland. In this location, the analyzed mycotoxins were not detected in the grain of cv. Wintergold following the biological treatment. The content of DON and CUL in grain was strongly positively correlated with DON-3-glucoside and 15-hydroxyculmorin. A positive correlation was also found between the content of CUL, DON and NIV. In northern Poland, the effectiveness of biological treatment in reducing the severity of FHB ranged from 19% to 91%, compared with the control treatment. In this location, the abundance of yeasts on the grain of cvs. Auradur, Karmadur, Spiradur and Tempodur was higher after the application of *D. hansenii* cell suspension than in the control treatment. In both locations (northern and southern Poland), the inoculation of wheat spikes with *F. graminearum* decreased yeast counts on grain, relative to the control treatment, and this decrease was not inhibited by the application of *D. hansenii* before inoculation. Biological treatment reduced the concentrations of mycotoxins in grain, but its efficacy varied depending on location and winter durum wheat cultivar.

This research was funded by the Smart Growth Operational Program, project number POIR.01.01.01-00-0251/17 (Development and implementation of a complex technology for obtaining high-quality pasta products with the addition of Polish durum wheat).

Index of presenting authors

Yeast Research Groups in Poland

GDAŃSK

University of Gdańsk

Research group: Laboratory of Protein Biochemistry, Intercollegiate Faculty of Biotechnology
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Molecular mechanisms of chaperones in control of protein aggregation and disaggregation
Investigators: Krzysztof Liberek, *krzysztof.liberek@ug.edu.pl*
Agnieszka Kłosowska

Research group: Laboratory of Evolutionary Biochemistry
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Our goal is to unravel the molecular mechanisms behind the Hsc20/Hsp70 function(s) in the process of FeS biogenesis. In our research, we combine biochemical experiments and evolutionary analyses, because only such an approach can comprehensively explain molecular and functional properties of proteins involved in this complex process. As model systems, we study yeast (*Saccharomyces cerevisiae*) and bacteria (*Escherichia coli*); differences between these systems have allowed us to investigate molecular mechanisms behind changes in JDP/Hsp70 partnership that took place during evolution.
Investigators: Jarosław Marszałek, *jaroslaw.marszalek@ug.edu.pl*
Rafał Dutkiewicz
Bartłomiej Tomiczek

LUBLIN

University of Life Sciences in Lublin

Research group: Department of Biotechnology, Microbiology and Human Nutrition
Yeast research model: *Saccharomyces cerevisiae*, - wine, distiller's yeast, *Saccharomyces cerevisiae var. boulardii* – probiotic yeast, *Candida parapsilosis*, *Pichia stipitis*
Research profile: ethanol production, food additives e.g. sweeteners (xylitol, arabitol) production, probiotic yeast as functional food component
Investigators: Monika Kordowska-Wiater, *monika.kordowska-wiater@up.lublin.pl*
Adam Staniszewski, *adam.staniszewski@up.lublin.pl*

The John Paul II Catholic University of Lublin

Research group: Institute of Biological Sciences
Yeast research model: *Candida albicans*
Research profile: mechanism of *C. albicans* pathogenesis, virulence factors, identification of new antifungal compounds and their molecular targets
Investigator: Monika Janeczko, *monika.janeczko@kul.pl*

Maria Curie-Skłodowska University in Lublin

Research group: Department of Molecular Biology, Institute of Biological Sciences
Yeast research model: *Saccharomyces cerevisiae*
Research profile: translation, protein biosynthesis
Investigators: Marek Tchórzewski, *marek.tchorzewski@mail.umcs.pl*
Przemysław Grela
Leszek Wawiórka
Eliza Molestak
Monika Szajwaj
Barbara Michalec-Wawiórka

Research group: Department of Immunobiology, Institute of Biological Sciences
Yeast research model: *Candida albicans*
Research profile: The mechanisms of insect innate immunity, on the example of the model organism *Galleria mellonella* and the honey bee *Apis mellifera*. Pathogen-host interaction studies using *Galleria mellonella* as model host. Characteristics and mechanisms of action of antimicrobial peptides and proteins. Biologically active molecules derived from *Dendrobaena veneta* earthworms.
Investigators: Małgorzata Cytryńska, *malgorzata.cytrynska@mail.umcs.pl*
Agnieszka Zdybicka-Barabas
Iwona Wojda
Mariola Andrejko
Aneta Ptaszyńska
Marta Fiołka

Sylwia Stączek
Magdalena Kunat

ŁÓDŹ

Lodz University of Technology

Research group: Institute of Molecular and Industrial Biotechnology (IBMiP)
Yeast research model: extremophilic yeasts (mainly psychrophilic and psychrotrophic; including *Candida* spp., *Debaryomyces* spp., *Rhodotorula* spp. and *Goffeauzyma* spp.) belonging to the Collection of Antarctic Microorganisms of TUL's IBMiP
Research profile: isolation and taxonomic identification of extremophilic yeasts; their genetic, biochemical and kinetic characterization, determination of potential applications, including production of unique industrial enzymes, antifreeze proteins (AFPs), pigments and biosurfactants.
Investigators: Aneta Białkowska, *aneta.bialkowska@p.lodz.pl*
Aleksandra Twarda-Cłapa, *aleksandra.trwarda-clapa@p.lodz.pl*
Iga Jodłowska, *iga.jodlowska@p.lodz.pl*
Aleksandra Olczak, *aleksandra.olczak@dokt.p.lodz.pl*
Marcin Sypka, *marcin.sypka@dokt.p.lodz.pl*

Research group: Department of Environmental Biotechnology
Yeast research model: conventional (*Saccharomyces cerevisiae*) and non-conventional (*Metschnikowia pulcherrima*, *Wickerhamomyces anomalus*, *Hanseniaspora uvarum*, *Yarrowia lipolytica*, *Kluyveromyces marxianus*, *Scheffersomyces stipitis*) yeasts
Research profile: production of bioethanol and protein from food industry by-products, yeast autolysates, yeast as biocontrol agents, low-temperature fermentation.
Investigators: Dorota Kręgiel, *dorota.kregiel@p.lodz.pl*
Joanna Berłowska, *joanna.berlowska@p.lodz.pl*

KRAKÓW

Jagiellonian University in Krakow

Research group: Faculty of Biochemistry, Biophysics and Biotechnology,
Yeast research model: *Candida albicans, Candida glabrata, Candida dubliniensis, Candida parapsilosis, Candida tropicalis*
Research profile: adhesins, proteases, moonlighting proteins, extracellular vesicles, yeast interactions with host cells, multispecies biofilm formation
Investigators: Maria Rąpała-Kozik, *maria.rapala-kozik@uj.edu.pl*
Andrzej Kozik, *andrzej.kozik@uj.edu.pl*
Justyna Karkowska-Kuleta, *justyna.karkowska@uj.edu.pl*
Marcin Zawrotniak, *marcin.zawrotniak@uj.edu.pl*

Research group: Evolutionary Genetics Group, Institute of Environmental Sciences
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Genetic basis of adaptation; frequency distribution of beneficial and deleterious mutations; constraints on protein evolution; phenotypic differentiation in adaptive evolutionary strategies; genome instability as a source of selectable variation
Investigators: Ryszard Korona, *ryszard.korona@uj.edu.pl*
Dominika Włoch-Salamon
Katarzyna Tomala

POZNAŃ

Poznan University of Life Sciences

Research group: Department of Biotechnology and Food Microbiology,
Yeast research model: *Yarrowia lipolytica, Komagataella phaffii (Pichia pastoris)*
Research profile: Production of heterologous proteins in the yeast expression systems. Engineering of the secretory pathway and resistance to stress. Understanding the molecular background of cellular stress response to environmental factors and / or internal bottlenecks upon overproduction of biomolecules. Construction of new yeast strains overproducing selected proteins and metabolites with their detailed characteristics at the molecular level.
Investigators: Ewelina Celińska, *ewelina.celinska@up.poznan.pl*
Monika Borkowska, *monika.borkowska@up.poznan.pl*
Wojciech Białas, *wojciech.bialas@up.poznan.pl*

Institute of Bioorganic Chemistry, Polish Academy of Sciences

Research group*:* Department of Functional Transcriptomics
Yeast research model: *Saccharomyces cerevisiae*
Research profile: identification, characterization and determination of the regulatory potential of small ribosome-associated noncoding RNAs (rancRNA) in Saccharomyces cerevisiae under various environmental conditions, with particular emphasis on heterogeneity of ribosomes,
Investigators*: Kamilla Grzywacz, kamilla.grzywacz@ibch.poznan.pl*
Anna Wasilewska-Burczyk, awasilewska@ibch.poznan.pl
Piotr J. Pietras, ppietras@ibch.poznan.pl

RZESZÓW

University of Rzeszów

Research group: Institute of Biology and Biotechnology, Department of Biotechnology
Yeast research model: *Ogataea polymorpha, Candida famata, Komagataella (Pichia) pastoris*
Research profile: The group works mostly with non-conventional yeasts, i.e. yeasts different from *Saccharomyces cerevisiae*. The main topics involve studying alcoholic fermentation of lignocellulosic sugars (glucose, xylose, L-arabinose) and glutathione synthesis in the thermotolerant yeast *Ogataea polymorpha*, bioproduction of higher alcohol isobutanol by *Magnusiomyces magnusii*; riboflavin, flavin nucleotides and antibiotic roseoflavin synthesis in *Candida famata* and *Komagataella phaffii* and studying the mechanisms of autophagy of peroxisomes and cytosolic proteins in *K. phaffii*. Finally, the group is involved in construction of the producers of protein subunit vaccine against COVID-19 in the humanized strain of yeast *K. phaffii.*
Investigators: Andriy Sybirny, *asybirnyy@ur.edu.pl*, *sibirny@yahoo.com*
Justyna Ruchała, *jruchala@ur.edu.pl*
Olena Dmytruk, *odmytruk@ur.edu.pl*
Alicja Najdecka, *awojtun@ur.edu.pl*

Yeast research model: *Saccharomyces cerevisiae, Saccharomyces paradoxus, Saccharomyces mikatae, Saccharomyces kudriavzevii, Saccharomyces arboricola, Saccharomyces eubayanus, Saccharomyces uvarum, Candida albicans, Candida glabrata and Candida tropicalis*
Research profile: mechanisms of chromosome stability, development of *in situ* techniques for detection of chromosomal instability, genomic plasticity, yeast aging
Investigators: Maciej Wnuk, *mwnuk@ur.edu.pl*
Anna Lewińska, *alewinska@ur.edu.pl*
Leszek Potocki, *lpotocki@ur.edu.pl*

Yeast research model: *Saccharomyces cerevisiae, Yarrowia lipolytica, Candida famata, Candida albicans*
Research profile: nanomaterials synthesis based on the microorganism and their metabolites.
Investigator: Małgorzata Kus-Liśkiewicz, *mkus@ur.edu.pl*

Research group: Institute of Biology and Biotechnology, Department of Biology
Yeast research model: *Saccharomyces cerevisiae*
Research profile: regulation of cell size and its reproductive capacity; processes modifying the redox status of the cell; mechanisms of the yeast cell's lifespan regulation
Investigators: Renata Zadrąg-Tęcza, *retecza@ur.edu.pl*
Magdalena Kwolek-Mirek, *mkwolek@ur.edu.pl*
Roman Maślanka, *rmaslanka@ur.edu.pl*
Mateusz Mołoń, *mmolon@ur.edu.pl*
Sabina Bednarska, *sbednarska@ur.edu.pl*

WARSZAWA

Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Research group: Laboratory of Genetic Stability Mechanisms
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Genome stability mechanisms, cellular stress response, DNA repair, mechanisms of action of various therapeutic compounds, and yeast as model of human diseases at the cellular and molecular levels.
Investigators: Adrianna Skoneczna, *ada@ibb.waw.pl*
Tuguldur Enkhbaatar, *tugdo@ibb.waw.pl*
Anna Długajczyk, *a.dlugajczyk@ibb.waw.pl*

Research group: Laboratory of DNA Replication and Genome Stability
Yeast research model: *Saccharomyces cerevisiae*
Research profile: DNA replication, mechanisms controlling DNA replication and cell cycle progression, genome stability
Investigators: Iwona J. Fijałkowska, *iwonaf@ibb.waw.pl*
Michał Dmowski, *mdmowski@ibb.waw.pl*
Karolina Makieła-Dzbeńska, *kmakiela@ibb.waw.pl*

Research group: Laboratory of Molecular Basis of Aging and Rejuvenation
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Post-translational mechanisms to maintain protein homeostasis upon oxidative stress and during aging; Impact of oxidation of ribosomal proteins for ribosome composition and translational output; Adaptation of the function of co-translational chaperones under cellular stress conditions
Investigator: Ulrike Topf, *utopf@ibb.waw.pl*

Yeast research model:. *Saccharomyces cerevisiae*
Research profile:; mutagenesis, DNA repair and DNA damage tolerance, genome stability
Investigators: Ewa Śledziewska-Gójska, *esg@ibb.waw.pl*
Agnieszka Hałas, *halas.agnieszka@ibb.waw.pl*

Yeast research model: *Saccharomyces cerevisiae*
Research profile: Mechanisms of mtDNA repair and maintenance
Investigators: Aneta Kaniak-Golik, *anetak@ibb.waw.pl*
Ewa Śledziewska-Gójska, *esg@ibb.waw.pl*

Yeast research model: *Saccharomyces cerevisiae*
Research profile: Import of proteins to peroxisomes, secretion of proteins to periplasm, stress response
Investigators: Marek Skoneczny, *kicia@ibb.waw.pl*
Zuzanna Frydzińska, *z.frydzinska@gmail.com*

Research group: Laboratory of Genetics and Molecular Biology of Yeast
Yeast model used in research: *Saccharomyces cerevisiae*
Research profile: Modelling of rare neurodegenerative diseases and searching for therapies
Investigators: Teresa Żołądek, *teresa@ibb.waw.pl*
Joanna Kamińska, *kaminska@ibb.waw.pl*

Yeast research model: *Saccharomyces cerevisiae*
Research profile: Yeast *S. cerevisiae* as a model to understand the pathogenesis mechanisms of mutations in mitochondrial genes *MT-ATP6* and *MT-ATP8*, encoding subunits of ATP synthase
Investigator: Róża Kucharczyk, *roza@ibb.waw.pl*

Research group: Laboratory of tRNA Transcription
Yeast research model: yeast *Saccharomyces cerevisiae*
Research profile: Using yeast *Saccharomyces cerevisiae* as model, our laboratory investigates the regulation of tRNA transcription by RNA polymerase III and its auxiliary factors. We aim to characterize molecular mechanisms that control RNA polymerase III biogenesis and activity. We use mainly genetic methods and classic molecular biology techniques. Protein interactions with chromatin are determined by immunoprecipitation (ChIP) followed by real-time polymerase chain reaction (RT-PCR). RNA levels are determined by non-radioactive Northern hybridization or RT-PCR. Protein-protein interactions are determined by co-immunoprecipitation (co-IP), followed by Western blot. A phos-tag approach has been used to detect phosphorylated forms of proteins.
Investigators: Magdalena Boguta, *magda@ibb.waw.pl*
Małgorzata Cieśla, *goges@ibb.waw.pl*
Alicja Armatowska, *a.armatowska@ibb.waw.pl*
Aleksandra Łopusińska, *o.lopusinska@ibb.waw.pl*

Research group: Department of Bioinformatics
Yeast research model:: *Saccharomyces cerevisiae*
Research profile: There are following main areas of our studies: apoptosis/apoptosis origin, the evolution of epistatic interactions, and the less-is-more hypothesis. Our collaborators from the Laboratory of Environmental and Evolutionary Systems Biology test our hypotheses using yeasts.
Investigator: Szymon Kaczanowski, *szymon@ibb.waw.pl*

Research group: Laboratory of Environmental and Evolutionary Systems Biology
Yeast research model: *Saccharomyces cerevisiae*
Research profile: The main focus of our research is comparative and evolutionary genomics. We investigate microbial consortia from natural and experimental environments and explore the history and evolution of apoptotic machinery.
Investigators: Urszula Zielenkiewicz, *ulazet@ibb.waw.pl*
Joanna Klim, *klim@ibb.waw.pl*
Vandana Kaushal, *vandana@ibb.waw.pl*

University of Warsaw

Research group: Institute of Genetics and Biotechnology
Yeast research model: *Candida albicans, Schizosaccharomyces pombe, Saccharomyces cerevisiae*
Research profile: Nucleo-mitochondrial interactions, evolution, transcriptomics, RNA degradation in mitochondria and cytoplasm
Investigators: Paweł Golik, *p.golik@uw.edu.pl*
Michał Małecki, *mg.malecki@uw.edu.pl*

Warsaw University of Technology

Research group: Faculty of Chemistry, Chair of Drug and Cosmetics Biotechnology, Microbiology and Bioengineering Group
Yeast research model: *Saccharomyces* sp., *Kluyveromyces* sp., red yeasts (*Rhodotorula, Sporobolomyces, Cystobasidium*)
Research profile: production and characterization of probiotic yeast extracellular vesicles as carriers of biologically active substances; production of natural cosmetic raw materials (incl. aromas, bioferments, carotenoid dyes); production of bioethanol from agri-food waste.
Investigators: Jolanta Mierzejewska, *jolanta.mierzejewska@pw.edu.pl*
Karolina Drężek, *karolina.drezek@pw.edu.pl*

Research group: Centre for Advanced Materials and Technologies
Yeast research model: *Candida albicans, Candida auris*
Research profile: action mode of new antimycotics; mast cells in candidiasis
Investigator: Monika Staniszewska, *monika.staniszewska@pw.edu.pl*

Mossakowski Medical Research Institute, Polish Academy of Sciences

Research group: Neuromuscular Unit
Yeast research model: *Saccharomyces cerevisiae*
Research profile: Yeast- based models of the mutations causative for hereditary motor and sensory neuropathies
Investigators: Andrzej Kochański, *akochanski@imdik.pan.pl*
Weronika Rzepnikowska, *wrzepnikowska@imdik.pan.pl*
Dagmara Kabzińska
Katarzyna Binięda

WROCŁAW

University of Wrocław

Research group: Faculty of Biotechnology, Department of Biotransformation
Yeast research model: *Candida albicans*
Research profile: Multidrug resistance in *Candida albicans*
Investigator: Anna Krasowska, *anna.krasowska@uwr.edu.pl*

Research group: Faculty of Biological Sciences, Department of Genetics and Cell Physiology,
Yeast research model: *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis*
Research profile: molecular mechanisms of response to arsenic stress, regulation of gene expression, sorting and endocytosis of membrane proteins, DNA damage response, regulation of the cell cycle
Investigators: Robert Wysocki, *robert.wysocki@uwr.edu.pl*
Ewa Maciaszczyk-Dziubińska, *ewa.maciaszczyk-dziubinska@uwr.edu.pl*
Donata Wawrzycka, *donata.wawrzycka@uwr.edu.pl*

Research group: Faculty of Biotechnology
Yeast research model: *Schizosaccharomyces pombe*
Research profile: replication stress response
Investigator: Dorota Dziadkowiec, *dorota.dziadkowiec@uwr.edu.pl*

Research group: Academic Excellence Incubator - Research Centre for DNA Repair and Replication, Faculty of Biological Science
Yeast research model: *Saccharomyces cerevisiae, Schizosaccharomyces pombe*
Research profile: mechanisms of DNA repair and replication stress tolerance, regulation of sister chromatid cohesion process, mechanisms of metal genotoxicity
Investigator: Ireneusz Litwin, *ireneusz.litwin@uwr.edu.pl*

Wroclaw University of Environmental and Life Sciences

Research group: Department of Biotechnology and Food Microbiology
Yeast research model: *Yarrowia lipolytica*
***Research profile**:* production of fodder yeast, production of organic acids, production of polyhydroxy alcohols, production of pigments and aromatic compounds, bioremediation, degradation of plastics, utilization of waste materials in biotechnological processes using yeast, analysis of metabolism regulation, construction of new biosynthetic pathways of valuable compounds production using synthetic biology and systems biology
Investigators: Waldemar Rymowicz, *waldemar.rymowicz@upwr.edu.pl*
Anita Rywińska, *anita.rywinska@upwr.edu.pl*
Zbigniew Lazar., *zbigniew.lazar@upwr.edu.pl*
Magdalena Rakicka-Pustułka, *magdalena.rakicka-pustulka@upwr.edu.pl*

Ludwika Tomaszewska-Hetman, *ludwika.tomaszewska-hetman@upwr.edu.pl*
Piotr Juszczyk, *piotr.juszczyk@upwr.edu.pl*
Dorota Rzechonek, *dorota.rzechonek@upwr.edu.pl*

Research group: Laboratory for Biosustainability
Yeast research model: *Yarrowia lipolytica*
Research profile: In the research we focus on the biotechnological application of yeast *Yarrowia lipolytica* employing synthetic biology and metabolic engineering. The main aim of the study is biodegradation of plastic by the genetically engineered strains directly in the culture. The second target is a production of the single cell oils (SCO) by the engineered yeast *Y. lipolytica*. The research focus on the extension of the substrates range for SCO production by yeast.
Investigators: Aleksandra Mirończuk, *aleksandra.mironczuk@upwr.edu.pl*
Adam Dobrowolski, *adam.dobrowolski@upwr.edu.pl*

Abstract Book cover and illustrations by Maria Anna Ciemerych-Litwinienko, stem cell biologist from Department of Cytology, Faculty of Biology, University od Warsaw. Collages include fragments of photographs from www.polona.pl, www.nac.gov.pl, Wikipedia, and other sources. ©Ciemerych2022 ciemerys

University of Rzeszów would like to present a

International Artistic and Scientific Project ART & LIFE 2

We welcome all scientists that are interested in join passion to science and art