

16th HORIZONS IN MOLECULAR BIOLOGY

9th - 12th September 2019 MPI for Biophysical Chemistry Göttingen, Germany



Presented by the students of the IMPRS for Molecular Biology at the Georg-August Universität Göttingen

16th Horizons in Molecular Biology

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International PhD Student Symposium and Career Fair for Life Sciences

9th-12th September 2019 Göttingen, Germany

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Acknowledgements

It took almost twelve months to get us where we are now. Started with a big and extremely motivated team, we organized ourselves in smaller groups, each responsible for another field of organization. All those up and downs, deadlines and unexpected problems, they kept us busy but also motivated. Horizons would not be Horizons if not many things have to be solved last minute by a creative, innovative and smart team. Horizons is a great success, every year, not because we have a lot of experience in organizing conferences, but because that is what we live for in those twelve months before Horizons.

However, burning desire alone does not make a conference. There are many people involved in this adventure, people, which are essential for the success of our conference. Of course, we have to thank Dr. Steffen Burkhardt and Kerstin Grüniger from the Molecular Biology Coordination Office. Without their experience, network, ideas and motivation, Horizons would have never taken place. Many thanks for your input and problem solving skills.

We also want to thank the Georg-August University of Göttingen and the Max Planck Institute for Biophysical Chemistry for their support and for letting us use their space and resources to set up all the talks, booths, posters and events. We specially thank also the directors of the Max Planck Institutes, because without them and their support, the exchange of knowledge and intense interactions with the speakers and the campus would not be possible. We also benefit from the administration people, thanks for processing our questions and reimbursement issues.

As you will see in our booklet, we have again various supporters from Göttingen and other places in Germany, from single people to global companies. Without their help and resources, Horizons would be neither that large nor that entertaining. We would not have food or breakfast, we would not have these great prizes nor anything for the conference bags, not even this booklet. It is fascinating how many different groups and companies are already willing to support this student-organized event. During the last weeks, even more companies wrote to us and asked whether they can participate. This is a good sign, not only for us but also for the entire region of Göttingen and southern Lower Saxony. It is not without reason that even the federal ministry for education and research, represented by Anja Karliczek, honors us with their patronage. Also, thanks to the local media like the Göttinger Tageblatt but also German journals like the Labor-journal, more and more people learn about our symposium. We feel that Horizons is changing the scientific atmosphere in Göttingen and beyond and we hope that this process will progress in the future.

Further thanks to the people "behind the scenes", i.e. Michael Hartig who is the master of sound and light, together with Peter Lösel and the porters. Without you, we would sit in a dark lecture hall and the speakers would have to shout to get heard. We also thank Frederick Köpper, Claus-Peter Adam, Hartmut Sebesse and Irene Böttcher-Gajewski for their professional contribution to our booklet, for making stunning photos of Horizons for years already and for printing our posters, schedules and putting them on the screens. Also thanks to the university and UMG for letting us use their screens to promote this conference. Thanks to Uwe Krüger for serving us delicious food and a wide variety of drinks throughout the conference. We appreciate the work and effort of our MPI drivers. Without you, our honorable speakers would never reach the campus.

Last but not least, we want to appreciate our mentors, previous organizers and friends who are greatly involved in Horizons. Thank you for staying with us all the time and doing work that no one will appreciate as much as we do. Thanks to our colleagues and labs, the material and resources that we were allowed to use and that we can be on this great adventure. Finally, thanks to this enthusiastic team of organizers. It was a wonderful time, we will remember every up and down and we are looking forward toward the next Horizons already, with new energy, with a burning fire and our minds wandering out beyond the Horizon.

From the Horizons Organizing Committee 2019

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An International Scientific Conference from PhD students for PhD students



Welcome to the 16th Horizons in Molecular Biology!

Horizons in Molecular Biology symposium was conceived by the graduate students of the International Max Planck Research School in Molecular Biology to widen their own horizons beyond the rut of classes and lab work. Ever since, Horizons has grown from a humble idea to one of the most sought-after events in Göttingen and Germany. Horizons aims to bridge the gap between young scientists and experienced researchers by promoting them to engage in a productive dialog and exchange information. This year, we have planned four exhilarating days of presentations, poster sessions, panel discussions, and workshops aimed to inform, inspire and pique the interest of the young, scientific minds. One could describe Horizons as a platform built to facilitate interactions between participants and speakers! We therefore invite you to engage with our roster of distinguished speakers from diverse fields of work during their presentation or even over a cup of coffee. Do not miss this opportunity to network with your favourite speakers and participants from around the world. Should you require an introduction, we are glad to help you! From academia to far and beyond! Our Career Fair is specially tailored to meet the needs and expectations of a budding researcher. In addition to our informative talks and educational workshops, we also have a 'Speed Dating' event where you can gain valuable insights from the diverse speakers in a personalized and informal setting. Make sure to sign up for this unique experience!

Life in science can be gratifying and fun, but is often punctuated with times of hardships, doubt and disheartening lows, too. Graduate life serves as a training ground where failures are also important in moulding one as a capable scientist. However an unlucky streak of failures can leave one doubting their caliber. This is when science may become less of a passionate pursuit and more of a job that needs to be done. Being all too familiar with the experience, we have organized a *Panel Discussion on "Being a Scientist: Job or Passion?*", to get advice from our diverse speakers on how to face such adversities. We have planned the discussion in a highly interactive format so our participants get to ask many questions, too. Learning from the experiences of accomplished scientists is of vital importance, however, here at Horizons, we believe it is equally imperative to support and network with our peers. The Poster Sessions and Awarded Student Talks provide just such an opportunity. We encourage everyone to actively participate in the sessions and vote for whichever poster appealed to you the most! We look forward to having you here with us and hope to make this not only an educational experience, but also a memorable one.

Horizons in Molecular Biology 2019 Organizing Team

Organizing Committee

The 16th PhD Student Symposium Horizons in Molecular Biology is organized by a group of PhD students of the International Max Planck Research School for Molecular Biology in Göttingen.



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Partners

The 16th International PhD Student symposium is financially supported by the following institutions through the International Max Planck Research School for Molecular Biology.





Solutions For Life Sciences

Donors

The PhD Student Organizing Committee would like to thank the following companies and organizations for their support:





















Program Overview

Monday, September 9th

- 09:15 Registration Career Fair
- 09:45 **Opening Ceremony Career Fair**
- 10:00 Workshop Deb Koen Presentations that Pop: Bringing Science to Life
- 10:00 Simone Mayer Hertie Institute for Clinical Brain Research
- 10:30 Michael Becker Bayer

Coffee Break

- 11:20 Christine Mieck Springer Nature Communications
- 11:50 Andreas Laustsen Technical University of Denmark & Biotech Entrepreneur
- 12:20 Speed Dating / Lunch break
- 13:00 Workshop Angelika Hofmann The Art of Grant Writing
- 13:30 Beata Mierzwa Beata Science Art
- 14:00 Magdalena Wienken Definiens GmbH
- 14:30 Bernd Reichert EU Commission

Coffee Break

- 15:30 Horizons Opening Ceremony
- 15:45 Michael Rosbash The Circadian Rhythm Story: Past, Present and Future
- 16:45 **Robert Ernst** Emerging roles of the Unfolded Protein Response in membrane homeostasis
- 17:30 **Argyris Papantonis** Impaired chromatin remodelling links misregulation of autophagy and DNA damage response to a rare Progeroid Syndrome
- 19:30 Join us for a beer! (Z.A.K., Am Wochenmarkt 22, 37073 Göttingen)

Tuesday, September 10th

- 09:00 Registration
- 09:30 **Randy Hampton** Mallosteric Misfolding and Rhomboidal Retrotranslocation: Lessons From Regulated ERAD
- 10:15 Gaia Pigino Mechanistic understanding of cellular processes by cryo-EM

Coffee Break

- 11:30 Christopher Vollmers Sequencing for transcriptome analysis
- 12:15 **Leonie Ringrose** Epigenetics meets mathematics: The fusion of experiment and theory brings insights beyond intuition

Lunch

- 13:50 Miki Ebisuya Human Time vs. Mouse Time: *In vitro* segmentation clock as a model system
- 14:35 Needhi Bhalla A tale of two checkpoints

Coffee Break

- 15:35 Awarded Student Talk
- 15:55 **Zoya Ignatova** Translational control: probing dimensionality beyond linear sequence of mRNA
- 16:40 Asifa Akhtar Epigenetic regulation by histone acetylation
- 17:25 **Poster Session 1** (wine and cheese)

Wednesday, September 11th

- 09:00 Registration
- 09:30 Jen Heemstra A chemical biology toolbox for specific RNA modification and capture
- 10:15 **Tatjana Kleele** Degradation and proliferation are outcomes of distinct types of mitochondrial division *Coffee Break*
- 11:30 **Sjors Scheres** The atomic structures of Tau filaments from Alzheimer's and Pick's disease brains
- 12:15 Max Cryle Understanding the biosynthesis of the glycopeptide antibiotics
- 13:00 Poster Session 2 / Lunch break
- 15:00 Margaret McCarthy Surprising origins of sex differences in the brain
- 15:45 **Pavel Tomancak** A new force awakens: comparative approach to tissue morphogenesis in insects *Coffee break*
- 16:50 Awarded Student Talk
- 17:10 Leo James Epigenetic regulation by histone acetylation
- 19:15 City tour
- 20:15 Horizons Dinner (Paulaner, Düstere Straße 20A, 37073 Göttingen)

Thursday, September 12th

- 09:00 Registration
- 09:30 Food for Thought: Horizons Breakfast
- 10:15 **Naama Barkai** Gene transcription as a limiting factor in protein production and cell growth

Coffee Break

- 11:00 Awarded Student Talk Break
- 11:40 **Panel Discussion:** Being a scientist: Job or Passion? Lunch & Coffee
- 14:30 James Williamson Ribosome Assembly in Bacteria
- 15:15 Michael Levine Enhancer-promoter communication in living drosophila embryos
- 16:00 Closing Ceremony
- 16:25 Champagne and Goodbye



Admission to Plenary Lectures

Admission to plenary lectures is restricted to registered participants.

Name Badges and Conference Package Vouchers

You will receive your name badge and vouchers for the conference package (if you chose it) during registration. The conference package includes lunches, city tour and conference dinner and party. Please wear your name badge at all times during the symposium. It will be required for admission to both scientific and social programs.

Poster Sessions

If you are presenting a poster, you will be given your poster number during registration. Make sure that you put up your poster on the right poster stand. The number will serve as an identification mark for our poster committee. Pins for putting up the poster will be available at the registration desk.

Poster Session I: Tuesday, 17:25-18:55, (*Wine and Cheese*), Poster Session II: Wednesday, 13:00-14:30, (*Lunch Buffet*).

Poster Prizes

Prizes will be awarded to the top two participants selected by our juries. All registered participants presenting a poster are eligible for the prize. All participants must be available during the first poster session and present their poster to a jury of peers. Short-listed posters will be marked with a ribbon the following morning and these participants are expected to present their work to a jury comprised of Speakers and IMPRS faculty members.

Additionally, a prize will be awarded to the participant whose poster receives the most votes in our anonymous 'People's Choice Award' ballot. So be sure to drop off your ballot (found in your nametag) in one of our ballot boxes!

Travel Grants

One of the main objectives of Horizons is to give young scientists a platform to present and share their research findings at various stages of their career. The awarded student talks give selected PhD students the opportunity to present their work in the plenary lecture of the conference. This year we received more than 50 applications from all over the world representing 21 countries over 5 continents. After a rigorous selection process, we would like to congratulate this year's awardees.

Awarded Student Talks

- Angel Santiago-Lopez (10th September, 15:35) Mobilization of cellular stress signaling in protein misfolding diseases for controlled gene delivery - *Georgia Institute of Technology, USA*
- Julie Trolle (11th September, 16:50) Engineering Prototrophy In Mammalian Cells - *New York University, USA*
- Triana Amen (12th September, 11:00)

Stress Granules regulate cell metabolism through clustering of mitochondrial membrane protein VDAC - *Universitätsmedizin Göttingen, Germany*

Poster Presentation

- Tomas Lagunas Washington University School of Medicine, USA
- Rima Siauciunaite Karlsruhe Institute of Technology, Germany
- Priscilla Gonzalez Pontificia Universidad Catolica de Valparaiso, Chile
- Ivana Borovska Slovak Academy of Sciences, Slovakia
- Abhishek Upadhyay Humboldt University of Berlin, Germany
- Alina Stein Universität Basel, Switzerland

Diversity of applications received for Horizons 2019



Social Events

Horizons is not only about scientific exchange but also about building personal contacts with people in your field. A long standing tradition of the symposium is to organize social events so that the participants and speakers get a chance to interact in an informal setting. This year we will be hosting the following activities:

Social Mixer: Join us for a Beer!

After an engaging day of career fair and scientific discussions, we invite the participants and speakers to join us in exploring the colorful nightlife of Göttingen. We will visit one of the local's favorite bars to experience the lively atmosphere. It is a great opportunity to meet new people over a glass of traditional German beer and food from 19:30 on Monday, September 9th. All participants are invited to join, the event is not included in the conference package.

Location: Z.A.K. (Am Wochenmarkt 22, 37073 Göttingen)

Wine and Cheese

During one of the poster sessions, a wine and cheese tasting is organized, where a great variety of cheese from different places will be served. With a glass of wine in hand, you can have forthright discussions about the research of young scientists from all parts of the world. Tuesday, September 10th, 17:25 (during Poster session 1)

City Tour

Göttingen is known as the city of Science, it dates back to the Middle Ages and boasts its well preserved half-timbered houses. Join us in a short city tour combining the fantastic architecture and the scientific historical overview. The guided tour will take place on Wednesday, September 11th, starting at 19:15 h. The tour is in English. We will meet at the Gänseliesel fountain in the city center and end the tour at *Paulaner* where we will have the conference **dinner and party**. The tour is for participants who opted for the conference package and the speakers.

Starting point: Gänseliesel fountain in the city center

Dinner and Party

As every year, we invite you to a night of informal interaction with dining and merriment. It is a good occasion to get to know the other participants and speakers and to celebrate new friendships over a couple of drinks, food, games and music and dancing starting at 20:15 on the 11th of September (only included in conference package).

Location: Paulaner - Zur alten Brauerei Göttingen (Düstere Straße 20A, 37073 Göttingen)

"Food for Thought" Breakfast

On Thursday morning we invite you to our science breakfast, to enjoy bread, pastries and Castagnes with a cup of coffee or tea on our last conference day! We thank our sponsors *Ruch* and *Küsters* for sponsoring the breakfast.

History of Horizons

December 4th, 2003. The seminar room in the Göttingen Center for Molecular Biosciences was bustling with activity. Organizers darted in and out of the room, making sure everything was in order and as it should be. Speakers attempted to look through their talking points, whilst entertaining the many curiosities of the next generation gathered around them. The graduate students themselves, whispered to each other and waited apprehensively, with thinly veiled excitement, for the program to begin. There were just under a hundred eager minds crowded into that one room. Students and researchers; all brought together by a shared passion: a passion for science. This was the inaugural Horizons in Molecular Biology Conference: An International PhD Student Symposium!

The conference has come a long way since then. Over the years, the number of participants grew steadily and increased in variety. Horizons now regularly attracts around *three hundred participants from over thirty countries*. The symposium has featured numerous renowned researchers, representing a wide range of fields in the life sciences. This exhaustive list also included several Nobel Laureates, namely: Professors **Martin Chalfie**, **Sir John Walker**, **Ada Yonath**, **Thomas Südhof**, **Kurt Wüthrich**, **Carol Greider**, **Venki Ramakrishnan**, **Sir John Gurdon**, **Tim Hunt**, and this year **Michael Rosbash**.

Horizons has also evolved continuously as each new batch of student organizers brings a fresh perspective and new ideas, proving consistently that you *can* make a good thing better! In 2006, the organizers introduced a **Poster Session** accompanied by **Student Talks**. PhD students presented *over a hundred posters* and a select few were given the chance to present their work to an audience of peers and professors. Building on this, in 2007, Horizons launched its first **Career Fair** in conjunction with the conference. The fair offered budding scientists with an opportunity to connect with industry and network with life science professionals with a different backgrounds and perspectives. Representatives from over *twenty companies* were present at the inaugural Career Fair, conducting live interviews and CV checks. Today, the Career Fair has adopted a major role in the conference, offering **workshops** held by professionals, and interactive discussions with key figures in the modern life science industry. All catered to the needs of a young scientist.

At the heart of Horizons, is an idea: **An International Scientific Conference** *from* **PhD students** *for* **PhD students**. Organized by students from Göttingen's International Max Plank Research School for Molecular Biology, Horizons in Molecular Biology provides an interactive experience in a relaxed environment. It aims to bridge the gap between young scientists and experienced researchers and bring together experts and novices from various fields of the life sciences to engage in a productive dialog and exchange information. Modern discovery is persistently progressing from exploring rainforests in faraway lands to observing the nature of life under a microscope. We are the modern explorers, and are committed to keep progressing forward, in the pursuit of science, towards the Horizon.

IMPRS for Molecular Biology

For the 16th time, the Horizons symposium is organized by students of the International Max Planck Research School (IMPRS) for Molecular Biology at the University of Göttingen. The Molecular Biology Program is jointly conducted by various departments of the University of Göttingen, the University Medical Center Göttingen, the Max Planck Institutes for Biophysical Chemistry and Experimental Medicine, and the German Primate Center. The common aim is to offer an intensive, research-oriented education in order to prepare the students for a professional career in the life sciences. This broad spectrum of topics is mirrored in the fields covered by Horizons. In addition to the small classes and specialized hands-on laboratory courses, the intercultural experience is extraordinary. Excursions, culture nights and workshops with students from all over the world naturally become a "seminar" on intercultural communication. Students can join the program after completing their Bachelor studies. During the first year, students receive a broad education in molecular biology through lectures, lab rotations and methods courses delivered by the participating institutions. From the second year, the students conduct their own research during their MSc or PhD thesis projects and receive further training in specialized methods and skills courses within the Göttingen Graduate Center for Neurosciences, Biophysics and Molecular Biosciences (GGNB), which is funded by the German Excellence Initiative.

In September 2000 the first brave students from as far away as Ghana, China, Mexico and Malaysia came to Göttingen to join the new International MSc/PhD Molecular Biology Program. Today, with the twentieth batch of twenty students joining the IMPRS, the program has become very successful as demonstrated by excellent evaluations, awarded prizes, and applications from all over the world. By now, more than 175 students have successfully defended their PhD theses within the program and kissed the Gänseliesel. Kissing this statue that stands on a fountain on the market place in the middle of the city and giving her flowers is an old tradition for Göttingen PhD students. For more detailed information about the International MSc/PhD Molecular Biology Program in Göttingen, please take a look at our website:

www.gpmolbio.uni-goettingen.de

About Göttingen

The renowned university town of Göttingen lies in the center of Germany in southern Niedersachsen (Lower Saxony) between the Harz mountains and the Weser river. Its establishment dates back to 953, it was chartered in 1210 and flourished as a member of the Hanseatic League.

A glimpse of this hanseatic opulence is still present today in the medieval Town Hall, the splendid churches and quaint half-timbered houses in Gothic, Renaissance and Baroque styles. Göttingen's landmark is the "Gänseliesel", an art nouveau statue of a girl herding geese on top of the fountain on the market square, outside the Old Town Hall. Traditionally, all new doctoral graduates of the University kiss the cheeks of the statue after passing their examinations, making her "the most kissed girl in the world".

The Georg-August University was founded in 1734 and today has 13 faculties with 32,000 students. The university sends forth many famous scientists – amongst its alumni are 45 Nobel laureates. Especially many famous mathematicians come from Göttingen: Carl Friedrich Gauss, Bernhard Riemann and David Hilbert, to name only a few.

Apart from the university, the city is also home to many research institutes, such as Max Planck Institutes, and international companies.

Nearly untouched by bombings in World War II, the city center is now an attractive place to live in with many shops, small cafes and chic bars. Consequently many students live here, giving Göttingen a young face: in 2003, almost half of the population of the inner city was between 18 and 30 years of age. Today Göttingen has approximately 130,000 inhabitants including 24,000 students, giving it the charm of a small and lively university city.

Practical Information

Certificates of attendance will be handed out during registration upon request.

Insurance

The Organizing Committee accepts no responsibility for accidents or damage to participants' private property. Please make your own arrangements for all the necessary insurance.

Internet

Free Wi-Fi internet (eduroam) is available throughout the symposium in the lecture facilities. If you do not have access to eduroam, please contact the registration desk.

Parking

Participants can use the parking lots available near the MPI for Biophysical Chemistry.

Buses

The buses number 21, 22 and 23 to the city center leaves just outside the symposium venue at the bus stop "Faßberg". Bus schedules are available at the registration desk.

Taxi

Taxis to the city center can be arranged by calling one of the many Göttingen taxi companies:

- Göttinger-Funk-Taxi-Zentrale: 0049-551-69300
- puk minicar: 0049-551-484848

Please note that taxis are considerably more expensive than public transport. Also, keep in mind that taxis only accept cash.

Organizers

In case you have difficulties ordering a cab, getting around town or else, you are always welcome to ask for help one of the organizers. Just look out for students wearing a **Horizons t-shirt** and get in touch with us regarding your issue.

Services

Registered participants are entitled to the following: Admission to plenary lectures, conference material (certificate of participation, name badge, conference bag) and refreshment during coffee breaks.

Participants who opted for the conference package, in addition to the above get a voucher for lunch, city tour, conference dinner and party and a hardcopy conference booklet.



Career Fair - Schedule



Welcome to the 13th Career Fair for Life Sciences

Your PhD is the starting point of your career in science. But have you ever found yourself asking: what is a career in science? A Post-Doc and tenure track to become a group leader? A position in a big biotech company? What are my options?

The answer is that with a PhD within the natural sciences, you have an ocean of options. Certainly more than two! The truth is, during a PhD you will acquire skills and a mind-set sought after everywhere.

In this year's Horizons Career Fair, we introduce you to seven people, all with a background in the natural sciences, who have chosen seven different career paths. These speakers represent the fields of science policy, academic research, industry, startups and entrepreneurship, publishing, consulting, and science art. Our goal is to present a broad range of possible future paths for our participants and to create an informal environment in which students can explore their possibilities after a PhD or a Post-doc in the natural sciences.

In addition, we give you the opportunity to learn the "dos" and "don'ts" of research proposal writing and a chance to learn clever tricks to become a more confident speaker in our two workshops.

The Horizons career fair 2019 will give you a taster of what opportunities are out there and broaden your Horizons!

Career Fair Organizers

Notes

Career Fair



Monday 10:00 am

Insights: Academia

Following one's own curiosity and pushing forward the frontiers of human knowledge is a dream for many PhD students. But how do you make this dream come true? And is the grass really so much greener on the other side? Simone Mayer will share her strategies and decision points that crucially contributed to her longterm goal of becoming a principal investigator. Having only recently started her own research group, she will share the unexpected joys as well as the unforeseen hurdles involved in this endeavor. For Simone, heading your own research lab is a bit like having your own company. As the CEO, one does a diverse array of jobs including being the head of Human Resources, the Head of Acquisitions, the Head of Public Relations as well as the Head of Finance and Research Strategy. For these reasons, heading her own group has been a very fun experience that has allowed Simone Mayer to grow as a person in many different areas already. Simone Mayer is looking forward to developing her research endeavor further in the coming years to push the frontiers of human knowledge on human brain development and evolution.

Simone Mayer Hertie Institute for Clinical Brain Research



Dr. Simone Mayer is a molecular neurobiologist interested in understanding the development of the neocortex, the brain structure that has significantly contributed to the evolution of advanced cognitive functions in mammals. Simone conducted her doctoral work at the Max Planck Institute of Experimental Medicine focused on inhibitory synapses. Being interested in both academia and science policy, Simone decided to get practical experience beyond the lab during a traineeship at the European Research Council Executive Agency in Brussels, Belgium. After 5 months in an office, however, she was happy to be back at the bench starting her postdoctoral work at the University of California, San Francisco. Being based in the Bay Area, in collaboration with a biotech company, Simone established a novel method to determine physiological and transcriptomic features of single cells simultaneously. Since September 2018, Simone is heading an independent research group at the Hertie Institute for Clinical Brain Research in Tübingen, Germany. Her research questions focus on how environmental impacts affect brain development, for example by modulating the neurotransmitter system.

Baver



Michael Becker

Michael Becker is heading a laboratory in the area of kidney disease research at Bayer AG. In addition, he is leading multiple preclinical research projects partly involving other companies or academic institutions. After studies in Biochemistry at Tübingen University and Harvard Medical School, he did his PhD at the German Cancer Research Center in Heidelberg. He joined Bayer in 2015 in the area of Lead Discovery, where he worked on uHTS assay development and switched to his current position in 2016. In addition to his passion for drug discovery he has a strong interest in innovation. He won the Merck Innovation Cup in 2014 and is supporting innovation at Bayer in his role as Innovation Coach.



Preclinical Research in the Pharmaceutical Industry

Michael will talk about his personal career path and decisions who led him to his current position. Moreover, he will give an insight into his everyday work and the differences between research in pharma and academia. At the end of his talk, perspectives into other positions in pharma R&D and beyond will be given.

Career Fair



Insights: Publishing

Do you love science but feel that a career at the bench isn't enough to sate your desire to learn more about the natural world? Do you enjoy reading papers outside your chosen area of research? If the answer is 'yes' to any of these questions, consider an editorial career!

To give you an insight in the publishing world and help you decide whether this could be a career choice, Christine will talk about recommended skills for being an Editor and how the daily life at Nature Journals looks like. Please bring all your questions regarding career options with publishers, editorial workflows and decision making along. Christine Mieck Nature Communications



Christine obtained her PhD from the IMP Vienna where she studied kinesin motors using in vitro reconstitution and structural biology approaches. Fascinated by 'bottom-up' approaches to rebuilt dynamic biological systems, she decided to move from motor proteins to studying the dynamic organization of membrane proteins. Her post-doctoral work at the IST Austria explored the biochemical and biophysical bases of cellular polarity in an in-vitro setup. After starting her editorial career as an Editor at Scientific Reports for 3 months, she joined Nature Communications in September 2017. Christine handles submissions on the biochemistry and structural biology of membrane proteins, computational structural biology, and general and single molecule biophysics. Christine is based in the Berlin office of Springer Nature.



Andreas Laustsen

Technical University of Denmark & Biotech Entrepreneur

Associate Professor Andreas H. Laustsen heads the Tropical Pharmacology Lab at the Department of Biotechnology and Biomedicine, Technical University of Denmark. The Tropical Pharmacology Lab is an academic research group focusing on developing biotherapeutics and diagnostics against neglected tropical diseases. Andreas himself is specialized in antibody discovery, toxinology, antivenom, and neglected tropical diseases, and holds a PhD from the University of Copenhagen. He is an Advisor to the World Health Organization's Working Group on Snakebite Envenoming, and he is a co-founder of the biotech companies Biosyntia, VenomAb, Antag Therapeutics, Chromologics, Bactolife, and VenomAid Diagnostics. Andreas is recognized as Denmark's Coolest Engineer, and he was on Forbes 30 under 30 list for 2017 and MIT Technology Review's list of the 35 Top Innovators under 35 in Europe 2017. Andreas spends most of his time with academic engagements, but also still plays an active role in most of the companies he co-founded.



Venturing into biotech entrepreneurship as a young academic

Upon graduation, the majority of PhD graduates nowadays transition from a daily work life in academia to one in industry. This switch may involve a steep learning curve. However, often PhD graduates will have easy access to mentors and older colleagues that may help them make this transition. In contrast, venturing into biotech entrepreneurship is completely unchartered territory for many young graduates, who may not as easily find access to advice and mentorship. This is both because a much smaller number of biotech entrepreneurs exists in comparison with biotech industry professionals, but also because the young biotech entrepreneur will typically have to work in a very small startup team with only few co-founders and colleagues due to the often limited resources of a young biotech startup. In my talk, I will present my own story from how I co-founded my first biotech company (Biosyntia) during my master studies in 2012, over why I choose to go back to university to pursue a PhD, and end with describing why and how I chose to pursue a career as a university academic, while co-founding 5 additional companies alongside my academic career. I will reflect on the benefits and drawbacks of venturing into (biotech) entrepreneurship, as well as continuing your career in academia, and present my views on how one can merge or bridge these fields. Finally, I hope to share some personal advice for aspiring biotech entrepreneurs, as well as younger academics..

Career Fair



Monday 1:30 pm

Communicating Scientific Concepts Through Art

Creativity is an integral part of both science and art, and using it to combine these two seemingly different disciplines creates unique and effective ways to communicate scientific information. Along my classical academic career path, I have been exploring the visual aspect of science communication using hand- drawn illustrations and science fashion. My science art aims to convey complex biological concepts using both abstract imagery and real scientific data. Each illustration requires breaking down the essence of complex scientific findings and translating them into aesthetic visuals using metaphors that describe abstract biological processes in intuitive ways. This form of visualization provides a powerful tool to facilitate communication between scientists from diverse fields, spark fascination and curiosity, as well as inspire future scientists. In this talk I will share my journey towards combining science and art, and discuss how cultivating passions outside of the lab creates beautiful and unexpected opportunities with the potential to open up new and creative career paths.

Beata Mierzwa Beata Science Art



Beata is a postdoctoral researcher in the labs of Karen Oegema and Arshad Desai at the Ludwig Institute for Cancer Research and University of California San Diego, where she explores the variation of the cell division machinerv in different cell types. After studying Molecular Biology at the University of Vienna, Beata completed her master's thesis at the ETH Zürich. During her PhD in Daniel Gerlich's lab at the Institute of Molecular Biotechnology (IMBA) at the Vienna BioCenter, she focused on the final step in cell division, exploring the dynamics of the ESCRT-III machinery that separates the cells during cytokinetic abscission.

Beata made her first science art drawing to depict her own PhD research, and her illustrations quickly developed into a mission to communicate science. She started spending her evenings drawing in addition to her daily experiments in the lab, making artwork for many conferences, journal covers and research groups. She also designs science-inspired clothes printed with drawings and microscopy images. You can find a gallery of her work at www.beatascienceart.com or follow her on social media (@beatascienceart).



Magdalena Wienken

Definiens GmbH

Magdalena received a B. Sc. and Honours degree in Applied Biology with a focus on Genetics and Immunology from the University of Applied Sciences in Bonn as well as the University of Aberdeen in Scotland. She continued her studies with an M. Sc. in Molecular Medicine at the University Medical Center Göttingen and finished her PhD in Molecular Oncology as a scholar of the GGNB graduate school. Within her PhD she focused on the p53 independent functions of Mdm2 as a stemness promoting factor and chromatin modifier. After her PhD, Magdalena decided to pursue her career in strategic consulting, working for Accenture Technology Strategy with a Pharma and Biotech focus. She specialized in data-driven strategies, working for the top-ranked pharma companies in the DACH region. She only recently left consulting, continuing her career as a manager at Definiens, a middle-sized, Munich based company, which specializes in tissue phenomics in the context of innovative immunotherapy of cancer.



Disrupting Biomedical Science Through Data and Al

Finishing a PhD in Science raises a lot of questions and opens many different doors. Am I going to stay in academia? Shall I change into industry? Which alternative job opportunities are out there? Magdalena decided to leave academia for consulting due to 2 reasons: 1) get a broader understanding on the current challenges and opportunities of the Pharma industry and 2) get the opportunity to quickly gain industry and strategic expertise, and work with top management clients in innovative and short-cycled projects.

This talk will outline the pros and cons of a career in consulting, experiences as well as the challenges that might come along with it. In addition, it will highlight why she decided to change back to science again.

Career Fair



Monday 2:30 pm

Eurocrat in spite of oneself, or; love yourself and it doesn't matter who you marry

A short outline of my path into the European institutions and why I stayed there for over 25 years. In this talk, Bernd Reichert will talk about the work within the European Union and the prospects of young scientists within the field of science policy. Bernd Reichert EU Commission



University studies and PhD in physical chemistry and quantum physics at the Humboldt University of Berlin. After Post-Doc studies at the Technical Universities of Berlin and Munich, he joined the European Commission, Directorate General for Research, in 1992. There he has held several assignments as programme officer in the areas chemistry, biotechnology, nanotechnology as well as science and society, until he became Head of Administration of the directorate "Industrial Technologies" in 2003. From 2007 until the end of 2013 he was heading the unit "Small and Medium-Sized Enterprises" in the Commission's Research and Innovation Directorate General, Until the end of 2018 he was responsible for setting-up the activities "Horizon 2020: Small and Medium-Sized Companies", in particular the implementation of the SME Instrument under the EU Research and Innovation Framework Programme Horizon 2020, within the Executive Agency for Small and Medium-Sized Companies (EASME).



Deb Koen

Account manager and career coach for Corning Incorporated, and Senior consultant for HR Works, Inc. and for BadFish Consulting.

Deb Koen, Career Strategist and Professional Development Coach, is a recognized leader in the field of career development. She conducts peak performance programs to develop individual and organizational potential. Along with having been a preferred presenter for The Wall Street Journal's Executive Diversity Career Fairs, Deb coaches, consults and presents on career management, transitions, job search strategies, and women in the workforce. She regularly conducts leadership development programs for the International Visitors Leadership Program (IVLP) sponsored by the U.S. Department of State. Deb brings her expertise and enthusiasm to each and every engagement to create innovative solutions.

Deb previously led the private nonprofit organization, Career Development Services, and she has consulted for numerous corporations and counseled in higher education. She is currently the account manager and career coach for Corning Incorporated, and senior consultant for HR Works, Inc. and for BadFish Consulting.

Deb is a co-author of Career Choice, Change & Challenge (Jist, 2000). She has been a columnist for The Wall Street Journal's WSJ.com, for the business section of Gannett's Democrat & amp: Chronicle and for the scholarly scientific journal, Nature. She holds a certificate in nonprofit management from Harvard Business School and M.A. in Counseling from Colgate University. Deb also serves on the Board of Trustees for The Nature Conservancy. In 2016, she received the Corporate Enlightenment Award from Rochester Global Connections for her work with emerging leaders "for exemplifying the ideals of inclusiveness, respect for diversity, global understanding and citizen diplomacy."



Presentation that Pop: Bringing Science to Life

Workshop

Communicating science through presentations plays a large role in delivering messages within the scientific community and beyond to the general public. The most effective speakers can design and present a scientific talk that conveys scientific information of value in a way that engages the audience.

This workshop provides practical approaches that are immediately applicable to:

- Prepare the content and delivery for a meaningful and lively scientific presentation that best represents you and your research
- Structure the introduction, core content, and conclusion to engage the audience aurally and visually
- Identify ways to adapt your formal presentation to more casual situations (e.g. networking and informal conversations) to tailor your message and reflect favorably on your career.

Join in this opportunity to increase your confidence and your impact through these key presentation practices.

Career Fair

Monday 1:00 pm

The Art of Grant Writing

For a successful grant, basic grant writing principles are a prerequisite, as is clear scientific writing. Learn to organize and compose your thoughts and apply winning writing principles within your next proposal through this workshop. Participants will also hear about common mistakes to avoid and receive guidance on best practices and the review process. Key sections of a grant will be highlighted, and hands-on exercises will be included. Emphasis will be on how to achieve maximum impact and success in grant writing with the reader and reviewer in mind. The workshop is presented by Dr. Angelika Hofmann, Strategic Projects and Communications Advisor to the Vice Provost for Research at Yale University and author of two major textbooks on the topic: Scientific Writing and Communication - Papers, Proposals, and Presentations (Oxford University Press) and Writing in the Biological Sciences (Oxford University Press.)

Angelika Hofmann Office of the Vice Provost for Research



Before joining the VPR team in 2018, she was Director, Corporate and Foundation Relations, Science and International Initiatives, in Yale's Office of Development where she managed some of Yale's most important prospects and played a key role in fundraising from leading international corporations and foundations. As part of this position, she also oversaw a team of professional scientific writers and the operational management of the Corporate and Foundation Relations office.

Angie holds a PhD in molecular biology and biochemistry from the University of California, Irvine, and was a post-doctoral fellow at the Max-Planck-Institute of Molecular Genetics in Berlin, Germany. She is renowned in the scientific writing field and teaches workshops on scientific communication around the world. She is also the author of two books that have become standards in the field: Scientific Writing and Communication -Papers, Proposals, and Presentation (Oxford University Press, 3.ed., 2016) and Writing in the Biological Sciences (Oxford University Press 3.ed., 2018).





Workshop
Speed Dating

Along with talks and exciting workshops, we have a 'Speed Dating' event in the lineup. Here, the participants get a chance to talk to the speakers of choice face to face. During the lunch break, this event gives you an opportunity to interact with the speakers in a personal and informal environment. In small groups, you will get to meet two speakers and discuss for half an hour all the questions that pop in your heads.

PLENARY LECTURES







Michael Rosbash

Brandeis University Waltham, Massachusetts, USA



Michael Rosbash is a Professor of Biology and the Peter Gruber Professor of Neuroscience at Brandeis University. He is also an Investigator of the Howard Hughes Medical Institute. Rosbash went to the Newton public schools in greater Boston and then to Caltech, graduating in 1965 with a B.S. in Chemistry. He spent the 1965-1966 academic year in Paris as a Fulbright Scholar in the lab of Marianne Grunberg-Monago and then entered the Ph.D. program at MIT in the fall of 1966. Rosbash worked there in the lab of Sheldon Penman and received a Ph.D. in Biophysics in 1970. After a brief stint at the University of St. Andrews, he was a post-doc in the lab of John Bishop in the Department of Genetics at the University of Edinburgh from 1971-1974. Rosbash joined the faculty of Brandeis University in 1974 and was promoted to Professor of Biology in 1986. He became a Howard Hughes Medical Institute Investigator in 1989.

Rosbash has made fundamental contributions to our understanding of the post-transcriptional regulation of gene expression, especially RNA metabolism in yeast. He is best known however for his work in Drosophila that illuminated our current understanding of the molecular mechanisms that underlie circadian rhythms, the intrinsic clock that controls the cyclic behaviors of all animals. These same molecules, molecular machines and biological principles not only control Drosophila circadian clocks but also the ubiquitous process of circadian rhythmicity throughout

The Circadian Rhythm Story: Past, Present and Future

The last 35 years has seen a sea change in the field of circadian rhythms. This modern era began with work in Drosophila (fruit flies), which has been a leading genetic system for more than 100 years. My colleagues and I discovered the clock mechanism that underlies circadian timing, and it turned out that the genes and mechanism are conserved in all animals. This circadian system governs a large fraction of all gene expression, once again extending from fruit flies to humans, which explains why so much animal physiology (biochemistry, metabolism, endocrinology, behavior, sleep, etc.) is under temporal control. The broad reach of circadian biology indicates that it will continue to be important to many aspects of human well-being and will become increasingly relevant to medicine as more knowledge and applications accrue. Time permitting, I will also touch on the role of the Drosophila brain clock in controlling sleep and locomotor behavior, which remains a central focus of my current lab.

the animal kingdom. This circadian clock also controls much of cell physiology and metabolism, again in all animals - from humans to Drosophila (fruit flies).

Rosbash and his Brandeis colleague Jeff Hall as well as Mike Young of the Rockefeller University have received numerous awards for their circadian work, including most recently the 2017 Nobel Prize in Physiology or Medicine. They previously received the Shaw Prize in Life Science and Medicine (2013), the Wiley Prize in Biomedical Sciences (2013), the Massry Prize (2012), the Canada Gairdner International Award (2012), the Louisa Gross Horwitz Prize for Outstanding Basic Research (2011), and the Peter and Patricia Gruber Foundation Neuroscience Prize (2009). Rosbash also received the Caltech Distinguished Alumni Award (2001), and he is a Member of the National Academy of Sciences, a Fellow of the American Association for the Advancement of Sciences and a Fellow of the American Academy of Arts and Sciences.



Robert Ernst



Saarland University Saarbrücken, Germany



Robert Ernst studied Human Biology at the Philipps-University Marburg and moved for his Diploma thesis to the Goethe University Frankfurt, where started his PhD thesis at the Institute of Biochemistry. He defended his thesis at the Heinrich-Heine University Düsseldorf in 2007. He was a postdoctoral fellow at the Whitehead Institute for Biomedical Research in Cambridge, MA before joining the group of Kai Simons at the Max-Planck Institute for Molecular Cell Biology and Genetics in Dresden, Germany. He then started his own group as Emmy-Noether fellow at the Goethe University Frankfurt. Since 2017, he holds the Chair for Molecular Biology at the Medical Faculty of the Saarland University. His laboratory is interested in how the organelles of eukaryotic cells can sense their own composition and physical properties in order to maintain their identity. Using cross-disciplinary approaches that combine cell biology, biochemistry, biophysics, and theoretical physics, his group uncovered the molecular mechanism of central machineries controlling key aspects of membrane biogenesis: 1) The decision between making more membranes or storing fat, 2) The proportion of saturated versus unsaturated lipids, and 3) The rate of membrane protein and membrane lipid production. These machineries form an intricate regulatory circuit that jointly controls membrane biogenesis. Currently, his laboratory is developing new approaches to isolate organelles

Emerging roles of the unfolded protein response in membrane homeostasis

Lipids and proteins shape the physicochemical properties of cellular membranes as a collective, thereby affecting membrane protein function and contributing to organelle identity. Eukaryotic cells face the challenge of maintaining the complex composition of several coexisting organelles. The molecular mechanisms underlying the homeostasis of subcellular membranes and their adaptation during stress are only now starting to emerge. We have studied three membrane property sensors of the endoplasmic reticulum (ER), namely OPI1, MGA2, and IRE1, each controlling a large cellular program impacting on the lipid metabolic network. OPI1 coordinates the production of membrane and storage lipids (Hofbauer et al., 2018), MGA2 regulates the production of unsaturated fatty acids required for membrane biogenesis (Covino et al., 2016), and IRE1 controls the unfolded protein response (UPR) to adjust the abundance and the folding capacity of the ER (Halbleib et al., 2017). Although these three machineries use remarkably distinct sensing mechanisms, they are functionally connected via the ER membrane and cooperate to maintain membrane homeostasis. Now, it is time to their cellular role by investigating their impact on the molecular composition of isolated organelles from stressed and unstressed cells. Based on a discussion of the sensing mechanisms and the integrative functions of the above-mentioned machineries, we propose that IRE1 and the unfolded protein response can sense the protein-to-lipid ratio in the ER membrane to warrant a balanced production of membrane proteins and lipids via the UPR.

- Covino R., Ballweg S., Stordeur C., Michaelis J.B., Puth K., Wernig F., Bahrami A., Ernst A.M., Hummer G., Ernst R. (2016) A Eukaryotic Sensor for Membrane Lipid Saturation. Molecular Cell 63:49-59
- [2] Halbleib K., Pesek K., Covino, R., Hofbauer H.F., Wunnicke D., Hänelt I., Hummer, G., Ernst R. (2017) Activation of the Unfolded Protein Response by Lipid Bilayer Stress. Molecular Cell 67:673-684
- [3] Hofbauer H.F., Gecht M. Fischer S.C., Sybert A., Frangakis A.S., Stelzer E.H.K., Covino R., Hummer G., Ernst R. (2018) The molecular recognition of phosphatidic acid by an amphipathic helix in Opi1. Journal of Cell Biology, 217:3109-3126

and to study their molecular compositions in order to generate a quantitative cellular map with subcellular resolution.





Argyris Papantonis

University of Göttingen Göttingen, Germany



Argyris Papantonis received his PhD on Biochemistry and Molecular Biology in 2008 by the University of Athens, Greece. He went on to study transcriptional organization as a postdoctoral researcher at the University of Oxford, where he later took up a Lecturer of Biochemistry position at University College. In 2013 he started his own lab on Chromatin Systems Biology at the University of Cologne, and in 2018 he was appointed as a Professor of Translational Epigenetic with the Medical Faculty of the University of Göttingen. His laboratory focuses on understanding the basic rules behind chromatin and transcriptional organization in 3D space and time with an emphasis on models of cellular senescence, ageing or pro inflammatory signaling

Impaired chromatin remodelling links misregulation of autophagy and DNA damage response to a rare progeroid syndrome

Members of the Chromodomain-Helicase-DNA binding (CHD) protein family are chromatin remodelers critically implicated in human pathologies. CHD6 is the least studied member of this family, and we were motivated to dissect its in-cell roles by discovering a mutated CHD6 allele in a patient suffering from the rare Hallermann-Streiff premature aging syndrome (HSS). We generated isogenic iPSC lines carrying (or not) this single point mutation in the CHD6 SANT/SLIDE domain, which allow studying HSS-relevant cell identities. Using these lines, we show for the first time that CHD6 binds the promoters of a cohort of autophagy and stress response genes across cell types. The mutation in CHD6 impairs its ability to recruit co-factors and regulate genes in response to DNA damage and autophagy stimulation, thus leading to accumulation of unresolved DNA damage burden. By combining genomics and functional assays, we describe a molecular mechanism underlying chromatin control of autophagic flux and genotoxic stress surveillance that is broadly applicable to human cell types and can explain HSS onset and progression.



Randy Hampton

University of California San Diego, California, USA



Randolph Hampton was born in New York State just before cars evolved fins. He grew up in Scarsdale, NY, attended Ohio Wesleyan University, and by a circuitous route that included research at University of Michigan, running the office at Zingerman's Delicatessen, and doing field work in music and comedy, finally earned a Ph.D. in Biochemistry in the laboratory of Christian Raetz at University of Wisconsin-Madison and at Merck & Co. in Rahway, NJ. He then headed west to do a postdoctoral fellowship with Jasper Rine at U.C. Berkeley, in one of the few laboratories in the world dedicated to both yeast and dog genetics, where his work on regulated degradation of HMG-CoA reductase (in yeast, not dog) began. He next took a position in the Division of Biological Sciences at U. C. San Diego, where he remains to this day. His research interests include cellular management of misfolded proteins, sterol regulation and the cholesterol pathway, metabolism, and ubiquitin in health and disease. His pedagogical interests involve finding ways to communicate to tomorrow's physicians and biomedical professionals the critical differences between sickness and wellness, and the role of science in exploring that boundary. In addition, Randy (which is what everyone calls him except when in he is in trouble) is also deeply involved in the recovery movement, with 36 years clean and sober so far. His interests outside the academe include running, 5 string banjo, secular spirituality, and the complex synergies that govern the lives of us all. Most recently,

Mallosteric Misfolding and Rhomboidal Retrotranslocation: Lessons From Regulated ERAD

ERAD is highly selective, allowing destruction of proteins subtly perturbed from their native conformation. Biology has capitalized on this selectivity to regulate the stability of normal native proteins. Understanding how the cell harnesses the specificity of ERAD to accomplish protein regulation can provide new routes for experimental alteration of quality control, useful both for basic and therapeutic applications. We have been studying the regulated ERAD of Hmg2, an integral membrane protein by the HRD ERAD pathway. The sterol pathway molecule GGPP directly causes Hmg2 to undergo a reversible transition to a HRD pathway substrate. GGPP regulates Hmg2 ERAD at the mid nanomolar range, and antagonism can be observed with closely related molecules. This appears to be an example of ligand-dependent misfolding, which we refer to as mallostery in reference to the classic mode of ligand mediated regulation of protein function. We believe that this mode of regulation has broad and untapped applications in chemical biology and pharma.

A subject of great interest in ERAD is the exit mechanism(s) for retrotranslocation of ERAD substrates to the cytosol. Using a new self-ubiquitinating substrate we have recently discovered a dedicated route for removal of integral membrane ERAD clients, referred to a ERAD-M substrates. This route of dislocation affects both HRD and DOA substrates, and is mediated by the Dfm1 rhomboid protein, which had previously been discounted as a participant, by us and others. This dichotomy is resolved with the realization that dfm1 nulls undergo rapid suppression that depends on Hrd1, and the current best candidate for lumenal ERAD-L retrotranslocation. We have discovered that the stress imposed by the dfm1 Δ null state results in dramatic remodeling of the ERAD machinery and the creation of a new retrotranslocation route for ERAD-M substrates. I will report on the primary function of Dfm1 in retrotranslocation, the features of the Hrd1-mediated retrotranslocation of ERAD-M substrates, and the novel stress that allows this suppression to arise.

(Aug 2018) he got married for the first time, to his lovely now-wife Marrie (with 2 r's), making him a newlywed who is applying for Medicare. The message: life is full of surprises.



Gaia Pigino

MPI of Molecular Cell Biology and Genetics Dresden, Germany

Dr. Gaia Pigino received her PhD in Evolutionary Biology from the University of Siena, Italy in 2007 for her studies on bio-indicators for contaminated soil. Electron microscopy (EM), one of the tools she used for this work, guickly became the central method for her research ever since. After her first postdoc in the EM Lab of the Department of Evolutionary Biology in Siena, she moved to ETH Zurich and the Paul Scherer Institute in Switzerland and was awarded an EMBO fellowship to continue her work on the cryo-EM structural analysis of ciliary components. In 2012, Dr. Pigino started her own lab at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany. The work of her lab is centered around the question how complex cellular machines self-organize. Cilia remain the main focus, where the Pigino Lab investigates fundamental functional aspects of the dynamic process required for the assembly of the cilium. Learning about self-organization and functional implications of structural aspects naturally requires to combine and further the latest imaging technologies (3D cryo-EM, tomography, correlative light and electron microscopy (CLEM), and various light microscopy techniques) with tools from biochemistry, in vitro reconstitution, and genetic engineering.



Mechanistic understanding of cellular processes by cryo-EM

New cryo-EM technologies enable to investigate protein structures in the native physiological context of the cell. We use these technologies to study the self-organized assembly of cilia, ubiquitous organelles of eukaryotic cells.

Assembly of the cilium requires the rapid bidirectional intraflagellar transport (IFT) of building blocks to and from the site of assembly at its tip. This bidirectional transport is driven by the anterograde motor kinesin-2 and the retrograde motor dynein-1b, which are both bound to a large complex of 25 IFT adaptor proteins. We have recently developed a millisecond resolution 3D correlative light and electron microscopy (CLEM) approach to show that anterograde and retrograde IFT trains use separated microtubule tracks along the microtubule doublets of the cilium (Stepanek & Pigino, 2016). With this method at hand we showed that the spatial segregation of oppositely directed trains ensures a collision free transport in the cilium. However, it remained to be explained how competition between kinesin and dynein motors, which are both found on the same anterograde trains, is avoided. In bidirectional transport systems in the cell, other than IFT, the presence of opposing motors leads to periodic stalling and slowing of cargos moving along the microtubule. No such effect occurs in IFT. To address these questions, we take advantage of the most advanced technologies in cryo-electron tomography and sub-tomogram averaging. After obtaining the 3D structure of IFT train complexes in the cilia of intact Chlamydomonas cells, we showed that a tug-

of-war between kinesin-2 and dynein-1b is prevented by loading dynein-1b onto anterograde IFT trains in an inhibited conformation and by positioning it away from the microtubule track to prevent binding. These findings show how tightly coordinated structural changes mediate the behavior of such a complex cellular machine.





Christopher Vollmers

University of California Santa Cruz, California, USA



Christopher Vollmers graduated with a M.S. in Biomedical Sciences from the University of Wuerzburg (Germany). He then pursued my Ph.D. through a shared program between the University of Heidelberg (Germany) and the Salk Institute for Biological Studies in La Jolla, CA. After graduating, he joined the lab of Stephen Quake at Stanford University as a postdoctoral fellow. In the Quake lab, he worked on developing genomic tools to analyze B cells on population and single cell levels. He now continues this work with a focus on long read sequencing technologies in his own lab which he started in 2014 in the Biomolecular Engineering Department at UCSC.

Sequencing for transcriptome analysis

Recent advances in sequencing technology have the potential to enable a new generation of tools for bulk and single cell transcriptome analysis. In particular, long-read sequencing makes it possible to, at high throughput, sequence full-length transcripts as opposed to sequencing transcript fragments using shortread based RNA-seq. I will outline the big potential of long-read sequencing technology for transcriptome analysis and annotation and talk about my lab's work on overcoming inherent limitations of this technology.



Leonie Ringrose

Humboldt University of Berlin Berlin, Germany



Leonie Ringrose is a British molecular biologist on a journey towards theoretical biology. She has been a professor at the IRI for Lifesciences at Humboldt University, Berlin since May 2015. From 2006 to 2014 she was a junior group leader at the IMBA (Institute of Molecular Biotechnology) in Vienna. In the time between her junior and senior positions she did a short sabbatical in the group of Martin Howard at the John Innes Centre, UK, to learn about theoretical approaches to epigenetics. Her interest in mathematical modelling and quantitative biology began during her PhD at the EMBL, Heidelberg (1992-1997), where she used modelling to investigate quantitative aspects of DNA recombination.

Her group works on epigenetic regulation by the Polycomb and Trithorax group proteins in Drosophila and mouse development. These proteins are highly conserved and essential to life, but many aspects of their function are enigmatic. Leonie believes that theoretical approaches, in combination with quantitative experiments, offer great potential for unlocking some of the mysteries of epigenetic regulation. We use a combination of quantitative live imaging, mathematical modeling, computational approaches and molecular and developmental biology to understand the interaction of the Polycomb and Trithorax proteins with their chromatin targets. We aim to unravel this fascinating epigenetic gene regulatory system in terms of the design, function and dynamic behaviour of its components. Our goal is to understand

Epigenetics meets mathematics: The fusion of experiment and theory brings insights beyond intuition

Epigenetic gene regulation is highly stable: epigenetic memory of gene expression states can persist over many cell generations and potentially for longer. However, epigenetic regulation is also flexible: genes that are subject to epigenetic regulation can respond dynamically to environmental and developmental signals. How can epigenetic regulation be both stable and flexible? I propose that the key lies in the highly dynamic nature of epigenetic systems. Over the last two decades it has become clear that the nucleus is an extraordinarily busy and noisy place: many proteins, including epigenetic regulators, are in constant motion, exchanging rapidly between chromatin bound and free states. Quantitative aspects of this motion are highly regulated. I propose that that to fully understand this regulation, epigenetics needs mathematics. We need "moving models" built of mathematical descriptions, which we can feed with measured values of quantities and mobilities of the components. A good model makes testable predictions that tell us whether our hypothesis makes sense. If it does not, we change the model. There has never been a better time to combine theoretical approaches with quantitative experiments. On the theoretical side, the last decade has seen a quiet revolution in the application of models built by physicists to the deep questions of epigenetics. On the experimental side, the advent of technologies that allow real time analysis at the single cell and single molecule level, together with those that enable targeted genome editing, allow precise perturbation and quantitative measurements at an unprecedented level.

It is time for epigenetics to meet mathematics. I will give examples from work in the field and in my own lab, of how the fusion of experiment and theory has brought fresh insights into epigenetic regulation that go beyond intuition.

how a system whose components are in constant flux can ensure both stability and flexibility of gene expression states.





Miki Ebisuya



EMBL Barcelona, Spain

Miki Ebisuya did her under graduate and PhD research at Kyoto University, Japan. After getting her PhD in 2008, she became a group leader at Kyoto University in 2009. Her lab moved to RIKEN in 2013, and then moved again to EMBL Barcelona, Spain, in 2018. The research interest of her group is reconstituting developmental mechanisms in cell culture.

Human time vs. Mouse time: *in vitro* segmentation clock as a model system

Different species have different tempos of development: larger animals tend to grow more slowly than smaller animals. My group has been trying to understand the molecular basis of this interspecies difference in developmental time, using the segmentation clock as a model system.

The segmentation clock is the oscillatory gene expressions that regulate the timing of somite formation from presomitic mesoderm (PSM) during embryogenesis. We have recently succeeded in inducing PSM from both human iPS cells and mouse ES cells, detecting the oscillation and traveling wave of segmentation clock in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system and simple mathematical models, we have been comparing the genome sequences and molecular processes of the segmentation clock between human and mouse to explain the interspecies difference in the oscillation period.





Needhi Bhalla

University of California Santa Cruz, California, U.S.A.



Needhi Bhalla received her undergraduate degree in biochemistry from Columbia College and her PhD in biochemistry from University of California, San Francisco. She performed her post-doctoral work at University of California, Berkeley and Lawrence Berkeley National Labs. She started her lab at University of California, Santa Cruz in 2008, where she studies chromosome structure and function in both meiosis and mitosis in C. elegans. She is particularly interested in how chromosome dynamics are monitored to ensure proper chromosome segregation during cell division. The work in her lab has demonstrated a surprising connection between the regulation of meiotic prophase events and the spindle checkpoint. In addition to her scientific research, she is interested in increasing diversity, equity and inclusivity in science, technology, engineering and mathematics (STEM) at all levels.

A tale of two checkpoints

How do cells ensure that the correct number of chromosomes is maintained with every cell division? Having an incorrect number of chromosomes, also called aneuploidy, is associated with cancer, birth defects, miscarriages and infertility, underscoring the importance of this question to human health. We combine genetic and biochemical approaches with high-resolution microscopy and cytological techniques in the nematode worm C. elegans to better understand how chromosomes are partitioned correctly during both sexual reproduction (meiosis) and development (mitosis). Currently, many of our studies focus on the conserved ATPase, PCH-2/TRIP13, which is required to monitor how homologous chromosomes interact in meiotic prophase as well as whether chromosomes are properly attached to the spindle during chromosome segregation. We are interested in determining whether common mechanisms underlie these diverse but essential chromosomal events.



Zoya Ignatova

University of Hamburg Hamburg, Germany



PhD with Volker Kasche within the Graduate School of TU Hamburg Harburg and University Hamburg. Postdoc with Lila Gierasch at the University of Massachusetts, Amherst, USA. Group Leader at MPI for Biochemistry, Martinsried. Tenure-track professor for Biochemistry at University of Potsdam and since 2014 full professor in Biochemistry and Molecular Biology at the University of Hamburg. 2016-18 Managing Director of Institute of Biochemistry and Molecular Biology. Prizes and awards: Heisenberg fellowship award; Lundbeck foundation, Denmark; Joliot professorship at ESPCI, Paris, France; Innovation award, MDI BioLab, Maine, USA; Hamburg teaching award; Claussen-Simon-Stiftung award for Mentorship.

Translational control: probing dimensionality beyond linear sequence of mRNA

The ribosome is a central molecular machine that translates the genetic information into a corresponding polypeptide in an mRNA template-directed manner. Thereby, the mRNA is not a mere messenger for translation of codons into amino acids but bears additional layers of information for folding, solubility and expression level of the encoded protein. We use cellwide approaches, including quantification of translational components, probing mRNA secondary structure, mRNA abundance and translation efficiency by means of deep sequencing to extract features that are selected to control translation and influence protein folding and function.



Asifa Akhtar

MPI of Immunobiology and Epigenetics, Freiburg, Germany



Asifa Akhtar obtained her bachelor's degree in Biology at University College London (UCL), UK, in 1993 and her Ph.D. in 1998 at the Imperial Cancer Research Fund in London, studying transcription regulation in Richard Treisman's laboratory. She continued in the field of chromatin regulation as a postdoctoral fellow at the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, and the Adolf Butenandt Institute, Munich, Germany, in Peter Becker's laboratory until 2001. From 2001, she led her own research as a group leader at the EMBL. Currently, Asifa Akhtar is a director at the Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany, heading the Department of Chromatin Regulation. Her laboratory primarily studies chromatin and epigenetic mechanisms, especially focusing on the regulation by histone acetylation. In 2008, Asifa Akhtar received the European Life Science Organization (ELSO) award for significant contribution in the field and in 2013 she was elected as an EMBO member. In 2017, she received the prestigious Feldberg Prize.

Epigenetic regulation by histone acetylation

Our lab is studying the chromatin and epigenetic mechanisms regulated by histone acetylation using evolutionary conserved complexes associated with MOF, a MYST family of histone acetyltransferases. In flies and mammals, MOF is associated with the MSL and NSL complexes, which are important regulators of gene expression. In flies, the MSL complex is well known for regulation of the X chromosome by the process of dosage compensation, while the NSL complex regulates expression of house-keeping genes. In mammals, both complexes appear to be involved in regulating diverse cellular processes. The recent progress of our work will be presented.



Jen Heemstra



Emory University, Atlanta, Georgia, USA



Jen received her B.S. in Chemistry from the University of California, Irvine, in 2000. At Irvine, she performed undergraduate research with Prof. James Nowick investigating the folding of synthetic beta-sheet mimics, which instilled in her a love of supramolecular chemistry. Jen then moved to the University of Illinois, Urbana-Champaign, where she completed her Ph.D. with Prof. Jeffrey Moore in 2005 studying the reactivity of pyridine-functionalized phenylene ethynylene cavitands. After a brief stint in industry as a medicinal chemist, she moved to Harvard University to pursue postdoctoral research with Prof. David Liu exploring mechanisms for templated nucleic acid synthesis. In 2010, Jen began her independent career in the Department of Chemistry at the University of Utah, and was promoted to Associate Professor with tenure in 2016. In 2017, Jen and her research group moved to the Department of Chemistry at Emory University. Research in the Heemstra lab is focused on harnessing the molecular recognition and self-assembly properties of nucleic acids for applications in biosensing and bioimaging. Outside of work, Jen enjoys spending time with her husband and two sons, as well as rock climbing, cycling, and running.

A chemical biology toolbox for specific RNA modification and capture

Nucleic acids are exquisitely adept at molecular recognition and self-assembly, enabling them to direct nearly all of the processes that make life possible. These capabilities have been fine-tuned by billions of years of evolution, and more recently, have been harnessed in the laboratory to enable the use of DNA and RNA for applications that are completely unrelated to their canonical biological roles. In our lab, we seek to use DNA and RNA for applications in biosensing and biomolecular imaging. We have developed RNA sequences that are capable of recognizing specific small molecule fluorophores and promoting covalent selflabeling with these fluorophores. We anticipate use of these self-labeling ribozymes for imaging of RNA in living cells. We have also demonstrated selective enrichment of inosine-containing RNAs using EndoV for non-covalent capture, and we anticipate this method will enable the identification of new sites of A-to-I editing in cells.



Tatjana Kleele

Laboratory of Experimental Biophysics, Lausanne, Switzerland

2015-today: Postdoc with Suliana Manley, Laboratory of Experimental Biophysics (EPFL, Switzerland)

- Live-cell super-resolution imaging of mitochondrial fission
- Physiological properties preceding mitochondrial fission at the single organelle level
- Development of a biophysical model for mitochondrial fission

2011-2014: PhD thesis with Thomas Misgeld, Institute of Neuronal Cell Biology (TU Munich)

- Development of an assay to study microtubule dynamics *in vivo* in the healthy and diseased nervous system
- In vivo microscopy techniques to study subcellular dynamics in mammalian neurons
- PhD program: Medical life science and technology

2008-2010: Research assistant with Bert Sakmann (Max-Planck-Institute of Neurobiology, Martinsried)

 Reconstruction of cortical neurons using bright-field mosaic scanning microscopy and semi- automated image processing

2004-2010: Studies in Biology (Neurobiology, Human Genetics and Organic Chemistry) at Ludwig- Maximilians-University Munich

Tatjana.Kleele@epfl.ch



Degradation and proliferation are outcomes of distinct types of mitochondrial division

"Mitochondria originated through the integration of an a-proteobacterium as a permanent organelle into a eukaryotic host cell. They became vital components of eukaryotic cells, executing critical functions, ranging from ATP production and Ca2+ handling to pro-apoptotic and reactive oxygen species signaling. Mitochondria are dynamic organelles constantly changing shape and size, depending on the metabolic demand of the tissue. The role of mitochondria in energy production makes them particularly vulnerable to reactive oxygen species, which can disturb protein structure and cause mtDNA mutations. Mitochondrial quality control mechanisms are counteracting this damage and regulate mitochondrial turnover: damaged mitochondria are selectively removed via mitophagy, and biogenesis of new mitochondria balances the removal of damaged mitochondria. Most of the molecular machinery regulating fission has been identified, but it remains obscure what distinguishes dysfunctional mitochondria from proliferating ones and how they are recognized by the division machinery.

Using live-cell super-resolution Structured Illumination Microscopy (SIM, iSIM) for ultra-fast multi-colour acquisition in Cos-7 cells and primary mouse cardiomyocytes, we analysed more than 2000 fission events. We discovered a key morphological determinant of the decision to proliferate or degrade: the positioning of the division site, giving rise to either "asymmetric" or "symmetric" division. By imaging fluorescent metabolic sensors, we find that asymmetric fissions show signs of stress and dysfunction and are followed by mitophagy. Symmetric fissions do not



Sjors Scheres

Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

Sjors studied chemistry at Utrecht University, The Netherlands, where he also obtained his PhD in protein crystallography. He was a post-doc in the group of Jose-Maria Carazo in Madrid, before he started his group at the LMB in 2010. His main interest is currently in the development of new methods for high-resolution cryo-EM structure determination and in the cryo-EM structure determination of amyloid fibrils.

Wednesday 11:30 am

The atomic structures of Tau filaments from Alzheimer's and Pick's disease brains

The assembly of microtubule-associated protein tau into abundant filamentous inclusions underlies many neurodegenerative diseases called tauopathies. Tau inclusions display distinct neuroanatomical and cellular distributions between different tauopathies. Morphological and biochemical differences suggest that tau filaments adopt disease-specific molecular conformations. There are six tau isoforms - three isoforms with four microtubule-binding repeats each (4R tau) and three isoforms lacking the second repeat (3R tau). This gives rise to tauopathies with filaments composed of only 3R tau, only 4R tau or both 3R and 4R tau. Molecular conformers of filamentous tau may give rise to different neuropathological phenotypes, similar to prion strains, but the underlying structures are not known. Using electron cryo-microscopy (cryo-EM), we previously determined the structures of tau filaments from Alzheimer's disease, which contain both 3R and 4R tau. More recently, we also determined the structures of tau filaments from Pick's disease and show that they define a second tau protein fold consisting of residues K254-F378 of 3R tau, thereby proving the existence of molecular tau conformers. I will present both folds and discuss how they explain differences in isoform incorporation and phosphorylation observed between Alzheimer's disease and Pick's disease.



Max Cryle

EMBL Australia, Monash University Melbourne, Australia



Prof. Max Cryle is an NHMRC Career Development Fellow and EMBL Australia Group leader based in the Biomedicine Discovery Institute at Monash University. After obtaining his PhD in chemistry from the University of Queensland in 2006, he moved to the Max Planck Institute for Medical Research in Heidelberg as a Cross Disciplinary Fellow of the Human Frontiers Science Program. He was subsequently awarded funding from the German Research Foundation (Deutsche Forschungsgemeinschaft) to establish his own group to investigate glycopeptide antibiotic biosynthesis as part of the Emmy Noether program. His group works at the boundary of chemistry and biology, where they apply a multidisciplinary approach including synthetic chemistry, biochemistry, structural biology and enzyme catalysis. In 2016, he joined EMBL Australia to continue his research into understanding the biosynthesis of important natural antibiotics and developing new antimicrobial agents. His group has made a number of important breakthroughs in understanding how nature synthesises the glycopeptide antibiotics, which are clinically relevant and synthetically complex molecules. For this work, he was awarded the 2016 Otto Schmeil prize by the Heidelberg Academy of Arts and Sciences. Currently, his group is investigating the biosynthesis of several important antibiotics as well as investigating novel strategies and targets for antimicrobial development.

Understanding the biosynthesis of the glycopeptide antibiotics

The glycopeptide antibiotics (GPAs) are a structurally complex and medically important class of peptide natural products that include the clinical antibiotics vancomycin and teicoplanin. They contain a large number of non-proteinogenic amino acids and are produced by a linear non-ribosomal peptide synthetase (NRPS) machinery comprising seven modules. Furthermore, GPAs are extensively crosslinked late in their biosynthesis on the NRPS assembly line by the actions of a cascade of Cytochrome P450 enzymes, a process which contributes to the rigidity and structural complexity of these compounds. Due to the challenge of synthesising GPAs, biosynthesis remains the only means of accessing GPAs for clinical use, which makes understanding the biosynthesis of GPAs of key importance.

In this presentation, I will detail results from our studies into the NRPS machinery, the P450-catalysed cyclisation cascade and the interplay of these two important biosynthetic processes during GPA biosynthesis. This includes the characterisation of key enzymatic processes during NRPS-mediated peptide biosynthesis (chlorination, thioesterase activity and reconstitution of peptide synthesis) as well as the P450-mediated cyclisation cascade (substrate specificity of P450 enzymes and cascade reconstitution) from a number of different GPA biosynthesis pathways. Overall, our results demonstrate how selectivity during GPA biosynthesis is mediated through the careful orchestration of critical modification steps and interactions between the peptide-producing NRPS machinery and trans-modifying enzymes.



Margaret McCarthy

University of Maryland, Baltimore, Maryland, USA



Margaret McCarthy received a PhD from the Institute of Animal Behavior at Rutgers University, and postdoctoral training at Rockefeller University. She was an NRC Fellow at NIAAA and joined the faculty of the University of Maryland School of Medicine in 1993. She was a professor in the Department of Physiology before becoming the Chair of the Department of Pharmacology in 2011. She has received numerous awards and recognition for her research and for mentoring of graduate students. McCarthy has a long standing interest in the cellular and molecular mechanisms establishing sex differences in the brain. She uses a combined behavioral and mechanistic approach in the laboratory rat to understand normal brain development and how this process might go selectively awry in males versus females, discovering numerous novel signaling processes along the way.

Surprising origins of sex differences in the brain

Elucidating the cellular and molecular mechanisms by which sex differences are developmentally programmed into the brain has been a central goal of neuroendocrinology since the discipline was formed half a century ago. Neuroanatomical sex differences range from cell number, to phenotype, to dendritic morphology and synaptic patterning. Research emphasis has largely been on the intersection of steroids, neurotransmitters and growth/survival factors. Our laboratory has found a central role for a different source of neuromodulation, the neuroimmune system, and has discovered that at least two immune cells, microglia and mast cells, are critical partners in the process of brain masculinization. We further find that membrane derived signaling molecules, in particular the prostaglandins and endocannabinoids, are also essential drivers of the sexual differentiation process by modulating the activity of immune cells in the brain. Why males have higher levels of all of these is a mystery but may have its origins in the maternal immune system and its response to male fetuses during mammalian gestation.



Pavel Tomancak

MPI of Molecular Cell Biology and Genetics, Dresden, Germany

Pavel Tomancak studied Molecular Biology and Genetics at the Masaryk University in Brno, Czech Republic. He then did his PhD at the European Molecular Biology Laboratory in the field of Drosophila developmental genetics. During his post-doctoral time at the University of California in Berkeley at the laboratory of Gerald M. Rubin, he established image-based genome-scale resources for patterns of gene expression in Drosophila embryos. Since 2005 he leads an independent research group at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden where he became senior research group leader in 2013.

His laboratory continues to study patterns of gene expression during development by combining molecular, imaging and image analysis techniques. The group has lead a significant technological development aiming towards more complete quantitative description of gene expression patterns using light sheet microscopy. The emphasis on open access resulted in establishment of major resources such as OpenSPIM and Fiji. The Tomancak lab is expanding the systematic analysis of gene expression patterns to other Drosophila tissues and employing the comparative approach in other invertebrate species to study the evolution of early development.

A new force awakens: comparative approach to tissue morphogenesis in insects

Wednesday 3:45 pm

During gastrulation, physical forces reshape the simple embryonic tissue to form a complex body plan of multicellular organisms. These forces often cause large-scale asymmetric movements of the embryonic tissue. In many embryos, the tissue undergoing gastrulation movements is surrounded by a rigid protective shell. While it is well recognized that gastrulation movements depend on forces generated by tissue-intrinsic contractility, it is not known if interactions between the tissue and the protective shell provide additional forces that impact gastrulation. Our recent work has shown that a particular part of the blastoderm tissue of the red flour beetle Tribolium castaneum tightly adheres in a temporally coordinated manner to the vitelline envelope surrounding the embryo. This attachment generates an additional force that counteracts the tissue-intrinsic contractile forces to create asymmetric tissue movements. Furthermore, this localized attachment is mediated by a specific integrin, and its knock-down leads to a gastrulation phenotype consistent with complete loss of attachment. Moreover, analysis of another integrin in the fruit fly Drosophila melanogaster suggests that gastrulation in this organism also relies on adhesion between the blastoderm and the vitelline envelope. Together, our findings reveal a conserved mechanism whereby the spatiotemporal pattern of tissue adhesion to the vitelline envelope provides controllable counter-forces that shape gastrulation movements in insects. It also provides a new perspective on evolution of early gastrulation processes impacted by patterned contacts with the constraining extra-embryonic envelopes.


Leo James

Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom

Leo received his Ph.D. from Cambridge University in 2000, where he worked on antibody structure and design including the first humanised antibody CAMPATH. He post-doc'd in the labs of Prof. Dan Tawfik and Sir Greg Winter, investigating molecular mechanisms of antibody pathogenicity. In 2007, Leo established an independent group at the Laboratory of Molecular Biology in Cambridge. His lab studies intracellular host-pathogen interactions using a broad range of in vitro and in vivo techniques. In 2010, Leo discovered TRIM21, the most conserved and highest affinity antibody receptor in mammals and unique for its cytosolic expression. Since then, his lab has shown that TRIM21 prevents infection by intercepting viruses, bacteria and pathogenic proteins inside the cell and targeting them for rapid degradation. This work has also led to the development of 'TrimAway', a technique which exploits TRIM21 for the rapid and specific degradation of cellular proteins. Leo's lab is now working to define the molecular mechanisms that underpin TRIM21 activity to further develop this technology and understand how intracellular antibody immunity is regulated. Leo also investigates HIV post-fusion biology; recent work includes identifying the HIV capsid interface used to recruit cofactors for nuclear import and discovering dynamic pores in the capsid that are essential for HIV infection.



Trim-Away: Targeted degradation of pathogens and proteins by the cytosolic antibody receptor TRIM21

TRIM21 is a recently discovered mammalian Fc receptor and E3 ubiquitin ligase expressed in the cytosol of all cells. The highest affinity IgG receptor in man, it mediates an intracellular humoral response against antibody-opsonized pathogens that invade the cytosol during infection. Upon detection, TRIM21 activates key immune transcription pathways to induce a potent antiviral state. Simultaneous with, and independent of, immune activation, TRIM21 recruits cellular degradation machinery, which catalyse the disassembly and destruction of cytosolic virions to prevent their replication. This rapid virion destruction also exposes the genomes of RNA and DNA viruses, allowing TRIM21 to potentiate the activity of RIG-I and cGAS to promote a rapid antiviral response. TRIM21 is effective against diverse viruses but also bacteria and proteopathic agents like tau. In my talk I will summarize key aspects of TRIM21 biology and focus on recent work showing how its inflammatory signaling is regulated and how we have repurposed its activity to perform targeted protein depletion. This latter technology, called 'Trim-Away', uses off-the-shelf antibodies to rapidly and acutely degrade cellular proteins in diverse cells.

Notes



Naama Barkai

Weizmann Institute of Science, Rehovot, Israel

Naama Barkai was born and raised in Jerusalem. During her doctoral studies in the Physics Department of the Hebrew University, she became interested in biological computation systems and began using tools of statistical physics to study models of neural networks. Her interests then shifted to computation systems that function within cells, and she joined a group at Princeton University that was studying a bacterial sensing system. Her theoretical studies, and the experimental studies that followed, established this bacterial sensing pathway as a main model for Systems Biology studies. Barkai joined the Weizmann Institute in late 1999, just as the genomic revolution was getting underway. She was fortunate to be among the physics-trained biologists who were applying tools and concepts from the quantitative sciences to study how cellular computation systems are designed. This group was the founding core of the emergent field of Systems Biology. Barkai's work contributed to two subfields of Systems Biology: understanding the design principles of biological circuits on a relatively small scale, and understanding principles of gene expression at the genomic level. A central contribution of Barkai's work is the formulation and application of the robustness principle. Biological circuits, in contrast (perhaps) to manmade computation devices, work within what is inherently a highly noisy environment, as manifested in different dimensions. The robustness principle suggests that the biological circuits selected by evolution are robust; that is,



Gene transcription as a limiting factor in protein production and cell growth

Cell growth is driven by the synthesis of proteins, genes and other cellular components. Defining processes that limit biosynthesis rates is fundamental for understanding determinants of cell physiology. We address this challenge by combining mathematical modeling with the analysis of cells forced to express high levels of inert proteins as a tool to examine the consequences of increasing biosynthetic demands. I will describe the parameters which define the maximum possible values by which cells can translate proteins or transcribe genes, and present results suggesting that cells work close to these 'hard' limits. I will them discuss the possible consequences of these 'hard' limits on cell growth rate and cell size, the interplay between these two parameters, and the potential evolutionary benefits of maximizing transcription rates.

they perform their function reliably in a noisy environment, showing minimal dependency on the kinetics of quantitative parameters. Over the years, Barkai has applied this principle to reveal design principles and operational mechanisms of multiple circuits that work in diverse contexts. In particular, her work has revealed mechanisms that function during multicellular development to pattern the body plan.

Barkai is a faculty member in the Department of Molecular Genetics at the Weizmann institute. She has received several awards in recognition of her work, including the EMBO award for women in science and the Rothschild Prize.



James Williamson

The Scripps Research Institute, California, USA



Dr. James Williamson is the Executive Vice President of Research and Academic Affairs at The Scripps Research Institute, as well as a Professor in the Departments of Chemistry and Integrative Structural & Computational Biology. His research involves the study of RNA structure, RNA-protein interactions and RNA-ligand interactions using biochemistry, biophysics and structural biology approaches.

Among his many early scientific career accomplishments, Dr. Williamson was named a Searle Scholar, a Rita Allen Scholar, a Camille Dreyfus Teacher-Scholar, a Sloan Research Fellow and was awarded a Jane Coffin Childs Postdoctoral Fellowship. His studies have appeared in more than 200 peer-reviewed publications and in 2010, he was elected to the American Academy of Arts and Sciences. He currently serves on editorial boards for a number of top scientific journals and professional societies.

At Scripps Research, Dr. Williamson continues to successfully execute multiple leadership roles, especially those encompassing academic planning and training. In 2001, he was named Associate Dean for the Chemistry program and from 2008-2017, he was the Dean of Graduate and Postdoctoral Studies for the Institute's nationally ranked graduate program. He was named Vice President of Academic Affairs in 2015 and appointed Executive Vice President in 2017.

Ribosome Assembly in Bacteria

Ribosomes are responsible for all protein synthesis in cells, and the process of assembling ribosomes accounts for one third of the energy budget for rapidly growing bacteria. The assembly process involves coordination of synthesis of three large ribosomal RNAs and fifty ribosomal proteins in an incredibly efficient process that requires about two minutes in cells. Assembly is guided by over fifty assembly factors that are chaperones and modification enzymes, but we have a basic and emerging understanding of the steps involved in making a ribosome. Our laboratory uses a wide variety of biophysical methods to characterize the structure and dynamics of assembling ribosomes in vitro, and in cells. Quantitative mass spectrometry reveals the composition of intermediates, electron microscopy reveals the heterogeneous distribution of assembly intermediates, and single molecule fluorescence reveals the dynamics of structural transitions that occur during assembly. The overall picture that is emerging is that there are parallel pathways for installation of various structural units, but that certain events must happen in a particular order. The parallel and sequential nature of assembly appears to be an optimal balance for efficient and accurate assembly. This talk will describe recent advances in our understanding of ribosome assembly in bacteria, focusing on general principles that are emerging about the extensive RNA folding that is at the heart of the process.

Dr. Williamson earned his doctorate in chemistry at Stanford University in 1988. Following postdoctoral work at the University of Colorado, he joined the faculty of the Chemistry department at the Massachusetts Institute of Technology, where he attained the rank of associate professor with tenure. He accepted an appointment to The Scripps Research Institute as a professor in 1998.





Michael Levine

Princeton University, Princeton, New Jersey, USA



Professional Experience:

July 2015: Director, Lewis-Sigler Institute for Integrative Genomics, Princeton University.

July 2015: Anthony B. Evnin Professor, Dept Molecular Biology, Princeton Univ.

July 2012-June 2015: Chair, Chancellor's Advisory Council for Biology, UC Berkeley

July 2007-June 2011: Head, Division of Genetics, Genomics, & Development, Dept MCB, UCB

July 2002-June 2015: Co-Director, Center for Integrative Genomics

July 2001-June: 2002 Associate Director, Functional Genomics, Joint Genome Institute, DOE, Walnut Creek, CA

Sept. 1999-Aug. 2000: Visiting Professor, Zoological Institute, University of Zürich, Switzerland

July 1996 - present: Professor, Department of Molecular and Cell Biology, UC Berkeley

Jan. 1991-June 1996: Professor, Department of Biology, University of California, San Diego

July 1988-Dec. 1990: Professor, Department of Biological Sciences, Columbia University, New York (NY)

July 1986-June 1988: Associate Professor, Department of Biological Sciences, Columbia, University, NY

July 1984-June 1986: Assistant Professor, Department of Biological Sciences, Columbia University, NY

Enhancer-promoter communication in living Drosophila embryos

Transcriptional enhancers are short segments of DNA (~100 bp to 1 kb) that switch genes on and off in response to intrinsic and external cellular signals. Whole-genome assays suggest that the human genome contains ~400,000 enhancers (~20 per gene). There is emerging evidence that sequence polymorphisms in enhancer DNAs are a major source of population diversity and predilection to disease. My lab uses quantitative live imaging methods to visualize the activities of enhancers that control the development of the early *Drosophila* embryo. I will discuss transcriptional bursts, coordinate activation of linked genes, and transvection, whereby an enhancer located on one chromosome can activate the expression of a transcription unit located on the other homologue.



Oct. 1983-June 1984: Postdoc, Department of Biochemistry, University of California, Berkeley; Dr. G.M. Rubin, Supervisor

Apr. 1982-Sept. 1983: Postdoc, Department of Cell Biology, Universität Basel; Dr. W. Gehring, Supervisor

Education:

B.A., 1976 University of California, Berkeley, Department of Genetics

Ph.D., 1981 Yale University, Department of Molecular Biophysics & Biochemistry

Honors:

1976 Phi Beta Kappa; Departmental Citation in Genetics. UC Berkeley

1982-1984 Jane Coffin Childs Postdoctoral Fellow

1985-1988 Searle Scholars Fellow

1985-1987 Alfred P. Sloan Research Fellow

1996 Award in Molecular Biology, National Academy of Sciences

1996 Elected to the American Academy of Arts & Sciences

1998 Elected to the National Academy of Sciences

1999 Harvey Lecture

2002 F. Williams Chair in Biology, UC Berkeley

2006 Gavin Borden Fellow, Cold Spring Harbor Labs

2009 Faculty Research Lecturer, UC Berkeley; Wilbur Cross Medal, Yale University

2010 Einstein Professor, Chinese Academy of Sciences

2012 President, Society for Developmental Biology

2015 Conklin Medal, Society for Developmental Biology

2017 Elected, Associate Member EMBO





Panel Discussion

The panel discussion has been organized every year in Horizons since its conception. It provides a channel for lively interactions in a less formal setting. Typically, we have five guests from diverse disciplines on the panel taking questions from a moderator and from the audience. The idea is to illuminate different aspects of the topic from various perspectives. Horizons strives to have a diverse range of speakers to guide us with their unique experiences. For the 16th Horizons in Molecular Biology symposium, the following speakers will join the panel:

- 1. Needhi Bhalla
- 2. Michael Levine
- 3. Andreas Laustsen
- 4. Christine Mieck
- 5. Katrin Wodzicki

Being a scientist: Job or Passion?

Many of us were driven to pursue science because we were fascinated by the marvels and beauty of the natural world around us. But what keeps us in the race? Is it purely our quest to answer challenging scientific questions? Or is it a more career oriented desire to succeed in academic life? Or should we think of science more as a 9-5 job? The creative process of science is rarely a straightforward "Eureka" moment. Obstacles faced by scientists include long workhours, pressure to publish and get grants and the need for success at each career stage. The accompanying stress can affect social and personal life and in extreme cases can lead to mental health issues.

In the panel discussion, we will discuss the ebb and flow of academia – from the exciting quest for knowledge to the harsh reality – emphasizing some aspects of the vast landscape of what it means to be a scientist. Topics chosen for this year's discussion are:

- Being a scientist, Job or Passion?
- How critical is the choice of scientific project and group in which to pursue it? How can a productive PI-student relationship be established? How can the power and responsibility of a PI be used to benefit this relationship?
- Work-Life Balance: Who decides what is reasonable? When things go wrong what help is available and from whom?
- Communication in science. What forms does it take and how important is it?

This year, Emeritus **Professor Mary Osborn** has once again kindly agreed to moderate the discussion. She will be joint in the moderation by three students from the organizers team of the symposium: Ida Jentoft, Tarana Nigam, and Panagiotis Poulis.

Furthermore, we would like to announce the participation of **Katrin Wodzicki**, who is the head of the *Human Resources and Organization Development Unit* of the University of Göttingen.

Mary Osborn

Mary Osborn received her BA in Physics from the University of Cambridge and a PhD in Biophysics from Pennsylvania State University. She was a post-doc in Jim Watson's lab at Harvard University and a staff scientist in Sydney Brenner and Francis Crick's division at the MRC Laboratory of Molecular Biology in Cambridge, U.K. She then joined the staff at Cold Spring Harbor Laboratory. In 1975, she moved to the Max Planck Institute for Biophysical Chemistry in Göttingen and in 1989 was appointed an honorary professor at the University of Göttingen.

Her research has focused on different areas in the life sciences. Her 1964 paper with Klaus Weber, on the use of SDS gels to determine molecular weight is a Citation Classic. Her work on the use of antibodies in immunofluorescence microscopy allowed the definition of the arrangements of microfilaments, microtubules and intermediate filaments in cells and tissues. The 1978 demonstration of a 1:1 correspondence between microtubules viewed in immunofluorescence and electron microscopy in the same cell was a major breakthrough as was the work showing that antibodies specific for the different types of intermediate filament proteins are powerful reagents in human tumor diagnosis.

She was a trustee of the Swedish Foundation on the Environment, MISTRA, and has chaired the Scientific Advisory Boards of the European Molecular Biology Laboratory (EMBL) in Heidelberg and the Pette Institute in Hamburg. She also chaired the Cell Biology Section of Academia Europaea. She was a member of the European Strategy Forum on Research Infrastructures (ESFRI) Group. She has participated in several juries concerned with awarding substantial grants to young scientists including the BioFuture program and the Helmholtz Young Investigator Program and was a member of the Helmholtz Senate. She was President of the International Union biochemistry and Molecular Biology from 2003 to 2006. She chaired the ETAN expert working group appointed by the European Commission that produced an influential report on Women in Science published in 2000.

She has been an EMBO member since 1978 and she holds an honorary doctorate from the Pomerian Medical Academy in Sczeczin, Poland.

She has been awarded the Meyenburg Prize and the L'Oreal/UNESCO Prize for her work on the cytoskeleton and the use of antibodies in tumor diagnosis. Her research and her efforts for Women in Science, were honored by the award of the Bundesverdienstkreuz (1st class) in 2014.

Katrin Wodzicki

K atrin is head of the HR and organization development unit of the University of Göttingen since 2017, responsible for the development of scientific, technical and administrative staff as well as for the implementation of the tenure track programme and the further development of the structure of academic career paths in general. Katrin studied Psychology at the University of Jena, and completed her Ph.D. at the University of Zurich, followed by five years of postdoc at the Leibniz-Knowledge Media Research Center (KMRC) in Tübingen. During her time in Tübingen, she took part in the think tank of the "Stiftung Neue Verantwortung" in Berlin, which aimed at encouraging an intersectional dialogue between science, business, politics, and society. Moreover, she is one of the founders of the group blog wissensdialoge. de on which we discuss and report about psychological research relevant for practitioners in knowledge management and organizational learning. From 2012 to 2016, she established the GGNB Career Service for postdocs and late-stage PhDs (continued as GAUSS Career Service) in Göttingen.

Katrin has always liked pushing the development of others - as of herself - forward. Everyone has specific abilities, skills, and talents. And there are numerous career options within and outside academia. Finding the right place to make use of one's own talents is challenging whilst at the same time exciting. By taking part in the panel discussion, she aims at providing useful information, answer questions and support informed individual decision-making. You can follow her on Twitter (@KatrinW).



Aydın Banu

The effect of epidermal growth factor and cholinergic receptors agonist / antagonists together with anticancer drug 5-FU in hepatocelluler carcinoma cell line

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The second leading cause of cancer-related death worldwide is hepatocellular carcinoma (HCC). Currently, there is no specific drug for anti-metastasis treatment in HCC. Drugs used both for the treatment of primary HCC tumors and even for fighting with tumor metastasis are very similar. The cytotoxic drugs used are cisplatin, doxorubicin and 5-FU. Epidermal growth factor is an important mitogen for hepatocytes. Hepatocellular carcinogenesis is promoted by its overexpression. Moreover the EGFR pathway plays an important role in promoting hepatocellular carcinoma (HCC) metastasis although the mechanism remains unclear. Acetylcholinesterase and acetylcholin receptors which comprise the cholinergic system have been detected in HCC. Furthermore ACh promotes HCC cell proliferation, which correlates with the tumor aggressiveness, and low survival rate. However, the function and molecular mechanism of cholinergic system in hepatic carcinogenesis remain unknown. Different researcher suggested that long chain saturated fatty acids induce apoptosis and reduce cell viability in liver cells. They showed that exposure of human HepG2 hepatoma cells to palmitate result in apoptosis.

The aim of the present study was to investigate the role of EGFR-AChR signalling pathway in the human hepatocellular carcinoma (HCC) regarding proliferation and apoptosis. Using the hepatoma cells as a model system, we analyzed the combined effects of EGFR, cholinergic receptors and 5-FU on cell proliferation and effects of the apoptotic pathway including BCL/ BAX and caspase-3. We suggest that activation of M3R and EGFR pathway might be a potential mechanism for ACh induced cell growth. Additionaly M₃AChR antagonism of 4-DAMP combined with 5-Fluorouracil (5-Fu) might effect the cell viability and apoptosis in HepG2 cells and steatotic HepG2 cells.

Keywords: Muscarinic receptor, Caspase 3, Liver cancer cells

Abhishek Upadhyay

An Inactivation Switch Enables Rhythms in a Neurospora Clock Model

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Autonomous endogenous time-keeping is ubiquitous across many living organisms, known as the circadian clock when it has a period of about 24 h. Interestingly, the fundamental design principle with a network of interconnected negative and positive feedback loops is conserved through evolution, although the molecular components differ. Filamentous fungus Neurospora crassa is a well-established chrono-genetics model organism to investigate the underlying mechanisms. The core negative feedback loop of the clock of Neurospora is composed of the transcription activator White Collar Complex (WCC) (heterodimer of WC1 and WC2) and the inhibitory element called FFC complex, which is made of FRQ (Frequency protein), FRH (Frequency interacting RNA Helicase) and CK1a (Casein kinase 1a). While exploring their temporal dynamics, we investigate how limit cycle oscillations arise and how molecular switches support self-sustained rhythms. We develop a mathematical model of 10 variables with 26 parameters to understand the interactions and feedback among WC1 and FFC elements in nuclear and cytoplasmic compartments. We performed control and bifurcation analysis to show that our novel model produces robust oscillations with a wild-type period of 22.5 h. Our model reveals a switch between WC1-induced transcription and FFC-assisted inactivation of WC1. Using the new model, we also study the possible mechanisms of glucose compensation. A fairly simple model with just three nonlinearities helps to elucidate clock dynamics, revealing a mechanism of rhythms' production. The model can further be utilized to study entrainment and temperature compensation.

Keywords: circadian clock; mathematical modeling; molecular switch; Neurospora crassa; glucose compensation

Sarvinoz Atakulova

The impact of ABCB1, SLCO1B1, CYP3A4/5 polymorphisms on efficacy of ticagrelor in patients with acute coronary syndrome

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Introduction: Ticagrelor is a newer direct-acting P2Y12 inhibitor which produces high levels of platelet inhibition. According to the studies, several single nucleotide polymorphisms (SNPs) of genes including ABCB1, SLCO1B1, CYP3A may affect the dual antiplatelet therapy with ticagrelor.

Objectives: The aim of this study was to determine the influence of ABCB1 rs1045642, SLCO1B1 rs4149056, CYP3A5 rs776746 and CYP3A4*22 rs35599367 genetic polymorphisms on the antiplatelet effect of ticagrelor in patients with an acute coronary syndrome (ACS) who have undergone percutaneous coronary intervention (PCI).

Materials and methods: The study included 83 patients (mean age = 58,11±10,08) with an ACS. All patients underwent PCI and were prescribed dual antiplatelet therapy with aspirin and ticagrelor. Genotyping of ABCB1, SLCO1B1, CYP3A5 and CYP3A4*22 polymorphisms was performed by real-time polymerase chain reaction. To assess the functional activity of platelets the test-system VerifyNow (Accumetrics, USA) was used. The SPSS Statistics 20.0 software was used for the statistical analyses. A P value of <0.05 was considered as significant.

Results: The genotype distributions of the polymorphisms were as follows: ABCB1 rs1045642 CC genotype (n=12), CT genotype (n=38), TT genotype (n=33); SLCO1B1 rs4149056 TT genotype (n=43), TC genotype (n=31), CC genotype (n=9); CYP3A5 rs776746 AG genotype (r=13), GG-genotype (n=70); CYP3A4*22 rs35599367 CC-genotype (n=81) and CT genotype (n=2). All distributions are consistent with Hardy-Weinberg equilibrium. There were significant differences in the levels of platelet reactivity units (PRU) between TT and TC+CC genotype carriers by the SLCO1B1 polymorphism (mean PRU = $24,85\pm6,46$ vs $11,54\pm3,44$; p = 0,024). Thus, antiplatelet effect of ticagrelor was lower in patients with TT-variant. In comparison, ABCB1, CYP3A5 polymorphisms had no effect on the PRU value. For the CYP3A4*22 no statistical processing of the data was performed due to the low frequency of CT heterozygotes in this sample.

Conclusion: We revealed the influence of SLCO1B1 polymorphism on the pharmacodynamics of ticagrelor. Comparison of genetic variants by the ABCB1 and CYP3A5 polymorphisms showed no significant differences in mean PRU value. The role of CYP3A4*22 SNP remained unclear because of the low frequency of CT allelic variant among the patients.

Oleh Rymarenko

Composition and properties of XPO-1 dependent nuclear export signals

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Exportin 1 (XPO1, also known as Crm1) mediates one of the most versatile protein transport pathways of the eukaryotic cells. It transports hundreds of different proteins and protein complexes from the nucleus to the cytoplasm. Recognition of such a vast and diverse set of cargos is possible due to the ability of XPO1 to bind a peptide motif called a nuclear export signal (NES). Despite more than two decades of extensive research, the features that contribute to NES's ability to recruit XPO1 remain poorly understood.

To gain the comprehensive understanding of the composition rules for the NESs, we employ the M13 phage display combined with the high-throughput DNA sequencing. Such approach allows us to cover the entire sequence space for the peptides of appropriate length and to analyze a contribution of any residue at each position of the motif. We discovered numerous features that contribute in a positive or negative way to the ability of a peptide motif to bind to XPO1. We confirm the discovered contributions of various residues at specific positions in vivo.

Armed with the dataset covering the entire sequence space for an NES motif, we rationally design peptides that should exhibit the highest possible propensity for binding XPO1. Indeed, we confirm that the interaction of the designed peptides with XPO1 cannot even be disrupted using a competitive suicide inhibitor of XPO1. We characterize the interaction of one of these peptides with XPO1 using X-ray crystallography. We see numerous interactions from residues at different positions contributing to an unprecedentedly strong interaction with XPO1. We show that the designed peptides act as XPO1 inhibitors *in vivo*.

Finally, we propose an inclusive way of sequence-based NESs prediction. By assessing a clearly defined contribution of each residue at each position of a potential NES, we can provide an accurate predictions of XPO1 binding propensity for any sequence of residues. Ability to predict potential NES for any protein based on its sequence is very useful in studying new proteins and characterizing their localization, transport, and logistics.

XPO1 is one of the major drug discovery targets for treatment of different cancer types and is involved in various viral infections including HIV-1. Thus, we expect that our data might prove useful in medical research.

Fiene Daniel

Analysis of mitochondrial homeostasis and damage via femtosecond laser-based nanosurgery

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The development of novel therapeutics on the cell level requires a profound understanding of the function and structure of the individual cells' organelles. Mitochondria have been known for over a century and play an important role in the intrinsic signaling pathway of apoptosis, in the ageing process, and in various diseases. However, their division, fusion, and degradation by mitophagy has not yet been sufficiently investigated.

In this study, we use femtosecond laser-based ablation to individually disrupt single mitochondria, to better understand the dynamics of the mitochondrial network and its relation to the whole cell. The employed femtosecond laser system enabled a spatially confined mitochondria ablation during multiphoton microscopy in HeLa cells. In a first step, the viability of the cells and the change in mitochondrial morphology are investigated. Secondly, biosensors are introduced into the Hela cells by lentiviral transduction. These serve to monitor mitochondria, cytochrome-C and the concentration of reactive oxygen species using multiphoton microscopy. Ablation of individual mitochondria is performed at a wavelength of 730 nm. The change in mitochondrial morphology is examined at two times, 20 hours and one minute after ablation. For long-term measurements, hourly images of the cells are taken in the first five hours. To check the cell viability, we stain the cells with Calcein AM after 20 hours.

There was an increased mortality with an increasing number of ablated mitochondria in the HeLa cells. The mitochondria of the dying cells fragmented in the first hours after ablation. Therefore, we assume that the loss of low numbers of mitochondria could already induce apoptosis. This might be due to cytochrome C release or an increased amount of reactive oxygen species or both. In order to detect a difference in the concentrations of the reactive oxygen species present in the cell, images of the cells were taken before ablation, immediately after ablation and after one minute. Currently, we are extending our experiments to monitor the change in concentration and localization of cytochrome C. This will enable us to better interpret the underlying dynamics.

Maryam Mehdipour

How could semen microscopic factors use as a predictable item for Sperm DNA Fragmentation Index in IVF laboratories?

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Sperm DNA Fragmentation Index (DFI) assumes to be one of the main factors in men infertility which affects embryo formation and embryo development. Many investigations showed semen microscopic characters could affect sperm DNA fragmentation.

In this study, we evaluated the effects of semen microscopic factors on Sperm DNA fragmentation in large scale in order to find a new method to assess how we could use the correlation between semen microscopic factors and Sperm DNA Fragmentation Index (DFI) as a predictable item in IVF laboratory.

For this purpose, 819 semen samples were divided into two groups. Those samples were analyzed based on the WHO 2010 manual, and DNA fragmentation Index for each sample was assessed with Sperm Chromatin Dispersion method. For both groups based on semen analysis items mentioned on the WHO 2010 manual, Sub-groups were defined.

In first group and its sub-groups we just surveyed the correlation between DFI, count, and motility. In second group and its subgroups, we overlooked the correlation between count, motility, morphology, non-sperm cells and DFI in semen samples.

Our data showed DNA fragmentation sperms have raised with reduction of count, motility, morphology. In particular when sperm count and motility fall to half of the normal range mentioned on the WHO 2010 manual (C<10, m<25) the amount of DFI was dramatically increased.

In conclusion, we strongly suppose in semen samples with sperm count and motility below the half of the normal range published in 2010 WHO manual, it is better sperm DNA fragmentation treats start immediately after doing a simple analysis and there is no need for more tests. In such cases, in IVF laboratories sperm preparation method should select based on the procedures which separate sperms with a minimum of level of DFI.

Anjana Ghelani

Next-generation Sequencing based Microbiome Analysis of Oxygen Minimum Zone of Arabian Sea Coast

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In the ocean's major oxygen minimum zones (OMZs), oxygen is effectively absent from sea water and life is dominated by microorganisms that use chemicals other than oxygen for respiration. Arabian sea near Indian coastline is largest OMZ in Asia need to characterized using novel Next-generation sequencing tools. The advanced genomic techniques are defining the different metabolic niches that microorganisms can occupy in OMZs. Characterizing such niches, the microorganisms that dwell them and their effect on marine biogeochemical cycles is vital as OMZs expand with increasing seawater temperatures. We are presenting the microbial composition and metabolic profiling of three sites of Gulf of Cambay, western India, i.e., Dandi (DA), Nargol (NA) and Umargam (UA) using whole shotgun sequencing by Illumina Miseq platform. The total reads from DA were 3.7, 6.6 and 4.0 million for DA, NA and UA respectively. Total 18, 38 and 36 phyla were reported in DA, NA and UA metagenome. The most abundance community phyla Proteobacteria are 48%, 37.69% and 33.77% in DA, NA and UA respectively. Unidentified and unassigned sequences were also detected in all three samples, which indicate the possibilities to translate the hidden unculturable microbes. The detection of various key biological processes including response to biotic stimulus, stress response genes are the signs for adaptation against stresses exist in a marine ecosystem. Based on the detection of various molecular and cellular traits, nutritional versatility was also indicated in the marine microbial community of each site. Due to the detection of the diverse gene pool, cultivation approach has been used to trap the various traits and 8 potential species of actinomycetes. The result enlightens the profile of bacterial species with detection of pollutants and possibilities of commercialization the marine microbial products.

Cesar Cordero Gomez

Generation of myelin specific hybridoma cell lines of preserved specificity but with different isotypes using a CRISPR-cas9 approach

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Multiple sclerosis (MS) is the most common neurodegenerative, demyelinating and inflammatory condition of the central nervous system (CNS) among young adults. Despite there is a consensus that T cells are the main drivers of the disease, recently B cell-driven pathogenic mechanisms such as antigen presentation, production of inflammatory cytokines and chemokines, and antibody production, were additionally coming into the focus of attention. Our research group has recently shown that antibodies targeting myelin structures accelerate the onset and enhance the clinical severity of the MS mouse model experimental autoimmune encephalomyelitis (EAE), presumably by increasing the ability of myeloid cells to present antigens to invading myelin-reactive T cells, which is a prerequisite for T cell reactivation within the CNS. For these experiments, an anti-myelin oligodendrocyte glycoprotein (MOG) IgG1 antibody derived from the 8.18c5 hybridoma was used. The antibody class, or isotype, is defined by the structure of the immunoglobulin heavy chain and determines antibody effector functions. Mature B cells normally express molecules of the IgM isotype on their surface, but upon antigen recognition, they rapidly proliferate and undergo class switch recombination (CSR), thereby selecting new heavy chain constant domains and producing antibodies of different isotypes. In order to elucidate the role of the immunoglobulin isotype in EAE modulation while preserving antibody specificity, we designed a CRISPR-cas9 based system in order to exchange the isotype of aMOG-antibody producing hybridoma cell lines. To this end, we designed guide-RNAs targeting the actually used genomic DNA regions of the immunoglobulin heavy chain locus by the hybridoma to allow the switch to the next heavy chain locus, resulting in its expression and therefore the switch of the isotype. Our CRISPR-cas9 approach enabled the generation of MOG-specific isotype switched hybridoma cell lines, which will allow us to unravel the relevance of the antibody isotype for the modulation of CNS autoimmunity.

Dominik Müller

Mechanistic investigation of sarcomeric elements in cardiomyocytes via femtosecond laser-based nanosurgery

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A well-defined sarcomere arrangement is unalterable for controlled and homogeneous contraction in cardiomyocytes (CMs). Abnormalities in sarcomeric elements, such as the Z-disc, are found in several diseases like cardiomyopathies. Gold standards to investigate the functions of cytoskeletal elements are chemical treatments, transgenic animal models, or knockout and overexpression studies. However, revealing the functions of distinct structures down to the scale of a single element is challenging. Therefore, we established a femtosecond (fs) laser-based setup to physically ablate structures in a precise, non-invasive manner. For the first time, a single Z-disc in neonatal as well as in human pluripotent stem cell (hPSC)-derived cardiomyocytes was ablated via fs laser-based nanosurgery. The viability of treated CMs was unaffected by the loss of a single Z-disc in the sarcomeric pattern. Furthermore, more than 42% of neonatal rat CMs and 68% of hPSC-derived CMs recovered a regular pattern within 24h. Significant alterations in CM morphology, in particular, cell perimeter, x- and y-expansion were not found over a time period of 24h. Only hPSC-derived CMs, which were cultured for a shorter period (14 days), react with a significant decrease in the cell area, x- and y-expansion 24 h past nanosurgery. In addition, calcium homeostasis as an indicator for the periodic contraction of CMs, was analyzed. The data show, that the frequency of calcium oscillations before and after single Z-disc ablation is comparable. This demonstrates that CMs can compensate for the loss of a single Z-disc and recover a regular sarcomeric pattern during spontaneous contraction and Ca2+ handling. It also illustrates that fs laser-based nanosurgery is a powerful tool to physically micro manipulate CMs to investigate cytoskeletal functions and organization of single elements. Thus, this technique can help to elucidate the role of single elements in a cytoskeletal network, which can ultimately lead to novel ideas for therapeutic treatments or drug testing strategies for intrinsic regeneration processes.

Eman Abbas

Chromatin Remodeling BAF Complexes Control Oligodendrocytes Development in the Embryonic Telencephalon

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In the developing mammalian brain, the ventral telencephalic neural stem cells (NSCs) produce distinct cell types such as interneurons, astrocytes, and oligodendrocytes (OLs). The epigenetic regulatory mechanisms, which control the oligodendrogenesis are still not fully understood. In this study, we focused on the role of the chromatin remodelling BAF complex in the OL production. Our findings revealed that the BAF complex is highly expressed in the OL lineage including oligodendrocyte precursor cells (OPCs), immature oligodendrocytes (iOLs) as well as, mature oligodendrocytes (mOLs). The transcriptomic analysis displayed significant downregulation of the OL linked genes in the BAF155/BAF170_hGFAP-Cre dcKO pallium relative to the control counterparts. Therefore, we deleted the BAF 155/170 subunits specifically in the Olig2-Cre line that is expressed in the ventral NSCs in developing forebrain to generate the conditional BAF155/170 double knockout mice. Noticeably, this conditional BAF complex loss resulted in a depleted pool of iOLs and mOLs in the ventral and dorsal telencephalon. Furthermore, our data exhibited that the BAF complex is required for the proper proliferation of OPCs. Thus, our findings substantiated that the BAF complex has a crucial function to regulate the OL development during the embryogenesis.

Francesca Lugarini

Initiation of Autophagy signalling by HORMA domain proteins

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Eukaryotic cells remove protein aggregates, pathogens and damaged organelles from the cytosol through the autophagic pathway. Autophagy is an intracellular degradation system that represents both a protective system as well as a source of energy and different biosynthetic precursors. This quality control system of the cell occurs at low basal levels but is dramatically upregulated under stress conditions. These signals trigger a cascade of events that leads to the formation of a cup-shaped double membrane structure. This structure elongates to engulf the cargos and matures as autophagosome, a mega-vesicle whose formation is mainly regulated by a family of autophagy-related proteins (ATG proteins)^{1,2}.

The first step of this well conserved mechanism is the recruitment of the ULK1/2-kinase complex to a few distinct sites on the ER membrane. However, its role and regulation within the human autophagy initiation machinery is still largely unknown. Intriguing components of the ULK1/2-kinase complex are the HORMA domain proteins ATG13 and ATG1013^{4,5}. HORMA domains proteins work as signal-responsive elements at the initiation of signalling cascades (autophagy, mitosis, cell damage etc.). They mediate protein-protein interactions through a structurally unique mechanism involving a reversible remodelling of its topology, which enables the protein to wrap around specific binding partners. Previous biochemical reconstitution efforts focussed on the HORMA domain MAD2 in mitosis yielded a list of principles that are likely to be conserved in all HORMA domain proteins⁶, as in this case ATG13 and ATG101.

ATG13 contains an N-terminal HORMA domain which interacts directly with the HORMA domain of ATG101⁴. The interaction between ATG101 and ATG13 is essential for the function of the initiation complex, since mutating the ATG101-ATG13 interacting interface showed a pene-trant autophagy inhibitory effect⁷, highlighting the importance of this interaction in regulating the pathway.

In order to understand the spatio-temporal control of autophagy initiation, we propose to biochemically reconstitute the ULK1 complex. Particular attention is given to the identification of interactors of HORMA domain proteins ATG101 and ATG13 and the characterization of the interaction dynamics which we hypothesise will be at the centre of the induction of autophagy.

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Fouzia Qamar

Bioefficacy of selected Isolates against Insects Pests

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Hazardous impacts of chemical insecticides on ecosystem and on targeted populations necessitate the development of alternate eco friendly pest control Strategies. Actinomycetes, the Gram positive bacteria with high G+C content in their DNA are known to produce variety of secondary metabolites that possess different biological activities and exhibit the potential to be developed as effective biocontrol agents against different pests. Different strains were isolated from diverse ecological niches and then screened for their insecticidal attributes. The selected isolates were characterized for their morphological, biochemical, physiological and cultural characteristics and by 16S rRNA gene sequencing. The biological screening revealed the potent metabolites of the selective isolates exhibiting larvicidal/anti-insect properties. Chemical screening by thin layer chromatography (TLC) and HPLC-UV, the crude extracts obtained from the culture broths of these isolates, are known to exhibit an impressive diversity of the chemical constituents. Isolates were found to be rich source of bioactive secondary metabolites which may provide valuable alternatives to chemical insect-control agents.

Flávia Rezende

Renal Nox4 contributes to systemic redox homeostasis by controlling glutathione, methionine, cysteine and folate metabolism

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The NADPH oxidase Nox4 produces H2O2 and is highly expressed in the kidney. Its expression is reduced in diabetic nephropathy and renal inflammation, suggesting that Nox4 is particularly important for normal renal function. As demonstrated by in situ hybridization (RNAscope) combined with immunofluorescence, Nox4 is selectively expressed in the proximal tubule, a part of the kidney responsible for mass transport. To study this function, WT (wild type) and Nox4*/* (tamoxifen-inducible, global Nox4 knockout mice) were put on an artificial fiber and protein-free diet, with low sodium (130 mg/kg chow) and low micronutrients. Urine samples (day 0, 3, 14), renal cortex and plasma (day 14) were analyzed by global untargeted LC/MS for metabolites. Genome scale metabolic reconstruction using fastcore showed a significant downregulation of extracellular and mitochondrial transport; metabolism of nucleotides, inositol phosphate and folate in response to the deletion of Nox4. Moreover, metabolites of histidine catabolism (urocanate and formiminoglutamate), which depend on folate, were significantly elevated in Nox4*/* compared to WT mice. Marked reduction in metabolites of methionine, cysteine and glutathione were observed in plasma and renal cortex of Nox4*/* compared to WT animals. Results from kidney phenotyping, protein expression and metabolomics indicate that the physiological function of Nox4 in the kidney is to control reabsorption and metabolism of amino acids and vitamins of the complex B that are important for redox homeostasis.

Franziska Grüner

Identification of B Cell Receptor Regulated MicroRNAs in a Model of Aggressive B Cell Lymphoma

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The most common B cell derived non-Hodgkin lymphomas (B-NHL) are diffuse large B cell lymphomas (DLBCL). DLBCLs can be divided into two major subtypes, the activated B cell like (ABC) and germinal center B cell like (GCB) DLBCL, respectively. Patients with the ABC subtype show in comparison to patients with GCB-DLBCL worse clinical outcomes and are often characterized by chronic active B cell receptor (BCR) signaling. Growth of ABC-DLBCL typically depends on BCR signaling which amongst others drives NF-kB activation, thus promoting proliferation and survival. The BCR signaling cascade also affects microRNA (miRNA) expression. Importantly, expression of these small non-coding RNAs is tightly regulated during B cell development and maturation. De-regulation of miRNAs has been reported for several B-NHL entities. Furthermore, miRNA expression profiles can distinguish ABC-DLBCL from GCB-DLBCL.

Despite these facts, the miRNA regulome as a result of chronic BCR signaling remains unknown. Thus, the aim of this Master's project is to identify miRNAs in ABC-DLBCL that are regulated by BCR signaling. A model cell line for ABC-DLBCL (U-2932), consisting of two subclones (R1 and R2), was chosen for BCR stimulations that were combined with perturbations using the inhibitors ibrutinib and copanlisib that both target BCR downstream kinases. The Bruton's tyrosine kinase (BTK) inhibitor ibrutinib showed good results in several clinical trials with B-NHL patients, however therapy resistances could already be observed. The phosphoinositide 3-kinases α and δ inhibitor copanlisib showed promising trial results in ibrutinib-resistant ABC-DLBCL. For this project the sensitivity (IC50) of both inhibitors was determined in the U-2932 subclones by live cell imaging using an IncuCyte[®] S3 System. BCR crosslinking and successful inhibitor treatments were verified via detection of phosphorylated kinases (e.g. BTK) downstream of the BCR via immunoblotting. In order to select a suitable timeframe of BCR stimulation for global miRNA expression profiling, expression of a known BCR-regulated miRNA was analyzed by quantitative PCR. Afterwards, smallRNA libraries are prepared from total RNA isolated from BCR-stimulated cells in presence and absence of the inhibitors utilizing the QIAseq miRNA Library Kit (Qiagen). After quality control libraries are subjected to next generation sequencing using a NextSeq (Illumina).

Herein data will be presented that show the impact of BCR stimulation as well as ibrutinib and copanlisib treatments on proliferation and BCR signaling activity in an ABC-DLBCL model cell line. Furthermore first results from miRNA library preparations, quality controls and miRNA expression profiling will be presented. Future studies need to address if the identified BCR-regulated miRNAs can be verified by means of qPCR and if they are also regulated by BCR signaling in other ABC-DLBCL cell lines.

Nicole Horn

Investigating the role of mature microRNAs identified in the nucleus of malignant B cell lymphoma cell lines

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MicroRNAs (miRNAs) are small non-coding RNAs that are important for normal B cell development and that are frequently de-regulated in cancer, including B cell lymphomas. Global downregulation of miRNAs is observed in cancer tissues compared to corresponding normal tissue. According to textbooks mature miRNAs are localized to the cytoplasm, where they are incorporated into the RNA induced silencing complex (RISC) to guide the interaction with their target mRNAs by targeting the 3' untranslated region. This may lead to degradation of the target mRNA or repression of translation. Besides this canonical function, other roles for miRNAs have been reported in recent publications. Importantly, mature miRNAs and components of the RISC complex have been detected in the nucleus suggesting novel nuclear functions for miRNAs.

In line with this, we observed enrichment of specific miRNAs in the nucleus when we analyzed the subcellular distribution of mature miRNAs in a panel of B cell lymphoma cell lines. However, the functions of these miRNAs in B cell lymphoma are yet unknown.

Thus, the aim of this Master's project is to elucidate the biological relevance of the nuclear miRNA candidates in B cell lymphoma cell lines. Several nuclear miRNA candidates were chosen to determine their impact on proliferation and survival after overexpression and knock-down in two different B cell lymphoma cell lines representing follicular lymphoma (SC-1) and mantle cell lymphoma (MINO), respectively. For overexpression the miRNAs were transfected both as an expression plasmid and as Locked Nucleic Acid (LNA) miRNA Mimics. Inhibition of miRNAs was achieved by transfection of anti-miRNAs (LNAs). Cell proliferation and survival of transfected cell lines were investigated by life-cell-imaging using an IncuCyte* S3 System. In addition, gene expression analyses of miRNAs across B cell lymphoma cell lines and from nuclear and cytoplasmic fractions were performed. Herein data will be presented that show the impact of the tested miRNAs on proliferation and survival of B cell lymphoma cell lines to elucidate their potential role as oncomiR or tumor suppressor miRNAs. Future studies need to address if the role of the nuclear miRNA candidates can also be confirmed in other B cell lymphoma cell lines and if the function is specifically related to their nuclear localization.

Yaroslav Faletrov

Novel Steroids with Artifical Side Chains as Substrates for Mycobacterial Enzymes

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Synthetic steroids, saving the 3β -hydroxy-5-androsten core of cholesterol, but with artificial side chains (substituents at C17) have been found applications as cholesterol analogues (22-NBD-cholesterol, BODIPY-cholesterol) or anti-cancer drugs (abiraterone). From the other hand, mycobacteria are known to convert cholesterol. The process is associated with pathogenicity of Mycobacterium tuberculosis (Mtb) and, thus, corresponding proteins are potential drug targets. In spite of the facts, little is known about ability of mycobacteria to convert steroids with artificial side chains [1, 2]. Previously we reported about ability of cholesterol-like molecules with N-heterocyclic 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)- fluorophore in side chains to be converted by mycobacteria or bacterial cholesterol dehydrogenase [1, 3]. Now we report that abiraterone acetate (Abira) and novel 2-aminobenzimidazole-16-dehydropregnenolone acetate fusion (JM1) [4], 17-(3-picolylamino)-3β-hydroxy-5-androsten (DAPyc) as well as hydrazones of DHEA with isoniazid and NBD-hydrazine are converted by M. smegmatis and M. tuberculosis to free 3β-hydroxy- forms (for Abira and JM1), 3-keto-4-en derivatives and 3-keto-1,4-diene derivatives as judged by TLC, HPLC-UV/Vis-FL and HPLC-ESI-MS data. Ability of mycobacteria to hydrolyze 3-hydroxysteroid esters seems to be not reported. This new fact is interesting with respect to known ability of mycobacteria-infected macrophages to accumulate cholesterol esters. BLAST analysis pointed out on Mtb LipH (Rv1399c) as a homologue of human neutral cholesterol ester hydrolase (Identity 32 %), a cholesterol acetate-hydrolyzing enzyme. In silico virtual screening of the compounds with Mtb's P450s and steroid-converting enzymes with known 3D structure have helped to predict new possible substrates for the enzymes, in particular for 3-ketosteroid-1-dehydrohenase relevant for Mtb virulence [5].

The findings shed light on applicability of the compounds as probes for investigations of steroids' impact on Mtb physiology and pathogenicity.

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Keywords: steroids, fluorescence, mycobacteria, NBD, synthesis, docking.

Nadine Brandes

Lineage tracing analyses reveal the potential of CD4+ stem cells in skin homeostasis and regeneration

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Basal cell carcinoma (BCC) is the most common neoplasia of the human skin. Thereby the most important driver mutations that lead to the development of this tumor entity are mutations that result in an over-activation of the Hedgehog (Hh) signaling pathway. To date, the cellular origin of BCC is controversial discussed, since stem cells of the hair follicle, of the interfollicular epidermis as well as bone marrow-derived cells might be potential candidate cells. Recently our group described the existence of a skin-resident cell population, which can give rise to BCC and that express stem cell-like markers as well as the CD4 protein, which typically is found on immune cells (e.g. T cells, monocytes). However, a hematopoietic origin of the BCC has been ruled out. Thus, the BCC originating cell type can be described as a CD⁴⁺ non-hematopoietic, stem cell-like cell with tumorigenic potential. Since the knowledge about such cells might have critical effects on the understanding of skin cancer development, we characterized the CD⁴⁺ non-hematopoietic population as well as their progeny by analyzing the skin of CD4Cre R26-tdTomato reporter mice. Our approaches revealed that CD4+ nonhematopoietic cells and/or their progeny harbors enormous regenerative potential since they repopulate the stem cell niche of hair follicles and differentiate into all layers of hair follicles and of the interfollicular epidermis during normal skin homeostasis. Upon experimentally induced wound healing as well as during increasing age of the mice the numbers of hair follicles and/or epidermal cells that descent from CD⁴⁺ non-hematopoietic cells and/or their progeny highly elevate. Thus, we concluded that regenerative processes (e.g. healing processes after incisional wounding) are inducers of the invasion/expansion of CD4-expressing cells and/or their progeny in hair follicles and epidermis. Currently, the cellular identity of CD4-expressing cells that seem to play a significant role in hair follicle and epidermal regeneration remains unclear, but several lines of evidence hint towards a subpopulation of epidermal stem cells or bone marrow-derived mesenchymal cells.

Zuzana Kadlecova

Temporal control of endocytosis by phosphorylation of the main endocytic adaptor protein AP2

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The levels of transmembrane proteins of widely differing functions must be tightly, but dynamically, controlled. This is achieved by precisely regulating the rate of internalization of each and every transmembrane protein from the cell's surface by clathrin-mediated endocytosis (CME).

Phosphorylation is a highly effective way for reversibly regulating protein activity. In clathrin-mediated endocytosis the major phosphorylation event is the phosphorylation of the AP2M2 subunit. AP2 is the most abundant adaptor, which orchestrates CME by coupling the clathrin to the inner membrane and transmembrane cargo. Surprisingly, the physiological significance and consequences of this phosphorylation remain unknown and highly debated. In this work we solved this conundrum:

We show that phosphorylation of AP2 forms a new, transient binding platform for the recruitment of scaffold proteins to the growing clathrin-coated pits (CCPs). In turn, these scaffold proteins called NECAPs recruit BAR domain proteins, which are drivers of later stage events in CCP formation. By using NMR spectroscopy, crystallographic and biochemical approaches, we describe the molecular and structural basis for both events. We find that AP2 phosphorylation is at the center of a major decision in clathrin-mediated endocytosis. Either the formation of a CCP proceeds to completion, including the energy-costly process of vesicle scission. Or it will abort.

We also combined quantitative proteomics with pharmacological perturbations together with quantitative automated live cell microscopy. We reveal that failure to phosphorylate AP2 disrupts the temporal regulatory system of endocytosis and results in CCP formation being stalled, causing global endocytic rates to drop. In conclusion, phosphorylation of AP2 appears to be an important regulatory mechanism of endocytosis.

Ivana Borovská

Mutations in RNA Recognition Motifs of PUF60 and U2AF65 Occuring in Cancer and their Impact on 3' Splice Site Selection

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U2AF65 and PUF60 are splicing factors involved in the early steps of spliceosome assembly – more specifically in splice-site recognition1. These factors play an important role in recruitment of the U2 small nuclear ribonucleoprotein to branch point sequence and selection of 3' splice sites (3'ss). These two proteins are critical recognizers of the highly variable 3'ss consensus motif known as the polypyrimidine tract (PPT)2,3. Protein - RNA interaction is mediated by central tandem RNA recognition motifs (RRMs)2.

Next generation sequencing studies of cancer cells identified mutations affecting important protein domains in RNA processing factors involved in 3'ss selection, including U2AF65 and PUF60. Using combination of RNA sequencing and transfection and other studies, we identified impact of cancer-associated mutations located in RRM on the selection and usage of correct 3'ss, RNA binding and protein properties. Different RRM substitutions produced a spectrum of splicing alterations, indicating that they increase the mRNA isoform diversity in cancer cells. We also uncovered new residues required for correct folding/stability of each protein. Together, these results reveal RRM residues in PUF60 and U2AF65 required for 3'ss selection and provide new and relatively simple tools to identify clonal substitutions in RRMs that may drive cancer initiation or progression.

Key words: pre-mRNA splicing; 3' splice site; PUF60; U2AF65; cancer; driver mutation.

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Ivonne M Bartsch

Bioinformatic Tools for Peptides Design with Phagocytic Activity for Pharmacological Porpuses

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Introduction: Opsonin C3b is an important member of the complement system. Cascade of the alternative pathway includes many other proteins, although C3b is the main one capable of opsonizing antigens. After this, the opsonin can be able to trigger phagocytosis by binding to complement receptors CR1 in macrophages¹.

Cells coating like bacteria or apoptotic cells with C3b would put them at risk of destruction.

The main goal of this research is to design peptides capable of activating CR1 and trigger the phagocytosis process. Bioinformatic tools for crystallographic analysis structures of CR1 and C3b are needed to create peptides based on protein sequences and binding sites.

Materials and Methods: We used UniProt, NCBI PROTEIN BLAST, and PDBsum as databases. Patchdock and FireDock for Docking realization. All of them of free access2.

Fmoc chemical synthesis strategy was applied for peptides shyntesis3,4. THP-1 monocytes cell line was used to analyze peptides binding to CR1. Red fluorescent latex beads coated with streptavidin for phagocytosis test was needed.

Conclusions: It was possible to obtain three agonist peptides to the CR1 receptor from interaction sites between C3b and CR1. Peptides were synthesized and subjected to quality control analysis and all the sequences match with designed ones.

Only one of them was capable of binding to CR1 receptor. This was proved with biotin peptides labeling and incubation with Streptavidin Alexa 647 and washing steps. Confocal microscope identified fluorescence by incubating THP-1 cells with peptides.

Phagocytosis test showed that there are no significant differences between the opsonin C3b effect and the peptide used.

Opsonin model could allow as to create hybrid peptides capable of opsonizing unwanted cells. The peptide tested in this work which is capable of trigger phagocytosis linked it to other peptides with affinity to specific proteins of other cells, can be used to eliminate cancer or tumor cells.

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João Nuno da Rocha Oliveira-Gomes

Metabolomics of Muscarin – Clinical Targets and Toxicological Effects

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Introduction: Muscarin [C⁹H²⁰NO²]⁺ is a tetrahydrofuran-containing toxic alkaloid found in several species of mushroom-forming fungus of the genera Inocybe, Mycena, Clitocybe and Omphalotus [1].

This substance doesn't occur naturally in the human body; however, it binds to and stimulates cholinergic receptors (mAChRs) in the plasma membranes of the autonomic nervous system cells mimicking the effects of acetylcholine (ACh).

It is the first parasympathomimetic substance ever studied, having been isolated from the fly agaric mushroom Amanita muscaria in 1869, however, its toxic effects are still being studied nowadays and potential pathways for the treatment of pathologies were left open by the studies regarding this molecule.

Aims: This work aims to review the state-of-the-art literature of muscarine, particularly its chemical structure, pharmacokinetics, pharmacodynamics, acute intoxications and therapy of intoxications.

Material and methods: The metabolism of muscarin, pharmacological- and toxicological-related effects were reviewed in books, PubMed (U.S. National Library of Medicine), Google Scholar and in PLOS (Public Library of Science) without a limiting period. Bibliographic results were intercrossed between sources for further accuracy in the data presented.

Results: Muscarine accounts for a dose-dependent toxic symptomatology that is characterized by a rapid onset, within 15 minutes to 2 hours. Its effects are exerted across several tissues, depending on the concentration of substance in the extracellular space and the subtype of mAChR. These receptors range from M1 to M5 and are non-selective G-protein coupled: the M1, M3, and M5 mAChRs couple predominantly to Gq proteins that activate the enzyme phospholipase C; the M2 and M4 receptors couple to Gi proteins that inhibit adenylyl cyclase, as well as to G proteins that directly regulate K⁺ and Ca²⁺ channels [2].

It is associated with several adverse effects on cardiovascular, gastrointestinal, neurological, psychiatric and genitourinary systems, among others [3]. that can potentially lead to shock and death. However, despite the resistance to acetylcholinesterase (AChE), supportive care and atropine – dose 1-2 mg IV in adults (0.02-0.05 mg/kg IV in children) – can counteract these effects by binding to and consequently blocking mAChRs [4].

Conclusions: Muscarine is a well-studied substance and currently analogues of this molecule are being used for the treatment of glaucoma, xerostomia, urinary retention and nonobstructive gastrointestinal hypomotility, among others. It is expected that knowing the metabolomics of muscarine may provide further insights regarding individual contribution for muscarine pharmacodynamics and toxicological effects, as well as the approaches to its adverse effects.

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Joachim Maier

NMR studies on peptide-protein interactions contributing to phase separation

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During B-cell receptor signaling, signal transducers assemble at the plasma membrane and eventually realize a humoral immune response. The adapter proteins SH2-domaincontaining leukocyte protein of 65 kDa (SLP65) and Cbl-interacting protein of 85 kDa (CIN85) function as transducer components within the BCR signaling pathway⁽¹⁾.

Both proteins need to be present close to the BCR at sufficiently high concentrations to function. Classified as adapter proteins, it's likely that they provide a scaffold for the effectors of the signaling cascade. The spatial and temporal organization and the underlying molecular interactions are coded in the sequence. This work focuses on the structural aspects of the interactions of SLP65 and CIN85; both proteins contain intrinsically disordered regions (IDRs).

In resting B-cells, SLP65 and CIN85 are clustered at VAMP7-positive vesicles^(1,2) and form a pre-signaling complex. To structurally characterize this signaling cluster, in vitro reconstitution of CIN85, SLP65 and small unilamellar vesicles (SUVs) were prepared resulting in tripartite phase separation at physiological relevant protein concentrations. The vesicle distribution within the dense phase has been visualized by cryo-ET. Also, the molecular interactions have been studied by NMR in our lab. It was shown previously that SLP65 interacts with SUVs by its N-terminal domain and that CIN85 trimerizes by its coiled coil domain reaching a higher valency (9 SH3 domains in the CIN85 trimer)⁽³⁾ while the three SH3 domains of CIN85 bind promiscuously to multiple PRMs of SLP65.

To elucidate the nature of the promiscuous interactions in more detail, we set out to determine the individual binding affinities between 21 combinations of SH3 domains and peptides bearing single PRMs. Designer constructs with different affinities were expressed to study the phase separation process dependence on affinity.

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Samar Mamdouh Shawki

Liraglutide improves 3-NP- induced Huntington's disease: Impact on BDNF/TrkB/PI3K/Akt/GSK-3 β / β -catenin/CREB trajectory and miR-130a

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Introduction: Huntington's disease (HD) is a dominant autosomal neurodegenerative disease associated with progressive motor and cognitive deficits that lacks a definitive symptomatic therapy. The antidiabetic liraglutide possesses a neuroprotective potential against models of neurodegenerative disorders; however, its role in HD remains elusive which is the aim of this work.

Methods: Liraglutide (200 μ g/kg, s.c) was administered to rats intoxicated with 3-nitropropionic acid (3-NP) for 4 weeks post HD model induction.

Results: Liraglutide abated the 3-NP-induced neurobehavioral deficits of rats (open field and elevated plus maze). Biochemically, it enhanced striatal miR-130a gene expression and TrKB protein expression, its ligand BDNF, and modulated the downstream targets; viz., p-PI3K/p-Akt/p-GSK-3β/p-β-catenin/p-CREB. Liraglutide also reduced the striatal protein content and mRNA expression of the death receptors sortilin and p75NTR, respectively in the 3-NP treated rats. Additionally, its anti-oxidant/-apoptotic capacity were verified by enhancing the antioxidant transcription factor Nrf2 and abrogating MDA, besides, upregulating both Bcl2 and Bcl-XL and downregulating Bax along with decreasing caspase-3 activity. To further emphasize its neuroprotective role, liraglutide displayed a marked downregulation in the striatal mRNA expression of HSP 27, PBR and GFAP, while it upregulated that of DARPP32. The molecular results were reflected also on the histopathological findings, where 3-NP-treated rats revealed focal neuronal degeneration with gliosis in the outer area of the cerebral cortex, while the inner deep area showed focal gliosis with no histopathological alterations in the subiculum of the hippocampus. Furthermore, most of the neurons of the fascia dentate and hilus of the hippocampus showed degeneration with nuclear pyknosis. Moreover, multiple focal eosinophilic plaques were detected in the striatum along with diffuse gliosis in between nuclear pyknosis and neuronal degeneration and congestion of the blood vessels was noticed in the cerebellum.

Liraglutide administration ablated most of the histopathological alterations induced by 3-NP, as evidenced by normal histological structures of the cerebral cortex, subiculum of the hippocampus, fascia dentate and hilus of the hippocampus, the striatum, the cerebellum and the medulla oblongata.

Conclusion: Based on these findings, liraglutide exerts a neuroprotective effect on 3-NP treated rats that is at least partially attributed to the increase of miR-130a and activation of BDNF/TrkB signaling pathway, beside its anti-apoptotic, antioxidant, and neurotrophic activities.

Key Words: Huntington's disease; 3-nitropropionic acid; BDNF/TrkB; neurotrophic; liraglutide

Lubos Janotka

Development of Resistance to HMAS through Apoptosis Regulation

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Cytidine analogs 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC) belong to group of hypomethylating agents (HMAs). The DNA demethylation promoted by these agents may lead to re-expression of tumor suppresor genes and normal differentiation of cancer cells. These two agents have been aproved for the treatment of high-risk MDS and AML patients. However big cohort of patients will not respond to this treatment. Prognosis after HMAs failure is poor and except clinical trials, there are currently no other treatment options.

Therefore first aim of our study was to establish HMAs resistant cell models. We have used human AML cell line MOLM-13 (Leibniz-Institut DSMZ GmbH, Germany) in our study. This cell line showed sensitivity to both observed HMAs. We have detected induction of apoptosis by both AZA and DAC in MOLM-13 after 72h incubation. The proportion of apoptotic and necrotic cells was detected by double staining using an annexin V/propidium iodide kit after which the samples were analyzed by flow cytometry. Sensitive cell line MOLM-13 was then adapted to DAC and AZA. We have cultivated MOLM-13 cells with step-wise increasing concentration of either AZA or DAC, that led to development of DAC-resistant MOLM-13/DAC and AZA-resistant MOLM-13/AZA cell variants.

We have confirmed resistance of MOLM-13/DAC and MOLM-13/AZA by flow cytometry after 72h treatment with DAC or AZA respectively. Interestingly, the development of resistence did not lead to cross-resistance, as we have detected induction of apoptosis in MOLM-13/DAC cells after treatment with AZA and otherwise.

Next we observed changes in apoptosis regulation on methylation and mRNA level. EpiTect* Methyl II Signature PCR Array Kit was used to determine methylation status of 22 promoter regions of genes associated with apoptosis (APAF1, BAD, BAX, BCL2L11, BCLAF1, BID, BIK, BIRC2, BNIP3L, CASP3, CASP9, CIDEB, CRADD, DAPK1, DFFA, FADD, GADD45A, HRK, LTBR, TNFRSF21, TNFRSF25, TP53). The most significant changes were observed in methylation status of genes BCL2L11, BCLAF1, BID, BIK, CASP3, DAPK1, GADD45A, TNFRSF25.

Subsequently we have evaluated transcription of these genes by qRT-PCR. We have observed upregulation of GADD45A and TNFRSF25 in correlation with HMAs effect in all cell variants. Interestingly, it led to constitutive expression of TNFRSF25 in MOLM-13/DAC, which correlates with decrase in its methylation status. On the other hand, we have observed downregulation of GADD45A in MOLM-13/DAC and MOLM-13AZA compared to parental cell line MOLM-13. Further we have observed correlation between overexpression of DAPK1 and effect of DAC and also downregulation of BIK in MOLM-13/DAC.

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Priscilla Gonzáleza

Effect of overexpression of the global transcription factor Xbp1 on the specific production and glycosylation patterns of erythropoietin produced by CHO cells

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Introduction: Erythropoietin (h-EPO) is a recombinant glycoprotein used for the treatment of chronic anemia. By 2020 the global market of h-EPO is expecting to reach 11.9 billion USD. The most used cell line in the pharmaceutical industry is the Chinese hamster ovary cells (CHO) because they produce a protein with a high degree of glycosylation, similar to humans. However, its production is limited by the secretory pathway/post-translational modifications (PTM). Affecting the viability, productivity, and quality of recombinant proteins (r-protein). Even more, there has been found disparities in h-EPO glycosylation patterns (GP) between different clones or under different cultivation condition, harming molecular aspects such as stability, immunogenicity and biological activity in vivo and in vitro. GPs occurring in the Endoplasmatic reticulum (ER) are dependent on its machinery and the unfolded protein response (UPR), a complex signaling system emanates from the ER membrane. One of the elements of the UPR is the transcription factor Xbp1, responsible for inducing the production of chaperones, folding and trafficking proteins1. We evaluated the effect of the overexpression of Xbp1 on q_p and glycosylation patterns of h-EPO produced by CHO cells².

Materials and methods: The homologous recombination technique (RMCE) was used to obtain a stable CHO cell line producing h-EPO (CHO- hEPO). Subsequently, it was transfected (chemically and/ or virally) with modified pCMV5-Flag-Xbp1s (addgene, # 63680, and hygromycin resistance). From the population (CHO-X) obtained, three clones were selected. All the clones (including CHO-hEPO) were adapted to serum-free culture and suspension, to be characterized kinetically, metabolically and productively. "DIG Glycans Differentiation Kit" (Roche, Germany) will be used to identify specific carbohydrate motives.

Results and conclusion: CHO-hEPO behaved kinetically and metabolically similar to previous reported, however, its specific production increased four times compared to previous publishing. Between three selected clones, just one of them showed an increase over the qp, enhancing in 218% far exceeding what was obtained on the other two clones selected. The second one didn't show any effect and the third one decreased by 62.3% the q_p . In addition, an effect on the metabolism of glutamine was evidenced, showing a general tendency of all clones to decreased ammonia production. Regarding the glycosylation patterns results, at this moment they are in the stage of processing samples. We expect to produced h-EPO with a high content of sialic acid and improvement on the sugar content because of the overexpression of Xbp1. These results are a breakthrough in biopharmaceuticals nationally and internationally. In this context, we have obtained cell lines in a quick way through the RMCE technique and the expression regulator showed a positive effect on the productivity of recombinant proteins.

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Rashi Goel

New Approaches for Studying Neurotransmitter Transporters

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Synapse is a functional nanomachine where neurotransmission is mediated by neurotransmitter filled synaptic vesicles (SV). To sustain a fast and potent synaptic response, SVs have to be filled with 1000's of neurotransmitter molecules within seconds. Neurotransmitters are transported by specialized transporters that draw the energy from the electrochemical gradient across the vesicular membrane. We want to decipher the mechanisms of neurotransmitter filling, however, due to lack of tools, this field is quite understudied. Thus, this project is about building new fluorescence based tools such as pH and chloride sensors to solve the unknown in synaptic vesicle transporter biology.

Rima Siauciunaite

Multiple clocks regulate amino acid levels in zebrafish cells

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The circadian system plays a pivotal role in orchestrating amino acid metabolism and makes it possible for daily rhythms in amino acid absorption, storage and transport to be temporally coordinated with the sleep-wake cycle and feeding behavior. At the molecular level the rhythmic expression of genes involved in amino acid biosynthesis is regulated by elements of the core clock mechanism in peripheral tissues, including the liver. Hence, asynchrony between the external environment and endogenous circadian rhythms results in disruption of amino acid homeostasis and can lead to an increased risk of cancer and diabetes mellitus. Nevertheless, the major challenge remains to unravel the molecular pathways through which the circadian clock and metabolism respond to light and food. In this study, we are exploring crosstalk between the circadian clock and the regulatory circuits involved in amino acid metabolism in zebrafish (Danio rerio). By applying multi-omics approaches we have discovered for the first time that daily changes in amino acid concentration are infradian, whereas clock gene expression exhibits a circadian rhythm. How can amino acid levels cycle with an infradian period? Are there alternative clock mechanisms underlying temporal control of metabolism? In order to understand this, we are now beginning to investigate candidates that are responsible for infradian rhythmicity. Additionally, by a comparative approach involving blind cavefish as a model, we hope to gain unique insight into how evolution under extreme environmental conditions can influence the mechanisms which time metabolism.

Georgy Selihanov

Crystallization in lipid mesophase can induce changes in crystal structure

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Membrane proteins are problematic objects for X-ray structural analysis of their spatial structure. One of techniques created for membrane protein crystallization is a crystallization using lipid mesophases. It turned out that this technique has a certain disadvantage. Using photosynthetic reaction center (RC) of Rhodobacter sphaeroides we have shown that crystal grows in lipid mesophase can affect the pigment composition in the resulting x-ray structure.

We obtained three types of RC crystals: (i) from detergent conditions without lipids; (ii) from lipid cubic phase conditions; (iii) from lipid sponge phase conditions. Spatial crystal structures were solved and refined with a final resolution of 2.0 Å for RC from LSP, 2.1 Å for RC from LCP and 2.3 Å for RC from detergent conditions.

Analysis of spatial structures derived from RC crystals grown in the presence of lipids showed two significant differences compared to RC structures obtained by crystallization with detergents.

First is the absence of carotenoid spheroidene in structures derived from mesophase crystals. Second is the presence of electron density in the secondary ubiquinone QB pocket, which does not correspond to the ubiquinone. We assumed that during the crystallization process foreign molecules displaced natural carotenoid and ubiquinone. The foreign molecule is probably an isomer of 1-monoolein that is a mesophase matrix lipid. This isomer (2-monoolein) can spontaneously form over time from 1-monoolein.

Since the spheroidene and secondary ubiquinone are important for the proper functioning of reaction center, their absence complicates studies of photosynthetic processes.

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Mariam Ahsan

Serum Osteopontin, as a Biomarker Predicting the Magnitude of Disease Severity & Progression to Hepatocellular Carcinoma in Hepatitis C Virus Infected Patients in Pakistan

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Background: Hepatitis C virus (HCV) infection is a leading public health problem in Pakistan, making it the 2nd worst affected nation around the globe. Patients carrying persistent viremia ultimately lead to cirrhosis making them more prone to have hepatocellular carcinoma (HCC) with an annual incidence of 7.9% which accounts for about 10-80% HCV. Moreover available treatments for HCV and there varying therapeutic efficacy were scrutinized.

Objectives: Osteopontin (OPN) is a highly phosphorylated pro-inflammatory cytokine essential for initiation of Th1 immune response in HCV infection and contributes to degree of disease progression. Hence serum OPN levels may be used as a predictable biomarker affirming the magnitude of disease severity & progression in correlation with hepatitis C viral load.

Methodology: In current investigation blood was collected from 117 patients with HCV infection. HCV genotype and viral load were done by Real time PCR and serum OPN levels were analyzed by ELISA. Patients compatible with the inclusion criteria were categorized into three groups (i) HCV infected patients (ii) patients with chronic liver disease patients (CLD) & (iii) patients with hepatocellular carcinoma (HCC).

Results: OPN levels were significantly elevated in patients with HCV, CLD and HCC in comparison to control group. The serum OPN level has interrelated well with the severity of inflammation and liver fibrosis in patients with HCV infection independent of viral loads. The sensitivity and specificity of OPN for prediction of fibrosis (HCV, HCC and healthy control group) were 85%, 73% and 54% respectively, suggestive of its diagnostic accuracy in predicting hepatic fibrosis and carcinoma.

Conclusion: Taken together, OPN levels were found as independent predictors of degree of damage & severity correlating with the disease progression in Pakistani population, thus can be serve as powerful diagnostic, prognostic and therapeutic biomarker against HCV viral infection and related hepatocellular carcinoma in future.

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Shaymaa Abdulmalek

Molecular Investigation of Natural Oil with Antidiabetic Drugs on Brain Insulin Signaling Pathway in Diabetic Rats

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Objectives: The present study was aimed to investigate the modifying effect of natural oils alone and combined with anti-diabetic drugs on brain insulin signaling pathway and to highlight their inhibitory effect on neurotoxicity induced in rats fed with a high-fat diet. Also, it aims to assess their anti-oxidative, anti-brain insulin resistance and anti-amyloidogenic activities.

Methods: Streptozotocin (STZ)- induced rats fed with a high fat diet were orally administrated 2.0 ml oils, 100 mg metformin (MET) and/or insulin, combination of 2.0 ml oils and 100 mg MET and/or insulin.

Results: We identified significant disturbances of insulin signaling in the brain of induced rats with a significant elevation in brain ROS was observed with an abnormal stimulation of neuro-inflammation. In addition, an attenuation in brain insulin signaling, formation of A β -42 plaques and hyperinsulinemia were also observed. Furthermore, the expression profile of some AD-related miRNAs in serum and brain displayed their neuroprotective role. The treatment with oils and the anti-diabetic drugs restored the antioxidant levels, suppressed neuro-inflammation, stopped amyloidgenesis process and stimulate the insulin signaling pathway in the brain.

Conclusions: These data shed the light on the synergistic modulating effect of oils with anti-diabetic drugs to intercept AD related-brain insulin resistance in diabetes with the promising use of miRNAs as biomarkers in early diagnosis of AD.

Yulia A. Sidorova

Small molecules mimicking biological activity of glial cell line-derived neurotrophic factor family ligands to target neurodegeneration.

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Diseases caused by the damage of neurons, such as Parkinson's disease (PD) and neuropathic pain (NP), impair the quality of life of patients and incur significant costs to the society. PD is caused by progressive degeneration of dopaminergic (DA) neurons in the brain, it affects 0.3% of general population and occurs more often in elderly people. NP is caused by the damage of dysfunction in sensory system, it often occurs as a result of traumatic nerve injury, disease (diabetes, cancer), infection (HIV, Herpes Zoster) or treatment (cytostatics) and affects 7-8% of people in the world. In the future the number of individuals suffering from NP or PD will grow due to the increase in human's life span, population aging and rise in prevalence of the conditions leading to NP development. Currently no cure for PD and NP is available, existing therapeutics alleviate symptoms of these diseases but do not protect or repair damaged neurons. We aim to utilize the potential of glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs), proteins able to support and regenerate DA and sensory neurons, to develop disease-modifying medications against PD and NP. GFLs have been shown to alleviate PD and NP symptoms and restore DA and sensory neurons in experimental animals, but clinical use of these proteins is complicated by their poor pharmacological properties (low bioavailability and biodistribution, difficult delivery to the site of action, quality variations between different isolates, special requirement to storage and handling). We chose to develop orally administered small molecules activating GFL receptors, similarly to GFLs supporting damaged neurons in vitro and in vivo and alleviating behavioural manifestations of PD and NP. We have develop a set of biological assays ranging from high throughput assay to animal models of PD and NP to select and validate compounds with desired biological activity. We also identified an unique set of compounds truly activating GFL receptors. Our results indicate that these compounds similarly to GFLs support DA and sensory neurons in vitro, alleviate symptoms of PD and NP in experimental animals and restore sensory neurons in vivo. Thus, we demonstrated that the selected target is druggable and established in vivo proof-of-concept showing that targeting GFLs receptors might be beneficial in PD and NP. Although promising, existing compounds acting similarly to GFLs need further optimization to progress to clinical practice. In addition, the chemical diversity of existing set of compounds should be increased by screening new chemical compound libraries. Afterwards, we plan to optimize efficacy and pharmacological characteristics of selected hits using rational drug design methods. We are looking forward to collaborating with medicinal chemists and chemists having diversity libraries of chemical compounds to proceed with our project.

Sopiko Kurdadze

Prevalence of High Oncogenic Risk HPV Genotypes 16, 31, and 33 in Georgian Women with Cervical Intraepithelial Neoplasia Grade 1

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Although the immune system clears most of the human papillomavirus (HPV) infections, in a few cases these infections persist. About 1 in every 200 women will develop cervical cancer during their lifetime, and almost all of them are caused by infections with high oncogenic risk HPV. There are at least 14 high-risk HPV genotypes and diagnosing their infections may provide increasingly significant health benefits. Unfortunately, very sparse data are available on the prevalence and distribution of high-risk HPV genotypes among Georgian women.

The present study aimed to detect and genotype high-risk HPV viruses in cervical swab specimens of Georgian women. In total, 95 women (ages 24 to 67) were tested and genotyped. All patients had cervical intraepithelial neoplasia grade 1 (CIN1) by Pap cytology or inconclusive colposcopy. DNA was extracted from the specimens and genotyping were carried out by RealLineTM HPV kit that detects 12 high-risk HPV genotypes 16, 18, 21, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

In this cohort of patients, we detected all tested genotypes, except HPV52. We found that 23% (18/95) of women tested positive for high-risk HPV with 33% (6/18) of all positives presented as multiple infections. Most prevalent genotype was HPV16 that was identified in 39% of positives (7/18) and 86% (6/7) of multiple infections. Other frequent infections were with HPV31 at 21%, HPV33 at 13% and HPV56 at 8% of all positive samples. Hence, we conclude that genotypes 16, 31 and 33 are the most frequent high-risk HPV in Georgian women.

Victor Tobiasson

The structure of the mitochondrial ribosome from T. thermophila

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The mitochondrial ribosome has its evolutionary origin in the bacterial ribosome but has an overall disparate structure and protein composition. In order to investigate the scope of ribosomal evolution we have determined the structure of the mitochondrial ribosome from the ciliate protozoan Tetrahymena thermophila. The structure revealed a highly divergent ribosome which defied convention with a small subunit with greater mass than the large subunit and harboring at least 26 previously uncharacterized ribosomal proteins. Among the identified ribosomal proteins are enzymes involved in amino acid synthesis, fatty acid catabolism, iron sulfur cluster biogenisis as well as components of the bacterial co-translational protein insertion machinery. In contrast to the overall divergent protein exterior the RNA core of the T.thermophila mitochondrial ribosome shows great structural conservation with its bacterial ancestor. The combination of structural conservation and divergence challenges previous observations and hints at the evolutionary path of the mitochondrial ribosome.

Svetlana Ohiienko and Anastasiya Bondar

Cu-Induced Liver Fibrosis Affects the Primary Culture of Rats Bone Marrow Cells

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The hypothesis of dependence of the functional characterizations of bone marrow cells (BMC) — proliferation rate, direction proliferation, etc. — not only by the age of animals, but also on features of BMC microenvironment was verified. Two methods of changing the microenvironment were used. There were *in vivo* (induction of liver fibrosis in young and old animals) and *in vitro* (transfer of young and old animals BMC obtained in intact animals and animals with fibrosis into the same standard culture system). CuSO₄-induced liver fibrosis had the effect on the ratio of cell types in the bone marrow in young and old animals. Thus, in young animals, regardless of the type of liver fibrosis inducer, the relative number of morphologically identifiable cell types decreased. This was accompanied by an increase in the number of identified cell types against the background of CuSO₄-induced liver fibrosis. The proliferative activity of BMC isolated from old animals and transferred to an *in vitro* culture was superior to that of young animals. This is due to the large number of lymphocytes in the bone marrow of old animals by 167% and the specific composition and characteristics of the BMC microenvironment in old animals.

Moritz W.R. Willmer

Ribosome recycling in a natural translation termination context

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Ribosome recycling is the last step of the translation cycle. During bacterial ribosome recycling, the large and the small subunit dissociate and the mRNA and the deacylated tRNA are released, preparing the ribosome for the translation of another mRNA. Recycling is the result of the combined actions of the ribosome recycling factor (RRF), elongation factor G (EF-G) and initiation factor 3 (IF3). The role of those factors and order of dissociation events are still controversial. The differences between the proposed models can reflect variations in experimental setups and model systems, such as the use of unnatural translation termination contexts and low temporal resolution of experiments. Here we present a study of ribosome recycling using rapid kinetic methods on native and structured mRNAs.

Victor Hugo Canela

Laser microdissection of human calcium oxalate monohydrate stones enables structurally specific exploration of the stone proteome

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Introduction and objective: Urolithiasis affects approximately 12% of the world population, and more than half of affected individuals will have recurrence within 10 years of the first episode. A detailed hypothesis of the mechanisms of stone disease etiology remains elusive and thus makes stone disease difficult to treat and to prevent. The present study assessed the feasibility of histologically sectioning human stones and using laser microdissection (LMD) to obtain samples for proteomic analysis.

Methods: Stones were imaged by micro-Computed Tomography, demineralized, embedded in paraffin, histologically sectioned and stained. Regions of the stone-matrix layers were dissected via LMD (Leica LMD 6) and collected in phosphate buffer. Extracted proteins were digested using Trypsin/Lysine C mix proteases and run in a Q Exactive Orbitrap High Field Liquid Chromatography (LC)/Mass Spectrometry (MS) system. Data were searched using MaxQuant (version 1.6.10.1) and filtered using False Discovery Rate for alpha=0.01.

Results: Histological sections of stones showed tightly packed layers of matrix that were clearly visible using bright field LMD microscopy (Figure 1A). Average area dissected by LMD for LC/MS was 1.64x10⁶ μ m², and these revealed and yielded an average of 629 distinct proteins each. Initial analysis shows proteins to be similar to those previously reported for kidney stones, including proteins involved in cell injury and repair as well as important mediators of the immune system.

Conclusion: Human kidney stones can be histologically sectioned and laser microdissected. A relatively small region of sectioned stone yields significant proteomic data. Utilization and optimizing of these novel methods and techniques in the future will pave the way into a deeper understanding of stone genesis. Future investigation of the stone matrix proteome should provide insight into the mechanisms underlying the pathogenesis of urolithiasis in an effort to develop effective therapies and prevention treatments.

Murali Aarthy

Investigations on the mechanistic insights of wild and mutant E6 Oncoprotein of HPV and its interaction with the tumor suppressor protein

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Cervical malignant growth is one of the foremost causes of death in women worldwide and the focal rationales of cancer progression entirely belong to the human papillomavirus (HPV) infection. Till date, HPV vaccines have been authorized in the medical society yet it is but it is very much essential for the development of drug based treatment against the infection and its oncogenic effects. The oncoproteins of HPV to be specific the E6 and E7 influence the cellular functions to promote the life cycle of virus and are essential for the cervical cancers induced by the "high-risk" mucosal HPV and plays an indispensable role in the ubiquitination and subsequent proteosomal degradation of p53. The wild type p53 is examined and observed in cervical malignancies though in different tumors the deactivation is done with the nearness of mutation. Thus, it is suggested that the E6's action regarding p53 is proportionate to an inactivating transformation of p53. Also, the high risk strains of human papillomavius (HPV) have been recognized as the etiological agent of some head, anogenital tract, and neck cancers. The E6 oncoprotein exclusively dimerizes through the N-terminal domain and that disruption of the dimer interface strongly increases E6 solubility. The homodimers interface mutations that disrupt E6 self-association inactivates the E6-mediated p53 degradation. Our study focuses on the identification of the small molecules that could inhibit the wild and mutant HPV 16 E6 protein which could disrupt the interaction with p53. Also, we strive for the understanding of the mechanistic insights of E6 oncoproteins of wild and mutant type along with the interaction of p53 and the small molecules through molecular dynamics simulation studies. The dynamic behaviour of the tumor suppressor protein p53 and the E6 oncoproteins analyzed helps in the understanding of p53 degradation mediated through E6 which in turn helps in the treatment of cervical cancer.

Key Words: p53, Degradation, High risk, Molecular dynamics simulation.

Mirko Minini

The activation of miR-125a-5p/IP6K1 axis in breast cancer cells upon treatment with myo-Inositol

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Several studies have been performed with the aim of identifying drugs able in inhibiting Epithelial-Mesenchymal Transition (EMT), chiefly by blocking PI3K/Akt pathway. We have already demonstrated that treatment with myo-Inositol at the pharmacological dose can block EMT in breast cancer cells by downregulating PI3K/Akt and inducing changes in cytoskeletal architecture. Herewith, we investigated the mechanism of action of myo-inositol in both highly (MDA-MB-231) and low (MCF-7) invasive human breast cancer cells.

After 30' and 24h from treatment, gene expression analysis revealed a significant downregulation of Pi3k and Psen1 after 30' in both cell lines. Psen1 downregulation was maintained in MDA-MB-231 at 24h. Likewise, we explored the modulation of Ip6k1, Dnmt3b, Isyna1 and p53. In MDA-MB-231, a strong downregulation of Ip6k1 expression was recorded at 30' and 24h, whilst Dnmt3b was reduced only at 30'. On the contrary, in MCF-7, Ip6k1 downregulation was unexpectedly associated to the upregulation of Dnmt3b at 30'. IP6K1 is a key enzyme of inositol metabolism, inhibits ISYNA1, probably inducing de novo DNA methylation (i.e., DNMT3B). Furthermore, IP6K1 inhibition correlates with a decrease of cancer cells motility. The upregulation of Isyna1 was observed in both cell lines at 30', together with p53. ISYNA1 activates myo-Inositol intracellular biosynthesis starting from glucose-6-phosphate. In this activation, p53 plays a key role in binding Isyna1 promoter and eventually enabling its expression.

Western-blot of MDA-MB-231 confirmed that changes in gene expression were also mirrored by concurrently modifications in IP6K1 and p53 protein levels, altogether with a decrease of both MDM2 and YAP/TAZ. It is worth noting that in MCF-7, no changes were observed in protein levels.

In-silico analysis was performed using TCGA miRNA-Seq data to identify differentially expressed miRNAs between normal and tumoral tissue in breast cancer patients. To further gain mechanistic insights on myo-Inositol effects, we compared these data with main differentially expressed cancer-related miRNAs in MDA-MB-231 cells after 30' from treatment. This analysis allowed to identify two mRNAs, downregulated in tumor tissues, that were significantly increased with myo-Inositol: miR-92a-3p and miR-125a-5p. Using DIANA tools, miR-92a-3p was predicted to interact with Notch-1 and PI3K, linking it to cytoskeletal rearrangement. Moreover, a strong interaction was predicted between miR-125a-5p and IP6K1 in 3'-UTR site. Indeed, the upregulation of miR-125a-5p is usually correlated with metastasis inhibition in breast cancer. In MDA-MB-231, miR-125a-5p upregulation was maintained at 24h, while in MCF-7 was slightly upregulated at 30' and downregulated at 24h.

Our results suggest that myo-Inositol causes early changes in gene expression, probably led by miRNAs and methylation remodeling. Elucidation of the role of miR-125a-5p/IP6K1 axis will reveal strategies for molecular targeted therapies in breast cancer.

Aleksandra Jarmolowicz

Finding New Influenza Inhibitors by Small Molecules Docking to Genomic Influenza RNA Structural Motif

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Influenza A virus is responsible for very dangerous and common respiratory infection called flu. Epidemics of flu occurred annually and there is no universal vaccine for flu and used therapeutics are not efficient since influenza strains are developing resistance to drugs. Therefore, searching for new influenza inhibitors and new strategies to fight flu is highly needed. Growing knowledge about influenza RNA structure and its importance for viral cycle gives opportunity for designing potential drugs targeting RNA.

Herein, secondary structure of influenza segment 8 vRNA (vRNA8) determined by chemical mapping coupled with free energy minimization was used for small molecules (SMs) target. Prediction confirming by experiments *in vitro* revealed a conserved vRNA8 motif as a binding site for specific SMs. vRNA8 secondary structure, and its selected motif of two strains, A/California/04/2009 (H1N1) and A/Vietnam/1203/2004 (H5N1), were folded to 3D using RNAcomposer . Molecular docking of SMs using AutoDock Vina were conducted to vRNA8 conserved motif showing tight binding to RNA target. Crucial interactions with vRNA8 for these SMs were determined and described. Results show new SMs that could very specific bind to certain influenza motif and are promising new influenza inhibitors. Structural conservation of target vRNA8 motif makes its a potential universal target for SM future therapeutics.

Pietro Busko

Analysis of the Interaction between RNA-Binding Protein SAM68 and Scaffold Protein ITSN1 *in vitro*

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The STAR family of proteins links signalling pathways to various aspects of post-transcriptional regulation and processing of RNAs. In particular, SAM68 is involved in several steps of mRNA metabolism, comprising transcription and alternative splicing. In addition, SAM68 participates in signalling pathways associated with cell response to stimuli. Recent studies showed that SAM68 interacts with scaffold protein ITSN1 involved in endocytosis, actin cytoskeleton remodelling, and cellular signalling. At the moment, the role of the ITSN1-SAM68 interaction in normal and pathological processes is unknown. Current work aimed to expand known SAM68 partners, to analyse the structural details of the interaction, and to identify the putative effects of ITSN1 on SAM68 functioning.

GST pull-down assay was used to obtain the interaction between proteins of interest. In brief, GST-fused SH3 domains of target proteins were used to precipitate endogenous SAM68 or its truncated forms HEK lysate. In addition, the assay was used to check if SH3-containing proteins could compete for the binding to SAM68. For the purpose, recombinant SAM68 was incubated with constant amounts of GRB2 and increasing concentrations of ITSN1 SH3 domains and vice versa. The precipitated proteins were visualized using Western blot analysis. Sedimentation assay was used to analyse the formation of SAM68 aggregates in the presence of ITSN1. A mix of recombinant ITSN1 and SAM68 was centrifuged at low speed. The protein content in pellet and supernatant fractions was estimated by SDS-PAGE and Coomassie staining.

GST pull-down assay demonstrated that SH3 domains of ITSN1, TKS4, TKS5, AMPN1, BIN, and CTTN precipitated SAM68. Using expression constructs encoding truncated forms of SAM68, it was found that SH3A domain ITSN1 and its neuronal isoform precipitated N-terminal fragment of SAM68, confirming the interaction between SH3A domain of ITSN1 and the class II proline motif of SAM68. Using purified recombinant proteins SAM68, GRB2, and the tandem of ITSN1 SH3 domains, it was found that GRB2 impaired the interaction between SAM68 and ITSN1 whereas high concentrations of ITSN1 SH3 domains displaced GRB2, revealing the competition between ITSN1 and GRB2 for the binding to SAM68. Sedimentation assay showed that ITSN1 facilitated solubilisation of SAM68 aggregates in vitro, demonstrating that SH3 domains could impair interactions between SAM68 low-complexity regions. The data is in line with the previous results showing that the accumulation of overexpressed ITSN1 in HeLa cells nuclei abolished the formation of SAM68 nuclear bodies.

The current work demonstrated that ITSN1-SAM68 interaction is mediated by the SH3A domain of ITSN1 and the N-terminal region of SAM68 which can also interact with other SH3-containing proteins involved in the cellular signalling and actin cytoskeleton remodelling proteins whereas, at high concentrations, ITSN1 induces the dissociation of SAM68 aggregates. The results suggest that scaffold protein ITSN1 could regulate SAM68 functioning as a part of signalling crosstalk between cytoplasm and nucleus.

Martin Cagala

L1210 mouse leukemic cell line resistance to endoplasmic reticulum stress inducers.

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P-glykoprotein (P-gp) is a transport pump localized in the plasmatic membrane of cells, responsible for multidrug resistance of leukemic cells. In our laboratory we determined, that P-gp positive L1210 cells are resistant to substances that induce endoplasmic reticulum (ER) stress tunicamycin, thapsigargin and also proteasome inhibitor bortezomib despite, these substances are not substrates of P-gp. The goal of this work was to prepare sublines resistant to ER stress inducers [tunicamycin (STu), thapsigargin (STh) a proteasome inhibitors bortezomib (SB) a MG-132 (SMG)] from P-gp negative L1210 parent cell line. We observed lower proliferation rate with the STu, STh and SB sublines compared to the parent sensitive S line, with the lowest proliferation rate observed with the STh subline. We didn't observe changes in the expression of ABC transporters (P/gp, MRP1 and BCRP) in any of the cell sublines adapted to ER stress inducers. We detected a decrease in the expression of Glutathione S – transferase Gstt2 in all sublines except for S and SB sublines. There is a decrease in the expression of Cytochrom P450 Cyp2d22 in all the sublines compared to the parental S line.

Key words: multidrug resistance; ABC transporters; glutathione S-transferase; cytochrome P450, endoplasmic reticulum stress

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Konstantin Chernov

Introducing inducible fluorescent split cholesterol oxidase to mammalian cells.

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Cholesterol oxidase (COase) is a bacterial enzyme catalyzing the first step in the biodegradation of cholesterol. COase is an important biotechnological tool for clinical diagnostics and production of steroid drugs and insecticides. It is also used for tracking intracellular cholesterol; however, its utility is limited by the lack of an efficient temporal control of its activity. To overcome this we have developed a regulatable fragment complementation system for COase cloned from Chromobacterium sp. The enzyme was split into two moieties that were fused to FKBP (FK506-binding protein) and FRB (rapamycin-binding domain) pair and split GFP fragments. The addition of rapamycin reconstituted a fluorescent enzyme, termed split GFP-COase, the fluorescence level of which correlated with its oxidation activity. A rapid decrease of cellular cholesterol induced by intracellular expression of the split GFP-COase promoted the dissociation of a cholesterol biosensor D4H from the plasma membrane. The process was reversible as upon rapamycin removal, the split GFP-COase fluorescence was lost, and cellular cholesterol levels returned to normal. These data demonstrate that the split GFP-COase provides a novel tool to manipulate cholesterol in mammalian cells.

Elijah Kolawole Oladipo

Comparative Structural Analysis of 3-D Predicted Matrix Protein of Influenza A /Swine/H1N2/Ibadan/2014 and A/Swine/H5N1/Ogbomoso/ 2014 in Nigeria

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Introduction: Variation of genome is very high in influenza A viruses due to antigenic shift and drift. However comparative structural analysis of protein structure can provide functional insight on antigenicity, pathogenicity and virulence.

Methods: The obtained nucleotide sequences through an epidemiological study for influenza A/Swine/H1N2/Ibadan and A/Swine/H5N1/Ogbomoso were translated to their corresponding peptide sequences using EMBOSS Transeq. Prediction of Homology model for the 3D structure Matrix gene protein of the influenza virus was constructed using CPH Model, validated using PROCHEK and viewed with PyMOL Molecular Graphics System. Extensive structural comparison was performed on their domains and sub-regions to investigate domain specific variations.

Results: The predicted 3D protein model shows the residues in the most favoured region for the two influenza isolates. The comparative study of the predicted 3D protein structure of A/H1N2/Ibadan/2014 and A/H5N1/Ogbomoso/2014 influenza virus matrix gene shows the same position of chains, segments but contains different elements, atoms and numbers of residues.

Conclusion: Evidence from this study suggests that integrating extensive structural comparison can help in understanding the biological characteristics of these viruses. In particular, the observed variations can provide information on drugs and vaccine development. Also, the predicted 3D protein structures has assisted to extract the important information related to the genes and protein structure.

Keywords: 3-D Protein, H1N2, H5N1, Ogbomoso, Ibadan, Matrix.

Dian Jamel Salih

Cytogenetic effects of Anabolic steroid hormones on bodybuilder athlete in Duhok province - Kurdistan region of Iraq

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Background: Cytogenetic analysis suggested that anabolic steroids hormones injection at different doses used by bodybuilders for long term had mutagenic effects on somatic and germinal genetic materials, cytotoxic effect on liver tissue and increased the sperm morphology abnormalities

Objective: The aim of this study was to evaluate the cytogenetics of anabolic steroids hormones in Athletes.

Materials and Methods: The study groups included forty five male participants and divided into three groups: fifteen bodybuilders who use anabolic steroid hormones and fifteen age matched bodybuilders who don't use anabolic steroid hormones and fifteen controls were recruited and evaluated for genetic damage.

Cytogenetic analyses were performed for each individual and Statistical analysis for data interpretation performed by Student's t-test, and P \leq 0.05 was considered statistically significant.

Results: Summarizing the results for all three analysed groups, we observed that the average structural chromosomal aberrations in the blood lymphocytes of Athletes who use Anabolic steroid hormones in the last three six months of training was statistically significantly higher (P≤0.005), in comparison with average structural chromosomal aberrations in the blood lymphocytes of Athletes who do not use Anabolic steroid hormones and adults who do not perform any physical activities not using Anabolic steroid hormones

Conclusions: The results of our study concluded that Anabolic steroid hormones consumption has a potential to increase frequency of structural chromosomal aberrations in blood lymphocytes in bodybuilders. The study also suggested that more protective strategies should be implemented by the concerned authorities to minimize the uses of these hormones.

Keywords: genotoxicity, cytogenetics, Ring chromosome, bodybuilders, anabolic steroid hormone.

Elinor Breiner-Goldstein

Semisynthetic macrolides can overcome resistance mechanisms.

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The ribosome, a multi component universal particle that is composed of RNA and proteins, translates the genetic code into proteins in all living cells. Active ribosomes are essential for cell life therefore inhibiting their function will damage the cell. Currently, about 40% of the clinically used antibiotic compounds target active sites of the prokaryotic ribosome.

The macrolides have been known as antibacterial drugs from the beginning of the 1950s and are used to treat gram positive as well as a limited number of gram negative bacterial infections. They were shown to inhibit protein biosynthesis by blocking the nascent peptide exit tunnel (NPET) in proximity to the peptidyl transferase center (PTC), thus interfering with nascent chain progression. Chemically, the macrolides are composed of a macrolactone ring that is substituted by one or more sugars as well as various short chemical groups.

Macrolides are well established as clinically used antibiotics and known for their broad antimicrobial spectrum as well as relatively high margin of safety. One of the undesired side effect of the antibiotic usage is the spread of antibiotics resistance strains of pathogenic bacteria in healthcare facilities and in communities. This is an increasingly serious threat to global public health. Among these pathogenic bacteria are the highly resistant, versatile and often aggressive bacteria Staphylococcus aureus (SA). Methycillin-resistant Staphylococcus aureus (MRSA), highly frequent in hospital-related infections, results in high mortality rate of up to 60%.

By tasting clinically isolated SA strains, we identified semisynthetic macrolides were able to overcome a few common resistance mechanisms in SA, including MRSA. The crystal structure of the D. radiodurans large ribosomal subunit in complex with three biosynthetic macrolides was determined, shedding light on their binding and inhibition modes as well as their selectivity and resistance aspects.

Hugo Garro

Inhibitory interaction of a Porphyrin derivative with the protein $\alpha\mbox{-synuclein}$

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The misfolding of proteins into a toxic conformation and their deposition as amyloid-like fibrils are proposed to be at the molecular foundation of a number of neurodegenerative disorders including Creutzfeldt-Jakob, Alzheimer, and Parkinson diseases. A detailed understanding of the mechanism by which proteins of wide structural diversity are transformed into morphologically similar aggregates is therefore of high clinical importance.

Alpha-synuclein (aS) aggregation is linked to the development of Parkinson's disease. Structurally, aS contains 140 aminoacids distributed in three different regions. The use of aggregation inhibitors as molecular probes of the structural and toxic mechanisms related to amyloid formation is an active area of research.

In the present study we applied a vast array of biophysical tools to investigate the binding and anti-amyloid properties of the small molecule Porphine (PfTS-3) on the amyloid fibril assembly of the protein aS. Moreover, a comparative analysis with the molecule Phthalocyanine tetrasulfonate (PcTS) was performed.

We recorded NMR spectra on a spectrometer equipped with a cryogenically cooled triple resonance ${}^{1}H({}^{13}C/{}^{15}N)$ TCI probe. 2D ${}^{1}H{}^{-15}N$ and ${}^{1}H{}^{-13}C$ HSQC experiments were used to map the interaction between aS (25, 50, 100 μ M) and the small molecules at a residue specific level of resolution. 1D ${}^{1}H$ NMR experiments were used to monitor the monomer consumption during the progression of the aS (25, 50, 100 μ M) aggregation process in the absence and presence of the studied compounds.

The results presented here allow us to conclude that: (i) PcTS and PfTS-3 compounds bind to the monomeric form of the protein aS; (ii) the N-terminal region of aS represents the binding interface for these compounds, mainly driven by aromatic and electrostatic interactions; (iii) Tyr at position 39 and Phe at position 4 constitute the most affected residues by PcTS binding, whereas no such specific interactions were observed with the PfTS-3 compound; (iv) formation of the aS-PcTS and aS-PfTS-3 complexes affect the amyloid fibril assembly of the protein in different ways; (v) the differences observed in binding capacity and anti-amyloid activity of PcTS and PfTS-3 compounds on aS might be attributed to their distinct ability to self-stack through π - π interactions; (vi) the structural and molecular mechanism behind the inhibitory effects of these compounds on aS amyloid fibril assembly are well different.

Amar Ranjan

Diagnostic dilemma in plasmablastic myeloma and plasmablastic lymphoma

Amar Ranjan, Harshita Dubey, Pranay Tanwar

Plasmablastic myeloma (PM) and Plasmablastic lymphoma (PL) share clinical presentations but have completely therapeutic plans.

Short clinical history:

1st case: A 31 year old HIV negative male presented with oral ulceroproliferative growth. FNAC showed atypical lymphoid cells with CD45+ve & HMB 45-ve, suggesting NHL. Biopsy showed lymphoplasmacytoid cells positive for CD79a, CD138, MUM1 & negative for CD20, CD3, CD56, CK & HMB45, suggesting PL. BMA smear & biopsy confirmed plasmablastic infiltration.

CHOP therapy showed no improvement. Repeat investigations showed raised serum free light chain ratio (27.00) & B2 microglobulin (3.263 mcg/mL), which changed our diagnosis from PL to PM.

2nd case: A 57 year old female presented with swelling in left lower alveolus. FNAC suggested Osteosarcoma. Punch biopsy showed sheets of plasmablasts, positive for CD 79a & CD138 and negative for CD 3, CD20, CD30 and CD 56 suggesting NHL.

PBF showed occasional Plasma cells with rouleux formation. BMA showed 10-12% Plasma cells. BM biopsy showed sheets of plasmacytoid cells positive for CD 138, negative for Pancy-tokeratin with proliferation index of 100% on staining with MIB. Serum M- band was positive & Beta 2 microglobulin was raised. FISH showed negative del 17p, 13q, t(4,14), t(14,18) & IGH rearrangement. Considering PM, executed therapy showed poor response. Repeat HIV test turned POSITIVE to change the diagnosis.

3rd Case: A 34 year old HIV positive male, on HAART for six months presented with swelling in neck and right axilla. FNAC suggested NHL. BMA showed sheets of plasmacytoid cell suggestive of PL. Immunophenotyping showed 35% abnormal lymphoid cells which were CD38+, CD138+, kappa light chain restricted, CD45+, CD20-, CD56-, CD19- and CD27dim. PL was considered.

4th **case:** 57 year old male with positive CARB criteria and lymphadenopathy showed 90% plasma cells in BMA. Serum M- band with raised free lambda light chain values. Lymph node biopsy confirmed Plasma cell dyscrasia (LCD).

Lymph node biopsy confirmed Plasma cell dyscrasia with plasmacytoid cells positive for CD 79a, MUM 1, CD 138, CD 38, CD 56, EMA & Cyclin D and negative for CD3, CD20, PAX 5, BCL6 &, CD10. Ki 67 proliferative index was 90%. Due to predominance of plasmablastoid morphology, the case was diagnosed as PM. However the patient didn't appear for follow up, so further work up couldn't be made.

Discussion: Despite advancement in novel diagnostic techniques, the distinction between PM and PL is still a challenge.

Cabadak Hülya

The effect of combination of cholinergic agonist and antagonist on apoptosis in megacaryocytic differentiated K562 leukemia cells.

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Many cells and tissue express a mixture of cholinergic receptors . Different studies have suggested that nonneuronal acetylcholine and cholinergic agonists alter cell growth and proliferation of T and B lymphocytes. Costa et al. demonstrated that acetylcholine released from T-lymphocytes acts via the M3 acetylcholine muscarinic receptor (mAChR) to trigger signaling pathways. Muscarinic cholinergic receptors activate stimulatory growth mechanisms in megakaryocytic differentiated K562 leukemia cells.

In this study, we investigated the levels of caspase 3,8 and 9 protein expression and caspase activity in megakaryocytic differentiated K562 leukemia cells. And we also investigated the effects of muscarinic agonist effects on apoptosis megakaryocytic differentiated K562 leukemia cells.

Megakaryocytic differentiation was induced by applying phorbol 12-myristate 13-acetate (PMA) (Calbiochem) at the concentration of 10 nM. Anti-Caspase 3,8 and 9 antibodies were purchased from Santa Cruz, Inc. .). For western blot assay, Proteins were electrotransferred to nitrocellulose membranes which were exposed to primary antibodies and then to alkaline phosphatase-conjugated secondary antibodies. The antibody-antigen complex was detected with NBT/BCIP. Caspase activity detected by Elisa technique.

We detected caspase 3,8 and 9 activity and expression in megacaryocytic differentiated K562 leukemia cells. Especially muscarinic receptor agonist and antagonist decrease caspase 3 and caspase 9 expression. These results suggest that CCh may be modulates megakaryocytic differentiated K562 leukemic cells apoptosis through muscarinic acetylcholine receptors

Keywords: Muscarinic receptors, caspase 3,9 and differentiated leukemia cells

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Hekmat El Magdoub

Betanin ameliorates cisplatin-induced kidney injury through TGF-β/Wnt/Notch3 pathways: Involvement of miRNA-135a & -135b

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Introduction: Clinical efficacy of platinum-based anti-cancer therapeutics like cisplatin cannot be abandoned. However, serious adverse effects like nephrotoxicity limit its use.

Transforming growth factor- β (TGF- β), a key pro-fibrotic mediator and its downstream TGF- β -activated kinase 1 (TAK1) are involved in type I collagen and fibronectin expression. TAK1 can transduce signals to nuclear factor-kappa B (NF-kB)-inducing kinase (NIK)-IkB kinase (IKK) and MKK3/6-p38 MAPK. NFkB activates notch3 signaling, hence, attracting activated macrophages with a rapid deterioration of renal function and structure. TGF- β activates Wnt1 signaling in a p38-dependent manner, thus, exacerbating myofibroblast proliferation.

Noteworthy miRNA-135a and miRNA-135b have been shown to play a role in activating Wnt/ β -catenin signaling and inducing the nuclear translocation of β -catenin.

Betanin, the most abundant betalain found in beetroot has recently attracted much attention owing to its high antioxidant, anti-inflammatory and antiproliferative effects.

The purpose of the current work was to investigate the anti-fibrotic, and anti-inflammatory effects of betanin on cisplatin-induced renal injury, and elucidate the involvement of TGF- β , Wnt/ β -catenin signaling, and notch3 pathways. We also aimed at investigating the involvement of miRNA-135a, and miRNA-135b in cisplatin-induced Wnt/ β -catenin activation and whether betanin administration will impact the level of these miRNAs or not.

Materials and Methods: Thirty two male Wistar rats were divided among four groups (Control, Betanin, Cisplatin and Betanin/Cisplatin). Betanin was administered daily (100 mg/kg/day, i.p.) for seven days. Cisplatin was administered on day four as a single 5 mg/kg i.p. dose. On day seven rats were sacrificed and kidneys were stored at -80°C for further analysis. Protein levels of TGF- β , NFkB, TAK1, p-38, notch3, Wnt1 and β -catenin were assessed by ELISA or Western blotting. The expression level of notch3, miRNA-135b were examined by real time PCR. Histological abnormalities were investigated through H & E stain.

Results: Cisplatin administration resulted in the presence of many degenerated tubular epithelial cells with occasional intraluminar cellular or eosinophilic casts with mild congestion of intertubular blood vessels. Betanin administration has shown moderate protection for renal tubular cells.

Cisplatin was able to significantly upregulate $TGF-\beta$, NFkB, TAK1, p-38, notch3, Wnt1 and β -catenin protein levels, as well as notch3 mRNA, miRNA-135a and miRNA-135b expression levels. Betanin administration was able to avert cisplatin-induced changes by significantly retaining all of the assessed parameters back towards their normal level. Yet and despite the significant improvement, betanin was not able to completely abolish the impact of cisplatin on the investigated markers.

Conclusion: The results of the current study depict the involvement of miRNA-135a and miRNA-135b in the pathogenesis of cisplatin toxicity and demonstrate the beneficial effects of betanin against the induced insult. Hence, signaling betanin's antiproliferative and anti-inflammatory effects, and promoting it as a potential future supplement for averting the serious adverse effects of platinum-based chemotherapeutic agents.

Esther Jimah

Investigation of Hepatitis C Virus among Blood Donors by Molecular Assay in Kwara State, Nigeria.

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Introduction: Hepatitis C virus (HCV) is one of the implicated etiologies of Human hepatitis which is a major public health burden. A good proportion of the world's populations are carriers of HCV with a seemingly increasing number annually. This study screened for Hepatitis C Virus Ribonucleic Acid (HCV-RNA) from pre-screened blood at University of Ilorin Teaching Hospital (UITH), and determined the circulating genotypes using phylogenetic analysis of sequence results and possible risk factors.

Method: The study was carried out between April and August 2018 with a total of 144 participating blood donors. Socio-demographic variables were obtained using a pre-tested self-administered interviewer-based questionnaire. Whole blood samples were obtained, and an enzyme linked immunosorbent assay (ELISA) was carried out on the blood samples. HCV-RNA was extracted using RNA extraction kit and was amplified using reverse transcriptase polymerase chain reaction (RT-PCR) with specific primers. The amplified region of 405 base pair was purified and sequenced using Sanger sequencer, and a phylogenetic tree was constructed.

Result: The prevalence of HCV antibodies by ELISA was 6.9 %(10/144) and the HCV-RNA was 18.1% (26/144), with genotypes 1(21.4%), 2(14.3%), 3(21.4%) and 42.9% uncharacterized strain of HCV respectively from the genetic analysis of the samples. The relationship between the possible risk factors and HCV-RNA was not statistically significant except for the possible risk of transmission (p= 0.023).

Conclusion: It is evident that the use of ELISA alone for blood screening may not be reliable, and could be suggested that to reduce the burden of transfusion transmitted HCV, molecular technique should be embrace for screening of blood donors. The knowledge of the prevailing genotype will help tailor treatment options and vaccine development in the population. Further research should be carried out on other possible risk of infections and route of HCV transmission besides the known risk factors in Nigeria.

Shuan-Su Chen and Wei-Hsuan Yu

Matrilysin Mediates Nucleolus Trafficking for γ-Catenin and p53 and Bulky-Ball-Like Nucleolus Assembly in Cancer Stem Cells

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The novel molecular mechanism underlying the silencing p53 in striking enlarged nucleolus in geftinib- resistant lung cancer stem cells remains elusive. Our new findings that MUC-1 C-ter participates in nucle- olus matrix assembly has further prompted the idea that the cell surface receptor MUC-1, via MMP-7 pro- teolytic cleavage of the MUC-1 SEA domain releases cryptic bioactive fragments that orchestrate nucleolar activities. Furthermore, MUC-1 C-ter induces a unique porous bulky ball-shaped, cage-like nucleolus that functions as a nucleus molecular "garage" for potent tumor supressor, such as p53. Specifically, this cage segregates the transcription factor from its target promoter. In addition, the intra nucleolus degradation of p53 by MMP-7 to generate a 35kDa p53 fragment suggests that the nucleolus could also facilitate the novel sub-nucleus compartment for proteolytic processing tumor suppressor protein, p53 in cancer stem cell, which implicate the novel molecular mechanism for destructing the p53 barrier and inducing cancer stem cell transformation. Salinomycin, an anti-cancer stem cell agent, can disrupt the nucleolus inducing trans- location of p53 and sensitize cancer stem cell to chemotherapy drug. These data provide important new insights into the dysregulations of cancer stem cells as well as highlight targets for cancer therapeutics.

Joanna Miszkiewicz

Designed RNA enzyme directed against bacterial mRNA

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Nowadays, antibiotic resistance is one of the greatest health threats. Therefore, there is a demand to look for new approaches to fight bacteria. One of the methods that is worth to investigate is the cleavage of a specific RNA sequence by RNA enzymes called ribozymes. Using ribozymes to cleave essential bacterial mRNA should hinder translation and as a consequence suppress the growth of pathogens. So far, using ribozymes to stop the progression of various diseases was proved in anticancer, antiviral and genetic therapies. The goal of this study was to achieve the successful cleavage of bacterial mRNA fragment.

For our research we have chosen model organisms. Designed hammerhead ribozyme aims to cleave the mRNA sequence encoding the acyl carrier protein (acpP) in the *Escherichia coli* K-12 MG1655 strain. Using isothermal titration calorimetry we proved the formation of tight binding of the ribozyme to the mRNA-acpP fragment in a 1:1 ratio. It was also confirmed by molecular simulations. Using gel electrophoresis we detected an effective cleavage of the chosen bacterial fragment by the designed ribozyme. Further, we plan to test the cleavage of mRNA-acpP by hammerhead ribozyme in bacterial cells.

Ana Carolina Schwarzer

Expression and Purification of Recombinant Human Wnt Signaling Modulators for Functional Analysis

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Wnt proteins comprise a large family of secreted signaling glycoproteins that regulate body-axis specification, morphogenesis, tumor development, metastasis, cell differentiation, senescence and death. Dysregulation of Wnt pathways has been implicated in many types of cancer, including different types of leukemia. Activation and repression of Wnt signaling pathways are determined by interactions between these proteins and different membrane receptors and secreted modulators, such as DKK3 and SFRP2, which can play a role in tumor promotion or suppression depending on the cellular context. The aim of this work is to obtain recombinant Wnt3a, DKK3 and SFRP2 proteins for functional studies. The cDNAs encoding human Wnt3a, DKK3 and SFRP2 were subcloned into the pIRES2-EGFP vector (Clontech) with a C-terminal His-tag. This vector allows simultaneous expression of the protein of interest and of a reporter protein (EGFP) from a single bicistronic mRNA transcript. EGFP translation is driven by an internal ribosomal entry site (IRES) and serves as reference for transfection efficiency using fluorescence microscopy. Expression of all proteins was tested in HEK293T, MDCK and CHO cell lineages following transient transfection with Lipofectamine. Expression levels of Wnt3a, DKK3 and SFRP2 were successfully detected by SDS-PAGE and Western Blot 24, 48, 72 and 96 h after transfection. However, the highest expression level was observed for SFRP2 in HEK293T cells. SFRP2 cell cultures were maintained in Opti-MEM medium containing 2.5% FBS in order to reduce the contaminants in the supernatant to facilitate purification of the recombinant protein. SFRP2 purifications were performed by affinity chromatography using heparin column (GE Healthcare) followed by Ni-NTA Superflow resin (QIAGEN) and good purity levels for SFRP2 were obtained. Wnt3a and DKK3 proteins have been partially purified and the purification protocols are being optimized. We concluded that the expression system was efficient to produce SFRP2 protein in the amounts needed for functional characterization. A purification protocol for SFRP2 was successfully established and the purified protein can be used for functional assays. Optimization of purification protocols for Wnt3a and DKK3 are in progress in order to achieve the purity grade required for the planned functional studies.

Indrani Mukherjee

Lipid dependent oligomerization of the ENTH domain drives membrane remodeling during clathrin mediated endocytosis

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Clathrin mediated endocytosis is a unique form of vesicle budding targeted at the Plasma membrane. It is a highly dynamic and sequenced process wherein more than 40 proteins come into play and can perform several different functions such as receptor internalization or recycling of synaptic vesicles. During this process the plasma membrane undergoes heavy remodelling within a minute. Essentially there is a formation of a Clathrin Coated Pit (CCP) which then pinches off from the plasma membrane in order to form the Clathrin Coated Vesicle (CCV). Previous research has shown the presence of several different important proteins being involved in the process of membrane deformation. One of these being the Epsin 1 protein that can be characterized by a membrane binding N-terminal domain and a C-terminal domain involved in protein-protein interaction. It has been shown that Epsin 1 can bind to $PI(4,5)P_2$ via a highly conserved N-terminal ENTH (Epsin N-terminal homology) domain. The mechanism for this binding involves the insertion of an amphipathic helix into the membrane which is perhaps the reason for the high degree of membrane curvature. Besides the requirement of $PI(4,5)P_2$ for binding of the ENTH domain to the membrane nothing else is known about the involvement of the lipid composition of the membrane that facilitates protein-dependent membrane deformation.

In this study, we have been able to identify an important phospholipid present essentially in the inner leaflet (Phosphatidylserine (PS)) to play a crucial role in the membrane curvature inducing ability of the ENTH domain. We found that this lipid triggers homo-oligomerization of the protein and that structure-function analysis shows that this assembly is essential for membrane deformation.

Mammals lack the biosynthetic machinery to produce 9 of the 20 proteinogenic amino acids. Phylogenetic studies indicate that the last eukaryotic common ancestor was able to synthesize all 20 amino acids and that the loss of ability to synthesize subsets of these pathways has been a result of multiple multi-gene deletion events throughout evolution. The reasons for these polyphyletic deletion events are not well understood. In our work, we take non-native amino acid biosynthetic pathways sourced from bacterial and fungal species, codon optimize their coding sequences for use in mammalian cells, and deliver them to Chinese Hamster Ovary (CHO) cells. Here, we successfully demonstrate the in yeasto assembly and delivery of constructs, which encode the missing steps towards the mammalian biosynthesis of isoleucine and valine. We show that CHO cells engineered to express *E. coli*-derived isoleucine/valine biosynthetic genes are able to proliferate in media lacking valine, and that they display a survival advantage in media lacking isoleucine provided that pathway intermediate 2-oxobutanoate is spiked into the culture media. To our knowledge, this is the first example of essential amino acid biosynthesis in an animal cell.

Sofiia Reshetniak

The dynamic organization of proteins in the synaptic bouton

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The synaptic bouton, a key structure in synaptic transmission, is one of the most intensely studied cellular compartments. The nature and the copy numbers of the molecules involved in synaptic vesicle recycling are known relatively well, and the mobility of some elements, such as the synaptic vesicles themselves, has been thoroughly investigated. Nevertheless, just like for other cellular systems, the dynamic organization of the synaptic bouton remains elusive and we only have a sketchy view of the overall dynamics of the synaptic proteins, which leaves open several fundamental questions.

We analyzed the motion of 45 synaptic proteins by combining fluorescence recovery after photobleaching and particle tracking with electron microscopy, nanoscale imaging and modeling. Our results suggest that simple and highly robust mechanisms, based on the synapse geometry and on binding to the synaptic vesicles, can account for the movement and distribution of many synaptic proteins, thereby also explaining how the composition of this compartment can be maintained over time.

This work provided the first visualization of overall protein motion in the synapse that illustrates measured in vivo mobility of synaptic proteins in their realistic copy numbers within realistic synapse geometry. This not only provides valuable insight in synapse biology, but would also be of interest for broad audience as the first such visualization of any mammalian cell compartment.

James Allen de Borja

Orthogonal assays and high-content imaging eliminate artefactual bioactivities in cardiometabolic natural products early drug screens

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The Philippines is one of the mega-biodiverse countries in the world, ranking high in the number of plant species and maintains about 5% of the world's flora. With high plant species endemism, natural products for pharmaceuticals are currently being sought at the national scale. The current early-stage drug discovery workflow starts with screening crude and semipure extracts in primary enzyme-based assays; cell-based hepatotoxicity, nephrotoxicity and cardiotoxicity testing; cell-based confirmatory orthogonal assays; purification and structure ID; and iterative structure activity relationship studies. Cardiometabolic disorders, a spectrum of diseases characterized by interrelated conditions, including Diabetes, hyperlipidemia and hypercholesterolemia, and hypertension, remain leading causes of morbidity & mortality worldwide. While there are several therapeutic options against these conditions, first-in-class or multi-action alternatives with fewer toxic liabilities are continually sought. Here, we report the use of cell-based orthogonal and secondary assays to validate primary hits initially screened for activity against these conditions. These include a luminescence-based glucose uptake assay for hyperglycemia, ACE inhibition for hypertension, and fluorescence-based cholesterol and LDL uptake assays aided by high-content imaging. Additionally, measuring lipid accumulation via triglyceride quantification was recently optimized. For our second-year outputs, we report additional confirmed bioactivity against hypertension for 9 out of an initial 15 non-cytotoxic positives in primary screens, and 8 out of 20 with confirmed ACE inhibitory activity. Four were found positive for bioactivity against hypercholesterolemia via Filipin staining, and confirmatory trials using an additional orthogonal assay, BODIPY FL LDL, are still ongoing. Several extracts positive for primary lipase activity are currently being tested in secondary lipid accumulation assays. Notably, false positive hits from primary screens were identified, highlighting the importance of hit validation via orthogonal, secondary as well as high-content imaging-based assays in the drug discovery workflow.

Sidney Allen Chua

Investigating the role of N-TAF1 and aberrant splicing in the pathogenesis of X-linked Dystonia Parkinsonism

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X-linked Dystonia-Parkinsonism (XDP) is a neurodegenerative disorder characterized by progressive loss of medium spiny neurons. The disease locus can be found within chromosome Xq13.1. Within the locus is the TATA-binding protein-associated factor-1 (TAF1), the largest subunit of the transcription factor IID complex. A neuronal isoform, N-TAF1, exists and differs from the canonical TAF1 by virtue of a 6-bp microexon inclusion. Additionally, both TAF1 and N-TAF1 seem to be dysregulated in XDP patients. Here, we present two different angles being investigated to elucidate the underlying mechanisms and functional consequences of N-TAF1 dysregulation, particularly in neuronal survival.

The first angle is a study on the effect of N-TAF1 expression on neuronal survival and the putative regulatory crosstalk between N-TAF1 and its splicing factor nSR100/SRRM4. We hypothesize that N-TAF1 and nSR100/SRRM4 may form an auto-regulatory feedback loop where each gene affects the other's expression. Knockdown and overexpression of both N-TAF1 and nSR100/SRRM4 were performed to assess their effects on neuronal viability, apoptosis, and neurite outgrowth. N-TAF1 knockdown led to increased apoptosis and decreased neuronal viability in SH-SY5Y neuroblastoma cells. Meanwhile, N-TAF1 overexpression was able to dampen the apoptotic effects of sodium butyrate treatment in SH-SY5Y neuroblastoma cells. Overexpression and knockdown of N-TAF1 has also shown that N-TAF1 expression may regulate neurite outgrowth. Similar to N-TAF1 downregulation, knockdown of nSR100/SRRM4 was shown to decrease neurite outgrowth. To provide evidence of the putative crosstalk between N-TAF1 and nSR100/SRRM4, knockdown studies and gene expression analysis were performed to assess their effects on each other's expression. Knockdown of nSR100/ SRRM4 resulted in a decrease in N-TAF1 expression, but N-TAF1 knockdown did not affect nSR100/ SRRM4 expression. However, results of nanoluciferase assays indicate that N-TAF1 preferentially binds to nSR100/SRRM4. These data suggest other endogenous transcriptional activators of nSR100/ SRRM4 that may compensate for the absence of N-TAF1. Additional experiments are needed to confirm the existence of the crosstalk between N-TAF1 and nSR100/SRRM4.

The second angle being investigated involves the functional consequences of increased TAF1 intron 32 inclusion observed in XDP patients. Here, we hypothesize that increased intron inclusion may affect neuronal survival. The strategy involves using Antisense Morpholino Oligonucleotides (AMOs) to simulate this phenomenon in SH-SY5Y neuroblastoma cells, by hindering splicing at the intron-exon junction. Additionally, AMOs are being used to prevent N-TAF1 splicing by obstructing the inclusion of the 6-bp microexon, with a view to determining the function of the neuronal isoform. Our preliminary results show efficient knockdown of N-TAF1 expression using AMOs while TAF1 expression was mostly unaffected. Initial apoptosis assays suggest interference with N-TAF1 splicing via the use of AMOs was able to promote apoptosis. Additional experiments are underway to elucidate both the specific functions of N-TAF1 and the consequences of aberrant TAF1/N-TAF1 splicing.

Evgeniia Ulas

The effect of nuclear receptors LXR and RXR agonists treatment on small GTPase ARL7 level in cultured HeLa and Vero cells

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The molecular mechanisms of microtubule (MT) organization on the Golgi apparatus (GA) need further clarification. Cultured Vero and BS-C-1 cells are both obtained from the green monkey kidney, but differ in activity of the GA as a MT-organizing center. We revealed distinction in expression level of the small GTPase ARL7 in this two cell cultures. We showed that mRNA and protein concentrations of ARL7 in BS-C-1 are approximately 4-fold higher than in Vero. It was earlier defined, that ARL7 interacts with MT tubulin (Wei et al., 2009). So we identified ARL7 as the candidate for involvement in the MT organization on the GA. According to previous reports, treatment with liver X-receptor (LXR) and retinoid X-receptor (RXR) agonists leads to increase of the ARL7 concentration in HeLa (Engel et al., 2004). We tried to reproduce the described effect in HeLa and then to apply this type of influence at the level of ARL7 in Vero for identification of possible MT organization changes. Cells were incubated with T0901317 and bexarotene, which are LXR and RXR agonists respectively. After HeLa treatment with LXR/RXR agonists for 2 days, the ARL7 level increased independently on the agonists concentration in the range of $0.1 - 10 \,\mu\text{M}$ for T0901317 and $0.5 - 2.5 \,\mu\text{M}$ for bexarotene. A more pronounced raise in the ARL7 concentration was achieved when extending the exposure time to 7 days and combining the both agonists. The cooperative treatment of HeLa with T0901317 and bexarotene for 7 days resulted in the approximately threefold elevation of the ARL7 level. In Vero the increase in ARL7 concentration was not observed under conditions identical to HeLa, in despite of the LXR/RXR activation detected by the increase in ABCA1 protein level, which gene is known to be under direct regulation of LXR/ RXR (Wagner et al., 2003). Thus, the difference in molecular mechanisms of ARL7 protein level regulation was revealed for Vero and HeLa cells. This work was supported by a grant from the Russian Foundation for Basic Research 18-04-00742.

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Yelyzaveta Zhyr

Changes in the Quantitative Composition of Plant Glycolipids during the Pathogenesis

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Lipids are one of the four major classes of compounds in living organisms. The membranes of chloroplasts are dominated by glycolipids: monogalactosyl-diacylglycerol (MGDG), digalactosyl-diacylglycerol (DGDG) and sulfohinovosyl-diacylglycerol (SHDG). They play an important role in the morphology and biochemical processes of chloroplasts. Recent studies indicate that under stress conditions the content of MGDG is a decreasing, although the concentration of DGDG remains relatively stable. SHDG plays the role of the adaptive protector of the CF1, F1, ATP-ase and the light-harvesting complexes of the photosystem II.

The purpose of the experiment was to determine the effect of the fungal infection of Pseudocercosporella herpotrichoides on the quantitative composition of glycolipids. Three types of winter wheat were used as plant material: Myronivska 808 (sensitive to *P. herpotrichoides*), Roazon (relatively resistant) and Renan (a product of the hybridization of the named varieties). Infection of seedlings occurred on the seventh day using a conidial suspension with a density of 106 CFO. The study was carried out using thin-layer chromatography.

The following patterns were revealed: there was a decrease in the content of glycolipids MHDG and DGDG during the 5 days after infection in the Roazon variety, although a sharp increase was observed on the 8th day of wheat growth both in the control and in the infected variant. Similar patterns in the Renan variety. Indicators for the Mironovskaya 808 variety were unstable: a significant increase of galactolipids in two variants on 8th day, reaching its peak on 10th day with following reduction. The increase in the content of SHDG was not detected in both variants for the Roazon variety. The decrease was detected from the 8th day; on the 9th day was a slight increasing, followed by a steady decline in Renan variety. In Myronivska 808 was found reduction of the content of SDHD on the 8th day in both variants, but the next day the growth of the content of sulfolipids began in the infected variant. When comparing controls, the highest content of all glycolipids was observed in the Roazon variety. General conclusion on the reduction of the number of glycolipids (relative to control for 5 days) in the development of fungal infection for the Roazon variety: 40% DGDG, 35% MHDG, 36% SHDG; for Myronivska 808: DGDG-10%, MHDG-14% and growth of sulfolipids by 10%; the indicators for the Renan variety were intermediate: DGDG-25%, MHDG-15%, SHDG-18%.

Thus, the Roazon variety showed a faster and more stable response in the development of fungal infection, as opposed to Myronivska 808, which reacted to the pathogen over a longer period. The Renan variety has confirmed its hybrid properties in intermediate indicators for the change in the quantitative composition of glycolipids.

Vasyl Velykyi

Antitumor Effectiveness of the Liposomal Form of the Cytotoxic Bacterial Lecitin of Bacillus Subtilis B-7025

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Background: previous works demonstrated that lectin of B. subtilis B-7025 has a high carbohydrate specify fructose-1,6-diphosphate, N-acetilneuramic, N-glycolyneuraminic and D-glucuronic acides [1]. Lectine had shown a high activity to agglutinate rabbit's erythrocytes and tumor cells (sarcoma C57, carcinosarcoma Walker W-256, Ehrlich ascites carcinoma, Geren carcinoma, lewis lung carcinoma) with furher it lysis [2]. Lectin stimulates specific cell-determinate immune response, through increasing titer IgG to tumor antigens, inhibit a primary tumor node growing, shown strong antimetastasis effect [3].

Aim: encapsulation of the cytotoxic bacterial lectin B. subtilis B-7025 in small monolamellar liposomes; to investigate antitumor activity of the liposomal form of cytotoxic bacterial lectin B. subtilis B-7025.

Methods: formation of big multilamellar liposomes by hydration of the bilipid layer, which consists of lecithin, cholesterol and cytotoxic lectin (mixing ratio is 1;0,15;0,3), hydration was cojoint with passive encapsulation of the lectin; ultrasound extrusion of the big multilamellar liposomes to the small monolamellar liposomes; description of the physical parameters of the created liposomes (size spreading, -potential spreading, electric conductivity, effectiveness of encapsulation of the lectin and leakage rate); hemagglutination; screening of antitumor activity of the liposomal form of lectin on in vivo model of tumor (carcinosarcoma Walker W-256, Geren carcinoma, Ehrlich ascites carcinoma).

Results: we have created liposomes filled with the cytotoxic bacterial lectin. Liposomes with the cytotoxic bacterial lectin have the average size of 314,8±113 nm, the size spreading is normal by Gauss law. -potential spreading has three peaks: 29,8 mV (49,1% liposomes), -41,3 mV (32% liposomes) and -108 mV (16,7% liposomes). 25,4 mS/cm the electric conductivity was shown. 75,89% effectiveness of encapsulation of the lectin was shown at first day. The leakage rate has shown declining value from 5,89% till -24,56%, 2-20 days were controlled (storage at 4C). The result of hemagglutination demonstrated an inhibitory ability of liposomal form of lectine to bind cells. Consequently, liposomal form of cytotoxic lectin car't induce cell lysis. Liposomal form of lectin in in vivo system showed high anti-tumor efficiency in comparison with soluble form of lectin. 75% of experimental animals with transplant of carcinoma W-256 have full resorption of primary tumors node. Average life expectancy of animals with model tumor of Geren carcinoma have increased by 34% to comparison with control group, with model tumor of Ehrlich ascites carcinoma have increased by 46% in compared to control group. Dynamics of primary tumor nodes growth have decreased reliable value of Geren and W-256 carcinoma, results has compared to control group. Results of the test of dynamic of primary tumor growth of Ehrlich ascites carcinoma do not have reliable value.

Conclusion: liposomal technique of encapsulation of cytotoxic bacterial lectin B. subtilis B-7025 opens up opportunities for elimination of unspecific cytotoxic effects of lectin without losses antitumor activity, moreover, antitumor effectiveness of the liposomal form of lectin increases. This fact can be explained by the decline cytotoxic effect of lectin on the immunity cells.

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Key words: tumor immunity, liposomes, bacterial lectin.

N. Frenkel

Chromatin modification effects on DNA replication

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DNA replication is a heavily regulated process that occurs in all living cells. In eukaryotes, replication is highly ordered with different genomic regions reproducibly replicated at different times in S phase. Replication is initiated at origins of replication during the s-phase (*S. cerevisiae* has about 200-300 origins), where the replication machinery assembles. Following initiation, this machinery progresses bi-directionality defining the replication fork. A large number of proteins are involved in this process. In addition, nucleosomes may also be involved in this regulation: indeed, nucleosomes need to be displaced and re-positioned as the fork progresses, making the chromatin environment an integral part of this process.

Here, we focus on the regulation of replication timing in budding yeast. Specifically, we ask how chromatin modifications affect the timing of origin firing and the velocity by which the replication fork progresses along the DNA. We analyze replication in mutants deleted of chromatin modifiers, as well as histone tail point mutants that mimic various histone modifications. Replication profiles of these mutants are defined using whole genome sequencing applied to unsynchronized cultures or to cultures that progress synchronously through S phase.

We find that deletion of Rtt109, a replication-specific histone acetyltransferase, increases replication velocity, and that this function of Rtt109 in limiting the speed of the fork depends on specific modifications in the histone H3 tail. By contrast, other components of the SAGA complex, which acetylate overlapping residues but do so throughout the cell cycle are needed for proper timing of origin firing, but show limited impact on the replication velocity. Of note, neither of these changes occurs via histone deposition defects. Our study reveals distinct contributions of histone modification on DNA replication dynamics, through both direct and indirect pathways. It further suggests a replication-specific function of Rtt109: by defining a wave of acetylation that precedes the replication fork, this factor reduces replication speed, probably as a way to limit replication error associated with rapid fork progression.

Mohamed A. Hassan

Production and Characterization of Metallo-Serine Keratinase from Bacillus amyloliquefaciens with Prospective Application in Leather and textile Industries

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Microbial biodegradation and valorization of keratinous wastes have been drawing considerable interests to convert the hard protein into several biotechnological products. This study intends to produce extracellular keratinase enzyme utilizing chicken feathers and human hair. A new keratin degrading bacterium was isolated from a cattle ranch soil and identified as Bacillus amyloliquefaciens MA12 with accession number (KT758733) following the phenotypic characteristics and 16S rRNA nucleotide sequences. The isolate was cultivated into a mineral medium containing feather or human hair as sole carbon and nitrogen source. It further revealed the potency to disintegrate the most substrate in case of feather and the whole substrate of human hair at 30 °C and pH 7 within 3 days. This action was accomplished via secretion of an extracellular keratinase enzyme that was characterized to determine its optimum conditions. The optimum pH and temperature of keratinase were pinpointed to be 9 and 55 °C, respectively, with maximum keratinase activity (786 U/ml). Moreover, the presented keratinase was active over a broad range of pH values and temperatures. The keratinase activity was perceived on SDS-PAGE at 48 kDa adopting zymogram analysis. Inhibitor studies exhibited that the keratinase from B. amyloliquefaciens lost 28% and 74% of its activity after treatment with PMSF and EDTA, respectively; thus, it belongs to metallo-serine keratinase. Furthermore, it was activated by Mg^{2+} and Zn^{2+} and retained the comparable activity in the presence of Ca^{2+} compared to the control. Similarly, the keratinase activities were steady in the presence of 1% triton X-100 and tween 20. These findings emphasize the conceivable implementation of keratinase from B. amyloliquefaciens MA12 in leather and textile industries.

Keywords: Metallo-serine keratinase, Bacillus amyloliquefaciens, Leather industry, Textile industry

Volodymyr Kavetskyi

Cloning and structural analysis of abiotic stress tolerance gene in *Deschampsia antarctica* E. Desv.

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Deschampsia antarctica Desv. is a unique plant, which has adapted to harsh conditions of maritime Antarctic. Transcriptional factors play an important role in regulation of abiotic stress response. Recently, we have predicted the genes of several transcription factors from DREB family in *D. antarctica.* The objectives of this study included cloning and structural analysis of a gene encoding DREB-like transcription factor from *D. antarctica.*

We used CTAB protocol for DNA extraction. PCR was performed with specific primers designed on the basis of the predicted sequence followed by gel-electrophoresis of amplified DNA. The DNA fragments of interest were recovered from the gel and blunt-end ligated into a SmaI-cut pUC18 vector for sequencing. Obtained recombinant plasmids were transformed into *E. coli* cells. The inserted clones were used for sequencing with M13-primers. Unipro UGENE software was used as alignment and analysis tool.

Analysis of the obtained sequences showed that the length of DREB-like cloned gene is 2891 bp. The gene contains two introns. The first intron begins at position 192 and ends at position 1030, and the second intron spans from 1087 and to 1899 position. The translated sequence is 364 amino acids in length. An analysis of the translated sequence revealed the conserved DNA-binding domain (AP2) specific for DREB-family, which consists of 58 amino acids and has [A-Z]*WV[A-Z]E[A-Z]R[AZ]*WLG[A-Z] consensus sequence. Furthermore, the translated amino acid sequence contain three conservative motives that were identified earlier in the members of the 4th group of DREB-family by Nakano et al. (2006). These motives includes CMIV-3, CMIV-1, CMVI-2. The presence of these motives as well as high homology with ortholog genes from other grass species allowed us to identify the cloned gene as *Deaschampsia antarctica* DREB2B gene.

Steve Thorne

The Copernican Project

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The Copernican Project considers how strain cycles associated with the earth's rotational and orbital motion become embedding into the frames we consider to be at 'rest' when we study cells under a microscope. Those non-isotropic background accelerations affect the fine-scale (10^-7) definition of equilibrium, and become expressed in the large-scale metabolic patterns found in cells.

To understand the role of altered gravitational environments on biological organisms, we have to be willing to go back and re-derive the equations of fluid dynamics from the reference frame of a moving earth. This may seem like a pedantic exercise, but it is critical to evaluate, from a mathematical perspective, how equilibrium should be defined in fluids that fundamentally possess small strain cycles due to the earth's orbital motions. Surprisingly, the equations of Bernoulli, the definitions of temperature, and the general form of the gas laws have never been upgraded to include the Copernican perspective.

Biological organism evolved within a gravitational environment that is most often treated as constant in both magnitude and orientation. But in realit – as Laplace, Lagrange, Fourier and Copernicus have taught us – gravitation is much more like a symphony, filled with a richness of notes and a complexity of periodicities. These periodicities are driven by the earth's rotation, its wobble about the earth-moon barycenter, and the earth's ever-changing orbital geometry.

Laboratory equilibrium for all earthbound fluids possesses strain cycles that vary over periods of hours, to weeks, to years. These cycles produce local accelerations as high as 6000 microns-per-second^2 with relative amplitudes of 10^-5. However, this richness in 'g' gets lost in thermal noise and is typically truncated from our models because the variations are perceived as small. But we may learn a lesson by the recognition that all tidal effects on earth are caused by relative changes in gravitational acceleration that are of order 10^-7 or smaller. Such strain cycles have near-perfect circadian periodicity, a 7% seasonal variation in strength, and even a chiral order to them.

We, as biologists and chemists and space scientists, are left to interpret the underlying flirtation that exists between gravity and biology from the small variations in metabolic order we see after organisms are placed in low earth orbit. That task has been complicated by the lack of readily available reference data pertaining to these strain cycles. The Copernican Project is providing an easily accessible database of these cycles for other biologists to reference when modeling cell dynamics. It will include comparisons between strain magnitudes and periodicities associated with both earthbound positions and with typical orbital reference frames. The results will be released in November 2019 during the ASGSR (American Society of Gravitation and Space Research) annual meeting.

Lena A. K. Bögeholz

Slow deformylation of nascent proteins on the ribosome

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In prokaryotes, proteins are synthesized with a formylated methionine at the N-terminus. In order to allow N-terminal processing and maturation of the protein, the formyl group has to be removed by the ribosome-associated protein biogenesis factor peptide deformylase (PDF). In eukaryotes, the N-terminus of nascent proteins is not formylated, which made PDF an appealing target for new antibiotics. The presence of PDF in mitochondria, however, requires that such inhibitors be highly specific to the bacterial homolog. Using a classic multiple turn-over assay with chromogenic dipeptides, we show that inhibitors based on the mycotoxin fumimycin have only modest antibacterial activity. To improve understanding of co-translational N-terminal processing on the ribosome, we are studying the deformylation kinetics of ribosome nascent chain complexes under multiple- and single-turnover conditions. We show that deformylation on the ribosome is slow under multiple-turnover conditions compared to the deformylation of dipeptides. While the single-turnover rate of deformylation is fast, there is a slow step after the chemical reaction that reduces the multiple-turnover rate by a factor of 100.

Sergey Zaitsev

Regulation of the Lipase Activity in Complexes with the Synthetic and Natural Polymers

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Both synthetic and natural polymers are promising carriers for immobilizing enzymes, including lipases from various sources. This direction is both of fundamental and applied importance, since immobilized lipases are widely used in various biotechnological processes. This work is devoted to study the effect of synthetic and natural polymers (polystyrene with different surfaces, polylysine, chitosan, etc.) on the catalytic properties of lipases from the hog pancreatic gland (LPP), *Candida Celyndracea* (LCC) fungi and wheat germ (LWG) during the hydrolysis of triacetin (as a substrate in all experiences). It was established that latex based on polystyrene (even without surface modification) significantly affects the activity of lipases of different origin, especially in the presence of large particles of about 1 micron in size (regardless of their concentration in the range of 1-10%). In these systems the activity of all three lipases (LPP, LCC , LWG) is significantly increased relative to the non-immobilized enzyme. Polystyrene latex with a carboxylated surface showed another effect on the catalytic properties of these same lipases.

The dependences of the lipase activity vs. chitosan concentrations (at their ratios from 100: 1 to 1: 1), as well as vs. the parameters of the medium (pH, temperature) were found. Measurement of the catalytic activity of lipase with polylysine showed that, at their ratios of 10: 1 and 5: 1 (excess LPP), there was an increase in the activity of the enzyme as compared to it free form by 17% and 9%, respectively. This is due to the interaction of a positively charged polylysine with an enzyme (having an excess negative charge). The results obtained are promising for use in biotechnology.

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Ismail El Shaffei

Ameliorative Effect of Betanin on Experimental Cisplatin-Induced Liver Injury; a Focus on SIRT-1/PGC-1α Signaling Pathways

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Albeit the evidenced-based anticancer efficacy of cisplatin (CIS), the concomitant organ toxicities remain a clinical challenge. The reported hepatotoxic effect of CIS is reported to be related to activation of oxidative stress and inflammatory pathways combined with apoptosis in hepatocytic structure and functions.

Objective: To investigate the effect of betanin on cisplatin-induced acute liver injury through the peroxisome proliferator activated receptor γ coactivator 1α (PGC- 1α) signaling pathway.

Methods: Forty-eight rats were divided into 4 groups; (1) control group, where rats are given water; (2) (control/ betanin); rats were given betanin (50 mg/kg/day, P.O for 7 days). (3) CIS group (5 mg/kg; i.p. at day 5) and (4) treated group; (betanin/CIS), in which betanin (50 mg/kg/day; P.O.) is given for 2 days after and 5 days before CIS treatment.

Results: Cisplatin-induced liver injury was evidenced by deregulated BAX/BCL2 ratio, decreased sirtuin-1 (SIRT-1) and AMPK-induced PGC-1 α . The latter significantly altered the nuclear factor erythroid-related factor-2 (Nrf2) and its associated antioxidants hemeoxigenase-1 (HO-1) and glutathione cysteine ligase modifier (GCLM). Intriguingly, betanin succeeded to correct the cisplatin-induced liver injury, through the upregulation of HO-1 and GCLM via activation of SIRT-1/PGC-1 α signaling pathway. These findings were supported by histochemical results pictures. The impact of miRNA 34a on SIRT-1/PGC-1 α signaling pathway provides a novel finding in the current research.

Katarzyna Więciorek

Defective endocytosis leads to mitochondrial malfunction

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Several links between endocytosis and mitochondrial structure and function have been reported, yet they are poorly understood. For example, inhibitors of clathrin mediated endocytosis, endosidin9 and tyrosine kinase inhibitor tyrphostin A23, uncouple mitochondrial oxidative phosphorylation. Furthermore, defects in both endocytosis and mitochondria have been linked with neurodegenerative diseases. Thus, advancing knowledge of interdependence between endocytosis and mitochondria would be advantageous. The goal of this work was to explore effects of impaired endocytosis on mitochondrial function using the murine models without endocytic proteins synaptojanin-1 (a PI(4,5)P2-phosphatase) and endophilin-As (curvature-sensors implicated in several types of endocytosis).

We performed transcriptomics and proteomics analysis of synaptojanin-1 and endophilin-A-knockout mouse brain, and mitochondrial functional assays. Next Generation Sequencing (NGS) data from mouse brain indicates that both knock-out (KO) of synaptojanin-1 and endophilin-As (Endophilin-A1/A2/A3 triple knockout, TKO) result in a decrease of transcript levels of genes encoding mitochondrial proteins. Yet, the levels of mitochondrial proteins were found to be up-regulated by proteomics. Mitochondrial malfunction was identified based on the following criteria: reduced membrane potential, decreased oxygen consumption rate, lower ATP production and elevated reactive oxygen species generation. The glycolytic pathway was also impaired. Moreover, PI3K/AKT/mTOR pathway was found to be significantly affected in synaptojanin-1 KO and endophilin-TKO cells and tissues. Abnormal activity of PI3K/AKT/ mTOR pathway may have multiple consequences since mTOR signalling coordinates key aspects of metabolism at both the cellular and organismal level.

In sum, lack of either synaptojanin-1 and endophilin-As result in mitochondrial malfunction. We speculate that the link between defective endocytosis and mitochondrial function is based on multiple components such as faulty recycling of plasma membrane-resident receptors and impaired mitophagy. The importance of these factors and their interdependence will be discussed.

Simon Henriet

Intron creation by miniature transposons and the emergence of a novel splicing code

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In eukaryotes, introns are present in almost all genes, and their removal by the spliceosome is generally a pre-requisite for gene expression. An overwhelming majority of introns have GT/AG ends, whose identity plays critical role for their recognition and removal by the U2 spliceosome, a well conserved complex of proteins and spliceosomal RNA (snRNA). Introns with other splice sites exist at very low frequencies in various genomes and some of them are processed by the U12 spliceosome.

Intron creation remains a fundamental issue in molecular biology. In many cases, introns were acquired early and they have been kept at the same locus during evolution. It is difficult to understand how these introns were created, as the constraints to keep their sequence intact are few. The whole-genome sequencing of the chordate *Fritillaria borealis* revealed a massive turn-over of introns, with the majority of old introns lost and replaced by non-canonical introns. These new introns have exceptionally diverse splice sites, though more frequently AG/AC or AG/AT, and features of thousands of them support an origin from transposons.

Our results show that novel mechanisms are responsible for mRNA splicing in *F. borealis*, where GT/AG introns represent less than 10% of all introns and they co-exist with non-canonical introns in the same genes. Using sequencing of lariat RNA and splicing inhibitor assays, we could confirm that non-canonical introns are removed by the spliceosome. We could not detect their splicing in human cells, unless the ends were changed to GT/AG. We examined the *F. borealis* snRNA complement with different approaches, and we could show that it corresponds only to the U2-type, without any significant divergence. We conclude that the spliceosome evolved to a different selectivity in *F. borealis*, with neither novel snRNA nor major remodeling of its core protein complement.

The genome-wide recolonization by non-canonical introns emphasizes the importance of transposons as a resource of novel introns in a context of massive intron loss. The emergence of novel intron recognition mechanisms could have taken place in parallel, thus contributing to neutralize harmful insertions. The molecular basis for non-canonical intron removal is still unclear, and it could involve previously unknown splicing factors.

Prashant Rawat

Role of Stress Induce Phase Separation in Transcriptional Attenuation

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Transcription is one of the most fundamental biological processes. It not only transcribes the genome to semi functional unit of cell (RNA) but also, gives enormous opportunity for regulation and fine-tuning of living systems. Transcription itself is fine-tuned and regulated at many steps and one of the steps, I particularly focus is Promoter proximal pausing. The phenomenon of Polymerase pausing at promoters plays a crucial role in many ways and one of them is very specific regulation upon stress like rapid induction of heat shock genes and molecular chaperones.

In the past our lab, showed the enrichment of pausing proteins at promoters during stress - reduces the gene body RNA Polymerase II and brings global transcriptional down-regulation. Metabolic and growth associated genes consists the major class of down-regulated genes. But what mechanisms drive this enrichment of these pausing proteins at chromatin remains poorly understood.

In this study, we discovered a role of Liquid Liquid Phase separation (LLPS) of pausing protein complexes in Stress induced transcriptional attenuation. We further dissected the factors and mechanism, which triggers and contribute to the LLPS of these complexes.

We also identified the signalling network that causes phase separation at chromatin and also, showed the implications of LLPS in Stress induced transcriptional attenuation.

Harshita Dubey

Clinical Evaluation of Procalcitonin in Gastrointestinal Cancer

Introduction: Apart from bacterial infections Procalcitonin (PCT) also increases in burns, trauma, and surgery.

Aim: Evaluation of PCT and thrombopoietin (TPO) after radiotherapy & surgery in cases of gastrointestinal cancer.

Material & Method: In a prospective study 45 cases (30 male and 15 female) of gastrointestinal cancer were taken, which were undergone surgery. It included the cases without (34) and with (11) prior radiotherapy. TPO and PCT were measured (ELISA) on day 1 preoperatively and day 3 (D3) & 5 (D5) postoperatively.

Result: At base level prior to surgery lower value of TPO was noted in the group not given RT (174.8 \pm 98.2 pg/ml) than the group who were given (220.3 \pm 120.7 pg/ml), although not statistically significant (p-value 0.2).

On D3, TPO value in the cases without RT was 287.2 ± 177.3 pg/ml and that of in the cases with RT was 472.6 ± 265.2 pg/ml, was statistically significant (p-value 0.01).

On D5: TPO in patients without RT was 409.57 ± 318.34 pg/ml (further increased) & in cases with RT 585.00 \pm 469.61pg/ml (further increased), P value- 0.2, was not statistically significant

The corresponding PCT preoperatively was $171.6 \pm 563.7 \text{ pg/ml} \& 100.0 \pm 42.08 \text{ pg/ml}$ in the cases without RT & with RT respectively (p-value 0.2, not statistically significant).

On D3, PCT value was $668.56 \pm 1114.09 \text{ pg/ml}$ and $400.63 \pm 660.64 \text{ pg/ml}$ in the cases without & with RT respectively; p-value 0.4, not statistically significant.

On D 5, PCT was $265.54 \pm 513.77 \& 648.27 \pm 1771.99 \text{ pg/ml}$ in cases without & with RT respectively; p-value 0.3, statistically not significant.

Discussion: In the cases after surgery following RT, TPO level was higher than in the cases who underwent surgery without RT. On D3, sharp rise in TPO was seen in both the groups, but RT cases it was statistically significant, which may be a predictor of better therapeutic response.

Conclusion:

- Higher TPO level after surgery or radiotherapy is an indicator of better response.
- TPO level may be an indicator therapeutic

Kristina Simonicova

Transport and Metabolism of Hypomethylating Agents and Development of Resistance

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In the treatment of two haematooncological diseases, myelodysplastic syndromes and acute myeloid leukemia, hypomethylating agents 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC) are often used, mostly in elderly patients unsuitable for intensive therapy. However, some patients do not respond to the treatment because of primary resistance, while others develop resistance during the treatment. In order to prevent or delay the development of resistance, it is necessary to identify its mechanisms first. It is very important, since there are no standard therapeutic opportunities for these patients after hypomethylating agents failure.

In our work, we focused on studying the potential mechanisms of resistance development in the human leukemia cell line MOLM-13 (Leibniz-Institut DSMZ GmbH, Germany). From this cell line, two resistant cell sublines were established in our laboratory by long-term cultivation in medium with increasing concentration of the drug – AZA-resistant cell subline MOLM-13/AZA and DAC-resistant cell subline MOLM-13/DAC. We found out, that these resistant cell sublines do not show cross-resistance to the second hypomethylating drug.

Since both hypomethylating drugs are administered to patients as prodrugs, to achieve effect, cellular uptake and subsequent intracellular metabolic activation are required. Therefore, we studied the expression of genes encoding proteins involved in transport and metabolism of hypomethylating agents in the sensitive MOLM-13 cell line and in the AZA and DAC-resistant sublines. We studied expression of genes SLC29A1, SLC29A2, RRM1, RRM2, RRM2B, UCK1, DCK and CDA. At the mRNA level, we observed changes that we believe are related to the effect of these drugs, but do not clarify the mechanism of acquired resistance. In our work we also incubated cell line MOLM-13 and resistant sublines in the presence of cytidine, respectively 2'-deoxycytidine, to confirm that the observed changes are not only the consequence of increased substrate concentration of the studied enzymes and transporters, but are a response to the drug.

According to our results we assume that acquired resistance to AZA and DAC in our cell sublines is not caused by changes in expression of selected transporters and enzymes involved in metabolism of these drugs, however, further study at the protein level is required.

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Khubaid Ur Rehman

TBA

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Cancer is the leading cause of mortality and morbidity. It is responsible for 8.2 million deaths per year according to WHO. Bladder cancer is the major urological problem, ninth most common cancer in the world. There are certain environmental and genetic factors involved in the development of cancer. Previous studies shown that ion channels are also involved in cellular proliferation and differentiation, apoptosis and helps the tumor cells in migration, invasion, and metastasis. Recently, cancer is considering as channelopathies. The current study was carried out to analyze the expression level of chloride channels including; Ano2, ClC3 and ClC4 in bladder cancer. For this study, we collected 14 paired tissue samples of bladder cancer patients including tumor and normal tissue samples. Along with these 03 unpaired samples were also collected which includes 02 tumors and 01 normal tissue sample. These samples were collected from two different hospitals of Islamabad. After RNA extraction, cDNA synthesis, qPCR was done for expression analysis. By relative expression analysis, we observed the upregulation of mRNA expression of Ano2, ClC3 and ClC4 in tumor as compared to controls. Ano2 expression analysis shown 3.6 fold upregulation in tumor samples as compare to control samples. ClC3 shown upregulation of 4.3 fold in tumor than controls. And ClC4 also 2.9 fold upregulated in tumor samples as compared to normal samples. Pearson's correlation tests shown strong positive correlation between the target genes in tumor and control tissue samples. Further investigation needed along with large sample size to understand the molecular mechanism of chloride channels. With increased understanding of molecular mechanism of chloride channels; it will help in the development of novel anticancer therapies by targeting the specific chloride channels involved in the initiation, invasion, and metastasis of cancer cells.

Aleksandr Kurahenko

New Tool for the TADs Prediction

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Various tools for the TADs prediction exist. However, all of them are defined by specific readjustable parameters, and therefore, show various results which are nearly impossible to compare. This leads to a problem of finding a unique algorithm that can provide the results that do not fluctuate depending on a threshold or a parameter. We propose an algorithm that does not search for the domains but builds hierarchical trees of the chromatin interactions. This algorithm does not rely on any changeable parameters, which allows it to be a universal tool for building trees on the Hi-C data. Thus, the current research aims to develop a new algorithm for building hierarchical trees and their comparison, and apply it on the different cell lines and conditions.

Suggested algorithm takes as an input a symmetric Hi-C matrix C (size $n \times n$). Entries of the matrix correspond to the contact frequencies between loci i and j. Each bin of a matrix has a particular value assigned to it. According to the Hi-C matrix characteristics the bins with higher values are located close to the main diagonal (they correspond to higher contact frequencies). Since the matrix is symmetric, in the algorithm described below we are only working with the upper triangle values.

The first part of the algorithm proposes the following: let's assign each bin from the first diagonal a specific direction. To do so, we need to calculate the sum of contact frequencies to the left (all values in the column) and the right (all values in row) of each bin.

The body of work presented in this thesis contributes towards the development of the novel tool for Hi-C data analysis. Specifically, the primary goal of the study was to devise an algorithm, which partitions chromosomes into hierarchical trees, and does not rely on any predetermined parameters. Then, the secondary task was to find methods for comparing the hierarchies.

The major result of the study is the implementation of the algorithm of one-dimensional clustering. This algorithm, which does not contain any changing parameters, lets us build a hierarchical tree on the entire length of a chromosome. Trees, delivered by the algorithm, correspond to Hi-C heat maps with very high precision. The algorithm identifies both small and large domains across the chromosome. The major feature of the algorithm is that, unlike the currently existing tools, it does not rely on any predefined value.

The study briefly covers how the algorithm works with the real data and does not deliver many significant biological results.

Summarizing, the study provides a strong and elegant tool for the analysis of Hi-C data. The algorithm does not contradict the biological intuition, moreover, confirms it. The advantages of the algorithm are its independence on any given parameters, robustness, and the ability to discover the domains on any scale and unite them into hierarchical trees.

Natia Tepnadze

Study of genetic diversity of *Aegilops tauschii* Coss. samples spread in Georgia using the chloroplast DNA

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Aegilops tauschii donated the third (DD) genome of hexaploid wheat (*Triticum aestivum L.*, genomes BBAADD). It is a consider hypothesis that *T. aestivum* originated by hybridization of domesticated tetraploid emmer with *Ae. tauschii ssp. strangulata* in south Caucasus. *Aegilops tauschii* natural range is from eastern Turkey to China. The Georgian part of the area is a specially interesting. Despite it is relatively small, an essential part of *Ae. tauschii* genetic variation was observed here. The three markedly different gene-pools of cpDNA of *Ae. tauschii* are designated as TauL1, TauL2 and TauL3. TauL3 lineage is the most ancient, Georgia is the only country where relict TauL3 is rather common.

The purpose of the research is to identify the genetic lines and diversity of the Aegilops tauschii subsp. tauschii and Aegilops tauschii subsp. strangulata collected in west and east part of Georgia.

TauL1 and TauL2 accessions have 27 bp insertion in the Rps15-ndhF intergenic spacer of chloroplast DNA. This indel feature is used to different TauL3 lineage among *Ae. tauschii* accessions by PCR Genetic variations of *Aegilops tauschii* accessions belonged to west and east part of Georgia were evaluated using PCR of spacer Rps15-ndhF (101,130-101,548) of cpDNA. Primers were designed accordingly, Forward primer: 5'-AATATGGGCCCTCAACACCC-3'. Reverse Primer: 5'-GGGTTAACCGAACTCACGGA-3'.

3 of 18 accessions were identified as a TauL3. One of TauL3 accessions belonged to west part of Georgia, while two accessions were collected in east part of Georgia.

Genetic variation of *Ae. tauschii* is an important natural resource. This study can be used to identify the material that has a higher conservation value, such as *Aegilops tauschii ssp. strangulata* forms that characterized by the resistance to some infectious diseases, characterized by the resistance to changeable climate conditions.

E. S. Solomatina

Focal adhesion dynamics: ROCK and MLCK have a similar effect in normal and tumor cells

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Adhesion and cell migration provide many crucial processes as cell division, differentiation, wound healing. These processes are mediated by focal adhesions (FAs). Dysregulation of normal FAs dynamics results in invasion and metastasis.

FAs are supramolecular complexes which links actin cytoskeleton to extracellular matrix and regulates cell response to the external signals^[1]. FAs formation and growth are the result of actomyosin contraction. Actomyosin interaction is regulated by two different phosphotyrosine kinases ROCK and MLCK from two different regulating pathways^[2]. The main attention in our research is payed to revealing the real role of kinases, which are able to provide an activating phosphorylation of non-muscle myosin II and regulate the FAs dynamics.

For the FAs visualization A549 (human lung carcinoma) and 3T3 (mouse embryo fibroblasts) were transfected with the vinculin-RFP construct. To study contribution of each kinase in FAs' dynamics we used inhibitory analysis. Control and treated cells (1-hour incubation with 10 μ M Y-27632 or 10 μ M ML-7) were captured by time-lapse during 400 min. We analyzed FAs lifetime and area (one frame from time-lapse sequence).

The majority (about 80%) of dynamic FAs for the both cell lines are localized at the cell periphery up to 10 μ m from the cell edge. Median of the FA lifetime is 50 min (range 10-235 min, n=100) for A549 cell and 33.5 minutes for 3T3 cell (6 – 164, n=60). After ROCK inhibition FA lifetime is increased in each cell line median is 75.2 min (15-180, n=50) and 49.50 (13-121, n=50) for A549 and 3T3 respectively, p<0.0001. The same effect has MLCK inhibition: median is 70 min (15-270, n=50) for A549 and 59.50 (18-271, n=50) for 3T3, p<0.0001.

The FAs area median for A549 control cells is $0.77 \ \mu m2$ (0.15-7.71 $\mu m2$, n=472) for 3T3 is 0.62 $\mu m2$ (0.07-5.2, n=750). After 30 min incubation with Y-27632 area is reduced in a both cell lines: 0.45 (0.03-4.25, n=476) for A549 and 0.31(0.05-1.6, n=687) for 3T3, p<0.0001. 30 minutes incubation with ML-7 also affects in FAs area reduction at the cell edge: 0.28* (0.06-4.59, n=372) for A549 and 0.26* (0.07-2.05, n=287) for 3T3, p<0.0001.

Our data shows high similarity of reaction to the myosin II kinases inhibiting in different cell lines. Both MLCK and ROCK kinases regulates FAs' dynamics at the cell edge. That disapproves existing hypothesis about different action of kinases on the FAs' in cell.

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Angel J. Santiago-Lopez

Mobilization of cellular stress signaling in protein misfolding diseases for controlled gene delivery

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Neurodegenerative diseases are characterized by the progressive accumulation of misfolded proteins and proteostasis dysfunction which negatively influence cell survival. Gene delivery of inducers of autophagy such as Beclin1 have shown promise in accelerating the clearance of misfolded proteins in vitro and in vivo. A major barrier for clinical translation, however, is that overactivation of autophagy-related signaling might lead to detrimental side effects. In this work, we introduce a gene delivery approach that captures intrinsic signals associated with the unfolded protein response (UPR) to control the expression of Beclin1 in cells. We designed a genetically encoded delivery system comprising 384 bp of the initial coding region of human ATF4 as a translational control operator fused to Beclin1 to enable ATF4-controlled Beclin1 delivery. In vitro validation of the molecular specificity revealed a marked increase in reporter expression when ATF4 levels were directly manipulated following chemical activation of eIF2a, an upstream effector of ATF4, and not in mock-treated cells. Furthermore, by conducting time-lapse fluorescent microscopy, we detected increased ATF4 levels in cells experiencing ER stress, a response that peaked at 20h post-induction of ER stress and that was abrogated by treatment with a PERK inhibitor. In cellular models of alpha-synuclein (Parkinson's) and huntingtin (Huntington's), the ATF4-regulated expression of Beclin1 is able to reduce aggregate accumulation to levels comparable to the constitutive expression of Beclin1. We expect the outcomes of this work to enable a strategy where a therapy is produced as needed by the affected cells, opening the possibility to intervene at an early stage for the treatment of otherwise intractable neurodegenerative conditions.

Elena Ryabova

Swiss Cheese Gene Knockdown Leads to a Glial Cells death in the Adult *Drosophila* Brain

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Recently significantly growth interest in glial cells due to their also participation in important development processes in the nervous system, not only in supporting and trophic functions. Glia dysfunction leads to various neurodegenerative diseases. For example, astrocytes and microglia dysfunction are considered to be one of the development of Alzheimer's disease, Parkinson's disease and Tauopathy causes [1, 3, 7].

The NTE gene (PNPLA6) mutations lead to a complex human syndrome, includes spastic paraplegia (SPG39), Gordon-Holmes, Boucher-Neuhäuser, Laurence-Moon and Oliver-McFarlane syndromes. The swiss cheese (sws) gene of Drosophila melanogaster is the NTE orthologue, mutations sws cause progressive neuronal degeneration of flies. SWS and NTE are expressed in neurons and glial cells and it is placed in neurons and glial cells [5, 6]. It has been shown that the loss of SWS in the pseudo-cartridge glia forms the lamina cortex multi-layered glial wrap [4]. In addition, the SWS expression knockdown in glial cells leads to the *Drosophila melanogaster* axons damages. However, their role in the NSP pathogenesis is still unknown.

In a previously published article [2], was shown neurodegeneration and changed the morphology of these cells as a result sws knockdown in the subperinerial and ensheathing glia.

In this work, using laser confocal microscopy was shown decrease surface and cortex glia cells number, blood brain barrier disfunction and the external structure change as result SWS knockdown in surface and cortex glia cells. These data may indicate that glial cells changes also contribute to the pathology development observed in human brain with mutations NTE.

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Nina Surina

Amyloid Beta Peptide Influence on Morphology and Functions of Drosophila Melanogaster Glial Cells

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Alzheimer's disease (AD) is the most common form of senile dementia. Approximately 5% of people over the age of 65 suffer from Alzheimer's disease, and their number increases to 20% after 75 years. It was estimated that there were 25 million diagnoses of Alzheimer's disease worldwide in 1998, and this number increases significantly due to the growing trend of population aging.

Symptoms of the disease include the degradation of social skills and emotional unpredictability, accompanied by serious and irreversible cognitive impairment. On average, the life expectancy of patients from the time of diagnosis is about 7-10 years and effective treatment has not been found yet. The main histopathomorphological markers of the disease are senile plaques and neurofibrillary tangles. The brain of the patient shows a pronounced neurodegeneration.

In AD, the functions of not only the neurons themselves, but also the glial cells, which are closely related to them, are disturbed. It is possible that amyloid beta peptide has a toxic effect not only on neurons, but also on glial cells. Thus, the massive death of neurons in Alzheimer's disease can be caused by disrupting the structure and reducing the number of neuroglia in the patient's brain. As it is known, the formation of amyloid beta peptide (Abeta) in humans occurs as a result of proteolysis of the APP protein by gamma and beta secretases. In this study, transgenic *Drosophila melanogaster* lines were used with the expression of APP and human beta-secretase, as well as the line with Abeta secretion itself. These lines reproduce the main pathological and clinical manifestations of AD well: formation of Abeta aggregates, decrease in synaptic density and neurodegeneration in the brain, decrease in the ability to learn and memorize, a violation of behavior etc. The expression of transgenes was carried out in different types of glial cells. Analysis of the morphology of *Drosophila* CNS glial cells: perineural, subperineural, cortex, astrocyte-like and ensheathing glia, was performed on the 5th, 15th and 30th day of life. Also, an assessment of the functional integrity of the fly blood-brain barrier was made, while the formation and deposition of amyloid beta peptide was induced.

Mizanurfakhri Ghazali

Comparative expression of the Microfold (M) cells between the alginate hydrogel beads and transmembrane 3D culture system.

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Human norovirus (HuNoV), a foodborne virus is the number one cause for acute gastroenteritis that can lead to death especially in children and elderly. Unfortunately, its inability to be cultivated in vitro imposed a major setback in the study of HuNoV biology. However, previous findings on presence of the microfold (M) cells within the human gastrointestinal tract showed their functions in facilitating HuNoV host cells entry. This could be attributable to the unique morphology of the M cells which allow the transcytosis process of the virus across epithelial barrier. To date, an efficient in vitro culture system for HuNoV cultivation is still lacking but recently, we have developed two models of 3D culture system expressing the M cells that could be used for HuNoV cultivation. Thus, this study aims to compare the efficiency of M cells expression between the two in vitro models; transmembrane well-plate and alginate hydrogel beads. Briefly, the Caco-2 cells were co-cultured with Raji B cells for a total of 21 days. The efficiency of both system was validated through cell viability assay of Trypan Blue method, followed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Immunocytochemistry and Western blot (WB) analysis were performed to detect specific protein markers expressed by the M cells in both systems. Results showed that both in vitro models supported the growth of Caco-2 and Raji B cells, indicated by the high cell number at post 21 days culture and the intact structure of the beads-entrapping cells. Successful formation of the M cells were shown in the SEM result of the alginate hydrogel beads, indicated by the smooth and regular apical surface of Caco-2 monoculture, while the co-culture showed a reduction of apical surface, which was a unique characteristic of the M cells. The TEM results of the Caco-2 monoculture cells grown in the transmembrane model showed columnar shape and brush border, with presence of the M cells indicated by lack of microvilli at their apical surface and desmosomes. Immunocytochemistry of the in vitro transmembrane model showed the up-regulation of alpha-5-beta-1 and sialylated Lewis A expression and down-regulation of Ulex Europeus expression. Meanwhile, WB of the in vitro alginate hydrogel beads showed down-regulation of Ulex Europeus expression. In conclusion, it is evident from the results that both in vitro 3D systems presented comparable expression of the M cells, indicated mainly by the similar trend of the M cells protein marker expressions. These promising results essentially prove that both of the newly developed in vitro 3D models are highly potential for the cultivation of the foodborne virus.

Zuzana Kočibálová

Atypical Expression of Latrophilin-1 in Acute Myeloid Leukemia: Balance between Multidrug Resistance and Immune Escape?

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Latrophilin-1 (LPHN1) is member of G protein-coupled receptors superfamily and is predominantly expressed in neurons where it is involved in neurotransmitter release. Most recently, it was found that it is atypically expressed also in the acute myeloid leukemia (AML) cells1. Since LPHN1 is expressed only in healthy CD34+ hematopoietic stem cells2, but absents in healthy leucocytes, it was proposed as a novel potential biomarker of AML1.

We confirmed LPHN1 expression in 2 cell lines derived from human patients with AML which developed after previous myelodysplastic syndrome (MDS). Although, we detected the massive downregulation of LPHN1 expression in cell sublines with developed multidrug resistance (MDR) in comparison with their sensitive counterparts. This downregulation occurred on both mRNA and protein levels in cells with upregulated probably best characterised ABC protein – P-glycoprotein (ABCB1 or P-gp). It is well known that P-gp overexpression is the most frequent molecular feature of MDR development. P-gp serves as ATP-dependent efflux pump with capacity to transport very wide group of chemical substances including anticancer drugs across plasma membrane. In our previous papers, we described several changes in proteins expression in relation with P-gp overexpression. Though, it is obvious that MDR development mediated by P-gp overexpression is complex phenomenon and attenuated LPHN1 expression is one of its features3.

However, the question to be answered is the potential function of LPHN1 in AML cells. To this date, there are only few available publications on this topic. It was proposed that LPHN1 serves as a potential regulator of immune escape mechanism of AML cells2. Because of that, it is essential to continue in the research in this field since there may be possibility of immune therapy application for patients with developed resistance to chemotherapeutics.

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Keywords: acute myeloid leukemia; latrophilin-1; P-glycoprotein; multidrug resistance; immune escape

Martin Škandík

Quercetin-Quinone Derivative Mitigates Microglial Inflammation and Senescence Markers by Redox Homeostasis Modulation

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Process of ageing relates to changes in redox homeostasis and proteostasis, which leads to chronic oxidative stress. Rising of oxi-inflammation affects mainly regulatory systems like immune or nervous system. Their damage and loss of their basal functions are responsible for development of many neuro-degenerative pathologies connected with decrease in motoric and cognitive functions.

Microglia are immune cells, of central nervous system and during physiological conditions maintain homeostasis in their environment. They can express different phenotypes based on the type and intensity of activation signal. Pro-inflammatory phenotype is presented after stimulation of receptors recognising molecular patterns, like Toll-like receptors (TLRs). This phenotype produces pro-inflammatory mediators and is responsible for elimination of harmful stimuli. On the other hand, microglia can present anti-inflammatory phenotype, which participates in clearance of debris from synaptic connections. Moreover, in aged brain, microglia cells are chronically activated under oxidative stress conditions and by aggregate proteins like β -amyloid. Primed microglia produce several pro-inflammatory molecules and themselves contribute to the neuroinflammation in vicious cycle. Therapeutic approaches focus on substances which can induce beneficial anti-inflammatory phenotype and rejuvenate microglia cells.

Furthermore, long-lasting defects in redox homeostasis and proteostasis have far-reaching impact on cell functionality. Gradual accumulation of oxidatively damaged cell structures and impairment of catabolic processes lead to development of senescent state. Senescent cells are characterised by loss of proliferative capacity, insensitivity to mitogenic signalization, defects in mitochondrial fusion and fission and alterations in lysosomal functions due to accumulation of non-degradable lipofuscin. Defects in mitochondrial and lysosomal conditions also negatively affect process of autophagy. Function and role of senescent cells is acute topic for research and essential effort to understand process of ageing.

Anti-ageing and rejuvenating effects have been described for electrophilic compounds, such as natural polyphenols or quinones. In our study, we investigated the anti-ageing and rejuvenating properties of natural quercetin and synthetic naphthoquinone derivative, 4-O-(2-chloro-1,4-naphthoquinone-3yloxy) quercetin (CHNQ). The derivative tested, more than its precursors, downregulated pro-inflammatory markers (TNF α , NO) in LPS-stimulated microglia and supported switch from harmful pro-inflammatory phenotype to more beneficial anti-inflammatory one. This switch is regulated by activation of Nrf2 signalization and suppression of Nf- κ B. Even more, CHNQ and quercetin showed neuroprotective effects. After long-term cultivation, we were able to develop replicatively senescent human fibroblasts. Our derivative overcomes senescent markers in lysosomes like senescent β -galactosidase activity and lipofuscin autofluorescence. Besides this, CHNQ improved mitochondrial membrane potential and downregulated production of reactive oxygen species in senescent cells without affecting their lifespan. CHNQ also restored flux of autophagic LC-3 protein, what along with improved mitochondrial and lysosomal conditions can support process of autophagy.

Accordingly to all mentioned, tested derivative represents a promising compound with anti-ageing and rejuvenating effects. This study was supported by VEGA 2/0041/17, APVV-15-0308, APVV-18-0336.

Marija Lj Medar

Immobilisation Stress Changes Expression Pattern of Core Clock and Steroidogenic Genes in Leydig Cells

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The ability of immobilization stress (IMO) to decrease Leydig cells steroidogenesis and serum testosterone level is well-known. However, the effects of IMO on circadian rhythm of Leydig cells endocrine function are unknown. This study was designed to evaluate the effect of acute (3 h daily) and repeated (3 h daily for 10 consecutive days) IMO, applied at different times during the 24h on circadian rhythm of testosterone secretion, expression of clock and steroidogenic genes in Leydig cells. The result showed that acute IMO decreased level and canceled circadian pattern of testosterone secretion, which is associated with changed transcription of some genes involved in steroidogenesis (decreased and canceled rhythm of Cyp11a1 and Cyp17a1 and decreased circadian pattern of Star) and some core clock genes (increased rhythm robustness and mesor of Perl and Reverba). Ten time repeated IMO also decreased and flattened oscillatory pattern of testosterone secretion and changed the rhythm of steroidogenic genes transcription (decreased rhythm of Cyp17a1, increased and initiated cyclic pattern of Hsd 3β 1/2). The transcription of clock genes were also deregulated (Bmal1, Per1, Cry1 and Cry2 decreased while Reverba increased). However, comparing the IMO effects at different periods during the 24 hours, it was found more pronounced IMO effect on the clock genes in the morning. Accordingly, presented data suggest the severe effect of IMO on Leydig cell endocrine function.

This work was supported by the MS173057 and APV2856.

IM Starovlah

Signaling Pathways Regulating the Mitochondrial Dynamic and Acrosomal Reaction are Disturbed in Spermatozoa from Stressed Adult Rats

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Mitochondria are key component of energy production, oxidative stress, calcium homeostasis, steroid biosynthesis, but also the key component of stress response. However, the molecular adaptation of spermatozoa from stressed males was not described well. The aim of this study was to determine the functionality and molecular adaptation of spermatozoa from stressed rat by applying in vivo and in vitro approach. For in vivo experimental model, psychophysiological stress by immobilization (IMO), was performed for 3 hours in different time during the day (ZT3, ZT11, ZT23), for one (1xIMO) or ten (10xIMO) consecutive days. For in vitro approach, epididymal spermatozoa from undisturbed rats were stimulated with stress hormones adrenaline and cortisol. Results showed that number of spermatozoa significantly decreased in all 10xIMO rats comparing to control. Acrosomal status (response to acrosome-reaction-inducer progesterone) significantly decreased in spermatozoa from 1xIMO and 10xIMO rats comparing to control. The same effect was observed in spermatozoa stimulated in vitro with stress hormones. RQ-PCR results revealed that transcription of the main mitochondrial biogenesis markers Ppargc1a, Nrf1 and Nrf2 increased in spermatozoa from 10xIMO rats in ZT3 time point. In the same spermatozoa samples transcription of main markers of mitochondrial architecture Opa1, Mfn1, Mfn2 increased in ZT3 while decreased in ZT11 time point. Incubation of spermatozoa with adrenaline decreased level of Ppargc1a and Nrf2a transcripts, while cortisol decreased expression of mitochondrial transcription factor TFAM. In summary, repeated psychophysical stress decreased the number and functionality of spermatozoa and disturbed transcriptional profile of their mitochondrial biogenesis and architecture markers.

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Jeremy McCallum-Loudeac

Sex Driven Changes in the Spinal Cord over Puberty and Adolescence: The Role of Hormones on Gene Expression

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The spinal cord, responsible for relaying information from periphery to brain contains many pathways each with unique roles including nociception, proprioceptive signalling and motor output. Synaptic pruning and modifications to the brain during puberty and adolescence are well documented, however the same processes have been overlooked in the maturing spinal cord. Puberty, driven by fluctuating hormones results in a large number of physical and molecular changes throughout the body. Hormones have been implicated in influencing gene expression directly through hormone response elements. The role hormones may play in the physical changes in the spinal cord are poorly understood. There are clear biological differences between juvenile and adult spinal cords and this may be due to the organisational effects of fluctuating sex-steroid hormones through puberty. Beyond these changes, the sex-specific changes may provide insight into sex-biased central nervous system diseases such as multiple sclerosis and neuromyelitis optica.

An examination into the molecular changes in the maturing spinal cord was carried out using C57BL/6 mice at pre-pubertal (<28 days) and post-pubertal (~56 days) stages. Total RNA was extracted from isolated spinal cords to perform RNA-sequencing. We identified 800 genes differentially expressed transcripts between pre- and post-puberty (589 decreased in expression, 211 increased), and 15 genes differentially expressed between male and female spinal cords at 4 weeks, 19 genes were differentially expressed at 8 weeks. RNA-sequencing results were confirmed by RT-qPCR analysis of nine differentially expressed genes with RNA collected from independent biological replicates, showing a strong correlation between RNA-seq data and RT-qPCR (Spearman r=0.87; P-value = 0.0005).

Gene Ontology analysis revealed an overrepresentation of biological pathways related to synaptogenesis, myelination and signal transduction. We also noted that genes expressed by progenitor cells, such as Hairless and Oligodendrocyte Transcription Factor 1, were downregulated in the adult spinal cord, suggesting that the adult spinal cord would heal poorly following injury, compared to the juvenile spinal cord, which supports current findings at a molecular level.

As we observed sex-specific differences in gene expression, we were interested in the effect of gonadal hormones on gene expression in the spinal cord. This was investigated by RT-qPCR on pre-pubertally (n=5) gonadectomised mice with hormone replacement at physiological levels. Female mice underwent ovariectomy, with estradiol replacement, male mice underwent orchidectomy and had replacement of either dihydrotestosterone (DHT) and estradiol (E).

Transcript levels in the spinal cord responded to hormone replacement following gonad removal, with most mRNAs examined showing an increase in levels following estradiol treatment. Further investigation is required to better understand how these transcript changes influence disease onset and progression between males and females.

Future directions include single-cell RNA-sequencing of the various spinal cord sections (cervical, thoracic, lumbar, sacral) spinal cord between sexes and across puberty with the aim of determining precise changes in synaptic connections, spinal pathways and total neuronal subtypes. This may provide insight into sex-biased disease etiopathogenesis and a better understanding of possible spinal cord treatments following injury.

Malgorzata Majewska

Characterisation of HMGR4 promoter from Salvia miltiorrhiza

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Salvia miltiorrhiza is one of the most commonly used traditional medicines in China. *In vitro* studies, animal models and clinical trials indicated that it may be a promising treatment for coronary heart disease, cerebrovascular disease, Alzheimer's disease, Parkinson's disease, neuropathic pain, diabetes mellitus, cancer, hepatocirrhosis, acute lung injury, renal injury, fibrosis, alcohol dependence. Secondary metabolites are considered to be responsible for the activity of *S. miltiorrhiza*.

The aim of the study is to investigate promoter region of 3-hydroxy-3-methylglutaryl coenzyme A reductase 4 gene (HMGR4) from *S. miltiorrhiza*. HMGR4 reductase plays an important role in the biosynthesis of mevalonic acid, the precursor of diterpenoid quinones.

At first, HMGR4 promoter was isolated using genome walking technique and for the first time sequenced. The sequence was then *in silico* analysed for the presence of TATA box, tandem repeats, CpG islands, target sites for miRNAs, cis-active elements and interacting transcription factors. Obtained results were verified by co-expression studies based on *Arabidopsis thaliana* microarray data. Moreover, the activity of HMGR4 promoter in response to abiotic factors is being evaluated using *in vitro* cultured transformed *S. miltiorrhiza* plants.

Obtained data related to the structure and activity of HMGR4 promoter are of importance for production of modified or synthetic promoters. As a result, greater concentrations of medically important secondary metabolites can be achieved.

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TBA

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Backgrounds: Amyloids are unique groups of protein fibrils that are unbranched with a typical spatial structure. Till date about more than 30 amyloid proteins associated with various incurable diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), diabetes type II diabetes (T2D) and Creutzfeldt-Jakob disease (CJD) progressed through an undefined biological basis pathway called amyloidosis are identified. Among these, Alzheimer's disease (AD) is a chronic neurodegenerative disease that is characterized by the presence of amyloid proteins including A β (amyloid- β peptide) and Tau in the cerebellar region of the brain, through which the severity of the disease would be analysed and it is mainly a result of degeneration and death of neurons controlling memory, cognition and behaviour. AD preferentially affects approximately 2% of individuals above the age of 65 years, with the incidence rate doubling every 5 years up to age 90 at which the incidence is over 50%.

Objectives: Similarly, Islet anyloid Polypeptide (IAPP) is also one such amyloid aggregate found in the pancreatic regions of the human body and these aggregates possess similar toxic effects to that of A β seen in AD patients during the later stages of the Type two Diabetes (T2D). Variant studies show evident research ideas and results in the recent years regarding the interconnected links between glucose levels and insulin resistance activities in the brain with the co-occurrence of human Islet amyloid polypeptides (hIAPPs) in the Islets of Langerhans Beta cells of T2D patients which might later lead to gradual progression of AD. The disease progression of AD can also be confined to the occurrence of Apo&4 allele of the apolipoprotein E (APOE) gene in a homozygous condition being one of the major risk factor for dementia and cognitive impairments. With this context there is very less data to relate the Apo&4 allele with T2D in various population. The present study is one such effort that has been put forth to conduct a population based analysis of the APOE genotype in AD, T2D and Control subjects in South Indian belt.

Methods: The study comprises of specific amplification of APOE gene from the genomic DNA isolated from the AD: 200; T2D: 200 and Control: 150 subjects to analyse APOE genotype using traditional RFLP method that digests the PCR product employing the restriction enzyme Hha I. The genotype results were further analysed using the Genetic Analysis in Excel (GENALEX) and the bioinformatics genotyping tool-Gene Marker.

Results: Interestingly the results showed a higher genotype frequency of 13.67% in T2D subjects; 3.0478% in AD subjects and a combined genotype frequency of 12.30% for C/T of Apoe4 allele in the total population.

Keywords: Alzheimer's disease, Type two Diabetes, Amyloid Beta, Amylin, Apo lipoprotein ϵ Genotype

Michał Marcinkowski

New partners of FTO protein – biophysical and biochemical studies

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FTO-two domain protein, part of alkylation dioxygenase family, which SNP in its first intron is responsible for increase of subject mass on approximately 20%. In vitro studies shows ability of FTO expressed in *E. coli* to demethylate 6-methyladenine, but role of this protein in whole metabolism is still subject of study. To evaluate role of FTO, series of biophysics analysis was performed on protein expressed in eukaryotic system. In contrast to prokaryotic source, BSE-derived FTO carries three phosphoserines in the N-terminal domain, measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS/MS). Also ability of FTO to dimerization and binding Fe^{2+} and 2-oxoglutarate (2-OG) were evaluate by microscale thermophoresis technique (MST). Low resolution structure of homodimer was obtain by Small-angle X-ray scattering (SAXS). Bioinformatics analysis show potential place of interaction between C-terminal domain of FTO and Calmodulin (CaM)- signaling protein presence in all type of cell, which substrate specificity may change upon calcium binding. This state was proved by MST. FTO and CaM form strong complex in presence of Ca2+, Fe2+ and 2-OG (Kd = 7,6 nM). Further analysis with usage of Hydrogen Deuterium Exchange technique shows significant changes of CaM conformation upon complex formation without serious changes on FTO structure. Also we state that, this interaction is dependent from calcium meta-bolic pathways because changes in CaM take places in calcium binding sites. The values of its complexes dissociation constant suggest that mentioned interaction may occur in vivo in human cells.

Alina Stein

Protein Engineering of Artificial Metalloenzymes with Unnatural Amino Acids and Rational Design

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Artificial metalloenzymes emerged as attractive alternatives to both chemical catalysts and natural enzymes over the past decade due to the great potential of protein engineering. To create such hybrid catalysts, an organometallic cofactor is anchored within a protein environment, thus extending the enzymatic repertoire with enhanced reaction rates, new-to-nature reaction and reaction cascade scopes and improved enantioselectivity. Meanwhile, site-specific incorporation of unnatural amino acids into protein scaffolds developed into a valuable tool in protein engineering. Using metal-chelating unnatural amino acids, metal ion binding sites can be selectively incorporated to create an artificial metalloenzyme with a sequence-encoded catalytic or redox site, structural element or fluorescent labeling site.

This project aims at the incorporation of metal-chelating unnatural amino acids to engineer a defined coordination sphere for a transition metal catalyst within the homotetrameric protein streptavidin (SAV) which has proven as a versatile host for artificial metalloenzyme design. The unnatural amino acids are genetically incorporated into SAV in *E. coli* in response to the amber stop codon, TAG, by stop codon suppression with a bioorthogonal cognate tRNA/tRNA synthetase pair.

A second approach towards the engineering of SAV as an artificial metalloenzymes within this project is the rational design of SAV. SAV serves as a potent scaffold since it binds tightly to its ligand, biotin. A biotinylated organometallic cofactor can thus easily be anchored tightly within the protein. Optimizing the catalytic performance of SAV can be achieved by chemical means and directed evolution, which is powerful but limited due to screening efforts. Rational design offers a potential solution to this shortcoming. In collaboration with the Correia lab at EPFL, insertions within the 3/4 loop of SAV that enhance the affinity of the protein for a biotinylated ruthenium cofactor were modeled. The resulting mutants are currently screened for improvements in catalytic performance during ring closing metathesis, an industrially relevant reaction without natural equivalents. The 3/4 loop is critical since it becomes ordered upon biotin binding and thus locks biotin and the attached cofactor in the binding pocket. We argued that a SAV variant with increased affinity for its cofactor compares favorably with wild type SAV in ring closing metathesis.

Nino Rostiashvili

Thrombocytosis in JAK2 V617F positive myeloproliferative neoplasms

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Mutations in JAK2, MPL, and CALR genes are defined by the World Health Organization as diagnostic criteria for major subcategories of myeloproliferative neoplasms (MPNs) which are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Although JAK2 V617F mutation is most prevalent and can be found in all subcategories, MPL and CALR mutations are not present in PV. All these mutations are mutually exclusive and provide significant prognostic and therapeutic value.

The purpose of our study was to evaluate the prevalence of JAK2, MPL and CALR mutations in a cohort of 32 patients, pre-diagnosed with myeloproliferative neoplasms. To detect JAK2 V617F and Exon 12ins/del, MPL W515K/L and CALR L367fs*46del/K385fs*47ins mutations in patient blood or bone marrow samples, we used Multiplex ligation-dependent probe amplification (MLPA) methodology. JAK2 V617F and MPL W515K/L mutation status were always confirmed by Competitive Allele-specific TaqMan PCR (CastPCRTM).

JAK2 V617F mutation was identified in 50% (16/32) and CALR L367fs*46del in 6.3% (2/32) of the patients. No patients were identified with MPL W515K/L or JAK2 Exon 12ins/del mutations. We also observed elevated complete blood count values in JAK2 V617F positive patients. Elevation was significant only in the case of platelet counts. Specifically, platelet counts in JAK2 V617F negative patients were 379+37X106 cells/ml and in JAK2 V617F positive patients 788+42 X106 cells/ml. The difference was highly significant (One-way ANOVA, p=6.6X10-8) indicating an association of the mutation with thrombocytosis.

Pravin Dudhagara

Microbiome study of sediment and water of polluted site by column based enrichment culture designed for the pesticides degradation

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The Winogradsky column is an ancient microbial ecosystem model to study environmental influences on microbial community structure and dynamics. This complex community can be maintained or manipulated under carefully controlled laboratory conditions with the enrichment of compounds in the column. In the present study, three different Winogradsky columns were prepared by adding water and sediment of the polluted site - Amala khadi (Ankleshwar, India). First column enriched with 2ml of guinalphos pesticide and second with 2 ml of monocrotophos. The third column was kept as a control without addition of xenobiotic compounds. All columns were maintained under sunlight with regular addition of minerals and water. Every week the observation was carried out. After eight weeks of incubation columns were broken and upper- aerobic zone and lower- anaerobic zone were separated to extract the DNA from each zone of each column. The DNA was subjected to quality checking followed by 16s rRNA sequencing using Illumina Miseq Platform. The data were analyzed by Kraken and One Codex and compared using Megan and STAMP. The result was also compared with the WGS data of the Amalakhadi metagenome. The Most reported Phyla was found Proteobacteria and Chloroflexi. However, both phyla proportion were different in aerobic and anaerobic zone of each column. The presence of phototropic bacteria with heterotrophic diversity indicates the site is rich with nutritionally versatile groups of microbes. The detection of xenobiotic-degrading photosynthetic groups of bacteria suggests the natural adaption of polluted environments. Anaerolineaceae bacterium and Sphaerobacter thermophiles were dominated in aerobic zone of control, quinalphos pesticide column. The detection of Steroidobacter denitrificans in aerobic zone of pesticides enriched column suggests the possible role in pesticide degradation. The study result suggests the detection of various important bacterial species useful for the bioremediation of the polluted site. The many potent bacteria species were isolated to prepare the consortium for the degradation of the pesticides and successfully, in lab scale 95%, degradation of both the pesticides were detected after 2 weeks. The degraded metabolites were not found toxic and identified by chromatography. Study suggests the analysis of stimulated microbiome of polluted sites is key tool to screen the potent pesticide degrading bacteria which are feasible to apply for the pesticide degradation.

Keywords: Metagenomes, Microbiome Winogradsky column, polluted site,

Julie Trolle

Engineering Prototrophy In Mammalian Cells

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Mammals lack the biosynthetic machinery to produce 9 of the 20 proteinogenic amino acids. Phylogenetic studies indicate that the last eukaryotic common ancestor was able to synthesize all 20 amino acids and that the loss of ability to synthesize subsets of these pathways has been a result of multiple multi-gene deletion events throughout evolution. The reasons for these polyphyletic deletion events are not well understood. In our work, we take non-native amino acid biosynthetic pathways sourced from bacterial and fungal species, codon optimize their coding sequences for use in mammalian cells, and deliver them to Chinese Hamster Ovary (CHO) cells. Here, we successfully demonstrate the in yeasto assembly and delivery of constructs, which encode the missing steps towards the mammalian biosynthesis of isoleucine and valine. We show that CHO cells engineered to express *E. coli*-derived isoleucine/valine biosynthetic genes are able to proliferate in media lacking valine, and that they display a survival advantage in media lacking isoleucine provided that pathway intermediate 2-oxobuta-noate is spiked into the culture media. To our knowledge, this is the first example of essential amino acid biosynthesis in an animal cell.

Nina V. Voronova

Five new mitochondrial genomes of aphids: Aphis affinis, A. craccivora, A. fabae mordvilkoi, Appendiseta robiniae and Therioaphis tenera

Nina V. Voronova

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Mitochondrial genomes in animals are believed to be evolutionary conservative, neutral, and independent of the details of biology and ecology of species. This fact allows using mitochondrial genes as important phylogenetical markers. On the other hand, even neutral evolution can unfold with different rates due to the complex combination of the pace of generation change and the various level of selection pressure, which, in turn, depends on biological and ecological traits. Understanding the major traits of molecular evolution of aphid mitochondrial genomes is an important step to reveal the principals of molecular evolution of aphids, mutational rate, the rate of genome reorganization, efficiency of reparative systems and other features important when you deal with such a severe pest as aphids.

We examined evolutionary patterns in 30 mitogenomes of aphids including 5 new ones, sequenced intentionally for this work. Aphids with newly sequenced genomes were *Aphis affinis*, *A. craccivora*, *A. fabae mordvilkoi (Aphididae: Aphidinae)*, *Appendiseta robiniae* and *Therioaphis tenera (Aphididae: Calaphidinae)*, all collected in Belarus from their typical host plants. Mitogenomes were extracted from the whole genome sequence data, assembled and annotated. Comparative mitogenome analysis was conducted using all available aphid mitogenomes on the state of 06/19/2019.

We found out that aphid mitochondrial genomes are very conservative in gene order, nucleotide composition and codon usage patterns comparing to other insects. At the same time, aphid mitogenomes have two non-coding regions which are highly variable in length and usually include several long-size tandem repeats or microsatellite tandem repeats. One region is situated straight after a control region and the second one is located between tR-NA-Glu and tRNA-Phe. Among our five aphid genomes tandem repeats in the repeat region were found in *A. affinis, A. craccivora, A. fabae mordvilkoi* and *Th. tenera*, but only in *Th. tenera* mitogenome they were longer than 60 bp. The repeat region of *Th. tenera* included a long (&&& bp) non-coding sequence with unusually small for aphid's mitochondrial DNA A+T content. Three from five mitogenomes: *A. craccivora, A. affinis* and *Th. tenera*, had repeats in the control regions longer than 100 bps. In *A. craccivora* and *A. affinis* these repeats cover the whole D-loop including conservative flanking regions. *Th. tenera* had the most complex combination of repeats in the control region the largest in length.

We constructed a phylogenetic tree based on different combinations of mitochondrial genes as well as the whole mitochondrial DNAs of 30 aphid species. We found out that despite the high conservativeness of aphids' mitogenomes they are a very good molecular marker for aphids' phylogeny constructing. Trees based on protein-coding sequences, protein-coding + RNA-coding genes, and even genes + non-coding regions let us get dendrograms with very high scores of a posterior probability of branch topology.

Zehra Kanlı

Investigation of the Role of M3 muscarinic receptor in Chronic Myeloid Leukemic Cells

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Objective: K562 cells are used to as model systems for normal myeloid, development of myeloid leukemia, control of differentiation studies. K562 cell line represent early differentiation stage in granulacyte leneage. Non-neuronal acetylcholine have a important role in migration, proliferation and differentiation of different types of cell. It was recently suggested that the non-neuronal cholinergic system has also a important role in different types of cancer cells. Muscarinic receptors are part of G protein coupled receptors . K562 cells express M2,M3 and M4 muscarinic receptors. Especially, muscarinic type 3 receptor (M3R) plays a important role in different cancer cells. Bax and Bcl-2 proteins are related to apoptosis.

Aim of this study firstly was to examine effects of pilocarpine, TNFalpha and other drugs on apoptosis. Secondly, we aimed to demonstrate the mitogenenic effects of FBS, TNF alpha and EGF in K562 cells proliferation. Effects of Pilocarpin on The AChE activity has been detected.

Materials and methods: Cell viability were evaluated by the 0.4% trypan blue exclusion test by using a hemocytometer. Proliferation assays were performed by measuring BrdU incorporation during DNA synthesis in proliferating cells. Effects of TNF alpha, EGF, M3 agonist and antagonist on bcl and bax expression were detected by western blotting method. AChE level was detected by the QuantiChrom Acetylcholinesterase Assay Kit (DACE-100).

This study, proposes that pilocarpine mediated M3R activation can promote apoptosis and inhibit cell proliferation.

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Keywords: Cholinergic receptors, pilocarpine, K562 cells, apoptosis
Nataliia Chornenka

Investigation of excess collagen formation in the conditions of alkaline esophageal burn and using of melanin

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Every year in Ukraine, more than 12 thousand children have had burn, the most affected group are children aged from 1 to 5 years old. Despite the primary closure of the wound, possible deviations from the normal healing process toward its chronicity, due to fibro-proliferative disorders. These deviations are characterized by excessive production and deposition of collagen, as well as a violation of the degradation process of the extracellular matrix. The correct approach for the treatment of burns may effectively influence the clinical outcome, without formation of a pathological scar. To the promising substances belongs melanin, which is produced of yeast fungi *Nadsoniella nigra* strain X-1.

In experiments were used immature rats in compliance with general ethical principles of experiments of animals. The animals were experimentally simulated the alkali burn of esophagus (ABE) with 20% NaOH. Scheme of the experiment was as follows: Group 1 - intact control; group 2 - rats, which modeled ABE, which was administered saline in the appropriate dose and timing (burn control); Group 3 - ABE rats, which was injected melanin at a dose of 1 mg/kg during 14 days. The material for the research selected at 7th and 21^v days. We had determined histochemical deposition of collagen fibers. In esophageal tissue investigated the level of matrix metalloproteinases (MMPs 1, 2, 8, 9) were analyzed by ELISA using sets of reagents Biotrack ELISA System. Also tested the gene expression (Col2a1) in esophageal tissue, were used Real-time PCR using sets of reagents Thermo Scientific Verso SYBR Green 1-Step qRT-PCR ROX Mix. To determine the reliability of the differences between the two samples we used the two-way ANOVA and Student test (t), differences p <0.05 were deemed reliable.

During the histological study, we determined the intensity of deposition of collagen fibers, and also measured the index of stenosis of the esophagus and assessed the effect of melanin on these processes, namely: less pronounced formation of collagen fibers in damaged esophagus tissues. Under the conditions ABE was increased the concentration of MMPs In esophageal tissue, comparison with the control. We have shown a decrease in the expression of a gene involved in the synthesis of Col2a1 collagen in esophageal tissues under the conditions of AEB 2. When melanin was administered, the level of expression of the Col2a1 gene in the esophagus tissues increased as compared to those in AEB 2.

In the application of melanin, the formation of collagen fibers were decreased, the normalization of the content of MMPs, increased expression of the Col2a1 gene, indicating the anti-fibrotic properties of these substances and demonstrates the promising use of melanin as a substance contributing to the healing of chemical burn of the esophagus without the formation of a pathological scar.

Tomas Lagunas

A massively parallel reporter assay to investigate the contribution of noncoding variation in autism spectrum disorder

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects ~1.7% of the population which leaves families and affected individuals with substantial lifetime costs. Current research has made significant investments in sequencing the genomes of ASD families as a diagnostic method and to further understand the genetic architecture of this complex disease. Whole Genome Sequencing (WGS) studies have revealed an enrichment of mutations in the untranslated regions (UTRs), which are noncoding regulatory regions, of ASD genomes. Assessment of these variations poses a challenge since these regions do not follow the triplet code and, even with prediction algorithms for RNA secondary structure or motif loss, these mutations must be defined experimentally. Furthermore, UTR sequences have been shown to have strong cell type dependency for their functionality which require assessment in appropriate cell types for context.

To address these challenges, I have used a Massively Parallel Reporter Assay (MPRA) to functionally assay several hundred mutations in parallel. MPRAs are a novel molecular genetic tool for assaying hundreds to thousands of predefined sequences for functional effects in a high-throughput manner. A library of 650 3' UTR elements, with and without variants, was synthesized in bulk. Each element was tagged with a unique sequence barcode and employed Post-Transcriptional Regulatory Element Sequencing (PTRE-seq) to enable counting of barcodes from the RNA of cells transfected with the entire library. This allows for massively parallel analysis of reporter activity. Additionally, I have coupled these technologies with Translating Ribosome Affinity Purification (TRAP) to facilitate analysis of ribosome bound transcripts which are indicative of translation efficiency. From a completed assay in a mouse neuroblastoma cell line that looked at 650 3' UTR mutations from ASD genomes, I have reported 10 candidate 3' UTR variations that appear to have functional effects on mRNA stability via PTRE-seq counts – indicating that some of these mutations may contribute to disease. Further, these candidate elements have been validated using a biochemical assay that measures expression of a reporter gene with these sequences in the 3' UTR position.

The benefits of MPRAs are that they are massively parallel, very flexible in design, high-throughput, and can be delivered to many cellular contexts; however, they are also limited in fragment size synthesis, do not provide the normal genomic context, and require selection of a cellular context. My research aims to use this technology to identify which elements in the genome are functional, identify which mutations can alter function, and test which class of elements contributes to ASD risk. These findings will justify the significant investments in sequencing patients with ASD by reporting on the burden of noncoding disease mutations and contribute to our understanding of ASD genetic architecture and UTRs.

Aster E. Pijning

TBA

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Background: Platelet aggregation is mediated by cross-linking of platelet aIIb β 3 integrin receptors by circulating fibrinogen. Numerous studies indicate that aIIb β 3 integrin function is controlled by vascular thiol isomerases. Five different vascular thiol isomerases (VTIs) are produced by activated platelets and endothelial cells and are essential for normal thrombosis in mice. We recently elucidated a function of one of the vascular thiol isomerases, ERp5. The Cys177-Cys184 disulfide bond in the β 3 subunit of aIIb β 3 integrin is cleaved by ERp5, which triggers release of fibrinogen from the activated integrin. We now report a functional role for an aIIb subunit disulfide.

Aims: To identify allosteric disulfide(s) in the aIIb subunit of platelet aIIbβ3 integrin.

Methods: The redox state of 7 of the 8 disulfide bonds in the aIIb subunit of aIIb β 3 was quantified by differential cysteine alkylation and mass spectrometry, both on the surface of human platelets and in purified protein, in the presence and absence of VTIs. To mimic the reduced disulfide, cysteines of interest were mutated to alanine and the integrin expressed on the surface of BHK cells. Activation of wild-type and disulfide mutant aIIb β 3 and binding to fibrinogen was measured.

Results: Two of the 7 disulfide bonds in the thigh domain of the aIIb subunit of the platelet integrin were 20-25% reduced in the purified protein. Analysis of aIIb disulfide bond mutants revealed a function for the Cys490-Cys545 disulfide in activation of the integrin and binding to fibrinogen. Expression of aIIb C490A or C545A mutant integrins was comparable to wild-type but the disulfide mutant integrin was insensitive to activation by Mn²⁺ and binding to immobilized or soluble fibrinogen was markedly impaired. No change in redox state induced by four VTIs; PDI, ERp5, ERp57, and ERp72 was observed. Involvement of a newly discovered VTI, TMX1, is currently being investigated.

Conclusion: We postulate that the redox state of the Cys490-Cys545 disulfide bond in the thigh domain of α IIb has a role in the conformational change associated with α IIb β 3 integrin activation. VTI control of this bond is unconfirmed and a current topic of investigation.

Sundararaghavan Pattabiraman

Vimentin protects differentiating stem cells from stress

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Vimentin is the first cytoplasmic intermediate filament to be expressed in mammalian cells during early differentiation, but its role in cellular fitness has long been a mystery. Vimentin is acknowledged to play a role in cell stiffness, cell motility, and cytoplasmic organization, yet it is thought to be dispensable for cellular function and organismal development. Here, we show that Vimentin plays a role in cellular stress response in differentiating cells, by directly binding aggregates and RNA-binding proteins, directing their elimination and asymmetric partitioning. In the absence of Vimentin, pluripotent embryonic stem cells fail to differentiate properly, with a pronounced deficiency in neuronal differentiation. Our results uncover a novel function for Vimentin, with important implications for development, tissue homeostasis, and in particular, stress response.

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RNA editing is a process of posttranscriptional modifications to the genetic information on RNA molecules. RNA editing by cytidine (C) to uridine (U) and Adenine (A) to Inosine (I) is common deamination phenomenon in animals and plants, whereas "reverse" U-to-C RNA editing due to amination is specific to plants. Pentatricopeptide repeat (PPR) proteins form a largest protein family that is particularly prevalent in land plants and includes 450 members in Arabidopsis thaliana. The DYW motif at the C terminus of many PPR editing factors contains residues conserved with known cytidine deaminase active sites, responsible for the Cytidine to Uridine RNA editing in 12-d-old seedlings. Because an amino transferase activity is reverse reaction of deaminase, the DYW motif in 12-d-old seedlings may be responsible for U-to-C conversion. In this method, Uridine aminotransferase catalyses the transamination of Uridine and aspartate, forming Cytidine and oxaloacetate. The oxaloacetate is then reduced to malate by malate dehydrogenase, while NADH is simultaneously converted to NAD. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the Uridine aminotransferase activity in the sample. And the artificial RNA Editing enzyme converting Uridine into Cytidine by Uridine aminotransferase activity, will also be transformed into the animal cell to restore the genetic code of mutated RNAs in vivo.

Temitope C. Ekundayo

Molecular characterisation and differential numerical delineation of DNA fingerprints of Plesiomonas shigelloides isolates into strain clusters

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Molecular signatures of Plesiomonas shigelloides strain specific to pathogenic and nonpathogenic variants are yet unknown. There is a fundamental need for the signatures to aid treatment of its infections and control of possible outbreak. Establishment of strain clusters would encourage comparative studies that could promote delineation of virulent strains from avirulent variants. In this longitudinal study, P. shigelloides were isolated from Tyhume, Kat and Kubusie river water samples in the Eastern Cape Province from February 2017 to December 2017. This study used a Plesiomonas-specific polymerase chain reaction method to characterise the isolates. Strain (dis)similarities were assessed using ERIC-PCR and (GTG)5-PCR DNA fingerprinting techniques. The DNA-fingerprinting products were electrophoresed, digitised and banding pattern documented via computer-assisted pattern analysis. Differential numerical analysis of the band occurrence matrices was performed for strains' clustering by neighbour-joining (NJ) using a Euclidean similarity index. Results revealed the 80% (44/55), 83.64% (46/55) and 80% (44/55) of water samples from Tyhume, Kat and Kubusie rivers, respectively were positive for P. shigelloides. The prevalence of P. shigelloides among isolates from sites ranged from 13.5% (12/89) to 88.9% (8/9). The NJ clustering of ERIC-fingerprints delineated 48 isolates to 8 and (GTG)5-fingerprints differentiated 34 isolates into 7 clades. The relative abundance of strains that made up each clade ranged from 6.3% (3/48, clade 3) to 22.9% (11/48, clade1) and 8.8% (3/34, clades 3,6&7) to 20.6% (7/34, clades 1&4) for ERICPCR and (GTG)5-PCR respectively. This study concludes that both fingerprinting approaches have good strain differentiating potential for P. shigelloides, however (GTG)5-PCR possessed higher resolution (20.6%) advantage over ERIC-PCR (16.7%).

Keywords: Plesiomonas shigelloides; DNA-fingerprinting; Neighbour-joining; Strains; Molecular characterisation

Swarna Kanchan

Responses of antioxidants to mitigate oxidative stress in cyanobacterium Scytonema spp.

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The present study aims to explore the responses of various antioxidants towards oxidative stress posed due to temporal changes in the cyanobacterium *Scytonema sp.* isolated from its natural habitat. Cyanobacteria, a group of ubiquitous photosynthetic organism are supposed to have evolved around 3.5 billion years ago and would have been the first oxygen evolving organism into the then oxygen free atmosphere leading to the development of aerobic metabolism.

Cyanobacterium at its natural habitat is exposed to various biotic as well as abiotic stresses posed by seasonal changes in ecophysiological conditions at its natural habitat. These stresses lead to the generation of Reactive oxygen species (ROS) that cause oxidative stress.

Results illustrated that *Scytonema spp.* under study possesses specific inbuilt potential to cope up with oxidative stress owing to the presence of an array of enzymatic and non enzymatic antioxidants which act in union to maintain the redox homeostasis. Native PAGE profile of catalase enzyme illustrated the presence of two isoforms of catalase enzyme during winter, summer and rainy season. However densitometric analysis revealed that the intensity of isoform I and II of the catalase enzyme were highest during summer season followed by winter and rainy season. Differential responses of non enzymatic polar and non polar solvent soluble photosynthetic pigments also elucidated their response to temporal variations at cyanobacterial natural habitat. Thus the responses and mechanisms employed by cyanobacteria may be implemented to design strategies elucidating their metabolic and genetic plasticity for further development of technologies for enduring stress tolerance potential of living organisms.

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Minu Kesheri

Temporal variations of Superoxide dismutase in bloom forming cyanobacterium Microcystis aeruginosa

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The present study aims to investigate the antioxidative response of the bloom forming cyanobacterium Microcystis aeruginosa inhabiting eutrophic pond at Durgakund Varanasi, India. Usually eutrophic to hypertrophic ponds readily support the flourishing growth of cyanobacteria due to the increase in nutrients, especially phosphorus and nitrogen in their diverse forms, the sources of which may vary from natural to anthropogenic ones. However a number of endogenous and exogenous stress factors such as high light intensity, hazardous radiations, carbon depletion, pollutant metal stresses etc. are known to cause an enhanced production of reactive oxygen species (ROS) leading to oxidative stress. Antioxidants reduce, neutralize, and prevent the damages caused by free radicals which are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures.

Results of native PAGE depict peculiar isoform patterns of the antioxidative enzyme superoxide dismutase owing to seasonal variations. Moreover variations in densiometric analysis of the isoforms depicted in native PAGE also illustrate the effect of temporal variations to which the cyanobacterium is exposed to in its own niche. Therefore it is evident that the cyanobacterium possesses stress tolerance potential owing to the production of enzymatic antioxidants as a part of defense response to the unfavorable environmental conditions existing in its natural habitat and may serve as source of antioxidants crucial in biotechnological applications.

The protein sequence of Fe-SOD in *Microcystis aeruginosa* retrieved from NCBI protein sequence database was used for *in silico* analysis. 3D structure of *Microcystis aeruginosa* was predicted by comparative modeling using MODELLER 9v11. Further, this model was validated for its quality by Ramachandran plot, ERRAT, and ProSA-web which revealed good structure quality of the model.

Niklas Mueller

Chemogenetic control of acute H₂O₂ production in cells with D-amino acid oxidase (DAAO)

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Background: Hydrogen peroxide (H_2O_2) has important redox signaling functions in the vascular system in response to growth factors, cytokines and calcium signals. These conclusions are often based on in vitro studies where exogenous H_2O_2 is added in supra physiological concentrations to cells or its production is induced by chemical compounds with uncharacterized effects. In order to define the consequences of endogenous H_2O_2 production on cell function, we here tested a chemogenetic way of controlled intracellular production.

Results: To induce acute intracellular production of H_2O_2 in HEK cells (human embryonic kidney cells) and HUVEC (human umbilical vein endothelial cells) D-amino acid-oxidase (DAAO) was overexpressed by a lentiviral system. This enzyme converts D-amino acids into imino acids and produces H_2O_2 as a byproduct. Subsequently, H_2O_2 production was increased by providing D-Alanine (D-Ala). Treatment with D-Alanine but not L-Alanine (1-10mmol/L) led to a dose-depend production of H2O2 as measured by chemiluminescence with Luminol/HRP and Amplex red^{*}. PEGylated catalase (250U/mL) reduced this signal by approx. 50% whereas the DAAO inhibitor 4h-furo[3,2-b]pyrrole-5- carboxylic acid (1µmol/L) completely blocked H_2O_2 .production. DAAO-derived H_2O_2 (3mmol/L D-Ala, 10min) had intracellular signaling functions as it increased oxidation of peroxiredoxin 3 and phosphorylation of p38 MAP kinase.

Conclusion: The DAAO system is a valuable tool to study dynamic changes in physiological redox signaling.

Parastoo Karimian

Functional analysis of the Arabidopsis non-characterized Leucinerich receptor-like kinase, LRR2, interacting with defence-related G-proteins

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The first step in plant immunity is the recognition of potential pathogens by receptors located on the cell surface that mostly belong to the receptor-like kinase (RLK) family. The largest subgroup of this family is the Leucine-rich receptor-RLK (LRR-RLK). heterotrimeric G proteins (G proteins) are conserved multifunctional signalling elements in Eukaryotes with an active role in the plant immune response. G proteins consist of three distinct subunits Ga, Gβ and Gγ. Upon activation, Ga and the Gβγ dimer independently trigger multiple downstream effectors mediating specific signal pathways. We have performed the initial characterization of LRR2, a LRR-RLK showing physical interaction with G proteins as well as other defence related proteins.

The lrr2 single mutant showed enhanced resistance to *Fusarium oxysporum*, however, it exhibited similar responses as wild-type to *Alternaria brassicicola*, and *Pseudomonas syringae pv. tomato* DC3000. The double lrr2 agb1 mutant showed increased sensitivity to *F. oxysporum* compared to WT, but identical sensitivity levels than the single agb1 mutant, suggesting that LRR2 and AGB1 might signal in the same defence pathway. In addition, we found that lrr2 mutant displayed reduced callose deposition in response to flg22 compared with WT. These results suggested that LRR2 might trigger multiple pathways in *Arabidopsis* defence. The functional characterization of LRR2 will be valuable to understand the involvement of plant-specific RLKs in plant immunity.

Keywords: Arabidopsis, receptor-like kinase, LRR2, plant immunity, G-proteins, F. oxysporum, A. brassicicola, P. syringae, Callose deposition.

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The objective of this study was to assess genetic diversity and population structure of 85 cowpea genotypes using DArTseq genotype by sequencing technique. From a total of 15 284 SNPs tested, only 640 (48.09%) were polymorphic. Chromosome 2 had the highest percentage of polymorphic loci (56.51%) while chromosome 5 had the lowest (39.12%). The highest and the lowest Ho, He and PIC values were recorded from Chromosome 10 and chromosome 2, respectively. The genetic distance value estimated on the basis of SNP markers ranged from 0.14 to 0.44, with a mean value of 0.35. The mean values of total Shonnan information index (I) for genetic diversity was 0.355 while average observed gene diversity (Ho) within genotypes per population was 0.050. Average gene diversity (He) within genotypes per population was 0.232. Average inbreeding coefficient (FIS) was 0.740 and overall mean percentage of polymorphic loci (%P) was 83.68. The Shonnan information index (I) values ranged from 0.325 to 0.381; Ho values varied from 0.047 to 0.050. He values were from 0.216 to 0.247; FIS from 0.717 to 0.770 while %P was from 73.59 to 92.31. Variance among populations was (8 % of the total variation), while the variance among individuals was high (78 % of the total variation). Red cluster only had four accessions (4.76% of the population) blue cluster had 29 accessions (34.5% of the population) while the black cluster had the majority of accessions at 51 (60.71%).GH43, GH45 and GH50 were from IITA while GH 47 was from South Africa.

Maria Fando

SmAP from Halobacterium salinarum has two different forms with the same structure but a different RNA-binding affinity

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Lsm (Sm-like) proteins are found in representatives of all the three domains of life. The functions of bacterial and eukaryotic Lsm proteins have been studied well. *Eukaryotic Sm/Lsm* are part of various RNP particles and are involved in the RNA processing. Bacterial Lsm protein Hfq exhibits RNA-chaperone activity, facilitate the interaction of egulatory rRNA with mRNA, thus regulating gene expression. Nowadays, there is a little information about the functions of the archaeal Lsm proteins SmAP, some data shows that they appear to be involved in the processing of some RNA (such as tRNA).

Our work concerns structural and functional studies of SmAP from *Halobacterium* salinarum. The protein has no unstructured N- and C-terminus or extended loops between secondary structure elements thus representing a minimal Lsm core. However, in the genomic data we have found a variant of the protein with nine amino acids length N-terminal extension. Both variants of the SmAP from *H. salinarum* have been isolated and their affinity to oligo(A) and oligo(U) RNA have been measured. A difference in the affinity of the alternatives to the RNA has been found. Besides, the proteins were crystallized and X-ray diffraction data has been collected at ERSF in Grenoble. SmAP from *H. salinarum* has the characteristic for the Lsm proteins doughnut-shape form with seven monomers organized into a torus.

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Triana Amen

Stress Granules regulate cell metabolism through clustering of mitochondrial membrane protein VDAC

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Stress Granules are membraneless organelles formed transiently during stress through phase separation of multiple RNA-binding proteins, including FUS, TDP43, and PABPC1, and clustering of other components (Buchan and Parker, 2009; Buchan et al., 2011; Kedersha and Anderson, 2007; Reineke et al., 2018). Stresses, including heat shock, starvation, oxidative stress, arsenite, and translation inhibition lead to formation of Stress Granules (Arimoto et al., 2008; Buchan et al., 2011). Stress Granules act as a signaling center during stress regulating a diverse set of cellular functions, including nucleocytoplasmic transport, mTOR kinase signaling, translation, and apoptosis (Kedersha et al., 2013; Takahashi et al., 2013; Zhang et al., 2018). We study how Stress Granules regulate cellular metabolism and energy production. In order to do that in live cells in a physiological setting we endogenously tag PABPC1 and TDP43 using CRISPR/Cas9 in human cell culture.

We present evidence of a novel metabolic function for Stress Granules. Using a Mass Spectrometry BioID approach (Roux et al., 2012) and confocal imaging we show that Stress Granules promote clustering of mitochondrial outer membrane porin – VDAC2. We demonstrate that Stress Granule-induced VDAC clustering is a remarkably conserved process from yeast to human cells, which depletes VDAC from the mitochondrial membrane and impairs its function. We show that VDAC2 clustering is regulated by GSK3 signaling, which in turn is regulated by Stress Granule formation. Finally, using metabolic analysis, we confirm a previously proposed function for VDAC2 in fatty acid trafficking across the mitochondrial membrane, which is necessary for cells to survive starvation stress.

Stress Granules are implicated in the molecular pathology of Amyotrophic Lateral Sclerosis (ALS) (Dewey et al., 2012; Lenzi et al., 2015; Li et al., 2013; Mackenzie et al., 2017). The role of Stress Granules in disease development is attributed to aberrant aggregation of its components, however the actual cause of disease pathology is unclear (Dewey et al., 2012; Mackenzie et al., 2017). Our model of SG functioning explains metabolic alterations, namely a high reliance on fatty acid oxidation, in the models of ALS and can be used to develop early bio markers.

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Ivan Sorokin

Effective formation of polyribosomes in mammalian cell lysate-based cell-free translation system

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The polyribosomes (or polysomes) are large macromolecular complexes consisted of several or dozens ribosomes associated with the single mRNA molecule. These beautiful assemblies may take a different form such as circles (most active in translation), plane spirals, 3-dimensional helixes (stalled polysomes), zigzags and others. Observation of polysomes was established long ago by an electron microscopy (EM). Contrasting techniques were used. Nowadays we can use advanced methods like a Cryo-EM to obtain nanometer- (by tomography) and ångström-scale (by single-particle analysis) structural models of ribosomes and polysomes. For example, cryo-EM studies revealed transition between different types of polysomes. However, in several cases structural data does not provide us information about intra-polysome movements of ribosomes, thus, about principles of different types of polysomes formation.

Recently we developed the way to visualize each ribosome into polysome by a fluorescence super-resolution microscopy (STORM/PALM). In order to study processes of polysome formation we needed an efficient system to mark each ribosome by a fluorophore. Genetically-encoded fluorescent markers such as photo-activated fluorescent proteins are preferable. Thereby, we would like to obtain genetically engineered cell-line that stably produce fluorescent ribosomes. The cell-free translation system based on those chimeric ribosomes should be capable of translation on endogenous polysomes or de novo formation of polysomes and their stable generation during long-time period. And here we meet an obstacle. The mammalian cell-free systems as widely known, basically does not generate new polyribosomes. Such cell-free systems mostly lacking the activity of initiation step limited by eIF2 α phosphorylation during lysis of cells. Another possible reason of poor polysomes in mammalian systems is a low concentration of polysomes in reaction mix (2-5 OE/ml, A260). Our previous studies on wheat germ extract (WGE) based systems shows that minimal concentration of polysomes should be 15 OE/ml or above.

And there are several ways to avoid eIF2 α inhibition caused by phosphorylation: usage of small chemical kinases inhibitors during lysate preparation; short-time treatment of cells with lysolecithin detergent; lysate preparation from cells with S51A mutation in eIF2 α gene (totally suppress phosphorylation); usage of cell-free system reconstructed from purified ribosomes from mammalian cell-culture and complemented with RLL (rabbit reticulocyte lysate) or extracted translational factors.

To obtain highly-active *in vitro* translation system with capability to stable formation of polysomes we used HEK293 culture and implemented lysis procedure with lysolecithin (LL) treatment. As a second way, purified ribosomes of HEK293 cells complemented with RRL were used. This way is more convenient for concentration control. These two systems were tested in batch and continuous exchange modes (CECF). Both systems supplied with dialysis are capable to generate polyribosomes during 1-2 hours of translation. Activity of LL cell-free assay is less than RRL-supplemented system. Thus, the cell-free system reconstructed from purified human ribosomes and RRL has more advantages and is more preferable.

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