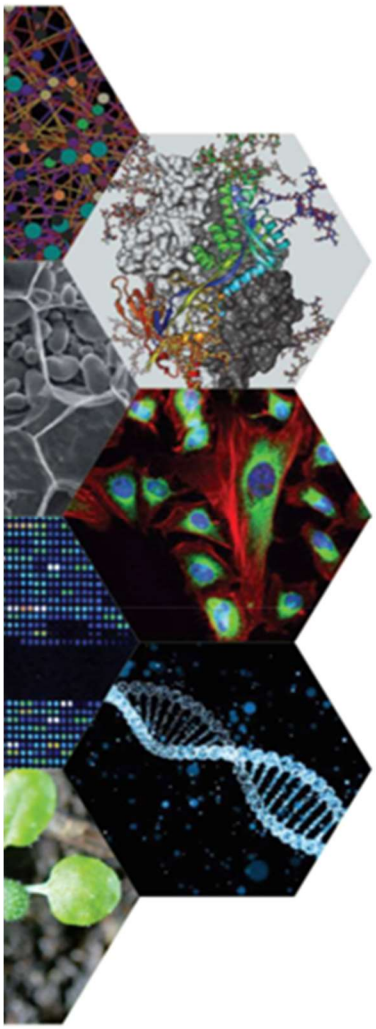
CEITEC



3rd Annual CEITEC Postdoc Retreat

22-23/09/2022

Zámek Křtiny

Dear Participants,

Welcome to the 3rd Annual CEITEC Postdoc Retreat, held 2nd time in the beautiful Chateau Křtiny in scenic South Moravia.

This event is connected to the Postdoc Appreciation Week (19-23 September) to support postdocs at different stages of their career. We hope that this meeting will help us to bring together postdocs from CEITEC consortium with our colleagues from partner institutions. We are looking forward to get an overview about the CEITEC Core Facilities and improve our presentation skills during a half-day science communication skills workshop with an excellent professional lecturer. During this meeting we will have an ample opportunity to get feedback, connect with fellow postdocs at CEITEC, foster networking, friendship, and new collaborations that will enrich the scientific community in this region. We look forward to the presentations, discussions, and interactions between attendees, and hope this will contribute to a successful meeting.

We thank our institutes and TWINNING project for their kind support that made this meeting possible. We hope this retreat will prove to be an exciting and stimulating time for all of us, and that we all do our part to realize this goal.

Sincerely,

3rd Annual CEITEC Postdoc Retreat Organizing Committee:

Khadija Hajji, Pavel Payne, and Maria Zlobina.

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Program

22nd September

Time	Event
9:00	Bus departure from Brno Campus Bohunice (CEITEC MU)
10:00	Registration with coffee break
10:30	Welcoming from PPC
10:45	Welcome lecture by CEITEC MU Director Jiří Nantl (online)
11:00	Zuzana Cieniková
11:15	Mateo Seoane Blanco
11:30	Ahmed H. Hassan
11:45	Lunch
12:35	Speed networking activity
13:00	Welcoming talk from Pavel Tomančák
13:15	CEITEC Core Facility overveiw by Daria Kuchařová
13:45	Vojtěch Bystrý
14:00	Petr Lepcio
14:15	Milad Takhsha
14:30	Hassan Zafar
14:45	Coffee break
15:15	Science communication in CEITEC by Ester Jarour
15:30	Workshop on presentation skills by Samuel Lagier 1st part
17:00	Coffee break
17:30	Poster Session
18:30	Dinner
19:30	Round table discussions
20:00	Evening Social

23rd September

Time	Event
8:30	Breakfast
9:30	Workshop on presentation skills by Samuel Lagier 2nd part
11:00	Coffee break
11:30	Neha Shukla
11:45	Vivek Raxwal
12:00	Barbara Wójcikowska
12:15	Aleš Obrdlík
12:30	Awards and closing remarks
12:45	Lunch
14:00	Hiking in the forest near the Venue
16:00	Bus departure from Chateau Křtiny

Information

Posters:

Please check the page of your poster in the abstract book and match it with the number on the stand. Please mount your poster after arrival at conference venue. Poster presentation will take place during and after the last coffee break in the first day of the retreat. Discussions and poster browsing will be possible both days at any time.

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Catering and retreat dinner:

The registration fee includes food and beverages during lunch and the retreat dinner buffet on September 22nd as well as the lunch on September 23rd, and coffee, tea, beverages and snacks during all coffee breaks throughout the meeting.

Hiking in the forest:

On Thursday September 23rd, in the afternoon after lunch, we offer a hiking in the forest in the nearby Moravian Karst. Departure from the reception at the retreat venue will be at 14:00.

Wi-fi internet connection:
Network name: zamek–krtiny
Password: zamekkrtiny

Welcoming talks

Pavel Tomančak

Executive Director of CEITEC

Pavel is renowned expert in evolutionary and developmental biology and has been research group leader since 2005 at the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden. His laboratory at the Max Planck Institute focusses on the study of regulation and evolution of gene expression during embryonic development of multi-cellular organisms. He continues to lead this research group also as a director of the CEITEC consortium. His research is among others unique in that it combines advanced molecular biology with state-of-the-art microscopy techniques and advanced computational analysis of microscopic images. Pavel Tomancak also has a very close relationship with the city of Brno, because it was here that he began his scientific career as a student of molecular biology and genetics at Masaryk University.

Jiří Nantl

Director of CEITEC Masaryk University

Jiří Nantl graduated in Political Science at the Faculty of Social Studies, Masaryk University, then Law at the Faculty of Law and Corporate Law at Nottingham Trent University. He was Director of Higher Education in the Ministry of Education, Youth and Sport, Czech Republic. Later, as a politician in the government of Petr Nečas, he served as First Deputy Minister of Education, Youth and Sports. He was an architect of higher educational legislation reform and the National Educational Strategy 2014-2020. Prior to his role as Director of CEITEC Masaryk University, at Masaryk University he worked as chancellor, director of legal and corporate relations, secretary of the Board of Trustees and chairman of the Editorial Board.

Invited speakers

Daria Kuchařova

Project manager
Grant Administration Department
CEITEC MU

The Core Facilities of CEITEC Masaryk University are scientifical technological units equipped with state-of-the-art instrumentation that is available to researchers from a range of fields, especially in life sciences. The aim is to provide the infrastructure for academic, as well as commercial users from the Czech Republic and abroad to allow them to effectively carry out their scientific projects, with support from experts.

Ester Jarour

PR and
Communication
Manager CEITEC MU

For all scientists it is important to know how to present their research and transmit their knowledge to non-specialists. Learning how to demonstrate the practical impact of research to the general public is considered critical in order to achieve long-term support for the scientific endeavours in member institutions and their home countries.

Presentation Skills Workshop

Samuel Lagier

Founder of SamSpeaksScience

Started his research as a PhD student at the Pasteur Institute, he performed a first post-doc at the Rockefeller University in New-York and a second post-doc at the University of Geneva.
SamSpeaksScience emerged from the aggregation of many interests. They have published articles in diverse research fields (neuroscience, biochemistry, biophysics, ecology, evolution…), they also perform improvised comedy and explore the interface between art and science. Their insights on how to face and engage an audience reflects their love for science, teaching and the performing arts.

A passion is a thing you talk about, not something you keep secret. Over the years, we have come to realise that a lot of driven scientists were struggling to share their passion and their vast knowledge, even with their close colleagues. Because enthusiastic experts are delightful to learn from, we created SamSpeaksScience to empower scientists to unleash their - too often confined - knowledge and passion. Science is a beautiful discipline, a noble endeavor that the world - not just a happy few - should benefit from. In a time where facts have become untrustworthy for some, it is important to know how to have an impactful discourse about science and scientific knowledge.

At this workshop you will learn how to face and engage an audience, how to work on the structure of the content, on the delivery or on the visual support of the presentations.

Participation of Dr. Samuel Lagier was supported from European Regional Development Fund-Project „HR4MU“ (No. CZ.02.2.69/0.0/0.0/16_028/0006244).

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

ORAL PRESENTATIONS

CEITEC Bioinformaticians and Where to Find Them

Vojtěch Bystrý,[a]

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Next-generation sequencing (NGS) is becoming the method of choice for an ever-growing number of molecular biology applications for research and clinics. With its significantly dropping costs, it recently started replacing the classic molecular biology laboratory techniques. The well-known bottleneck of NGS experiments is the subsequent bioinformatics analysis. Therefore, good bioinformatics support on the institutional level is a key element for molecular biologists and their labs to thrive and be successful.

Here we present the mysterious individuals working at CEITEC MU as bioinformaticians and the core facility that serves as a hub for their efforts. We would present what they work on, what tools they use, and, most importantly, how they can help **you** with **your research!**

Figure 1. The essential tool developed and used in CETIEC Bioinformatics Core Facility for NGS data analysis

Genome Anchoring, Retention, and Release by Neck Proteins of Phage 812

Zuzana Cieniková,[a] Marta Šiborová,[a] Barbora Břenková,[a] Tibor Füzik,[a] Martin Benešík,[b] Roman Pantůček,[b] and Pavel Plevka*[c]

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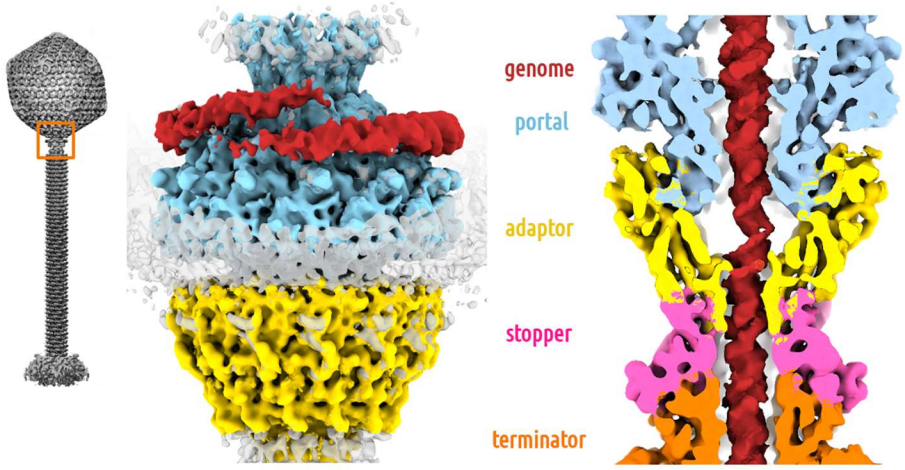
Virions of tailed phages are complex molecular machines that protect and deliver phage DNA into host cells to initiate infection. The virion of *Herelleviridae* phage 812 is formed by an icosahedral capsid and a contractile tail joined together by neck proteins. Despite the role of the neck proteins in virion assembly, DNA packaging, and regulation of genome release, their structures and mechanisms of action are not well characterized. We show that the neck of phage 812 consists of portal, adaptor, stopper, and tail terminator proteins. A dodecameric DNA-binding site on the portal complex anchors a segment of the phage genome inside the capsid. The DNA is recognized through minor groove base readout and wrapped around the portal complex by stabilization of narrowed minor groove segments. The circular anchoring affects the spatial organization of the packaged genome and may also prevent an accidental escape of the DNA during the initial stage of genome packaging. The adaptor complex forms a chamber that induces a local B-to-A-form transition in the DNA. The transition appears to be sequence-sensitive and dynamic and thus may serve to strategically pause the DNA progress through the neck. Gating loops of the stopper complex prevent premature genome ejection from fully packaged capsids by blocking the neck channel prior to the tail attachment. The binding of the tail terminator complex to the stopper complex induces opening of the gating loops and advancement of DNA towards the tail, priming the genome for release. Our results explain how the neck and tail proteins direct genome packaging, prevent premature genome release, and enable genome ejection upon infection in phage 812.



Translation Inhibition in RimM Deletion Bacterial Cell Lines

Ahmed H. Hassan [a], Yuko Nakano [b], Gregor Blaha [c], Ya-Ming Hou [b] and Gabriel Demo [a]

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[b] *Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, USA.*
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In stress or limited nutrients in the environment, bacteria need to save energy by slowing down protein synthesis, since synthesis of protein is a major energy draining source [1]. Ribosome silencing factor (RsfS) has a key role in decelerating the protein synthesis by blocking the formation of an intact 70S ribosome [2]. On the other hand, the ribosomal maturation factor RimM is one of the important factors for the maturation of the 30S small ribosomal subunit [3]. Here we use biochemical studies as well as cryo-electron microscopy to understand the close relationship of RimM with RsfS.

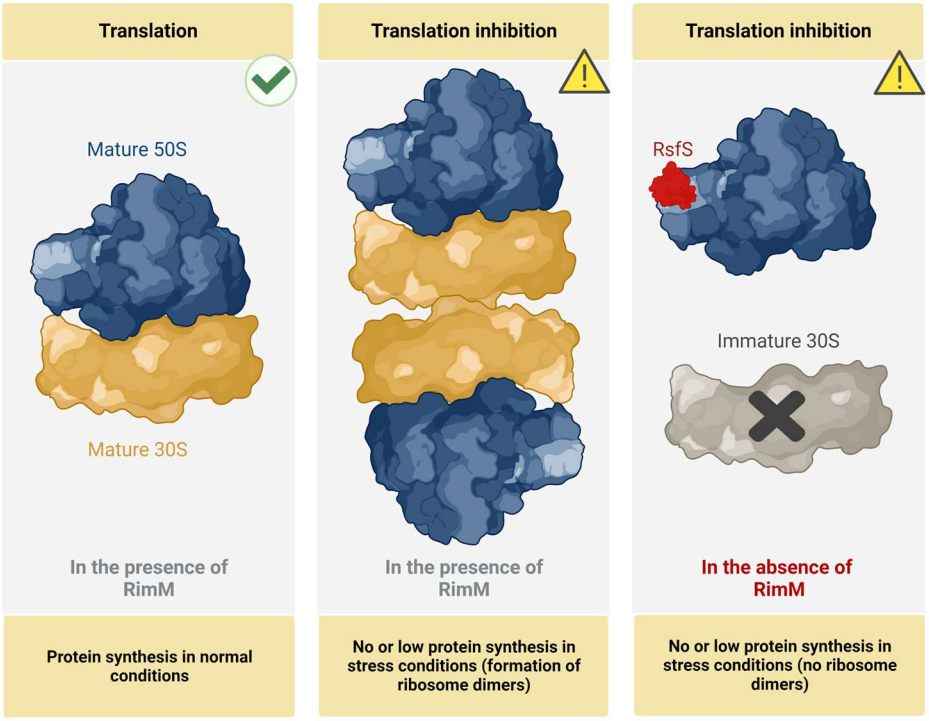

Figure 1. Translation inhibition in the absence of RimM. In normal conditions, bacteria with intact 70S ribosomes have normal protein synthesis. Under stress or nutrients deprived conditions, bacterial 70S ribosomes dimerize to form 100S and inhibit protein synthesis. Deletion of RimM result in accumulation of RsfS as well as inhibiting translation without formation of 100S.

References

[1] Starosta, A. L., Lassak, J., Jung, K. & Wilson, D. N. The bacterial translation stress response. FEMS Microbiol. Rev. 38, 1172–1201 (2014)
[2] Li X, Sun Q, Jiang C, Yang K, Hung LW, Zhang J, Sacchettini JC. Structure of Ribosomal Silencing Factor Bound to Mycobacterium tuberculosis Ribosome. Structure. 2015 Oct 6;23(10):1858-1865. doi: 10.1016/j.str.2015.07.014. Epub 2015 Aug 20. Erratum in: Structure. 2015 Dec 1;23(12):2387. Erratum in: Structure. 2015 Dec 1;23 (12):2387. PMID: 26299947; PMCID: PMC4718548.
[3] Suzuki S, Tatsuguchi A, Matsumoto E, Kawazoe M, Kaminishi T, Shirouzu M, Muto Y, Takemoto C, Yokoyama S. Structural characterization of the ribosome maturation protein, RimM. J Bacteriol. 2007 Sep;189(17):6397-406. doi: 10.1128/JB.00024-07. Epub 2007 Jul 6. PMID: 17616598; PMCID: PMC1951897.

Functional Nanofillers in Photoinitiated Polymerization and Vat 3D Printing

Petr Lepcio,[a] Martina Korčušková,[a] Veronika Sevriugina,[a] Juraj Svatík,[a] Wiktoria Tomal,[b] Joanna Ortyl,[b] and František Ondreáš*[a]

[a] *Central European Institute of Technology, Brno University of Technology, Purkyňova 656/123, Brno 612 00, Czechia.* [b] *Faculty of Chemical Engineering and Technology, Cracow University of Technology, Warszawska 24, 31 155, Cracow, Poland*
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Nanofillers can introduce or enhance functional properties of polymers such as thermal, mechanical, electric, magnetic, light absorption, or flame retardancy (Figure 1). Their addition to a 3D printing photocurable resin is an elegant and relatively simple approach benefiting from the good dispersing capability of the liquid medium, bringing the 3D printing versatility to a whole new level (Figure 1). On the other hand, nanofillers also interact with the photopolymerization reaction, the fundamental process in the vat 3D printing. Zinc oxide nanoparticles, for instance, cause a localized photothermal effect influencing the cure profile in their vicinity. Alumina doping suppress this effect of pristine zinc oxide and contributes to the curing by the own photoactivity of the doped nanoparticles. Simultaneously, nanoreinforcement and light scattering affect the critical energy and cure depths of the resin. Carbon nanofillers such as nanotubes, carbon black, graphene and its derivatives are favored for improving electrical conductivity and, sometimes, the mechanical properties. Yet, they often increase the viscosity and absorb light in broad range of wavelengths, making the printing particularly challenging. Most of these factors are closely tied to the quality of dispersion and its stability in time. For instance, aggregated carbon nanotubes have smaller adverse effect on the viscosity and printability of the nanocomposite resins than the dispersed ones, yet it contributes less to the conductivity either. Hence, the investigated effects together with our previous extensive research on nanoparticle spatial organization in freely adsorbing polymer solutions, i.e., systems analogical to a two- and more component monomer mixture, show the path towards the design of functional nanocomposite photocurable resins for vat 3D printing.

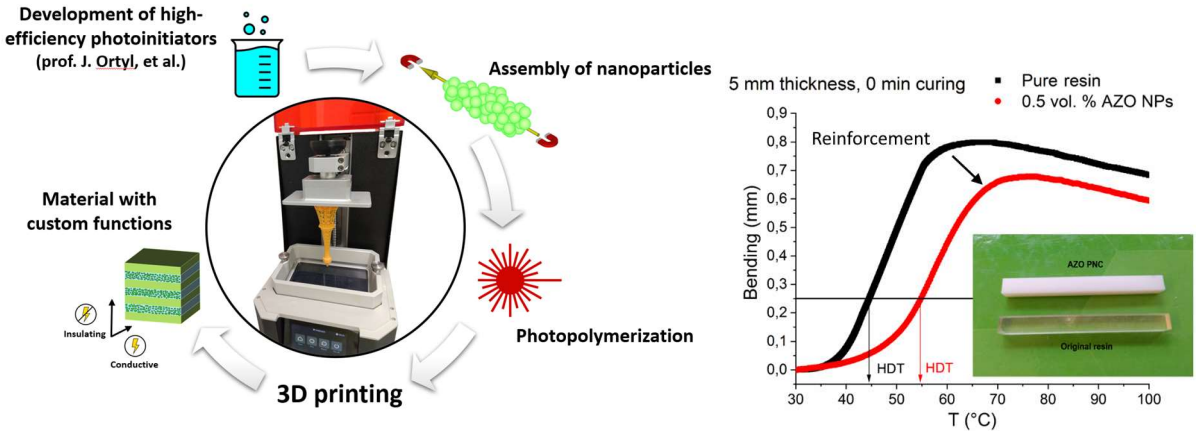

Figure 1. Scheme of the vat 3D printing process of functional nanocomposites (left), example of enhanced mechanical properties by AZO NPs.

Importance of N6-methyladenosine levels for cellular homeostasis and DNA repair

Ales Obrdlik[a], Veronika Rajecka[a], Praveenkumar Rengaraj, Veronika Kozlova[a], Linda Kasiarova[a], Martin Hamrik[a], Karolina Vavrouskova[a], Michal Smida[a], Stepanka Vanacova[a]

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N6-methyladenosine (m6A) and N6,2′-O-dimethyladenosine (m6Am) are modifications present across various classes of RNA in all higher eukaryotes. m6A(m) affect nearly every phase of the RNA life cycle, from nuclear processing to cytoplasmic RNA surveillance. We have recently characterized *in cellulo* protein interactomes of the key enzymes engaged in the m6A and m6Am pathway, using proximity labelling and mass spectrometry [1]. These studies pointed to physical interactions between the m6A and DNA replication/repair factors.
Here, we present follow-up studies addressing the relevance of the identified interactions in a genome, proteome, and RNA-interactome-wide context. While our findings widely confirm our data on the interactome of the m6A eraser ALKBH5, they also highlight new, unprecedented functions for the eraser protein FTO. We report here new isoform-specific functions of FTO in human cells, pointing towards its engagement at the intersection of cellular homeostasis and DNA repair.

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References

[1] Covelo-Molares H., Obrdlik A., Poštulková I., Dohnálková M., Gregorová P., Ganji R., Potěšil D., Gawriyski L., Varjosalo M., & Vaňáčová Š. *Nucleic acids research* **2021,** *49*, 10895–10910.

Transcriptional and post-transcriptional regulation of young genes in plants

Vivek Kumar Raxwal[1,2,], Somya Singh[1,], Manu Agarwal[1] and Karel Riha[2]

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2 Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic
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New genes continuously emerge from non-coding DNA or by diverging from existing genes, but most of them are rapidly lost, and only a few become fixed within the population. We hypothesized that young genes are subject to transcriptional and post-transcriptional regulation to limit their expression and minimize their exposure to purifying selection. We found that young genes in rice have relatively low expression levels, which can be attributed to distal enhancers, and closed chromatin conformation at their transcription start sites (TSS). The chromatin in TSS regions can be re-modelled in response to abiotic stress, indicating conditional expression of young genes. Furthermore, transcripts of young genes in Arabidopsis tend to be targeted by nonsense-mediated RNA decay, presenting another layer of regulation limiting their expression. Together, these data suggest that transcriptional and post-transcriptional mechanisms contribute to the conditional expression of young genes, which may alleviate purging selection while providing an opportunity for phenotypic exposure and functionalization.

Salmonella Virus Epsilon15 Tailspike Has Multiple Binding Sites and Two Catalytic Activities.

M. Seoane-Blanco[a]‡, A. Pereda-Hernández[b], N. K. Broeker[c], M. McConnell[d], J. Cañada[b], S. Barbirz[3], M. J. van Raaij*[1]

[a] *Dpt of Macromolecular Structure, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain.* [b] *Dpt of Structural and Chemical Biology, Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Madrid, Spain.* [c] *Dpt of Physical Biochemistry, Universität Potsdam, Germany.* [d] *Point Loma Nazarene University, San Diego, USA.* ‡ *Current address: Central European Institute of Technology, Masaryk University, Brno, Czech Republic.*

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Bacteriophages infect bacteria, including pathogenic ones, and have a huge influence on ecological processes. In the future, they may help to treat diseases caused by antibiotic-resistant bacteria. Many bacteriophages use their tailspikes to identify and to adsorb to its host bacteria. Consequently, they control the phage host range. The *Salmonella virus Epsilon15* tailspikes, homotrimers of gp20, bind to the O-antigen of the *Salmonella* serovar Anatum lipopolysaccharide[1,2].

In this project, we determined the structure of the O-antigen:tailspike complex. Each Epsilon15 tailspike monomer is composed of four domains, from the N- to the C-termini: the phage-binding domain, the β-helix domain, the β-sandwich domain and the petal domain. The petal domain contains a fold typical of the SGNH esterase family. Four oligosaccharides of the Anatum O-antigen oligosaccharides bind to three domains of gp20. Two oligosaccharides are located next to the endorhamnosidase site in the β-helix domain. Another oligosaccharide is placed in the petal domain esterase binding site (Fig 1.). NMR spectroscopy and site-directed mutation experiments revealed an endorhamnosidase active site at the junction of the two β-helix binding sites and an esterase site in the petal domain. The structural and functional data suggests a dual function for the bacteriophage epsilon15 tailspike: de-acetylation of the O-antigen, which may affect local structure and polysaccharide flexibility, plus cleavage of the O-antigen to allow the bacteriophage to approach the bacterial membrane.

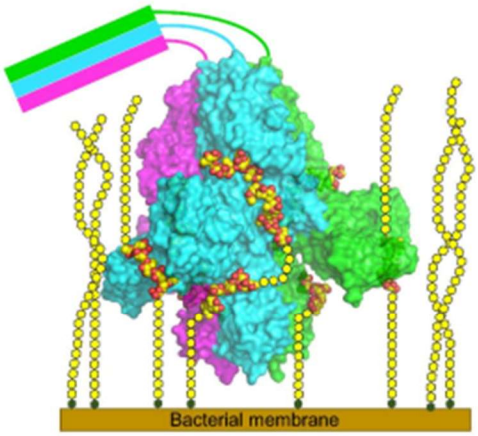

Figure 1. Epsilon15 tailspike in action. The chains of the trimeric tailspike are depicted in cyan, green and magenta. The fragments of the O-antigen are coloured yellow and red. The rest of the lipopolysaccharides were drawn as yellow and dark green hexagons.

References

[1] Murata, K., Liu, X., Danev, R., Jakana, J., Schmid, M. F., King, J., Nagayama, K., & Chiu, W. *Structure* **2010**, 18(8), 903–912.

[2] Sechter, I., & Sechter-Mooreville, R. *Arch. Virol.* **1990** 113(3), 297–297.

ROLE OF MRNA DECAPPING IN MEIOSIS PROGRESSION

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Meiosis is a specialised cell division that produces haploids from diploid cells. In plants, it marks the transition from the sporophytic stage to the gametophytic and requires extensive reprogramming of the cell division machinery from mitosis to meiosis and, upon formation of haploid gametes, back to mitosis. During the second meiotic division, a plant germline-specific protein TDM1 is incorporated into Processing bodies (P-bodies) through interaction with the nonsense-mediated RNA decay (NMD) factor SMG7 [1]. In addition to its role in NMD, SMG7 perform a crucial function in meiosis germline differentiation in plants. Interestingly, this function seems distinct from its role in NMD [2,3]. Mutations in SMG7 or TDM1, lead to third meiotic division and fail to produce microspores, resulting in male sterility. A forward genetic suppressor screen in the background of *smg7-6* mutants that exhibit reduced fertility was conducted, where we identified a mutation in the EVH-1 domain in decapping 1 (DCP1), a critical component of P-bodies. P-bodies are membrane-less, cytoplasmic ribonucleoprotein granules and are known to be hubs of RNA processing. Research in the past decade indicates that the fate of mRNAs associated with P-bodies is either degradation via decapping or storage for later translation. Control of cellular gene expression via mRNA degradation is crucial to maintain the abundance and life span of cellular mRNA. The DCP1 EVH1-like domain is highly conserved among eukaryotes and is predicted to have proline-rich sequences (PRS)-binding activity suggesting it acts like a protein-protein interaction module that recruits specific proteins for decapping, followed by decay. Our phenotypic analysis showed that the *dcp1-4* mutation is among the strongest suppressors of the meiotic defect of *smg7*. This indicates that RNA turnover is a critical determinant of meiotic progression and anther development in plants. Here, we aim to dissect the molecular function of SMG7 and DCP1 during meiosis progression in Arabidopsis.

References

[1] Cairo A., Vargova A., Shukla N., Capitao C., Mikulkova P., Valuchova S., Pecinkova J., Bulankova P., Riha K. *Science* **2022**, 377(6606):629-634. DOI: 10.1126/science.abo0904.

[2] Capitao C.*, Shukla N.*, Wandrolova A., Scheid O.M., Riha K. *Front Plant Sci.* **2018**, 9:1602. doi: 10.3389/fpls.2018.01602.

[3] Riehs N., Akimcheva S., Puizina J., Bulankova P., Idol R. A., Siroky J., et al. *J. Cell Sci.* **2008**, 121, 2208–2216. doi: 10.1242/jcs.027862.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic, European Regional Development Fund-Project “REMAP” (No. CZ.02.1.01/0.0/0.0/15_003/0000479)

Shape-Memory Alloys: The Material is a Machine

Milad Takhsha,[a] Jon Ander Arregi,[b] Francesca Casoli,[a] Vojtěch Uhlíř,[b,c] and Franca Albertini[a]

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Smart technologies tend to maximize their efficiency as well as to minimize the required material, human, and energy resources. Smart materials typically undergo extensive change of properties upon applied external stimuli. These materials are capable of converting different forms of energies as well as performing tasks in a partially or fully autonomous way. Among the smart materials, piezoelectrics, magnetostrictive materials, and shape-memory alloys (SMA) are of a special interest due to their high efficiency and versatility. In the SMA, the external stimuli, i.e., stress, magnetic field, and temperature gradient can lead to a reversible change of shape and properties of the material, which can be exploited towards a vast variety of applications. As a sub-class of SMA, there is a ternary Heusler compound, known as 'Ni_2MnGa', that is a representative member of a group called magnetic shape-memory Heuslers.

The concept of smart materials gains a special interest in small-scale environments (in the range of micro and nanometer), where the mechanical simplicity, compactness, intelligence, and efficiency of the material offer remarkable advantages for different application fields.

In the deemed presentation, we will briefly discuss the concept of the (magnetic) shape-memory effect (Figure 1) and different applications in biology, robotics, and aerospace. Then, we will briefly outline our activities at IMEM-CNR in collaboration with CEITEC BUT related to magnetic shape-memory Heuslers.

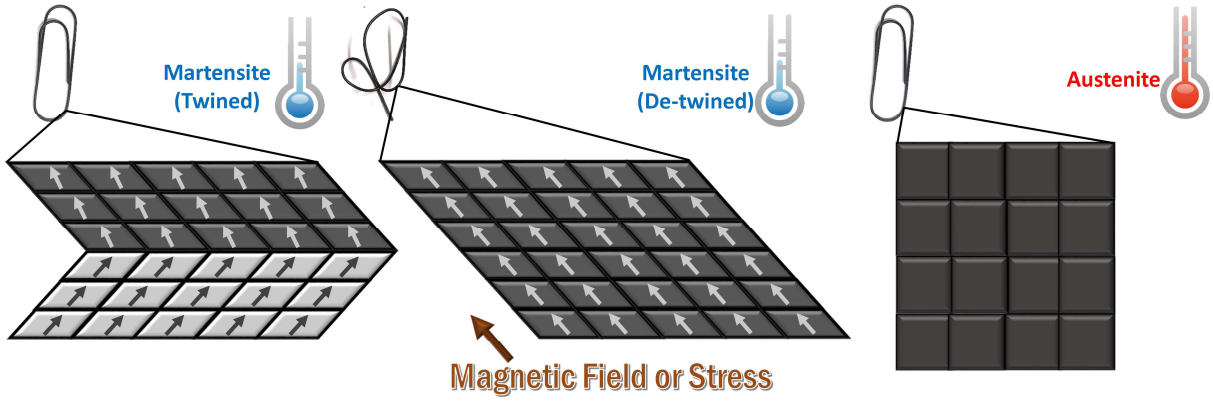

Figure 1. Simplified schematic representation of (magnetic) shape-memory effect.

References

[1] Jani J. M., Leary M., Subic A., Gibson M. A. *Mater. Des.* **2014**, *56*, 1078-1113.
[2] Heczko O., Seiner H., Fähler S. *MRS Bulletin* **2022**, *47*, 1-10.
[3] Zhang X. Qian M. Magnetic Shape Memory Alloys: Preparation, Martensitic Transformation and Properties; Ed. 1; Springer: Singapore, 2021; pp. 1-268.

The Power of Auxin - an Investigation of the Basic Auxin Signaling Component Auxin Response Factor5 Role in Embryogenic Transition Using Modern Tools of Molecular Biology

Barbara. Wójcikowska,[a,b] Contributor S. Belaidi,[a,c] and Principal Investigator Helene S. Robert*[a]
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The somatic embryogenesis (SE) process is widely used in biotechnology for the clonal multiplication of plants of agrobotanical importance. Plant cells are characterized by pluripotency. Under the influence of an inducer, they can form somatic embryos - clones of the mother plant. To learn about the genetic basis of the process of somatic embryogenesis and the developmental plasticity of plant cells, the model plant *Arabidopsis thaliana* is used. In this plant, it is possible to induce the process of embryogenic transition using the plant hormone - auxin. Auxin signaling is, therefore, crucial for the efficient induction of the SE process. The gene that plays a pivotal role in the SE process is *AUXIN RESPONSE FACTOR5* (*ARF5*), known as *MONOPTEROS*. *ARF5* encodes a transcription factor that regulates the expression of auxin-regulated genes. Ongoing studies investigate the relationship between the ARF5 transcription factor, the *MIR390A* and *MIR390B* genes encoding miR390 molecules, and auxin biosynthetic genes (*YUCCAs*) (Fig. 1A). The promoter regions of the studied genes include a *cis*-regulatory element Aux-RE recognized by ARF5. Genes encoding miR390 and YUCCA are active during embryogenic transduction, which was confirmed by transcriptomic analysis and studies using reporter lines. Additionally, ARF5 spliced variant called MP11ir is present during somatic embryogenesis. It may be necessary for regulating target gene expression in an auxin-independent manner, which expands current knowledge of the mechanism of auxin signaling in plants (Fig. 1B).

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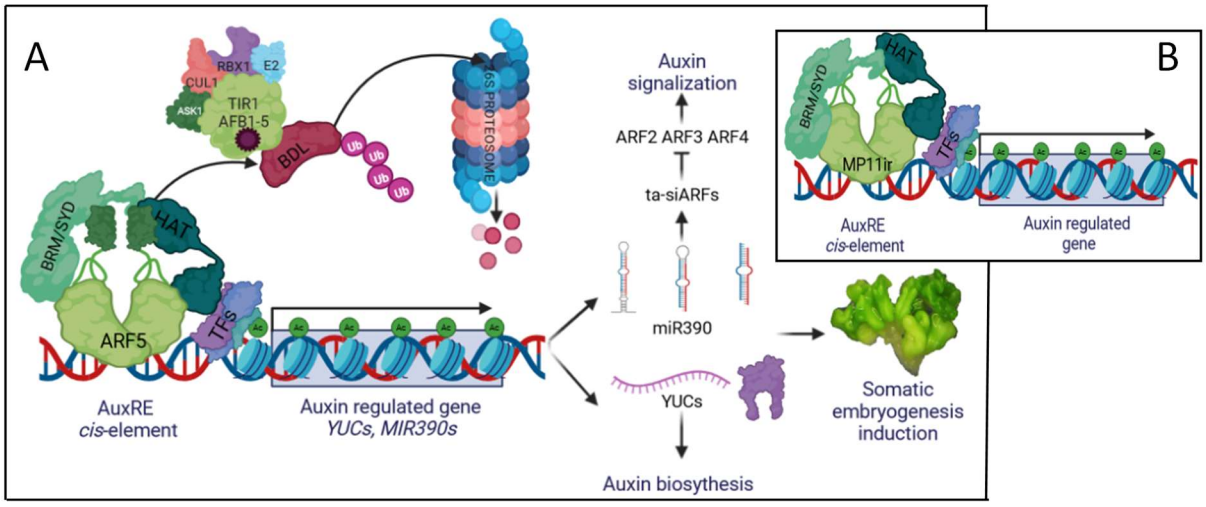

Figure 1. Mechanism of ARF5 (A) and its splice variant MP11ir (B) mechanism of action during process of somatic embryogenesis.

Liquid-liquid phase separation of translation factor in prokaryotes

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Compartmentalization is a hallmark of living cells that allow them to perform complex tasks by dynamically coordinating matter and energy fluxes in space and time. This compartmentalization of membraneless organelles in prokaryotes is driven by Liquid-Liquid Phase Separation (LLPS)[1]. These "biomolecular condensates" are comprised of proteins that are generally rich in intrinsically disordered regions (IDRs) and nucleic acids [2].

In prokaryotes, translation Initiation Factor-2 (IF-2) is a GTPase that binds the initiator tRNA and catalyzes the ribosomal subunit joining to form 70S complex [3]. Large portion of IF-2 is intrinsically disordered and might form LLPS in order to concentrate the translation in specific regions in bacteria. This study investigates IF-2 and its formation of LLPS to provide deeper insight into compartmentalized translation machinery in bacteria.

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References

[1] Azaldegui CA, Vecchiarelli AG, Biteen JS. The emergence of phase separation as an organizing principle in bacteria. Biophysical journal. 2021 Apr 6;120(7):1123-38.
[2] Alberti S, Gladfelter A, Mittag T. Considerations, and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell. 2019 Jan 24;176(3):419-34.
[3] Ling C, Ermolenko DN. Initiation factor 2 stabilizes the ribosome in a semi rotated conformation. Proceedings of the National Academy of Sciences. 2015 Dec 29;112(52):15874-9.

POSTER PRESENTATIONS

ncRNAS TRANSCRIPTS FROM RNA POL III REVELS INTERACTIONS WITH INTERFERON-STIMULATED GENES

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Interferon-stimulated genes (ISGs) frequently target RNA viruses by recognising specific RNA features associated with viral genomes, such as intermediate molecules during viral replication (dsRNAs) or missing molecular features (5′ caps or poly-A tail), typically associated with host mRNAs[1]. These ISGs can be induced even in the absence of IFN signalling, and some ISGs are expressed basally during homeostasis as well as being IFN-inducible, while others ISGs appear to be expressed only during an IFN response in some pathological conditions [2]. This complexity in expression opens a new opportunity to characterise other ISG functions. Our research group demonstrated that the ISG genes OAS3 and IFIT1 are expressed in two different human cell lines. In addition, these genes demonstrated RNA-protein interaction using orthogonal organic phase separation (OOPS). Immunoprecipitation of IFIT-1 in extracts of UV–cross-linked cells followed by RT-qPCR demonstrate interaction with the transposable elements, including Alu elements.

Because IFIT1 is an antiviral protein that recognises 5′ ends of viral RNA genomes, which are often 5' triphosphate or partially 5′ capped, and blocks their translation [3]. We hypothesised that transcripts from RNA Polymerase III (RNA pol III), which usually produce 5' triphosphate RNA [4], could be regulated by IFIT1 protein. For this, we established a stable IFIT1-short hairpin (sh)RNA cell line, which was confirmed by RT-qPCR. Then, we evaluated the relative expression of vault RNAs (vtRNAs), which have previously been demonstrated that are produced by RNA pol III and have a 5' triphosphate end [5]. Our results demonstrated an increase in the expression of these RNAs on the IFIT1-shRNA cell line, suggesting a new mechanism of regulation by IFIT1. Nevertheless, future studies were necessary to confirm our experiments.

References

[1] Yang, E., & Li, M. *Frontiers in immunology.* **2020,** 11, 605024.
[2] Schoggins, W. J. *Annual Review of Virology.* **2019**, 6:567-584
[3] Diamond M. S. *Cytokine & growth factor reviews.* **2014**, *25*(5), 543–550.
[4] Lata, E., Choquet, K., Sagliocco, F., Brais, B., Bernard, G., & Teichmann, M. *Frontiers in molecular biosciences.* **2021**, 8, 696438.
[5] Choi, J. H., Burke, J. M., Szymanik, K. H., Nepal, U., Battenhouse, A., Lau, J. T., Stark, A., Lam, V., & Sullivan, C. S. *Genes & development.* **2020**, 34(23-24), 1697–1712.

Kinase autophosphorylation: understanding CK1 regulation

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The human Casein kinase 1 family members (α, β, γ1, γ2, γ3, δ and ϵ) are Ser/Thr kinase with a highly conserved catalytic domain followed by C-terminal unstructured extensions which regulate the enzymatic activity. The autoregulation process, induced through phosphorylation of the disordered tail by the catalytic core, remains poorly understood over the last twenty years [1-3].

To understand the role of the disordered tail in the modulation of CK1 function, we performed structural and functional studies. NMR analysis of the free CK1ε C-terminal tail (aa 305-416) and in the context of the full-length enzyme (aa 1-416) allowed to map the core-tail interacting residues and to identify structural motifs (α-helices) in the tail.

Furthermore, combining MS and RT-NMR, we identified the phosphorylated residues, and we monitored the kinetic rates of autophosphorylation at different temperatures: our data suggest that CK1 activity is temperature-dependent.

In conclusion, our findings represent the first step in understanding the catalytic mechanism and self-regulation of Ck1 kinase family.

References

1. Cegielska, A., Gietzen, K. F., Rivers, A. & Virshup, D. M. Autoinhibition of casein kinase I epsilon (CKI epsilon) is relieved by protein phosphatases and limited proteolysis. *J. Biol. Chem.* **273**, 1357–1364 (1998).
2. Graves, P. R. & Roach, P. J. Role of COOH-terminal phosphorylation in the regulation of casein kinase I delta. *J. Biol. Chem.* **270**, 21689–21694 (1995).
3. Gietzen, K. F. & Virshup, D. M. Identification of inhibitory autophosphorylation sites in casein kinase I epsilon. *J. Biol. Chem.* **274**, 32063–32070 (1999).

Roles of antiviral dsRNA sensors in innate immune and neuronal defects in *Adar^{5G1}* null mutant flies lacking dsRNA editing

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Drosophila Adar is mainly expressed in the nervous system and carries out A-to-I RNA editing in dsRNA hairpins in pre-mRNAs. Edited mRNAs are numerous in CNS and enriched in ion channels and neurotransmitter receptor subunits which produce new edited proteoforms. Drosophila *Adar^{5G1}* null mutant flies show reduced viability and locomotion defects at eclosion, and develop increased neuronal excitability, pre-synaptic vesicle accumulation and neurodegeneration. All *Adar^{5G1}* mutant defects are suppressed by reduced expression of Tor or by overexpression of Atg5, which increase canonical autophagy initiation and reduce aberrant accumulation of synaptic vesicle proteins[1]. The loss of Adar RNA editing activity also leads to aberrant innate immune AMP induction[2]. Dicer-2 has an RNA helicase domain similar to that of MDA5, which senses unedited dsRNAs in mammalian Adar1 mutants and the aberrant AMP induction. The AMP induction is suppressed by silencing of Dicer-2 in cholinergic neurons2.We sought to determine whether knocking down the antiviral cGas-Like Receptor1(cGLR1), recently shown to be activated by dsRNA in *Drosophila,* or Sting receptor which acts downstream of cGLR1, can rescue aberrant AMP induction and other defects in *Adar^{5G1}* flies. We found that ubiquitous RNAi knockdown of cGLR1 *in Adar^{5G1,} arm>cGLR1 RNAi* flies rescues the aberrant immune induction in heads; however, it doesn't rescue locomotion or survival. Similar RNAi knockdown of Sting improves survival and rescues aberrant immune induction but not locomotion defects. Preliminary western blot data on whole flies with ubiquitous Sting RNAi knockdown shows a reduction in the aberrantly increased level of pre-synaptic Synaptotagmin-1 protein. Activated Sting may aberrantly activate antiviral autophagy and thereby interfere with canonical autophagy leading to accumulation of neurotransmitter synaptic vesicles, increased neuronal excitability and age-dependent neurodegeneration.

References:

[1]Khan A, Paro S, McGurk L, Sambrani N, Hogg MC, Brindle J, Pennetta G, Keegan LP, O'Connell MA. 2020. *Bmc Biol* **18**: 15.

[2] Deng P, Khan A, Jacobson D, Sambrani N, McGurk L, Li X, Jayasree A, Hejatko J, Shohat-Ophir G, O'Connell MA et al. 2020.*Nat Commun* **11**: 1580.

Influence of label and solid support on the performance of heterogeneous immunoassays

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Prostate cancer is the major cause of death in the male population caused by oncologic diseases. Sensitive detection of disease biomarkers, e.g., prostate-specific antigen (PSA), is essential for early-stage diagnosis and effective treatment. Conventional immunochemical methods widely used in clinical analysis are often not sensitive enough for early-stage diagnosis resulting in the need for novel assay formats. Here, we describe the systematic comparison of two kinds of solid phases for biomolecule immobilization in immunoassays utilizing three different labels. The two immunoassay surfaces included conventionally used microtiter plates (MTPs) and magnetic microparticles (MBs). A comparison of the different labels for immunochemical analysis together with the results obtained from conventional and MB-based immunoassays showed that the MBs can serve as a stable support for biomolecules immobilization. The three different immunoassay labels used in this study were horseradish peroxidase (HRP), 5(6)-carboxyfluorescein (FAM), and photon-upconversion nanoparticles (UCNPs); all of them being modified with streptavidin (SA). Among the three different labels used in this study, UCNPs were shown as the most sensitive and stable. The results obtained from conventional and MB-based upconversion immunoassays provided the highest signal-to-background ratios and the lowest limits of detection (LODs) compared to enzyme-linked immunosorbent assay (ELISA) and fluorescence immunoassay (FIA) performed under the same experimental conditions. The MB-based ULISA carried out with the PSA preconcentration provided the lowest LOD of 0.5 pg/mL achieved in this study. The results demonstrate that using micro- and nanomaterials in immunoassays for PSA detection improved the immunoassays sensitivity and working range.

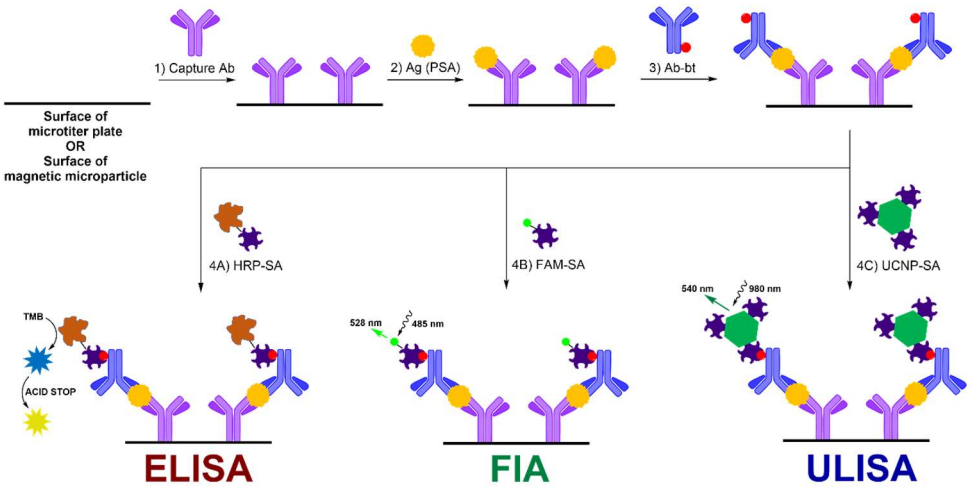

Figure 1. General scheme of the used immunoassay strategies. Different label types were used in the last step of each procedure, leading to three different types of readout.

CRISPR, herd immunity, and transduction allow bacteria to speed up adaptation by recombination

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Unlike most eukaryotes, bacteria reproduce by binary fission, which is devoid of recombination. Recombination is known to be able to speed up adaptation by bringing beneficial alleles arising in different individuals together. In a clonal bacterial population, however, the beneficial alleles compete with each other in a process called clonal interference, which slows down adaptation. Although bacteria lack recombination as it occurs in eukaryotes, they can occasionally exchange genetic information directly between individuals in a process called horizontal gene transfer (HGT). How much HGT affects evolutionary dynamics within bacterial populations, however, remains largely unclear. We experimentally determined that in bacterial populations of *Escherichia coli*, CRISPR-based herd immunity can lead to coexistence of CRISPR+ (resistant) and CRISPR– (susceptible) strains and the P1 phage in the population. As the P1 phage is capable of generalised transduction and the resistant strain is protected from lysis by the phage, gene flow from the susceptible to the resistant strain becomes continuous. As a result, this continuous transduction enables two different beneficial loci encoding antibiotic resistance to appear on the same background and thus circumvents clonal interference. In our system, this rate is one to two orders of magnitude higher than the rate at which a functionally similar mutation can arise de novo.

We thus show that a combination of CRISPR-based herd immunity and transduction can substantially limit clonal interference aid bacterial adaptation by frequent chromosomal recombination.

ADAR1 as a target for combating malaria.

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The Fulani ethnic group are protected from *Plasmodium falciparum* malaria. However, the genetic basis of their protection remains unclear. We performed RNA-sequencing analysis of the Fulani, and observed reduced rates of Adenosine-to-Inosine RNA editing by ADAR1. Therefore, we have investigated whether reduction in A-to-I RNA editing during infection with the *Plasmodium* parasite can confer protection against malaria.

A-to-I RNA editing by ADAR1 prevents or own 'self' RNAs from being recognized as 'non-self' viral RNAs and activating auto-immune responses. If ADAR1 is mutated, it can cause the deadly auto-immune disease *Aicardi-Goutieres syndrome*. However, whether ADAR1 can be temporarily targeted to promote immune responses during infectious diseases has not been investigated.

Analysis of publicly available RNA-sequencing data from different models of human malaria infection show that reduced A-to-I editing levels are associated with protection from symptomatic disease. Importantly, *Adar1*$^{+/-}$ heterozygous mutant mice are protected from malaria, with significantly reduced parasitemia during blood stage when they are infected with rodent malaria parasite *P.yoelii.*

Collectively our data support a model where ADAR1 activity and levels of A-to-I RNA editing are transiently reduced during *Plasmodium* infection. And, reduced levels of ADAR1 activity contribute to protection from parasitemia during malaria. Interestingly, this hints that ADAR1 inhibitors may have future utility in the treatment or prevention of infectious diseases.

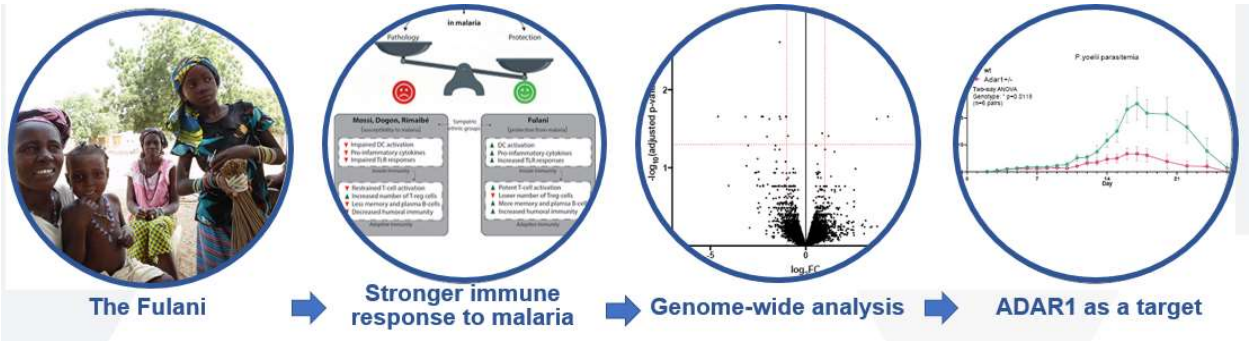

Figure 1. Overview of identifying ADAR1 as a target against malaria.

Chemical blockage of the mitochondrial rhomboid protease PARL by novel ketoamide inhibitors reveals its role in PINK1/Parkin-dependent mitophagy.

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The mitochondrial rhomboid protease PARL regulates mitophagy by balancing intramembrane proteolysis of PINK1 and PGAM5. It has been implicated in the pathogenesis of Parkinson's disease, but its investigation as a possible therapeutic target is challenging in this context, because genetic deficiency of PARL results in compensatory mechanisms. To elucidate this problem, we undertook a hitherto unavailable chemical biology strategy. We developed potent PARL-targeting ketoamide inhibitors and investigated the effects of acute PARL suppression on the processing status of PINK1 intermediates and on Parkin activation. This approach revealed that PARL inhibition leads to a robust activation of the PINK1/Parkin pathway without major secondary effects on mitochondrial properties, which demonstrates that pharmacological blockage of PARL to boost PINK1/Parkin-dependent mitophagy is a feasible approach to examine novel therapeutic strategies for Parkinson's disease. More generally, this study showcases the power of ketoamide inhibitors for cell biological studies of rhomboid proteases.

Stereoselective Synthesis of Biologically Important 5β-Steroids

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Our research is focused on the reduction of enone functionalities on steroids leading to biologically important 5β-steroid structures. For example, bile acids, neurosteroids and their synthetic analogues, neuroactive steroids possess a 5β-steroid skeleton. Bile acids play a role in the digestion and absorption of lipids. Neurosteroids endogenously synthesized from cholesterol in the nervous system. These steroids are involved in neuronal functions, such as cognition, memory processes and neuroprotection. Consequently, neurosteroids and neuroactive steroids have been implicated in the treatment of variety of diseases, i.e. anxiety, schizophrenia, neurodegenerative diseases and appear relevant to develop treatments against neuropathic pain that is refractory to current analgesic drugs.[1,2]

The asymmetric hydrogenation of unsaturated organic compounds is an important method to obtain optically active molecules. The organocatalytic reduction of enones with biomimetic reductants leads to high yields and stereoselectivities, which method was used for the diastereoselective synthesis of some 5β-steroids.[3] Ionic liquids (ILs) are attractive alternatives to conventional organic solvents due to their good chemical stability, negligible volatility and excellent solvation ability. In addition, a chiral ionic liquid as chiral additive can improve enantioselectivities in the palladium-catalyzed hydrogenation of enones.[4]

In our experiments, commercially available steroids – progesterone and testosterone were selected as starting materials. The above-mentioned strategies for the reduction of enone functionality on both steroids were tested and the selectivities affording desired 5β-isomers were evaluated.

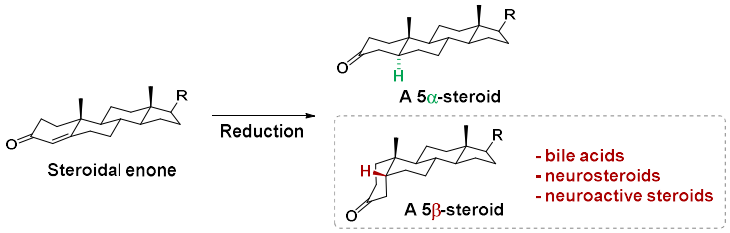

Figure 1. Synthesis of 5β-steroids.

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[1] Ratner, M.H., Kumaresan V., Farb, D.H. *Front. Endocrinol*. **2019**, *10*, 169.
[2] Meyer, L., Taleb, O., Patte-Mensah, C., Mensah-Nyagan, A.-G. *Front. Neuroendocrinol.* **2019**, *55*,100795.
[3] Ramachary, D.B., Sakthidevi, R., Reddy, P.S. *RSC Adv*. **2013**, *3*, 13497.
[4] Ferlin, N., Courty, M., Nguyen Van Nhien, A., Gatard, S., Pour, M., Quilty, B., Ghavre, M., Haiβ, A., Kümmerer, K., Gathergood, N., Bouquillon, S. *RSC Adv.* **2013**, *3*, 26241.

Role of MADS-box TFs in Regulating Auxin Biosynthesis During Embryo Development

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Survival of flowering plants is determined by the proper production of seed, whose shape and size are defined by the development of an embryo. Such mature embryo mimics the body pattern of the new plant that will develop after germination. Auxin is a key regulator of various aspects of plant development, including embryo morphogenesis. Cellular auxin gradients, maintained by auxin production along with signalling and transport, are essential for proper embryo development. The embryonic local auxin biosynthesis by TAA1-YUC pathway appears spatiotemporally controlled, necessitating the presence of regulatory networks. A yeast one-hybrid library screening using the promoter sequences of *TAA1*, *YUC1*, *YUC4*, and *YUC9* resulted in the identification of novel transcriptional factors (TFs) of auxin biosynthetic genes in seeds. Among them, several AGL TFs were found to bind *TAA1* and the analysed *YUC* promoters. We found one candidate, MADS-box AGL5/SHATTERPROOF2, regulating auxin biosynthetic genes using dual luciferase assay in tobacco leaves. *AGL5* is strongly expressed during early embryogenesis. We observed that over-expression of *AGL5* can upregulate the expression of *TAA1/YUC* genes in leaf tissue. Microscopic phenotyping of mutants is being carried out. These shreds of evidence demonstrate the involvement of AGLs in the regulation of auxin biosynthesis and in embryo development.

Genome characterization of novel lytic *Erwinia amylovora* bacteriophage Key

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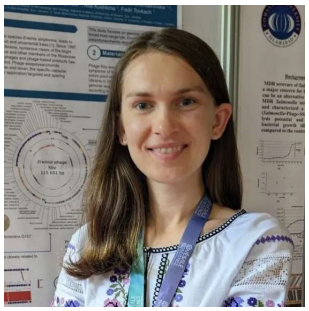

Fire blight, a plant disease caused by the bacterial species *Erwinia amylovora,* leads to significant economic losses in the cultivation of fruit and ornamental trees (Buttimer et al., 2017). Recently, the novel lytic bacteriophage Key was isolated from quince with fire blight symptoms. Transmission electron microscopy revealed its resemblance to members of the family *Siphoviridae*. Host range analysis indicated that this phage is able to lyse *P. agglomerans, E. amylovora* and *E. horticola* cells. Here, we report the genome sequencing and characterization of *E. amylovora* phage Key. Phage DNA was sequenced using the Illumina HiSeq 2500 platform at The Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada). The contigs were assembled using DNASTAR's SeqMan NGen12 software. Genes were predicted using DFAST with subsequent manual curation. tRNA encoding genes were identified using tRNAscan-SE and ARAGORN. Key phage has 115.651 kbp long double-stranded DNA genome with the G+C ratio of 39.03% encoding 182 proteins and 27 tRNA genes. The majority (69%) of predicted coding sequences (CDSs) encode proteins with unknown functions. The protein products of 57 annotated genes were found to have probable functions in nucleotide metabolism, DNA replication, recombination, repair, and packaging, virion morphogenesis, phage-host interaction and lysis. Furthermore, the product of gene 157 shared similarity of amino acid sequence and the conserved domain architecture with the exopolysaccharide degrading proteins of *Erwinia* and *Pantoea* infecting phages as well as bacterial EPS biosynthesis proteins. Due to the revealed genome synteny and similarity to the proteins of T5-like phages, phage Key together with its closest relative, *Pantoea* phage AAS21, was suggested to represent a novel genus within the *Demerecviridae* family, for which we tentative propose the name "*Keyvirus*". The obtained data expand the knowledge of *Erwinia* phages and may be helpful for the biocontrol of *E. amylovora*.

References

[1] Buttimer C., McAuliffe O., Ross R. P., Hill C., O'Mahony J., Coffey A. *Front. Microbiol.* 2017, 8, 1-15.

Mango aptamer as a tool for a high throughput RNA analysis *in vivo*

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A demand on new high throughput analysis methods raises with increasing popularity of *in silico* RNA structure modeling and design of small molecules interacting with RNA. Variety of cell-based and biochemical assays are in use already. However, RNA-protein interaction studies require some additional criteria. For example, simultaneous detection of mRNA of interest and protein expression level from the same mRNA in cell lysate is necessary for functional analysis of RNA-protein complexes *in vivo*. A possible solution can be a combination of techniques which let us analyze both specific mRNA integrity and resulting protein expression level. Here we suggest dual luciferase assay together with RNA detection using Mango aptamer [2]. Mango is a 39-nucleotide RNA aptamer which forms parallel-stranded G-quadruplex. This stabilized structure binds with nanomolar affinity to a set of thiazole orange (TO1) derivatives. Binding causes 1000-fold increase in fluorescence of TO1 dyes [1, 3]. This effect can be used for the fast qualitative and quantitative RNA detection in cell lysate. The described approach in parallel with luciferase assay presents another way of high throughput functional analysis of protein-RNA interactions and subsequent degradation or stabilization of RNA.

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References:

1. Autour, A., C. Y. Jeng, S., D. Cawte, A. et al. Fluorogenic RNA Mango aptamers for imaging small non-coding RNAs in mammalian cells. Nat Commun 9, 656 (2018).
2. Trachman RJ 3rd, Stagno JR, Conrad C, Jones CP, Fischer P, Meents A, Wang YX, Ferré-D'Amaré AR. Co-crystal structure of the iMango-III fluorescent RNA aptamer using an X-ray free-electron laser. Acta Crystallogr F Struct Biol Commun. 2019.
3. Trachman, R.J. 3rd, Autour, A., Jeng, S.C.Y. et al. Structure and functional reselection of the Mango-III fluorogenic RNA aptamer. Nat Chem Biol 15, 472–479 (2019).

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