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SYMPOSIA

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Abstracts submitted for the main call for abstracts to the 42nd FEBS Congress (Jerusalem, Israel; September 10–14, 2017) and accepted by the Congress Organizing Committee, as well as abstracts from invited speakers for the event, are published in this Supplement to *The FEBS Journal*. Late-breaking abstracts are not included in this supplement.

About these abstracts

Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are **not copyedited** prior to publication.
We are unable to make **corrections of any kind** to the abstracts once they are published.

Indexing

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* The Abstract number begins with either the letters S or ShT and can be found atop each abstract's title in the PDF file.

SYMPOSIA

Sunday 10 September
16:00–18:00, Oranim Hall 1

Synthetic Biology

S.1.1-001
CETCH me if you can: Bringing inorganic carbon into life with synthetic CO2 fixation

T. Erb
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Carbon dioxide (CO_2) is a potent greenhouse gas that is a critical factor in global warming. At the same time atmospheric CO_2 is a cheap and ubiquitous carbon source. Yet, synthetic chemistry lacks suitable catalysts to functionalize atmospheric CO_2, emphasizing the need to understand and exploit the CO_2 mechanisms offered by Nature. In my talk I will (1) discuss the evolution and limitation of naturally existing CO_2 fixing enzymes and pathways. I will (2) present strategies for the engineering and design of artificial CO_2 fixation reactions and pathways (*Peter et al. 2015*), and (3) outline how these artificial pathways can be realized and further optimized to create synthetic CO_2 fixation modules. An example for such a synthetic CO_2 fixation module is the CETCH cycle (*Schwander et al. 2016*). The CETCH cycle is an *in vitro* reaction network of 17 enzymes that was established with enzymes originating from nine different organisms of all three domains of life and optimized in several rounds by enzyme engineering and metabolic proofreading. In its version 5.4, the CETCH cycle converts CO_2 into organic molecules at a rate of 5 nanomoles of CO_2 per minute per milligram of protein. This is slightly faster than the Calvin cycle under comparable conditions and notably at 20% less energy per CO_2 fixed.

S.1.1-002
Cancer cell targeting: the signal and the noise

Y. Benenson
ETH Zürich, Basel, Switzerland

In the last decade, intensive research in mammalian synthetic biology has focused on gene circuits for precise targeting and identification of specific cells, and in particular cancer cells, for the development of more specific therapies with fewer side effects. Such circuits typically sense multiple endogenous molecular inputs and integrate them in order to generate a single molecular output that correlates with the cell lineage and/or pathological state. Multiple approaches have been proposed by our group and others to address this question. While the circuits operate sufficiently well on cell ensembles and generate selective outputs in the bulk, cell-to-cell variability in both the endogenous inputs and the circuit composition may introduce errors. In the talk I will describe the main approaches that we have been pursuing in order to target cancer cells specifically and selectively using microRNA and transcriptional inputs. I will also present the computational and the experimental tools that we have been developing to characterize and reduce the undesired effects of biological heterogeneity on circuit operation. I will argue that the anticipation and containment of biological fluctuations is the next frontier in synthetic biology.

S.1.1-003
Synthetic biology – between forward design and sophisticated guessing

S. Panke
ETH Zurich, Basel, Zurich, Switzerland

Synthetic biology is accelerating progress in the engineering of biology as well as in the generation of insights into its more fundamental aspects. At its heart is the claim of providing increasingly model-based blueprints of improved or alternative biological systems that can be put to work for human benefit, fueled by the increasing power in template-free DNA synthesis. One of the most interesting questions is to which extent biological design can be rationalized and which is the most appropriate design strategy for a given problem. We will illustrate different answers based on engineering problems recruited, broadly speaking, from the field of metabolic engineering, and the answers will range from a case of exact forward design of a metabolic system to the application of "natural design strategies", i.e. directed evolution based on varying degrees of naïveté regarding the fundamental constraints posed by the biological problem.

ShT.1.1-001
Inhibitory RNA aptamer against lambda cI repressor shows the transcriptional activator activity in vivo

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RNA aptamer is one of the promising components for constructing artificial genetic circuits. In this study, we developed a transcriptional activator based on an RNA aptamer against one of the most frequently applied repressor proteins, lambda phage cI. *In vitro* selection (SELEX), followed by *in vivo* screening identified an RNA aptamer with the intended transcriptional activator activity from an RNA pool containing a 40-nucleotide long random region. Quantitative analysis showed 35-fold elevation of reporter expression upon aptamer expression. These results suggest that diversity of artificial transcriptional activators can be extended by employing RNA aptamers against repressor proteins to broaden the parts for constructing genetic circuits.

ShT.1.1-002
Engineering fatty acid synthases (FAS) for custom compound synthesis

J. Gajewski[1], A. Rittner[1], K. Paithankar[1], M. Fischer[1], F. Buelens[2], H. Grubmüller[2], **M. Grininger**[1]
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Multienzyme type I fatty acid synthases (FAS I) provide compartmentalized reaction space for C-C bond formation. Natively used for the biosynthesis of C16 to C18 fatty acids, recent studies demonstrate the successful redesign of these proteins for the production of other and more complex compounds, among them short fatty acids, methylketones and lactons. Noteworthy, the production of the lactone product was achieved by the coupling of two FAS I modules, in which module 2 initiates its synthesis after loading the product of module 1. Key to the successful

design of FAS I is the well-established structural and functional description of these proteins, and the combined *in vitro* protein analysis and *in silico* description of the relevant enzymatic functions performed during this work. The approach of rewriting fatty acid synthesis also revealed the challenges of *in vitro* characterization and computational modeling of MDa-sized multienzymes, as well as the limitations and the perspectives of multienzyme engineering. As such, it has model character for the rational design of the evolutionally related, chemically highly versatile non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS).

Sunday 10 September 16:00–18:00, Oranim Hall 4

DNA Damage and Repair

S.1.2-003
Mechanism of UV-damaged DNA recognition in chromatin

N. Thoma
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Irradiation of DNA by UV-light causes covalent crosslinks of neighbouring pyrimidine bases. These lesions, mostly cyclobutane pyrimidine dimer (CPD) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PP), stall transcription and are replicated by error-prone trans-lesion polymerases only. Inability to repair these types of damages are known drivers of skin cancerogenesis. The human genome is constantly surveyed for the presence of UV-lesions by the UV-DDB complex, comprised of DDB1 and DDB2. While the mechanism of damage-readout on naked DNA is well-established, it is unclear how proteins recognize DNA damage on nucleosomes, given that nucleosome binding to DNA occludes a significant portion of the duplex. We present the structural basis of damage recognition on a nucleosome.

S.1.2-002
The DNA damage response: a matter of checks and balances

Y. Shiloh
Tel Aviv University, Tel Aviv, Israel

The DNA damage response (DDR) is a complex signaling network that is vigorously activated by double-strand breaks (DSBs). For all its complexity and breadth, the DDR is highly structured and meticulously regulated. Indeed, a close look at the function of various DDR players reveals the critical fine-tuning of this system. Two new such players currently investigated in our lab will be discussed: the first is nuclear poly(A)-binding protein 1 (PABPN1) – a novel ATM substrate, whose recruitment to DSB sites is required for optimal end-resection and timely DSB repair. It represents a functional module composed of several proteins that is borrowed by the DDR from the RNA processing arena. The second is the E4 ubiquitin ligase, UBE4A. We found that following initial ubiquitylation of proteins at the sites of DNA damage – mediated largely by the E3 ubiquitin ligases, RNF8 and RNF168 – the extent of ubiquitylation requires further tweaking by an E4 ubiquitin ligase before timely DSB repair can occur. E4 ligases bind to a single conjugated ubiquitin or an oligoubiquitin chain, and extend and regulate the length of the chains. We found that UBE4A modulates protein ubiquitylation at DSB sites, and this step is required for timely recruitment of critical DDR players, spatial organization of the proteins hubs formed at DSB sites, and maintaining the balance between DSB repair pathways. These findings reveal a critical regulatory level of the DSB response and underscore the importance of fine-tuning of this network for accurate and balanced execution of DSB repair.

S.1.2-001
Non-canonical role of ATM in the R-loop dependent-DNA Damage Response

W. Vermeulen, M. Tresini
Erasmus MC, Rotterdam, Netherlands

Environmental genotoxins and metabolic byproducts generate DNA lesions which can cause genomic instability and drive human pathology. To ensure genomic integrity, cells employ mechanisms that convert signals generated by stochastic DNA damage into organized responses including, activation of repair systems, cell cycle checkpoints and apoptosis. We have recently identified the key steps of a novel, replication-independent DNA damage response (DDR) pathway that initiates in response to transcription-blocking DNA lesions and activates the master DDR coordinator ATM, through mechanisms involving spliceosome remodeling and formation of potentially genotoxic R-loops. Our finding that in non-replicating cells damage-induced R-loops activate ATM exclusively through a non-canonical, dsDNA break-independent pathway, introduces the unanticipated concept that these atypical nucleic acid structures may act as signaling intermediates in the DDR after being "sensed" by a specialized, as yet unidentified mechanism(s). Importantly, R-loop-activated ATM, displays pan-nuclear localization and besides activating its canonical targets, impedes spliceosome organization. This dual ATM function is associated with a striking number of genome-wide UV irradiation-induced alternative splicing and transcriptome changes, highlighting the fundamental regulatory role of the R-loop/ATM signaling system in the cellular DDR.

ShT.1.2-001
NELF-E is recruited to DNA double-strand break sites to promote transcription repression and repair

S. W. Awwad, E. R. Abu-Zhayia, N. Guttmann-Raviv, **N. Ayoub**
Technion, Haifa, Israel

Double-strand breaks (DSBs) trigger rapid and transient transcription pause to prevent collisions between repair and transcription machineries at damage sites. Little is known about the mechanisms that ensure transcription block after DNA damage. Here we reveal a novel role of the negative elongation factor, NELF, in blocking transcription activity nearby DSBs. We show that NELF-E and NELF-A are rapidly recruited to DSB sites. Furthermore, NELF-E recruitment and its repressive activity are both required for switching off transcription at DSBs. Remarkably, using *I-Sce-I* endonuclease and CRISPR-Cas9 systems, we observed that NELF-E is preferentially recruited, in a PARP1-depenedent manner, to DSBs induced upstream transcriptionally active rather than inactive genes. Moreover, the presence of RNA polymerase II is a prerequisite for the preferential recruitment of NELF-E to DNA breakage sites. Additionally, we demonstrate that NELF-E is required for intact repair of DSBs. Altogether, our data identified NELF complex as a new component in the DNA damage response.

ShT.1.2-002
Enzyme processivity as a drug target: The case of poxvirus uracil–DNA glycosylase

D. Zharkov
Novosibirsk State University, Novosibirsk, Russia

Reaction processivity is the ability of DNA- or RNA-dependent enzymes to catalyze many consecutive steps, such as nucleotide addition or excision, without releasing the nucleic acid molecule. Another form of processivity, search processivity, relates to the ability of a protein to find its target in DNA by scanning the nucleic acid after a single random association. In many DNA polymerases, the reaction processivity is determined by special accessory proteins, such as β-clamp in bacterial replication complex or PCNA in eukaryotes. In the poxvirus replication complex, the processivity factor is believed to be the D4 protein encoded by the virus genome. This protein belongs to the uracil–DNA glycosylase structural superfamily, which includes DNA repair enzymes responsible for the removal of uracil from DNA. The assignment of D4 as a processivity factor is mostly on structural reasons, and the intrinsic processivity of this protein has never been studied. We have cloned and purified the D4 protein from vaccinia virus. The protein had robust uracil–DNA glycosylase activity, which allowed us to use the correlated cleavage of multiply damaged DNA as a quantitative measure of its processivity. Unlike *E. coli* or human uracil–DNA glycosylases, the efficiency of D4 protein transfer between two separate targets in DNA barely depended on the distance between them, indicating high search processivity. The D4 protein was able to traverse nicks and small gaps in DNA and to displace other bound proteins from its path. In a library of heterocyclic compounds, we have identified two inhibitors of micromolar affinity, one of which affected the catalytic activity of D4, while another interfered with its DNA scanning ability. Both inhibitors were also characterized in the virus-infected cell culture. We conclude that DNA scanning processivity is a viable, largely unexplored mechanistical drug target. The work was supported by RSF (grant 17-14-01190).

Sunday 10 September
16:00–18:00, Oranim Hall 2

Protein Dynamics and Interactions

S.1.3-001
The protein interactome provides insights into the disease association of missense mutations

M. Sternberg, A. David, C. Yates, S. Ittisoponpisan, I. Filippis, E. Alhuzimiu
Imperial College, London, United Kingdom

This talk will present a series of studies demonstrating how information in the interactome can assist in understanding the phenotypic effect of missense variants and of disease associated genes. First an analysis will be presented showing that on the surface of a protein chain a missense variant is more likely to be disease associated if it is involved in a protein-protein interaction. We will then describe our web server SuSPect (available at www.sbg.bio.ic.ac.uk/~suspect)which predicts the phenotypic effect of a missense variant incorporating information about sequence conservation, three-dimensional structure and the extent to which the protein is involved in numerous protein-protein interactions (i.e. if it is a hub protein). Our next study will describe how diseases can be associated with specific cell types. Here we use cell specific interactomes to relate genes to disease. Intriguing cell/disease associations are revealed. Finally, we will show that pleotropic genes (i.e. those involved in several distinct diseases) tend to have more interactions and greater flexibility than non-pleotropic proteins.

S.1.3-003
Molecular simulations of lipid membrane sensing dynamics

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Living cells need to exert tight control over their lipid membranes, to maintain their internal structure, to guard their outside boundary, to establish potential and concentration gradients as their energy source, or to transmit signals between their compartments and to the outside. As a consequence, elaborate protein machineries have evolved that allow cells to sense and regulate both shapes and physical characteristics of their lipid membranes. The molecular modeling of these machineries faces enormous challenges because of their complexity, size, and dynamic nature. To address these challenges, we combine atomistic and coarse-grained simulations of protein-membrane systems. In my talk, I will describe different mechanisms used by eukaryotic cells to sense and regulate the fluidity of their lipid membranes, as deduced from molecular dynamics simulations and experiment. In the case of the proteins Ire1 and Mga2, dynamic interactions of the transmembrane domains emerge as central to their membrane-sensing function.

S.1.3-002
Interactions and Dynamics of Ras

O. Keskin[1], S. Muratcioglu[1], H. Jang[2], R. Nussinov[3], A. Gursoy[1]
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The Ras superfamily of small GTPases, including the Ras, Rho, and Rab subfamilies regulate an astonishing diversity of cellular functions such as proliferation, differentiation, cell morphology, motility, intracellular trafficking and gene expression. It has been known for many years that the attachment of Ras superfamily proteins to cellular membranes is essential for their function. Ras interacts with its effectors to control the cellular activities. In this talk I will talk about Ras- PDEδ interaction and how dynamics govern this interaction. Experimental data indicate that PDEδ binds K-Ras4B as well as N-Ras; but no interaction was observed for K-Ras4A, which is a splice variant K-Ras4B. The hyper variable tail (HVR) of K-Ras4B and K-Ras4A are similar and both highly positively charged; N-Ras is neutral. K-Ras4B is farnesylated; K-Ras4A is farnesylated and palmitoylated as is N-Ras. Unlike farnesylation, palmitoylation is reversible. Here we investigated the binding modes of PDEδ with farnesylated and geranyl-geranylated K-Ras4B, and dissected the interactions. We modeled K-Ras4B structures and performed explicit-solvent molecular dynamics (MD) simulations using six different initial conformations.

ShT.1.3-001
Structural studies of the human procaspase-2 in the complex with 14-3-3ζ protein using HDX-MS and SAXS

M. Alblova[1], D. Kalabova[1], A. Smidova[1], P. Man[2], T. Obsil[3], V. Obsilova[1]
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14-3-3 proteins are family of highly conserved regulatory molecules which were found in all eukaryotes. They are involved in physiological processes such as metabolism, regulation of cell cycle or gene transcription. We focused on structural understanding of the 14-3-3ζ protein-dependent regulation of the human caspase-2 (EC 3.4.22.55). This cytosolic and nuclear enzyme comes from a family of cysteine-dependent and aspartate-specific proteases. Caspase-2 plays key roles in DNA damage response, cell cycle regulation, tumor suppression and apoptosis. It is synthetized as an inactive single-chain procaspase-2 which consists of a long N-terminal CARD prodomain and large and small catalytical subunits. Efficient amount of NADPH in cell induces phosphorylation of procaspase-2 and enables the 14-3-3 protein binding. 14-3-3 proteins block the binding of adaptor proteins, inhibit procaspase-2 dimerization, autoproteolysis and activation thus blocking the apoptosis through unknown mechanism. To investigate the mechanism the of protein-protein interactions between doubly phosphorylated procaspase-2 (dPproC2) and 14-3-3ζ dimer as well as to reveal the conformational changes of these two binding partners upon their complex formation, hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) and SAXS experiments were performed. HDX kinetics revealed structural changes of both dPproC2 and 14-3-3ζ upon their complex formation. Our results proved that 14-3-3 protein binding induces a rearrangement of the whole dPproC2 molecule. Conformational changes of 14-3-3 protein are present not only within its ligand binding groove but also outside the central cavity. Our study of the 14-3-3 protein complex with the human procaspase-2 may contribute to better understanding of inhibition of caspase-2 activity as well as the role of 14-3-3 proteins in the apoptosis and the regulation of other proteases. This work was supported by the Czech Science Foundation (Project 17-00726S).

ShT.1.3-002
Promiscuous protein assembly as a function of interface plasticity and protein stability

G. Schreiber, R. Cohen-Khait, O. Dym
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To maintain homeostasis in the cell proteins have evolved to balance between binding desired partners while rejecting others. Here, we investigated the plasticity of protein-interfaces that promote binding to their specific partner, and then selected for mutations required to bind a new partner. As experimental system we chose TEM1-b-lactamase binding with high affinity its protein inhibitor BLIP. Examining the plasticity of the interface was done by low stringency selection of a random TEM1 library using yeast surface display. We show that most interfacial residues could be mutated without loss in binding affinity or protein stability or enzymatic activity,, suggesting that many solutions support this high affinity binding. Interestingly, many of the selected mutations promoted faster association. Selecting even faster binders was achieved by drastically decreasing the library-ligand incubation time. Pre-equilibrium selection is a novel methodology for selecting faster binders. To select for TEM1 heterodimerization a random library of a pre-stabilized TEM1 mutant was selected against WT TEM1 using yeast surface display. Surprisingly, three mutations located far from the BLIP binding site were sufficient to achieve μM affinity binding specifically between the evolved variant and WT TEM1. The X-ray structure of the complex showed the selected mutations to cause an unwinding of the N'-terminal helix, which consequently allowed protein coupling via β-sheet mediated interaction in an amyloid like manner. However, the reduced stability of the evolved protein prevented its evolution on the WT TEM1, emphasizing a simple mechanism were selection of unwanted binding is purged by loss of thermal stability. These findings correspond with the observation that protein stabilization in secondary sites is often required for protein evolution and design, preventing the evolution of promiscuous, harmful protein-protein interactions on meta-stable WT proteins. PMC5206572.

Sunday 10 September
16:00-18:00, Oranim Hall 3

Proteomic Approaches in Cell Biology

S.1.4-002
Systems biology of oncogenic kinase signalling

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Barts Cancer Institute, QMUL, London, United Kingdom

Kinase inhibitors are revolutionizing the way most tumour types are treated. However, not all cancer patients respond to these compounds to the same extent, and relapse limits their efficacy. The work in my group aims to understand why some tumours respond to targeted therapies while others are resistant to the same treatments. Using label-free phosphoproteomics and computational approaches for inferring kinase activity from phosphoproteomics data, we found that the activities of pathways acting in parallel to PI3K determine whether or not primary leukaemia cells may respond to PI3K inhibitors. Using similar approaches, we have recently found that specific combinations of pathway activities explain the mode of action of MEK and FLT3 inhibitors in leukaemia. These data suggest that technology for measuring the signalling network as a whole (rather than just the pathway that is being targeted) may be able to predict clinical sensitivity to signalling inhibitors with high accuracy.

S.1.4-003
Using proximity biotinylation to map the human cell and understand the assembly of RNA granules and bodies

A. Gingras
Mount Sinai Hospital, Toronto, Canada

Compartmentalization is essential for all complex forms of life. In eukaryotic cells, membrane-bound organelles, as well as a multitude of protein- and nucleic acid-rich subcellular structures, maintain boundaries and serve as enrichment zones to promote and regulate protein function, including signaling events. Consistent with the critical importance of these boundaries, alterations in the machinery that mediate protein transport between these compartments has been implicated in a number of diverse diseases. Understanding the composition of each cellular "compartment" (be it a classical organelle or a large protein complex) remains a challenging task. For soluble protein complexes,

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approaches such as affinity purification other biochemical fractionation coupled to mass spectrometry provides important insight, but this is not the case for detergent-insoluble components. Classically, both microscopy and organellar purifications have been employed for identifying the composition of these structures, but these approaches have limitations, notably in resolution for standard high-throughput fluorescence microscopy and in the difficulty in purifying some of the structures (e.g. p-bodies) for approaches based on biochemical isolations. Prompted by the recent implementation *in vivo* biotinylation approaches such as BioID, we report here the systematic mapping of the composition of various subcellular structures, using as baits proteins (or protein fragments) which are well-characterized markers for a specified location. We defined how relationships between "prey" proteins detected through this approach can help understanding the protein organization inside a cell, which is further facilitated by newly developed computational tools. We will discuss our low-resolution map of a human cell containing major organelles and non-membrane bound structures, but also focus on a resolution map of the RNA-associated p-bodies and stress granules that illuminates the dynamic formation of these structures in mammalian cells.

S.1.4-001
The target landscape of clinical kinase drugs

B. Kuster
Technical University of Munich, Freising, Germany

Kinase inhibitors have developed into important cancer drugs because de-regulated protein kinases are often driving the disease. Efforts in biotech and pharma have resulted in more than 30 such molecules being approved for use in humans and several hundred are undergoing clinical trials. As most kinase inhibitors target the ATP binding pocket, selectivity among the 500 human kinase is a recurring question. Polypharmacology can be beneficial as well as detrimental in clinical practice, hence, knowing the full target profile of a drug is important but rarely available. We have used a chemical proteomics approach termed kinobeads to profile 240 clinical kinase inhibitors in a dose dependent fashion against a total of 320 protein kinases and some 2,000 other kinobead binding proteins. In this presentation, I will outline how this information can be used to identify molecular targets of toxicity, re-purposing existing drugs or combinations for new indications or provide starting points for new drug discovery campaigns.

ShT.1.4-001
Coding non-coding human telomerase RNA

Y. Naraykina[1,2], M. Rubtsova[1,2], D. Vasilkova[1], M. Meerson[1], M. Zvereva[1,2], V. Lazarev[3], V. Manuvera[3], S. Kovalchuk[4], N. Anikanov[3], I. Butenko[3], O. Pobeguts[3], V. Govorun[3], O. Dontsova[1,2]
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Telomeres are shortened after each round of cell division, and once telomeres reach a critically short length, the cell either dies by apoptosis or stops dividing and senesces. Telomeres are maintained in most eukaryotes by a specific enzyme called telomerase. The telomerase holoenzyme is a ribonucleoprotein complex (RNP) that possesses a reverse transcriptase activity and carries its own RNA template (TR, TER, TERC) for telomere repeat addition. Telomerase is repressed in most normal cells, but upregulated in many tumor progenitor cells, which enables the continued and uncontrolled proliferation of the malignant cells that provide tumor growth and progression. We have found that the human telomerase RNA (hTR), also known as noncoding RNA, contains open reading frame. The length of ORF in hTR is 366 nucleotides. This protein was named hTERP (human TElomerase RNA Protein). The molecular weight of hTERP is 13 kDa. We demonstrated the existence of hTERP in eukaryotic cells by different molecular biology techniques.*Elizabeth H. Blackburn et al.* have previously shown that hTR plays an anti-apoptotic role in CD4 T-cells independently from telomerase catalytic activity. Our data allow to propose that the hTERP may be a player in regulation of cell survival during apoptosis-autophagy crosstalk. Exogenous expression of wild type telomerase RNA protects cells from drug-induced apoptosis, but the expression of telomerase RNA mutated at start codon does not. In addition, we analyzed the biological role of endogenous protein encoded by human telomerase RNA using CRISPR/Cas system and investigated the potential interaction partners. Our preliminary data indicates that hTERP can influence an autophagosome formation. In conclusions, our results show that the human telomerase RNA translation product may be a player in the regulations of cell survival, which is crucial in cancer and aging treatment. This work was supported by Russian Foundation for Basic Research [16-14-10047].

ShT.1.4-002
Triple-stage mass spectrometry unravels the heterogeneity of endogenous protein complexes

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Many endogenous protein complexes are composed of a diverse collection of assemblies with distinct functions and regulation, which is enabled by various protein diversification mechanisms, such as alternative splicing, post transcriptional and post translational modifications (PTMs). The resulting proteins can assemble into compositionally distinct protein complexes, with different functional properties that may enable response and adaptation to changing conditions. Despite the biological importance of this layer of complexity, little is known about the compositional heterogeneity of endogenous protein complexes, mostly due to technical barriers of studying closely-related species. We developed a native mass spectrometry approach to unravel this inherent heterogeneity. Our approach relies on a modified Orbitrap mass spectrometer which is capable of multi-stage analysis across all levels of protein organization, from the quaternary structure through single subunits and up to peptide fragments and PTM mapping. We demonstrate our approach on the yeast homotetrameric FBP1 complex, the rate-limiting enzyme in gluconeogenesis, and the 20S proteasome complex. We show that the complex responds to changes in growth conditions by tuning post-translational modifications. Overall, this method deciphers, on a single instrument and in a single measurement, the stoichiometry, kinetics, and exact position of modifications, contributing to the exposure of the multilevel diversity of protein complexes.

Sunday 10 September
16:00–18:00, Teddy Hall

Molecular Basis of Diseases

S.1.5-003
Epilepsy: from genes to cures for the sacred disease?

S. F. Berkovic
University of Melbourne, Melbourne, Australia

Epilepsy is an ancient condition shrouded in misunderstanding, superstition and stigma. Understanding the basic causes is challenging. Whilst epilepsy may be a consequence of an acquired insult including trauma, stroke, and brain tumours, the genetic component to epilepsies has been under-estimated. Considerable progress has recently occurred in the understanding of epilepsy genetics, both at a clinical genetic level and in the basic science of epilepsies. The evidence for genetic components will be first briefly discussed including data from population studies, twin analyses and multiplex family studies. Research in simple systems, and utilizing animal models with mutant ion channel subunits observed in humans, is revealing mechanistic insights at cellular, network and whole animal levels. Examples will be given as to how discoveries beginning with clinical research in patient populations leads to molecular genetic discoveries with subsequent unravelling of pathophysiology that informs clinical care and provides a realistic pathway to new treatments tailored to the individual. Proof of principle that this is feasible is provided by examples of dietary therapy for GLUT1 deficiency, pyridoxine treatment in encephalopathy due to *ALDH7A1* or *PNPO* mutations, preliminary data that quinidine may be effective in some subjects with *KCNT1* mutation, and the use of rapamycin analogues in the expanding family of mTORopathies. A major challenge will be to establish how widely applicable this strategy may be, and how it might be applied to epilepsies with complex inheritance.

S.1.5-001
New players in the biogenesis of the mitochondrial respiratory chain

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The molecular dissection of human mitochondrial disorders can shed light in the factors and mechanisms operating the biogenesis of the mitochondrial respiratory chain. We found a homozygous mutation in TMCO6, standing for transmembrane coiled-coiled protein 6, in a patient with progressive encephalopathy and isolated complex I deficiency. We showed that this protein is localized in the inner compartment of mitochondria, its amount is reduced in patient's fibroblasts, and a newly created *TMCO6*$^{-/-}$ mouse, displays neurological impairment. This model is currently under investigation from the molecular and biochemical standpoints. Loss-of-function mutations in *TTC19* (tetra-tricopeptide repeat domain 19) are associated with severe neurological phenotypes and mitochondrial respiratory chain complex III deficiency. We previously demonstrated the mitochondrial localization of TTC19 and its link with complex III biogenesis. Using a *Ttc19*$^{-/-}$ mouse model, we have now demonstrated that TTC19 binds to the fully assembled complex III dimer, i.e. after the incorporation of the iron-sulfur Rieske protein (UQCRFS1). The *in-situ* maturation of UQCRFS1 produces N-terminal polypeptides, which remain bound to holocomplex III. These UQCRFS1 fragments are rapidly removed, but when TTC19 is absent they accumulate within complex III, causing its structural and functional impairment. Overall, these data indicate a role for TTC19 in a novel post-assembly quality control process. Finally, non-sense mutations in APOPT1 are associated with cavitating leukoencephalopathy and isolated mitochondrial cytochrome c oxidase (COX) deficiency. We generated an *Apopt1* knockout mouse which recapitulates the biochemical hallmarks found in human patients. Further features of this suitable in vivo model of APOPT1 deficiency are under investigation, concerning COX assembly and function in differentiated tissues.

S.1.5-002
STOP hereditary spastic paraplegia: from gene hunting to new therapeutic approaches

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Hereditary Spastic Paraplegia (HSP) is a group of neurodegenerative diseases of the spinal cord with a rapidly increasing number of causative genes offering new targets for molecular therapy. SPG5 is a subtype of HSP caused by loss of function mutations in *CYP7B1* encoding the cytochrome oxysterol-7a-hydroxylase that is involved in the synthesis of primary bile acids from cholesterol. CYP7B1 deficiency resulted in an about 6-fold accumulation of its substrates 25-hydroxycholesterol and 27-hydroxycholesterol (25-OHC and 27-OHC) in serum. As oxysterols can pass the blood brain barrier, we also assessed 27-OHC in CSF and found it about 25-fold increased compared to healthy controls. Moreover, 27-OHC levels in serum correlated with disease severity and disease duration. We studied neurotoxicity of oxysterols in iPSC-derived cortical neurons and found 27-OHC to interfere with metabolic activity and viability of human cortical neurons using LDH and WST assays at concentrations found in SPG5 patients. Furthermore, 27-OHC impaired axonal outgrowth in accordance with the most severe affection of the longest axons (to the legs) in human disease. As oxysterol levels are known to correlate closely with cholesterol levels, we performed a randomized placebo-controlled trial with atorvastatin 40 mg/die for 9 weeks in 14 SPG5 patients and aimed to lower 27-OHC levels as the primary outcome measure. Atorvastatin but not placebo reduced serum levels of both, 25-OHC and 27-OHC, by about 30% in serum. In CSF 27-OHC was reduced by 8% but this did not differ significantly from placebo. As expected, no effects were seen on clinical outcome parameters in this short-term trial. We thus demonstrate the first causal treatment strategy in HSP and recommend a follow-up study with a longer treatment period to explore the effects of statin therapy on CSF levels of oxysterols in the long run.

ShT.1.5-002
P32: a mitochondrial protein that affects FUS-mediated ALS

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder caused by motor neuron loss. Mutations in the RNA binding protein FUS are associated to familiar forms of the disease. An *in vivo Drosophila* model of FUS-mediated neurodegeneration has been established: FUS mutations reduce fly viability

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and alter its locomotion activity. In an affinity purification experiment, we have identified P32 as one of the proteins that specifically bind to FUS. P32 is expressed in motor neurons and has several mitochondria-related properties. RNAi-mediated downregulation of P32 in Drosophila reduces fly locomotion. More interestingly, upregulation of P32 expression in flies carrying FUS mutations partially suppress FUS-mediated neurodegeneration suggesting that alterations of P32 activity may be relevant for the pathogenesis of ALS due to FUS mutation.

ShT.1.5-001
Pathological increase of store-operated calcium entry in iPSCs-based model of Huntington's disease is caused by expression of the mutant huntingtin

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Huntington's disease (HD) is an autosomal dominant hereditary neurodegenerative disorder which manifests in neural loss predominantly of GABAergic medium spiny neurons in striatum. HD is caused by the mutation in gene encoding huntingtin protein that leads to polyglutamine expansion within N-terminus region of this protein. One of the most promising frontiers of the modern science is an investigation of disease models based on patient-specific induced pluripotent stem cells (iPSCs) that can be used in frames of the patient-oriented therapy paradigm. Our previous studies of deregulation of the calcium homeostasis in iPSCs-based HD model have indicated a pathological increase of calcium entry through store-operated calcium (SOC) channels. Here, we address the question whether this increased calcium influx is really caused by endogenous expression of the mutant huntingtin. We showed that the allele-specific knockdown of the mutant huntingtin in HD-specific neurons returns SOC entry from pathologically enhanced to the normal level. Further we demonstrated that lentiviral expression of the N-terminus of the mutant huntingtin (Htt138Q-1exon) but not the N-terminus of the normal huntingtin (Htt15Q-1exon) in wild type human neurons leads to pathological increase of calcium entry through SOC channels. Thus, we convincingly showed that the increased currents mediated by SOC channels are caused by the mutant huntingtin expression. Our data also indicate a validity and well-reproducibility of iPSCs-based HD model and strongly support previous findings of calcium deregulation in HD. Therefore, the iPSCs-based model could be considered as one of the most adequate HD models and may provide a useful platform for future fundamental studies of HD and drug development. The study was supported by the RSF grant No. 14-14-00720 and the fellowship of the President of RF.

Monday 11 September
9:00–11:00, Teddy Hall

Signaling Across Membranes: Receptors, Channels and Transporters A

S.2.1-006
The role of lipids in Macs

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Mechanosensitive channels allow cells to response to changes in external pressure, this is an important and perhaps ancient ability. We have focussed on the MscS (mechanosensing channel of small conductance) found in bacteria. Following on from the ground-breaking work of the Rees lab in determining the closed structure of MscS, we determined an open structure. Using this structure we designed mutants to probe the conformational change in the protein that accompanies gating. To resolve issues over the validity of crystal structures we then probed the protein in both detergent solution and bilayer. This work prompted us to look at the role of lipids in the sensing of pressure. Bringing together biophysics and a new higher resolution structure we have developed a model how pressure gradient across a membrane could be sensed by proteins. This model identifies lipid binding to protein as playing an active role in controlling the conformation of the protein and this gating.

S.2.1-005
Sacrificing efficiency to achieve promiscuity: re-examining the multi drug transport mechanism of EmrE

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EmrE is a small multi drug resistance transporter that exports polyaromatic cations from the cytoplasm of *E. coli*, conferring resistance to drugs of this type. We have previously studied how this transporter recognizes and transports a broad array of polyaromatic cation substrates. Now we have used solution NMR spectroscopy to map out the different drug and proton-bound states of EmrE and quantitatively measure the open-in/open-out exchange rate for each state. This data unambiguously demonstrates that EmrE violates the "rules" commonly invoked to explain coupled antiport of two substrates. We have used our NMR data to develop a new model for secondary active transport and compared our predictions with functional assays to understand how EmrE harnesses the proton motive force to drive drug efflux. Our results show that EmrE does not have a single transport stoichiometry, resulting in reduced coupling efficiency of drug/proton antiport. This energetic cost may be a consequence of the protein flexibility required for recognition and transport of diverse substrates, and must represent an acceptable biological tradeoff for multidrug resistance and survival.

S.2.1-001
G protein-coupled receptors: the structural basis for their pharmacology

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G protein-coupled receptors (GPCRs) activate intracellular signalling proteins (G proteins and arrestins) in response to extracellular signalling molecules. GPCRs are highly dynamic proteins that rapidly interchange between different conformational states. In order to understand the molecular mechanisms of GPCR activation, structures of GPCRs in different conformational states are required. We have determined structures for both the adenosine A_{2A} receptor ($A_{2A}R$) and β_1 adrenergic receptor (β_1AR) in a variety of different states bound to ligands ranging from inverse agonists to full agonists. The inactive states of the receptors are highly conserved, as are the active states bound either to a nanobody or an engineered G protein. However, the molecular consequences of agonist binding to $A_{2A}R$ differ to those in β_1AR, with $A_{2A}R$ shifting to an active-intermediate state whereas β_1AR shifts to a state primed for activation that is very similar to the

inactive state. Understanding the conformational changes in the receptors allows an understanding of the molecular basis for some of the receptors' pharmacology, such as the increase in agonist affinity upon coupling to a G protein and why some ligands inhibit the receptors whilst other activate them. This work was funded by core funding from the Medical Research Council and by grants from Pfizer, MRCT, European Research Council, Wellcome Trust, BBSRC and Heptares Therapeutics Ltd.

ShT.2.1-003
Plasma membrane distribution of the Gi proteins and dopamine D2 receptor

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Jagiellonian University, Krakow, Poland

G proteins are peripheral membrane proteins which interact with the inner side of the plasma membrane and form part of the signalling cascade activated by G protein-coupled receptors (GPCRs). Since many signalling proteins do not appear to be homogeneously distributed on the cell surface, they associate in particular membrane regions containing specific lipids. Therefore, protein-lipid interactions play a pivotal role in cell signalling, but still some key questions about this interaction are unanswered. It is unclear whether the spatial organization of the GPCRs and G-proteins in the cellular membrane or/and interactions with lipids and other proteins affect high specificity and rate of signalling. In the context of current data we investigated membrane distribution of dopamine D_2 receptor with cognate G-proteins (Gαi) in living cells. Our main goal was to elucidate the differences in the membrane localization of investigated proteins. We also tested the effect of D_2 receptor or G$\beta_1\gamma_2$ dimer presence on the dynamics of Gαi_1, Gαi_2, Gαi_3 in the cellular plasma membrane. Using fluorescence resonance energy transfer (FRET) detected by lifetime imaging microscopy (FLIM) and fluorescence recovery after photobleaching (FRAP) microscopy, we demonstrated that the membrane distribution of different inhibitory Gα subunits is modulated by the G$\beta_1\gamma_2$ dimer. Our results imply that the inactive G protein heterotrimers are localized in the low-density membrane phase, from where they displace upon dissociation into the other membrane regions where interact with its signalling partners.

ShT.2.1-004
Insights into ion channel structure and function by ultrafast magic angle spinning NMR

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A milestone towards understanding of transmembrane channel functionality is the site specific characterization of minimal changes in structure and dynamics due to the presence (or absence) of metal ions. NMR spectroscopy of proteins in solution provides the resolution and sensitivity to observe these effects but is inherently limited by the protein size. This size limitation is absent for solid-state NMR under magic angle spinning (MAS), for which new state-of-the-art equipment and new concepts allow nowadays the rapid acquisition of well-resolved, atomic-level fingerprint spectra of membrane proteins in lipid bilayers. We illustrate the capacity of this technique on the bacterial divalent cation channel CorA, a pentamer of 5×42 kDa, comprised of two transmembrane helices and a large cytoplasmic domain hosting a metal binding side (usually Mg^{2+} or Co^{2+}). We present strategies to obtain sequence specific chemical shift information, which give insight into the secondary structure, as well as novel experiments that report on motions of different time scales. We finally discuss how this unique residue-specific information can be used to follow the transport of ions through the CorA channel. The data presented is complementary to already available crystal and cryo-EM structures, and represents a powerful example of integrated structural biology.

Monday 11 September
9:00–11:00, Oranim Hall 1

CRISPR and RNA Processing and Regulation A

S.2.2-001
Ribosome rescue and homeostasis

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There are several mRNA surveillance pathways in eukaryotes (NGD, NSD and NMD) that moderate the effects of natural errors in the cell and more broadly regulate gene expression. We have previously defined biochemical parameters of the factors Dom34, Hbs1 and Rli1 using our *in vitro* reconstituted yeast translation system. We have correlated these biochemical observations with ribosome profiling experiments to broadly define the *in vivo* targets of these same mRNA surveillance pathways. More recently, studies in mammalian platelets and reticulocytes revealed the accumulation of ribosomes in the 3' UTR of mRNAs and we connected this phenomenon with diminished levels of ribosome rescue (DOM34/HBS1L) and recycling (ABCE1) factors in these systems. Our current focus is on understanding the critical role of ribosome homeostasis during development and stress in diverse eukaryotic systems.

S.2.2-003
Germline small RNAs for genome defense

R. Pillai
Department of Molecular Biology, Geneva, Switzerland

Germline small RNAs for genome defense Ramesh Pillai Department of Molecular Biology, University of Geneva, Switzerland ABSTRACT Epigenetic silencing of transposons by germline small RNAs called Piwi-interacting RNAs (piRNAs) constitutes an RNA-based defense mechanism to ensure genome integrity and fertility. The piRNA pathway can be compared to an innate immune system that seeks out transposon elements for silencing via both post-transcriptional and transcriptional means. Information on which transposons are to be targeted is already encoded in defined genomic loci called piRNA clusters that become transcribed into single-stranded RNAs, which are then converted into tens of thousands of 24–30 nucleotide piRNAs. Animal germlines contain millions of individual piRNAs and how they are generated is an area of active research. Very much like an immune system, germ cells possess a poorly-understood small RNA biogenesis machinery for mounting an adaptive response which can selectively amplify antisense piRNAs targeting active transposons. Finally, the mechanisms and identity of the small RNA-guided nuclear machinery that guides transcriptional silencing by deposition of histone or DNA methyl marks are beginning to be understood. Latest results from the field and our own research will be discussed.

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S.2.2-006
Networks of alternative splicing regulation in cancer

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Alternative splicing of mRNA precursors allows regulation of gene function and physiology in higher eukaryotes, from the speciation of vampire bats to the selection of eatable quinoa seeds in South America. Alterations in alternative splicing can impact every hallmark of cancer and provide biomarkers of prognostic value. For example, activation of alternative splice sites in the oncogene BRAF confers resistance to vemurafenib and facilitate the frequent relapse of melanoma tumors. Splicing alterations can be caused by cancer-associated mutations in splicing regulatory sequences or by other genetic alterations, including mutations or changes in expression levels of splicing factors. Splicing factor mutations are particularly common in hematological tumors, including myelodysplastic syndromes and chronic lymphocytic leukemia. While advantageous for cancer progression, splicing alterations appear to make cancer cells particularly sensitive to splicing inhibitory drugs. These and other observations suggest that mis-regulation of alternative splicing networks contributes to tumor progression and at the same time can confer vulnerability to cancer cells. I will summarize our recent efforts to systematically reveal splicing regulatory circuits altered in cancer cells and the potential of this knowledge to design novel anticancer therapies. These include methods for saturation mutagenesis of alternative exons, genome-wide identification of regulatory factors and reconstruction of splicing regulatory networks via profiling of alternative splicing after systematic knock down of spliceosomal components. Our results reveal highly dense regulatory content of alternative exon sequences and extensive regulatory potential of core splicing factors. They also uncover circuits of cell cycle and apoptosis control by spliceosomal components, reveal detailed molecular mechanisms of versatile splicing modulation by anti-tumor drugs and by modified antisense oligonucleotides, and provide insights into the impact of signaling pathways important for cancer cell proliferation on alternative splicing.

ShT.2.2-001
Characterizing by dicer knockdown the developmental roles of microRNAs and their biogenesis in the sea anemone nematostella vectensis

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The Hebrew University of Jerusalem, Jerusalem, Israel

MicroRNAs (miRNAs) are small RNAs that negatively regulate gene expression in animals and plants, and by that play a role in diverse functions including development. To gain a deeper understanding of the evolution of miRNA biogenesis and miRNA-mediated post-transcriptional regulation, particularly during development, we need to look into a wide repertoire of extant species, including non-bilaterian animals. One such group is Cnidaria (corals, sea anemones, hydroids and jellyfish), an ancient lineage that diverged from the rest of Metazoa more than 600 million years ago. Earlier research revealed that most miRNAs in the model cnidarian *Nematostella vectensis* are spatiotemporally regulated, suggesting that miRNAs may play regulatory roles in the development of *Nematostella*. However, annotation of *Nematostella* miRNAs is still lacking when compared to that of major animal and plant models. In order to improve *Nematostella* miRNA annotation we knocked down Dicer by injecting Morpholino oligonucleotides to embryos and sequenced small RNAs. As Dicer is an RNase III responsible for miRNA biogenesis, its knockdown reveals bona fide canonical miRNAs and the roles of the miRNA pathway in *Nematostella* development and physiology. Our preliminary results suggest that knockdown of *Nematostella* Dicer decreases mature miRNAs levels and inhibits development and prevents larval metamorphosis into primary polyp. This is the first evidence for the involvement of miRNAs in development of a non-bilaterian animal. Overall, this study will provide a deep understanding of miRNA biogenesis in *Nematostella* as well as a first glimpse into the developmental roles of miRNAs in Cnidaria.

ShT.2.2-003
Pervasive acquisition of CRISPR memory from multiple replicons during inter-species mating in archaea

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Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems provide sequence-specific immunity to prokaryotes against viruses and other selfish elements, based on acquired DNA derived from these invaders, known as spacers. Surprisingly, many archaeal genomes contain spacers that target chromosomal genes of related species rather than selfish DNA. We sequenced 15 genomes of haloarchaea that were isolated from the same sampling site and identified a total of 17 inter-species spacers, 13 of which were perfect matches along the entire spacer length. We show experimentally that such spacers are actively acquired during inter-species fusion between two CRISPR-containing *Haloferax* species, from locations throughout the main chromosome. Engineering the genome of one mating partner so that it matched the other's CRISPR-Cas machinery reduced the frequency of horizontal gene transfer by fusion events between species. These findings implicate CRISPR-Cas systems in limiting gene exchange between species.

Monday 11 September
9:00–11:00, Oranim Hall 2

Mechanisms for Protein Homeostasis A

S.2.3-003
A versatile chaperone network coping with protein aggregates and amyloids

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Misfolded proteins are sticky and tend to form intracellular aggregates underpinning age-related deterioration and diseases including cancer and neurodegeneration. Normally, multi-tiered cellular quality control systems monitor and repair protein damage, limiting aggregation. Severe stress however overloads these

systems allowing aggregates to accumulate. This activates a cellular machinery which mediates the organized aggregation of misfolded proteins as well as the subsequent solubilisation and refolding of aggregated proteins. This machinery plays a pivotal role in cell survival under protein folding stress and in counteracting disease and age-associated cell toxicities. Small heat shock proteins (sHsp) constitute an evolutionary conserved yet diverse family of chaperones acting as first line of defense against proteotoxic stress. They promote the storage of misfolded proteins in native-like conformation facilitating disaggregation by ATP dependent chaperone systems. In plants, fungi and bacteria the central disaggregation machinery is a powerful bi-chaperone system comprised by the AAA+ disaggregase Hsp100 (Hsp104, ClpB) and the cooperating Hsp70 chaperone system. Metazoan cells lack Hsp100 disaggregases, but have evolved a potent Hsp70-based disaggregation machinery which relies on synergistic action of Hsp70 and its co-chaperones. This activity has broad specificity and can even disassemble amyloid fibrils. This seminar provides insights into the working mode of the eukaryotic disaggregases in dealing with aggregates and amyloids.

S.2.3-004
Ribosome-associated quality control (RQC) and neurodegeneration

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We had previously found that mutation of a novel E3 ligase, Listerin, causes neurodegeneration in mice (Chu et al 2009, *PNAS* 106:2097). We subsequently discovered that the yeast Listerin ortholog, Ltn1, acts in a protein quality control pathway—now known as Ribosome-Associated Quality Control (RQC)—that targets nascent polypeptide chains associated with ribosomes that become stalled during translation elongation. In eukaryotes, stalled ribosomes are first rescued by factors that split the subunits, releasing the mRNA for degradation, the 40S subunit, and the 60S subunit—the latter still stalled with a nascent peptidyl-tRNA conjugate. The stalled 60S is the target of Ltn1. Ltn1 acts as a component of the RQC complex, along with Rqc1 and Rqc2/Tae2 (NEMF in mammals) subunits. We solved the first cryo-EM structure of this complex, which revealed that the stalled 60S is specifically recognized via the simultaneous binding of Rqc2 to the 60S and the tRNA moiety of peptidyl-tRNA. Rqc2 next recruits Ltn1, which, in turn, ubiquitylates the aberrant nascent chain for proteasomal degradation. More recently, Rqc2 has been found by others to additionally act in RQC by modifying stalled nascent chains with a carboxy-terminal, Ala- and Thr-containing extension—the "CAT tail." We have reported that CAT tails mediate formation of insoluble nascent chain aggregates. CAT tail modification and aggregation of nascent chains were conspicuous under conditions of limiting Ltn1 function, suggesting that inefficient targeting by Ltn1 favors the Rqc2-mediated processes. These findings uncovered a protein aggregation mechanism that may be relevant to the pathophysiology of neurodegeneration in Listerin-mutant mice. We will review these data and discuss how knowledge on yeast RQC can predict novel genes and mechanisms underlying mammalian neurodegeneration.

S.2.3-006
Selective inhibition of a phosphatase to correct protein quality control failures and treat neurodegenerative diseases

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MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The deposition of misfolded proteins is a defining feature of many age-dependent human diseases, including the increasingly prevalent neurodegenerative diseases. Why misfolding-prone proteins accumulate in aged cells remains largely unclear. Cells normally strive to ensure that proteins get correctly folded and have powerful and sophisticated protein quality control mechanisms to maintain protein homeostasis under adverse conditions. However, with age, the cellular defence systems against misfolded proteins gradually fail, leading to the accumulation of misfolded proteins with devastating consequences for cells and organisms. In principle, improving the cells' ability to deal with misfolded proteins should represent a generic approach to reduce pathology in diverse protein misfolding diseases. My lab has identified powerful strategies to help cells survive when protein quality control fails and implemented one of such strategies in mice. Exploiting the current knowledge on protein quality control systems, we have identified a small drug-like molecule that safely boosts the natural defence system against misfolded proteins. When given to mice, the small molecule completely prevents two unrelated neurodegenerative diseases, without any deleterious side effects. The small-molecule was named Sephin1 because it is a selective inhibitor of a holophosphatase. Our work demonstrates that generic approaches aimed at helping cells to survive protein quality control failures can be useful to prevent protein misfolding diseases, including the devastating neurodegenerative diseases. Moreover, whilst phosphatases were previously thought to be undruggable, our work establishes that phosphatases can be selectively inhibited by targeting their regulatory subunits. This finding has broad relevance because there are hundreds of phosphatases that could in principle be inhibited using the same paradigm. This opens up a broad range of possibilities to manipulate cellular function for therapeutic benefit.

ShT.2.3-001
Control of beclin 1-induced autophagy by polyglutamine repeats

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Nine polyglutamine (polyQ) diseases are caused by an expansion of the polyQ domain in different proteins. Examples include mutant huntingtin in Huntington's disease (HD) and mutant ataxin-3 in spinocerebellar ataxia type 3. Autophagy is a key degradation pathway for these aggregate-prone mutant proteins. In this study, we investigated how autophagy is modulated by wild-type ataxin-3, a deubiquitinating enzyme that is widely expressed in the brain. We show that the polyQ stretch in wild-type ataxin-3 is required for it to bind and to deubiquitinate beclin 1, a key autophagy inducer. This maintains the levels of beclin 1 by protecting it from proteasome-mediated degradation. Beclin 1 is critical for autophagy, which is compromised by ataxin 3-knockdown in neurons and in-vivo. Thus, these data provide a discreet and critical role for the polyQ stretch in a

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wild-type protein. Since the interaction of beclin 1 with ataxin-3 is dependent on the polyQ stretch in the latter, we tested and confirmed that they could be competed for by isolated polyQ stretches in trans, which impair starvation-induced autophagy in a beclin 1-dependent fashion. Importantly, we could demonstrate this phenomenon with wild-type, non-aggregating forms of huntingtin, suggesting that this can be modulated by soluble species via the polyQ interaction, compatible with the toxicity seen in HD cells without aggregates. Likewise, this phenomenon was compromised either in an HD mouse model or in fibroblasts from patients with different polyQ diseases. The mechanism we have described reflects a less obvious impairment of basal autophagy caused by polyQ disease proteins that is amplified under starvation conditions. This may contribute to late-onset polyQ disorders, where the toxic mutant protein itself is an autophagy substrate.

ShT.2.3-002
Hsp70 chaperone-substrate interactions

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Weizmann Institute of Science, Rechovot, Israel

Most proteins must fold into defined three-dimensional structures to gain functional activity. But in the cellular environment, newly synthesized proteins are at great risk of aberrant folding and aggregation, potentially forming toxic species. To avoid these dangers, cells invest in a complex network of molecular chaperones, which use ingenious mechanisms to prevent aggregation and promote efficient folding. We have studied the mechanism by which, Hsp70, one such molecular chaperone aids in protein folding, helps to refold misfolded protein and prevents protein aggregation. Using NMR spectroscopy we show that Hsp70-bound proteins are globally unfolded, but can start folding and forming local secondary structure even while bound to the chaperone. Moreover, Hsp70 binding prevents the client protein from forming long range non-native interactions that are otherwise present in the unbound, unfolded conformation and which can lead to protein misfolding and aggregation. Moreover, looking directly at the substrate residues situated in the Hsp70 binding pocket, we have shown that there are multiple conformations of substrate protein bound to Hsp70 and that there is a significant amount of heterogeneity in the bound ensemble. This promiscuous binding to client proteins may serve to generate different initial starting points for the client protein to start folding during the chaperone cycle, with some of the starting structures more amenable to proper folding than others. Protein molecules that do not fold to the native state upon release, still have a chance to re-enter the chaperone cycle by binding via another site, thereby increasing their chances of ultimately folding correctly.

Monday 11 September
9:00–11:00, Oranim Hall 3

Organelle Biogenesis and Dynamics A

S.2.4-003
Protein import into peroxisomes

R. Erdmann
Ruhr-University Bochum, Bochum, Germany

The peroxisomal protein import machinery differs fundamentally from most other translocons as it allows the membrane passage of folded, even oligomerized proteins. Cycling import receptors shuttle between the cytosol and peroxisomal lumen, recognize and bind the peroxisomal targeting signals (PTS1 or PTS2) of their folded cargo proteins in the cytosol and target them to a docking- and translocation-machinery at the peroxisomal membrane. At the membrane, the import receptors assemble with docking components and form a protein-conducting gated channel and import takes place in an unknown manner. In the following, import receptors are ubiquitinated and released from the membrane in an ATP-dependent manner. Here we report 1) on the adaptation of yeast cells to changes in environmental conditions by alternative protein import pathways and 2) a novel way to selectively kill human Trypanosoma parasites causing sleeping sickness and Chagas disease by blocking peroxisomal/glycosomal protein import. In cooperation with the group of Michael Sattler (Munich), we developed small molecules that efficiently disrupt the docking of cargo-loaded receptor to parasites organelles. This results in mislocalization of peroxisomal/glycosomal enzymes causing metabolic catastrophe and kills the parasite. Our data identify protein import into glycosomes/peroxisomes as an "Achilles' heel" of the Trypanosoma suitable for the development of novel therapies against trypanosomiases.

S.2.4-005
Biogenesis and maintenance of mitochondria through protein and lipid trafficking

T. Endo
Kyoto Sangyo University, Kyoto, Japan

Mitochondria are essential for the viability of all eukaryotic cells. They function as powerhouses for production of ATP, are crucial for the metabolism of amino acids, lipids and iron, and play a central role in apoptosis. Defects in mitochondrial biogenesis and functions lead to severe diseases in particular of the nervous system, heart and muscles. Since mitochondria are only generated by growth and division of preexisting mitochondria or require them as templates, mitochondrial growth relies on import of their resident proteins as well as transport and synthesis of phospholipids. Thus, protein import and lipid transport constitute the central processes of mitochondrial biogenesis and maintenance. Mitochondrial protein transport is mediated by the translocators in the outer and inner mitochondrial membranes and by soluble factors in the cytosol, intermebrane space, and matrix. I will discuss how these translocators and soluble factors cooperate to achieve precise as well as efficient transport of over 1,000 different mitochondrial proteins from the cytosol to different sub-compartments within mitochondria. How hydrophobic phospholipid molecules can traverse aqueous compartments to shuttle between different membranes is also a critical problem concerning the mechanism of organelle biogenesis. I will discuss how different lipid transport machineries mediate transport of hydrophobic phospholipids between the ER and mitochondria and between the outer and inner mitochondrial membranes by different mechanisms.

S.2.4-001
Biogenesis of mitochondrial outer membrane proteins in evolutionary context

D. Rapaport
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The mitochondrial outer membrane (MOM) mediates multiple interactions between the mitochondrial metabolic and genetic systems and the rest of the eukaryotic cell. Biogenesis of this membrane involves integration of newly synthesized proteins into the lipid bilayer. Among these precursor proteins are those that span the membrane once, twice or with multiple segments. Our aim is to fully define the biological processes and molecular mechanisms that underlie the biogenesis of MOM proteins. Employing

in vitro, *in organello* and *in vivo* experiments we could characterize unique import pathways for the various families of MOM proteins. Remarkably, the biogenesis of β-barrel proteins is conserved from Gram–negative bacteria to mitochondria and this conservation allows the organelle to recognized and process bacterial substrates.

ShT.2.4-001
Incorporation of mitochondria into cells – characteristics and therapeutic utility

E. E. Kesner, E. L. Kesner, V. Trembovler, A. Alexandrovich, A. Saada, E. Shohami, H. Lorberboum-Galski
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Mitochondria can be incorporated into cells by a simple co-incubation of isolated mitochondria with cells. We found that mitochondria can be incorporated into recipient cells very quickly, and co-localize with endogenous mitochondria. Mitochondrial-incorporation into mitochondria-defected cells results in increased cell viability and enhanced complex II+III activity. Transmission electron microscopy revealed that the isolated mitochondria interact directly with recipient cells, which engulf the mitochondria with cellular extensions in a way, which may suggest the involvement of macropinocytosis or macropinocytosis-like mechanisms in mitochondrial incorporation. Indeed, macropinocytosis inhibitors but not clathrin mediated endocytosis inhibition-treatments, blocks mitochondrial-incorporation. The integrity of the mitochondrial outer membrane and its proteins is essential for the mitochondrial incorporation; cells can distinguish mitochondria from similar particles and incorporate only intact mitochondria. Next, we tested mitochondrial-incorporation *in-vivo*. We found that mitochondrial-intravenous (I.V) administration into mice is safe, and that the administrated mitochondria incorporated into animals organs, including liver, hart, and, most surprisingly, brain. The therapeutic potential of mitochondrial-incorporation was tested in a mouse-model of close head injury (CHI), in which mitochondrial-dysfunction is a key player. We found that a 48 h-post trauma single I.V mitochondrial-administration treatment resulted in a significant improvement in mice motor and cognitive abilities. All together, our data characterize the intrigues phenomenon of mitochondrial incorporation, and may suggest that it effects mitochondrial dynamics. In addition, we highlight mitochondrial-incorporation remedial applications potential.

ShT.2.4-002
Investigating the role of lysosomes in mammalian cytokinesis

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Historically lysosomes have been predominantly considered cellular "waste bags" however new findings demonstrate that they are highly dynamic organelles tasked with a wide spectrum of biological functions.. One of these is their ability to fuse with the plasma membrane, enabling membrane donation during plasma membrane wound repair. Our group has previously identified a unique re-distribution of lysosomes during cytokinesis, the final stage of cell division. The organelles cluster at either side of the intercellular bridge separating the daughter cells. If this particular lysosome redistribution is disrupted, cytokinesis fails. A potential role for lysosomes during cytokinesis is to act as a membrane reservoir which is donated to the plasma membrane to permit daughter cell separation. Understanding cytokinesis regulation is of the utmost interest as failure in the division process can lead to aneuploidy, genetic instability and malignant phenotypes. Using a combination of imaging techniques, pharmacological, biochemical and genetic approaches, I have investigated lysosomal exocytosis in the context of cell division. Osmotic lysis of lysosomes with the agent GPN increases the frequency of polynucleate cells and cells undergoing cytokinesis, suggesting that lysosome integrity is essential for normal cytokinesis completion. PI4KIIIβ has been identified as a potential key player in this process and is able to influence both lysosomal exocytosis and cytokinesis completion. Furthermore, lysosomal exocytosis increases towards the end of cytokinesis in cells loaded with lucifer yellow. Finally, using a lysosome-targeted genetically encoded pH probe, I have discovered that the clustering of lysosomes during cytoplasmic division is accompanied by an alkalinisation of these organelles. Collectively these results indicate a novel function for lysosome exocytosis during mammalian cell cytokinesis opening a novel area of future investigation into this key biological process.

ShT.2.4-003
Perilipins protect against lipotoxicity but have no essential role in lipid droplet growth or maintenance

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Lipid droplets (LDs) are unique subcellular organelles for the storage of neutral lipids. Their hydrophobic core is surrounded by a phospholipid monolayer with a distinct set of embedded proteins. These are mainly enzymes of lipid metabolism and perilipin (PLIN) family proteins. Perilipins are found in various combinations on all mammalian lipid droplets and are therefore deemed essential LD proteins. PLIN-1 has been implicated in the regulation of lipolysis in adipocytes, and PLIN-5 plays a role in the ß-oxidation of muscle cells. For the ubiquitously expressed PLIN-2/adipophilin and PLIN-3/TIP47 proteins however no clear function has been assigned yet. Here, we applied CRISPR/Cas9 technology to knock-out both PLIN-2 and PLIN-3 in human osteosarcoma U2-OS cells, creating to our knowledge for the first time an essentially perilipin free mammalian model system. The number of steady state small LDs was strongly decreased in the double knock-out cells. Comparison to single knock-out cells suggested that this was mainly due to the deletion of PLIN-3, with PLIN-2 being able to compensate only partially. LD biogenesis was likewise impaired, with the number of newly emerging LDs reduced by half. Remarkably however, FACS analysis suggested that general LD content was only slightly reduced, and LD growth as observed by microscopy was also not affected dramatically. No major changes of lipid metabolism were observed as indicated by acetate labeling. Surprisingly however, the double knock-out cells were exceptionally sensitive to fatty acid induced lipotoxicity. The rescue of this phenotype required the presence of both PLIN-2 and PLIN-3. In conclusion, we have uncovered an unexpected function of perilipins in the protection against lipotoxicity.

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ShT.2.4-004
NME4 at the crossroads of mitochondrial dynamics and signaling: roles in shaping mitochondria, initiating mitophagy, and cell morphology

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The well-established function of the mitochondrial intermembrane space protein NME4 (also called NDPK-D or NM23-H4) is phosphotransfer activity as a nucleoside diphosphate kinase (NDPK). However, recent data have revealed a second function in lipid signaling that triggers mitophagy, a critical process for cell homeostasis. This latter function involves NME4-mediated intermembrane phospholipid transfer activity, leading to externalization of cardiolipin (CL) from the mitochondrial inner membrane to the mitochondrial surface. Interestingly, both functions seem to involve an interaction of NME4 with OPA1, a dynamin-like GTPase of the mitochondrial inner membrane. First, NME4 directly fuels OPA1 with GTP via its NDPK bioenergetic activity. However, also the CL transfer activity of NME4 is related to OPA1, since OPA1 seems to be a negative regulator of this NME4 CL transfer. Our current model suggests that NME4/OPA1 complexes exist in healthy mitochondria to maintain OPA1 functions in inner membrane fusion and dynamics. Upon OPA1 cleavage, an early step during mitophagy, NME4 may be released from these complexes, allowing simultaneous interaction of the hexameric NME4 complex with inner and outer mitochondrial membrane and CL transfer. Our most recent data on HeLa cells reveal that ablating either NME4 function, phosphotransfer or lipid transfer, affects cell morphology and motility.

Monday 11 September
9:00–11:00, Oranim Hall 4

Integrated Structural Biology for Innovative Translational Research

S.2.5-002
Structural virology from basic science to translation and back

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Viruses remain beautiful model systems for biology and major threats to health and agriculture. Whilst their diversity obstructs the development of broad silver bullet therapeutic strategies, the control of, for instance AIDS, has been built on a substantial amount of structure guided design. Vaccines can go a step further and have already allowed the global elimination of two viruses. Most current vaccines were developed using methods devised 50 years ago, essentially either relying on inactivating pathogenic viruses or deriving attenuated strains by multiple passages. Making virus protein coat mimics by recombinant methods has been used more recently and can produce much safer products. Combining this with structural knowledge should allow vaccine antigens to be produced with much better physico- chemical and immunogenic properties, however this will require strong basic science understanding. I will give examples showing some success from systems we are currently working on.

S.2.5-001

Withdrawn

S.2.5-003
NMR for understanding functional processes

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NMR spectroscopy constitutes a unique tool for describing functional biological processes at atomic level and in a cellular context. NMR is indeed suitable not only to characterize the structure and dynamics of biomolecules but, even more importantly, to describe transient interactions and functional events with atomic resolution, possibly in a cellular context. This approach requires the development of suitable methodologies capable of addressing multiple, specific, and sometimes non conventional aspects and amenable to characterize functional processes in living cells, also integrating these data with those obtained in vitro. Along a functional process, most interactions are transient in nature, suitably studied by NMR, which can also characterize processes in living cells with atomic resolution. Among processes involving transient interactions are the metal transfer processes, in which metal transfer, from metal transporters to the final recipient proteins, occurs through a series of protein-protein interactions. This transfer is determined by metal affinity gradients among the various proteins, with kinetic factors contributing to the selectivity of the processes. The presence of paramagnetic centers, such as iron-sulfur clusters, dramatically affects the NMR spectra, requiring the integration of multiple techniques, which are available within the H2020 iNEXT project, an infrastructure for NMR, EM and X-ray crystallography for transnational research. The power of NMR in describing cellular pathways at atomic resolution in a cellular environment will be presented for a few pathways responsible for copper trafficking in the cell and for the biogenesis of iron-sulfur proteins. New major advancements in in-cell NMR and in the characterization of highly paramagnetic systems will be also discussed within an integrated approach where, from single structures to protein complexes, the processes are described in their cellular context within a molecular perspective.

ShT.2.5-002

Building stable RNA tertiary structures with protonated residues

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In order to carry out their biological functions, RNA molecules adopt intricate tertiary structures. The diversity in RNA folds is astonishing given that they are built out of only four distinct entities. Protonation of cytidine at the N3 or adenine at the N1 position provides additional diversity in hydrogen bond donor and acceptor patterns. Considering the ever increasing number of protonated residues in the structures of functional RNA molecules observed by us and others, we here study the role of protonated nucleotides in functional RNAs and characterize their stability and structural context.

Using heteronuclear NMR together with specific isotope labeling, we readily identified a number of protonated adenine residues in the structures of several synthetic GTP aptamers.[1] We also investigated several functional RNA mutants carrying isosteric replacements of G and U residues by protonated A and C residues, respectively, such as in the U-turn motif and different riboswitch aptamer domains. Mutational data and sequence alignment among species suggest that isosteric replacements by protonated residues is a common theme in functional RNA. All of the structure building protonated residues reported here appear to be extremely stable with pKa values shifted significantly above neutrality. Base protonation can easily escape notice in routine structural investigations. The frequent occurrence of protonated bases in the structures of functional RNAs, however, suggests that its presence should be routinely considered both by experimental structural biology and in tertiary structure predictions.

ShT.2.5-001

High resolution snapshots of active macromolecular machineries by single particle cryo-EM

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Trypanosomatids are single cell eukaryotic protozoan parasites that pose a serious health concern afflicting more than 20,000,000 people worldwide. The most notable pathogens belonging to this order are *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* species which cause African sleeping sickness, Chagas' disease and Leishmaniasis, respectively. The often fatal outcome of infections, lack of effective vaccines, limited selection of available therapeutic drugs, and emerging resistant strains, underline the need to develop novel strategies to combat these pathogens. The Trypanosomatid ribosome has recently been highlighted as a promising therapeutic target due to structural features that are distinct from other eukaryotes. In a serious of structural studies exploiting the recent advances in the field of electron-cryo microscopy (cryo-EM) we revealed the unique architecture of the Trypanosomatid ribosome at extremely high resolutions (2.5–2.8 Å) identifying unique elements that play a significant role in ribosome assembly and function as potential drug targets. Additional studies in active Trypanosomatid ribosome complexes also provide atomic resolution snapshots into Trypanosomatid translation and unveil the mechanisms by which anti-ribosomal drugs with promising anti-protozoan properties induce their deleterious effects on the translation apparatus and inhibit parasite growth.

Monday 11 September 15:00–17:00, Teddy Hall

Signaling Across Membranes: Receptors, Channels and Transporters B

S.2.1-003

Ion channel-transporter interactions

G. Abbott
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Ion channels provide an often highly selective pathway for diffusion of aqueous ions across lipid bilayers, down their electrochemical gradient. Transporters facilitate movement of solutes up their electrochemical gradient, utilizing in some cases energy from concomitant downhill movement of another solute (e.g., sodium-coupled solute transporters). Ion channels and transporters must function in a coordinated manner to ensure ion homeostasis, electrical excitability, and other essentialy processes. Recently, we and others found that some ion channels form physical complexes with certain solute transporters, producing reciprocally regulating complexes permitting crosstalk and feedback. Here, we focus on interaction between voltage-gated potassium channels of the KCNQ (Kv7) subfamily and sodium-coupled transporters from the SLC5A family, including SMIT1 and SMIT2 (which transport *myo*-inositol) and NIS (iodide). The KCNQ1-KCNE2 heteromeric channel forms complexes with SMIT1 and NIS; disruption of these complexes in mice causes aberrantly increased neuronal activity and hypothyroidism, respectively. KCNQ2/3 channels form complexes with SMIT1 and SMIT2 in the brain and sciatic nerve; disruption in mice of either SMIT1 or KCNQ2 was previously shown to cause lethal hypoventilation. *Myo*-inositol is an important osmolyte and also a substrate for production of PIP2, a soluble lipid-derived signaling molecule required for normal function of many ion channels. Formation of KCNQ2/3-SMIT1 complexes preserves transporter function during cellular depolarization, and also appears to ensure a locally high PIP2 concentration for regulation of KCNQ2/3 activity. Even in the absence of *myo*-inositol, SMIT1 modifies key KCNQ functional properties. Specifically, SMIT1 alters the ion selectivity and pharmacology of KCNQ1 and KCNQ2, and shifts the voltage dependence and gating kinetics of the latter. Finally, biochemical studies suggest SMIT1 binds directly to the KCNQ2 pore module.

S.2.1-004

Modulation of the activity of a bacterial K^+ transporter

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The bacterial machinery involved in K^+ homeostasis is conserved across species and it involves pumps, channels and sensor proteins. Many of these proteins are also conserved in plants and fungi but are not present in animal cells. The KtrAB is a component of the K^+ homeostasis machinery of the bacterium, Bacillus subtilis. It is a protein complex with a known structure formed by the KtrB membrane protein, which displays the architecture of K^+ channels, and the KtrA cytosolic protein, a RCK domain. Regulation of KtrAB activity is dependent on ATP, ADP and c-

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diAMP binding to KtrA. I will present our most recent data on the mechanism of regulation of KtrAB activity.

S.2.1-002
Entropic clocks in the service of electrical signaling

O. Yifrach
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Electrical signaling in the nervous system relies on action potential generation, propagation and transmission. Such processes rely on precisely timed events associated with sequential voltage-dependent ion channel conformational transitions between the closed, open and inactivated states, as well as clustering at unique membrane sites. In the modular voltage-dependent potassium channel (Kv) protein, the activation, inactivation and clustering mechanisms are based on interactions involving either folded membrane-spanning domains or intrinsically disordered protein segments. While the role of the Kv channel in generating action potentials is primarily based on channel activation gating, mediated by tight electro-mechanical coupling between the channel voltage-sensing and pore domains, the regulation of action potential shape and frequency involves Kv channel inactivation and clustering, mediated by intrinsically disordered channel segments respectively found at the N- and C-termini of the protein. These IDP tails function as entropic clocks to time specific but distinct binding events, relying on what is traditionally referred to as a 'Ball and Chain' mechanism. In my presentation, I will discuss the experimental criteria that argue for an entropic clock function for IDP segments and delineate the expected thermodynamic signature of this function. Using the Kv channel example, I will summarize results demonstrating that Kv channel fast inactivation and clustering occurs according to a 'Ball and Chain' mechanism and will provide evidence for the compatibility of the two processes, while considering their similarities and differences. Finally, I will present evidence for thermodynamic coupling between the inactivation and clustering functions of the Kv channel and will discuss the enigmatic question of how such coupling is even possible, considering the entropic clock-based mechanisms underlying these functions.

ShT.2.1-001
Ca^{2+} signals originate at immobile IP3 receptors adjacent to the ER-plasma membrane junctions where Ca^{2+} entry occurs

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University of Cambridge, Cambridge, United Kingdom

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are intracellular Ca^{2+} channels that directly regulate Ca^{2+} release from the ER and thereby also control store-operated Ca^{2+} entry. IP_3Rs open when they bind IP_3 and Ca^{2+}, a property that allows them to propagate Ca^{2+} signals regeneratively. The spatial organization of IP_3Rs determines both this propagation of Ca^{2+} signals between IP_3Rs and the selective regulation of cellular responses. We use gene-editing with TALENs to fluorescently tag endogenous IP_3Rs in mammalian cells, and super-resolution microscopy to determine the geography of IP_3Rs and Ca^{2+} signals within living cells. We show that native IP_3Rs form loose clusters within ER membranes. Most IP_3Rs are mobile, moved by diffusion and microtubule motors. Ca^{2+} signals are generated by a very small population of immobile IP_3Rs. These IP_3Rs are licensed to respond, but they do not readily mix with mobile IP_3Rs. The licensed IP_3Rs reside alongside ER-plasma membrane junctions where STIM1, which regulates store-operated Ca^{2+} entry, accumulates after depletion of Ca^{2+} stores. IP_3Rs tethered close to ER-plasma membrane junctions are licensed to respond and optimally placed to be activated by endogenous IP_3 and to regulate Ca^{2+} entry.

ShT.2.1-002
Crystal structure of the pentameric ligand-gated ion channel ELIC in complex with a nanobody active as an allosteric modulator

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Pentameric ligand-gated ion channels (pLGICs) belong to a class of ion channels involved in fast synaptic transmission in the central and peripheral nervous system. Members of this family include the nicotinic acetylcholine receptors, 5-HT_3 serotonin receptors, $GABA_{A/C}$ (gamma-aminobutyric acid) receptors and glycine receptors. These receptors are the target for a wide variety of therapeutics, including benzodiazepines such as diazepam (Valium). From a drug discovery perspective much attention goes out to the development of novel positive allosteric modulators, which enhance receptor function while preserving the normal fluctuations of transmitter release at the synapse. In this study, we take advantage of the Erwinia ligand-gated ion channel, ELIC, which can easily be produced in large quantities for structural studies. Previously, we demonstrated that the benzodiazepine flurazepam occupies an allosteric binding site in ELIC, which lies opposite of the agonist-binding site, in the vestibule of the extracellular ligand binding domain. In this study, we generated single domain antibody fragments, also known as nanobodies, against ELIC. Using electrophysiological recordings of ELIC channels expressed in Xenopus oocytes, we demonstrate that nanobodies can either inhibit or potentiate agonist-evoked responses, suggesting that nanobodies can act as negative or positive allosteric modulators, respectively. The X-ray crystal structure of ELIC in complex with a nanobody reveals that the nanobody binds at a site near the vestibule binding site. In addition, we performed cysteine-scanning mutagenesis of key residues in the vestibule binding site and highlight the functional importance of these residues in allosteric modulation of ELIC. Given the structural conservation of the vestibule binding site between ELIC and eukaryote receptors our study provides further evidence in support of this site as a suitable target for development of novel allosteric modulators of pLGICs.

Monday 11 September
15:00–17:00, Oranim Hall 1

CRISPR and RNA Processing and Regulation B

S.2.2-004
Gene expression is a circular process

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Gene expression is traditionally viewed as a linear process divided into distinct stages (e.g., transcription, translation). We have shown that this view is oversimplified. First, RNA polymerase II controls mRNA translation and decay, via a mediator

– Rpb4/7. Second, many transcripts are "tagged" with factors co-transcriptionally, one of these tags is Rpb4/7, which later regulates the mRNA localization, translatability and decay. We name this tagging "mRNA imprinting". Promoters, which are DNA elements that control transcription, also regulate "mRNA imprinting", thereby affecting the mRNA fate in the cytoplasm. Third, we found that the major mRNA 'decaysome", known to degrade mRNAs in the cytoplasm, also functions as a transcription activator by physically associating with promoters (~30 bp upstream of transcription start sites). We propose that the interplay between mRNA decay, import of some decaysome components and transcription is important for understanding how expression of some genes is regulated. Our findings demonstrate that gene expression is a circular process in which the hitherto first and last stages are interconnected.

S.2.2-005
RNA editing is important for regulation of RNAi mechanism

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Technion, Haifa, Israel

A-to-I RNA editing is a conserved and widespread phenomenon in which adenosine (A) is converted to inosine (I) by adenosine deaminases (ADARs) in double-stranded RNA molecules. Although human RNAs contain millions of A-to-I editing sites, most of these occur in noncoding regions and their function is unknown. Knockdown of ADAR enzymes in *C. elegans* causes defects in normal development but is not lethal as in human and mouse, making *C. elegans* an ideal organism for studying the regulatory effects of RNA editing on the transcriptome. Previous studies in *C. elegans* indicated competition between RNA interference (RNAi) and RNA editing mechanisms, with the observation that lack of both mechanisms can suppress defects observed when only RNA editing is absent. To study the effects of RNA editing on gene expression and function, we established a novel screen that enabled to identify thousands of RNA editing sites in non-repetitive regions in the genome. These include many genes that are edited at their 3'UTR region. We found that these genes are mainly germline and neuronal genes and they are downregulated in the absence of ADAR enzymes. Interestingly, we observed that almost half of these genes are edited in a developmental-specific manner. Furthermore, we found that many pseudogenes and other lncRNAs are extensively downregulated in the absence of ADARs in embryo but not L4 larva developmental stage, while this downregulation is not observed in additional knockout of RNAi. Taken together, our results suggest a role for RNA editing in normal growth and development by regulating silencing by RNAi.

S.2.2-002
Transgenerational inheritance of small RNAs and chromatin marks

O. Rechavi
Tel Aviv University, Tel Aviv, Israel

In *C. elegans* small RNAs enable transmission of epigenetic responses across multiple generations. Different environmental challenges, including exposure to viruses, starvation, and heat stress generate heritable small RNA responses, that in certain cases could be adaptive. Heritable RNAi induced by administration of dsRNA lasts typically for 3–4 generations. Until recently the mechanisms that determine the duration of inherited silencing, and allow "forgetting" of the inherited epigenetic effects after a number of generations were unknown. We found that the duration of these transgenerational responses is governed by a number of feedback interactions, that together establish a "timer" mechanism. Chromatin marks, among other factors, affect the duration of heritable RNAi. Disruption of the chromatin landscape produces transgenerational effects, such as inherited increase in lifespan and gradual loss of fertility. Inheritance of histone modifications can be induced by dsRNA-derived heritable small RNAs. We show that the mortal germline phenotype found in a specific histone methyltransferase mutant depends on HRDE-1, an Argonaute that carries small RNAs across generations, and is accompanied by accumulated transgenerational misexpression of heritable small RNAs. We discovered that this histone methyltransferase inhibits small RNA inheritance, and as a consequence, induction of RNAi in mutants of this histone methyltransferase leads to permanent RNAi responses that do not terminate even after more than 30 generations. We found that this potentiation of heritable RNAi results from global hyperactivation of the small RNA inheritance machinery. Thus, changes in histone modifications can give rise to drastic transgenerational epigenetic effects, by controlling the overall potency of small RNA inheritance.

ShT.2.2-002
Gene targeting in zebrafish

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Here, we report the use of a type IIIA CRISPR complex for RNA knockdown in zebrafish (*Danio reiro*). Gene silencing in zebrafish is challenging, as RNAi is not amenable and the alternative, morpholino polynucleotide analogues have been shown to have off target affects. Therefore, zebrafish seems an ideal target organism to investigate the use of CRISPR techniques for specific RNA degradation. We decided to use the Csm complex, a type IIIA CRISPR system. Csm proteins and bound crRNA form multisubunit protein-RNA complexes that exhibit typical two-filament structures characteristic for class 1 CRISPR complexes. Csm complexes have RNA-guided, RNA-directed endoribonuclease activities cleaving substrate RNAs at multiple, regularly spaced sites. The Csm complexes have additional transcription dependent ssDNase activity. We chose to use Csm complex from *Streptococcus thermophilus* loaded with crRNA complementary to GFP. Csm complexes were purified from *E. coli* expression system and injected into zebrafish embryos expressing GFP under the control of a variety of different promoters. Possible suppression of GFP fluorescence by RNA knockdown was then monitored by imaging and flow cytometry. GFP knockdown was observed for strains expressing GFP under early development promoters. We also proved that the knockdown is dependent on the RNase, but not the ssDNase activity of the Csm complex. Overall, we described a novel CRISPR based strategy for targeted RNA knockdown in zebrafish embryos.

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ShT.2.2-004
A-to-I RNA editing introduce somatic mutations to the RNA

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Genomic mutations in key genes are known to drive many diseases, including cancer, and have been the focus of much attention in recent years. However, genetic content also may change farther downstream. RNA editing alters the mRNA sequence from its genomic blueprint in a dynamic and flexible way. Here, we provide a transcriptome-wide characterization of RNA editing across various human diseases from thousands of samples, and we show that A-to-I editing and the enzymes mediating this modification are significantly altered, usually elevated, in several autoimmune diseases and in most cancer types, where increased editing activity is found to be associated with patient survival. As is the case with somatic mutations in DNA, most of these newly introduced RNA mutations are likely passengers, but a few may serve as drivers that may be novel candidates for therapeutic and diagnostic purposes.

Monday 11 September
15:00–17:00, Oranim Hall 2

Mechanisms for Protein Homeostasis B

S.2.3-005
Neuronal caveolae orchestrate aging and proteostasis at the organismal level

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Department of Biochemistry and Molecular Biology, the Institute for Medical Research Israel – Canada, the Hebrew University School of Medicine, Jerusalem, Israel

Neurodegenerative disorders such as Alzheimer's disease and prion disorders share two key features; they emanate from toxic protein aggregation (proteotoxicity) and onset late in life. Why these maladies manifest late in life is not entirely clear however, various studies indicated that aging plays an active role in enabling their emergence in late stages of life and raised the prospect that the alteration of aging could postpone their onset. For decades aging was thought to be a stochastic, uncontrolled process, however, several genetic and metabolic pathways were found to regulate this process. The most prominent aging-regulating pathway is the Insulin/IGF signaling cascade (IIS). Reducing IIS activity extends lifespan, promotes proteostasis and elevates stress resistance of worms, flies and mammals, however, how this manipulation modulates cellular organiszation to mediate its functions is poorly understood. We asked how IIS reduction affects the protein content of lipid assemblies and discovered that in the nematode *Caenorhabditis elegans* this pathway positively regulates the expression of caveolin-1 (cav-1), thereby allowing the formation of caveolae, a subtype of lipid microdomains that serve as platforms for signaling complexes. Accordingly, IIS reduction lowers the expression of *cav-1*, and lessens the quantity of neuronal caveolae. Consistently, the knockdown of *cav-1* expression extends lifespan and protects model worms from neurodegeneration-linked proteotoxicity. Using deep sequencing we found that the knockdown of *cav-1* modulates the expression of genes that are known to regulate lifespan and stress resistance. Our findings define caveolae as aging-governing centers and suggest that reducing the expression of *cav-1* could be useful as an aging-altering treatment that can potentially postpone the onset of neurodegenerative maladies.

S.2.3-001
Ubiquitin sets the timer: coordination of aging and protein homeostasis

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Accumulation of damaged and aggregated proteins is associated with age-related neurodegeneration in Alzheimer's and Parkinson's patients. Protein homeostasis (proteostasis) is typically maintained by a balanced coordination between protein translation, folding, and degradation. The ubiquitin/proteasome-system (UPS) mediates turnover of damaged proteins supporting maintenance of the cellular proteome, organismal health, and lifespan. It is one major proteolytic route functioning in a cellular network that maintains the proteome during stress and aging. Degradation of damaged proteins is mediated by the 26S proteasome upon attachment of ubiquitin (Ub) proteins (ubiquitylation). Another proteolytic system supporting protein homeostasis (proteostasis) is the autophagy-lysosome pathway that degrades proteins inside activated autophagosomes. An age-related impairment of either of these systems causes enhanced protein aggregation and affects lifespan, suggesting functional overlap and cooperation between UPS and autophagy in stress and aging. Despite the progress made in searching for key substrates that are destined for degradation, the major challenge in the field is to understand how these proteolytic systems are mechanistically coordinated to overcome age-related proteotoxicity. The ultimate goal of our team is to assemble a global picture of the stress-induced proteolytic networks critical for aging and age-related diseases and to identify key regulatory, conserved degradation pathways that might be relevant for therapeutic approaches.

S.2.3-002
A differentiation transcription factor establishes muscle-specific proteostasis in caenorhabditis elegans

Y. Bar-Lavan, N. Shemesh, S. Dror, E. Yeger-Lotem, **A. Ben-Zvi**
Ben-Gurion University, Beer Sheva, Israel

In multicellular organisms, the composition of the proteome, and by extension, protein-folding requirements, varies between cells. In agreement, chaperone network composition differs between tissues. Here, we ask how cellular differentiation affects chaperone expression. Our bioinformatics analyses show that the myogenic transcription factor HLH-1 can bind to the promoters of muscle-expressed chaperones. When we employed HLH-1 myogenic potential to genetically modulate cellular differentiation of *C. elegans* embryonic cells, we found that HLH-1-dependent myogenic conversion induced the expression of HLH-1-regulated chaperones in differentiating muscle cells. Disrupting HLH-1 function in muscle cells reduced the expression of HLH-1-regulated chaperones and compromised muscle proteostasis, disrupting, in turn, the folding of muscle proteins and thus, myogenesis. We propose that cellular differentiation could establish a proteostasis network dedicated to the folding of the muscle proteome.

ShT.2.3-003
Chromatin remodeling factor integrates responses against nuclear and mitochondrial stress

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École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland

Age-associated changes in the chromatin structure have a major impact on longevity. Despite being a central part of the aging process, the organismal responses to the changes in chromatin organization remain unclear. By using *C. elegans*, we demonstrate that moderate disturbance of histone balance during development causes changes in histone levels and triggers a stress response typified by the increased expression of cytosolic small heat shock proteins. This stress response is dependent on the transcription factor HSF-1 and on the chromatin remodeling factor, ISW-1. In addition, we show that also mitochondrial stress at developmental stages modulates histone levels, thereby activating similar cytosolic stress response. These data indicate that histone- and mitochondrial perturbations are both monitored through chromatin remodeling and involve the activation of a cytosolic response that has an effect on longevity. Together with HSF-1, ISW-1 hence emerges as a central mediator of this multicompartment proteostatic response regulating longevity.

ShT.2.3-004
The organization of molecular chaperones across human tissues

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Chaperones play key roles in maintaining cellular function through *de novo* protein folding, refolding and prevention of aggregation. When chaperone function is disrupted protein homeostasis can be compromised, thus leading to protein misfolding and aggregation. As demonstrated by many protein-misfolding diseases, the sensitivity of the protein-folding environment to chaperone disruption can be highly tissue-specific. Yet, the tissue-specific organization of the chaperone system has received little attention. Here, we used RNA-sequencing profiles of over 40 human tissues to analyze chaperone expression and organization across tissues. We found that relative to protein-coding genes, chaperones were expressed more ubiquitously across tissues, and at significantly higher levels per tissue. These results agree with the fundamental roles of chaperones in living cells. Yet, chaperones were not expressed uniformly across tissues. We identified clusters of chaperones that were upregulated in certain tissues, suggesting that they have tissue-specific roles. For example, chaperones that were upregulated in skeletal muscle were enriched for homologs of muscle genes in worms, and included several chaperones associated with muscle diseases. Lastly, chaperones act by interacting with each other and with their targets. However, these relationships have been worked out only for a small subset of chaperones. To unravel functional relationships between chaperones we carried co-expression analyses. We identified known and novel relationships between chaperones associated with similar diseases, and among mitochondrial chaperones. Thus, while most chaperones are required in all tissues, their compositions vary between tissues in a way that could affect and illuminate tissue phenotypes and diseases.

Monday 11 September
15:00–17:00, Oranim Hall 3

Organelle Biogenesis and Dynamics B

S.2.4-002
New insights into COPI-mediated trafficking

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Coatomer (COPI) is a vesicle coat complex that enables retrograde vesicular traffic within the Golgi apparatus or from the Golgi to the endoplasmic reticulum (ER). Impaired COPI function is implicated in the molecular pathogenesis of neurodegenerative disease. Different types of peptide sorting motifs enable COPI to recognize its diverse cargo proteins with final destinations in different compartments of the secretory pathway – from the ER to the plasma membrane. Current progress in the structural biology of COPI allows the detailed dissection of the different cargo binding sites. As for other coat complexes, large conformational changes and coincidence detection of an appropriate donor membrane and cargo proteins emerge as important mechanisms in COPI function. We investigate the role of the different subunits of the COPI heptamer in cargo recognition and conformational control of COPI. To this end, we biochemically analyze the binding of manipulated mammalian or yeast COPI to different peptide sorting motifs such as the C-terminal dilysine and arginine-based COPI recognition motifs. Furthermore, we conduct systematic genetic screens of the yeast GFP-tagged proteome designed to test the functional relevance of the delta-COP subunit and its individual domains, an N-terminal longin domain, a linker helix, and the mu-homology domain. Thereby, we aim to elucidate the functional role of these individual domains in the dynamics of the COPI heptamer and in the recruitment of cargo or regulatory factors controlling the membrane association or dissociation of COPI.

S.2.4-006
Regulation of membrane function and identity by ubiquitin dependent proteolysis

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The endoplasmic reticulum (ER) is the largest organelle in eukaryotic cells carrying out several essential functions. Although delimited by a continuous membrane, the ER consists of functionally distinct domains. Among these is the inner nuclear membrane (INM), whose functions in nuclear organization require a unique proteome distinct from the rest of ER membranes. We and other recently identified a protein quality control system acting exclusively at the INM. Remarkably, this system shares many features with ER-associated degradation (ERAD), which performs protein quality control in bulk ER membranes. Among INM quality control substrates are damaged INM proteins as well as ER proteins that are mistargeted to the INM. I will discuss how these different classes of substrates are recognized and how protein quality control contributes to maintain the identity and functionality of the INM.

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S.2.4-004
Mapping organelle contact sites

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The flow of solutes and information within the eukaryotic cell requires inter-organelle communication. Membrane contact sites between two organelles can facilitate both signaling and the passage of ions and lipids from one cellular compartment to another. To uncover the extent of contact site formation between the cellular organelles we created a split fluorescence sensor of contact sites in yeast. In our sensor, one part of a fluorophore is fused to the outer membrane of each organelle in one mating type while the other is fused to the outer surface of the membrane of all organelles in the other mating type. Yeast can then be mated in all possible pairwise combinations and a fluorescent signal reports on the formation of a contact site between two organelles. We extensively validated that the sensor reports on bona fide contact sites and have discovered novel contact sites. I will discuss how these are now being used in high content screens to uncover novel tethers and regulatory molecules as well as the physiological roles of the contacts and how this is relevant to understanding sphingolipid regulation.

Tuesday 12 September
9:00–11:00, Oranim Hall 4

Molecular Machines in Action

S.3.1.A-003
Application of novel in vitro single-molecule approaches to the studies of chromatin and replication and transcription

N. Dekker
Department of Bionanoscience, TU Delft, Delft, Netherlands

Over the past few decades, there has been steady progress in both our ability to produce biological material and in our ability to manipulate matter at small length scales. These two developments merge in the fascinating research area of single-molecule biophysics. I will illustrate how developments of this interdisciplinary field allow us to shed light on genomic processes such as DNA compaction, replication, and (time permitting) transcription. By measuring the twist and length of single DNA molecules, we learn about DNA compaction into chromatin. We monitor the real-time loading of tetramers or complete histone octamers onto DNA and find, remarkably, that tetrasomes exhibit spontaneous flipping between a preferentially occupied left-handed state and a right-handed state. The application of weak positive torque converts left-handed tetrasomes into right-handed tetrasomes, whereas nucleosomes display more gradual conformational changes. These findings suggest that chromatin can serve as a ''twist reservoir'' and thereby regulate supercoiling. Using high-throughput single-molecule techniques, we examine the termination of DNA replication. In E. coli, replisome progression beyond the termination site is prevented by Tus proteins bound to asymmetric Ter sites. Structural evidence indicates that strand separation on the blocking (non-permissive) side of Tus–Ter triggers roadblock formation, but biochemical evidence also implicates protein-protein interactions. We perform DNA unzipping experiments showing that nonpermissively oriented Tus–Ter forms a tight lock in the absence of replicative proteins, whereas permissively oriented Tus–Ter allows nearly unhindered strand separation. Quantifying the lock strength reveals the existence of several intermediate lock states that can be impacted by mutations. Lock formation is highly specific and exceeds in vivo efficiencies. We postulate that protein-protein interactions may hinder, rather than promote, proper lock formation.

S.3.1.A-001
The type VI secretion system: an antibacterial nanoweapon

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Bacterial pathogens are facing fierce conditions to colonize their host and launch successful infections. Not only they have to escape the immune system but they should also compete with other invading organisms or resident bacterial flora. This warfare is crucial for winning access to scarce resources and pathogens employ a variety of strategies and molecular tools which make them successful. Here we discuss how the ESKAPE pathogen *Pseudomonas aeruginosa* eliminates other competitors by using a bacterial weapon called the type VI secretion system (T6SS). There are many examples of bacterial toxins which are transported by the T6SS and injected into prey cells, including peptidoglycan hydrolases, phospholipases or DNases. These T6SS toxins are co-produced with an immunity to avoid self-intoxication. The transport of the toxins involves the T6SS nanomachine which is made of 13 core components and 3 sub-complexes, the membrane platform, the baseplate and the tail. The latter is made of a contractile sheath that surrounds a hollow tube consisting of hexameric Hcp. On tip of the Hcp tube sits a VgrG trimer, whose torch-like structure is sharpened by a PAAR protein. The different toxins are accommodated within the Hcp tube or at the tip of the tail by interacting either with VgrG or PAAR, using or not adaptor proteins. Upon sheath contraction Hcp/VgrG/PAAR and the cocktail of toxins are expelled from the producing cell and injected into the prey cells resulting in intoxication. We will discuss key structural aspects in the assembly of the T6SS nanomachine and review T6SS toxins that are injected into bacterial preys. We will address how the characteriztion of novel T6SS toxins can be conducted and how these toxins may point at the identification of new antimicrobial targets which could be used for drug development. This is needed in a context where bacteria developed ways to overcome ancient drugs and become recalcitrant to available and sometime last-resort antibacterial.

S.3.1.A-002
Structural characterisation of the chaperone Hsp70 network

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In all cell types, molecular chaperones control cell proteostasis by preventing misfolding and aggregation or by directing proteins to degradation. The Hsp70s are a ubiquitous group of molecular chaperones involved in a large number of pathways that include folding, transport across membranes, and remodelling of specific proteins or complexes. These functions are executed by the Hsp70s with the help of a large number of co-chaperones with which they form transient complexes that are well suited for their analysis by electron microscopy and image processing. These studies provide a clear picture of the plasticity of these interactions and reveal in part the working mechanism of these nanomachines.

ShT.3.1.A-001
The effect of Ala155Thr substitution in tropomyosin on the modulation of actin-myosin interaction during simulated stages of ATPase cycle

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A substitution in *TPM3*-encoded tropomyosin (Tm), Ala155Thr, has been found in patient with nemaline myopathy, which is characterized by skeletal muscle weakness and hypotension. The substitution was found to lead to violation in forth alanine cluster of Tm, Ala151-Ala155-Ala158, by incorporation of non-canonical polar residue in the sequence of protein. It was suggested, that the substitution can affect the coiled coil structure, increase the distance between two polypeptide chains, change both the bending features and electrostatics of interface between actin and Tm. In the present work the effect of Tm with Ala155Thr substitution on the modulation of actin-myosin interaction during simulated stages ATPase cycle has been investigated. For this purpose actin, myosin subfragment-1 (S1) and Tm (wild type and with substitution) were modified at Cys374, Cys707 and Cys190, respectively, and incorporated in skeletal muscle fibre models, ghost fibers. Four intensities of polarized fluorescence of each probe were registered by polarized fluorimetry technique. The angles of orientation for each probe and the changes in the angle values at the simulated stages of ATP ($\pm Ca^{2+}$) hydrolysis cycle were calculated to assess actin, myosin S1 and Tm dynamics and flexibilities. It was found that in the presence of Ca^{2+} the substitution Ala155Thr leads to abnormal shift of tropomyosin strand to position on actin, characteristic of activated muscle, i.e. in open position, which stimulates both the transition of larger number of actin monomers from OFF to ON state and larger number of strong-bound S1 with actin. It was also found that in the presence of the substitution in Tm the troponin loses the ability to switch thin filaments off at low [Ca^{2+}]. These abnormalities can be a reason of defective contractile function during nemaline myopathy, associated with the substitution. The study was supported by the Russian Science Foundation (Grant 17-14-01224).

ShT.3.1.A-002
Bi-directional motility of mitotic biological nano-motors: from single molecules to intracellular function

L. Gheber
Ben-Gurion University of the Negev, Beer-Sheva, Israel

Kinesin motor proteins are biological machines that move along the microtubule filaments using ATP hydrolysis. The bipolar kinesin-5 motors perform essential functions in mitotic spindle dynamics by crosslinking and sliding apart antiparallel microtubules of the spindle. We recently demonstrated that the *S. cerevisiae* kinesin-5 Cin8 and Kip1, are minus-end directed as single-molecules and switch directionality *in vitro* under a number of conditions. These findings broke twenty-five-years old dogma stating that kinesin motors with N-terminal catalytic domain are exclusively plus-end directed. The mechanism of this directionality switch and its physiological significance remain unclear. We have also demonstrated that Cin8 is differentially phosphorylated during late anaphase at three cyclin-dependent kinase 1 (Cdk1) specific sites located in its motor domain. This phosphorylation regulates Cin8 activity during anaphase, but the mechanism remains unclear. To study the bi-directionality of Cin8 and Kip1, we examined their *in vitro* motile properties and *in vivo* functions by TIRF microscopy and live-cell imaging. Addition of negative charge in a phospho-mimic mutant of Cin8 affects its interaction with the microtubules, thus regulating its motile properties and directionality. We also found that *in vitro*, Cin8 not only moves to- but also clusters at the minus-end of the MTs. This clustering causes Cin8 to reverse its directionality from fast minus- to slow plus-end directed motility. Clustering of Cin8 at the minus-end of the MTs serves as a primary site for capturing and antiparallel sliding of the MTs. Based on these results, we propose a revised model for activity of Cin8 and Kip1 during mitosis and propose a physiological role for their minus-end directed motility.

Tuesday 12 September 9:00–11:00, Oranim Hall 1

Chromatin Structure and Epigenetic Modifications A

S.3.2-005
Cooperative and non-cooperative regulatory chromatin interactions uncovered by Multi-Contact 4C (MC-4C)

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Spatial chromatin organization is increasingly recognized as a regulator of nuclear processes such as gene activity. Most methods developed to unravel three-dimensional (3D) chromatin conformation analyze pair-wise chromatin contacts, but are incapable of identifying simultaneous associations among multiple loci. Yet, exactly this type of 'beyond two-way' contact information will provide the insights required to start composing a more complete picture of 3D genome organization at the level of the individual allele. We describe a novel chromatin conformation capture technology that allows direct identification of multi-way chromatin contacts. Multi-Contact 4C (MC-4C) applies third-generation long-read single molecule real time (SMRT) sequencing technology to an intermediate product of the conventional chromatin conformation capture (3C) protocol. For a given allele of interest, MC-4C can easily identify 4 to 8 spatial neighbors, based on proximity-ligation events. With many thousands of identified multi-way (>3) contacts per targeted locus, MC-4C presents a unique and promising new technology to distinguish co-operative from mutually exclusive 3D chromatin structures.

S.3.2-006
Topological characteristics of cohesin-mediated mammalian regulatory domain

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Research Department of Cancer Biology, Cancer Institute, University College London, 72 Huntley Street, London, WC1E 6BT, United Kingdom. The spatial organization of chromosomes

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in the nucleus has fundamental roles in genome stability and gene expression. Understanding the principles governing how the genome is structurally organized and how it dynamically functions represents a remarkable challenge for biomedical research. We have revealed that CTCF and cohesin complexes have key architectural roles in 3-dimensional genome organization. CTCF and cohesin collaborate to anchor chromatin loops throughout mammalian genomes and in so doing structure chromosomes in space (Sofueva S *et al*. 2013 EMBO J). We have also discovered that partitioning of the genome into spatial units (or 'TADs') is encoded in the DNA through high-affinity, directional CTCF sequences (Vietri Rudan M *et al*. 2015 Cell Rep). Our work joins a growing body of evidence that supports the notion that cohesin and CTCF are fundamental regulators of spatial genome architecture and are therefore key to our understanding of how topological configurations of chromatin influence gene expression and consequently cell states. We will discuss recent work examining the topological characteristics of mammalian spatial domains and how cohesin/CTCF-mediated nuclear organization contributes to, and is required for these.

S.3.2-002
Polycomb proteins and 3D genome architecture as regulators of development and heredity

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Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications, modulation of nucleosome remodeling activities and regulation of 3D chromosome architecture. These proteins can transmit epigenetic inheritance, but also bind dynamically to some of their target genes genes and regulate cell proliferation and differentiation in a wide variety of biological processes. Polycomb group proteins form two main complexes, PRC2 and PRC1, which coregulate a subset of their target genes, whereas others are regulated only by one of the complexes. We have recently described the 3D architecture of the genome and identified the Polycomb system as one of the fundamental folding and regulatory principles. Our progress in these fields will be discussed

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ShT.3.2-001
Histone acetylation drives the partitioning of the Drosophila chromosomes into topologically-associating domains

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Interphase chromosomes in multicellular eukaryotes are organized into arrays of megabase- and submegabase-scale self-interacting topologically-associating domains (TADs) commonly interpreted as chromatin globules. Despite extensive studies revealed crucial role of TADs in the genome function, molecular mechanisms of TAD formation and maintenance remain largely unknown. Recently, we have proposed that TADs in the Drosophila genome are formed by transient internucleosomal interactions predominantly within repressed chromatin. According to our model, histone N-terminal tail acetylation within active genome regions suppresses chromatin compactization that explains the location of transcribed genes within TAD boundaries and interdomain regions. To get further insights into this process, we have studied chromatin structure using Hi-C analysis coupled with profiling of acetylated histones and polyA-RNA in the Drosophila cultured cells treated with either trichostatin A (TSA, HDAC inhibitor) or curcumin (HAT inhibitor). We found that TSA-mediated suppression of HDAC activity leads to a considerable increase of histone acetylation predominantly within TAD boundaries and inter-TAD regions accompanied with a decrease of spatial interactions between adjacent TADs. Conversely, we observed that curcumin-mediated suppression of HAT activity leads to genome-wide increase of spatial interactions between adjacent TADs and even to TAD fusion resulted in the loss of TAD profile at certain genome regions. Moreover, we have observed similar effects when performed siRNA-mediated knockdown of histone deacetylase I and major histone acetyltransferase CBP. Our results suggest that internucleosomal interactions within repressed chromatin may represent a major driving force for the TAD formation in the Drosophila genome.

ShT.3.2-002
The Zinc finger-associated domain of ZAF1 protein is important for long distance interactions and insulation

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Architectural/insulator proteins are responsible for global chromosome architecture as well as local regulation of enhancer–promoter interactions. However, the mechanisms and protein domains involved in long-distance interactions are not well understood. ZAF1 (ZAD domain Architectural Factor 1 – CG6808) is a new architectural/insulator protein which contains an N-terminal Zinc finger-associated domain (ZAD) and a cluster of five C_2H_2 zinc fingers. ZAF1 has a structure which is similar to that of the previously studied insulator proteins Zw5, Pita and ZIPIC. ZAD domain of ZAF1 forms homodimers which suggests that it might be involved in long-distance interactions. ZAF1 interacts with CP190. CP190 is a cofactor of every known Drosophila insulator protein and is involved in the establishment of open chromatin, long distance interactions and insulation. ZAF1 zinc fingers binds a specific DNA motif which is predominantly located near promoters. ZAF1 is not expressed in the eyes which allowed us to make model system for studying the functional activity of this protein and its domains in vivo. Using this system we show role of ZAD in insulation and supporting long-distance interactions. This study was supported by the RSF 14-24-00166.

Tuesday 12 September
9:00–11:00, Oranim Hall 2

Redox Regulation of Biological Activities

S.3.3.A-001
Mechanisms of redox signaling

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Redox signaling is a process by which endogenous oxidants, derived from metabolism, reversibly modify particular thiols on particular proteins to change their functional behavior in an adaptive manner. However, the molecular mechanisms of redox signaling remain largely unknown. In particular, specificity and efficiency of redox signaling remain largely unexplained. The now emerging solution to this conundrum is that redox signaling is mediated and channeled by protein-to-protein redox relay chains. There is growing evidence that H_2O_2 signals are relayed through thiol peroxidases to neighboring proteins within supramolecular assemblies. Evidence now suggests that so far we may have just glimpsed the 'tip of the iceberg', in that redox relay chains are ubiquitous and also operate for reactive nitrogen and sulfur species.

S.3.3.A-003
Redox control of eukaryotic secretion by a novel pathway regulating the ER import of glutathione

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Disturbance of glutathione metabolism is a hallmark of numerous diseases, yet glutathione functions are still poorly understood. One key to address this question is to consider its functional compartmentation. In the endoplasmic reticulum (ER), protein folding requires disulfide bond formation catalyzed by the thiol oxidase Ero1 and protein disulfide isomerase (PDI). In the ER, glutathione is thought to counterbalance ER oxidation by the Ero1-PDI redox relay, which explain its relative more oxidized redox state (E_{GSH} -242 mV), relative to the cytoplasm (E_{GSH} = -295 mV). To access the function of GSH in this compartment, we first asked about the mechanism that maintains the ER E_{GSH} homeostatic value. As GSH is exclusively synthesized in the cytoplasm and there is no GSH reductase in the ER to recycle the GSSG produced by the activity of the ER Ero1-PDI oxidative pathway, maintenance of ER E_{GSH} likely depends on an ER import of GSH and export of GSSG. We found that GSH enters the ER by facilitated diffusion through the protein-conducting channel Sec61. We also found that an oxidized form of the chaperone Bip (Kar2 in yeast) inhibits this transport. We show that increased ER transport of GSH triggers Ero1 activation by reduction of its negative regulatory disulfides, which in turn leads to Bip oxidation by the H_2O_2 by-product of Ero1 activity. This regulated transport is strongly activated during ER stress by the UPR induction of Ero1 and an increase in GSH biosynthesis. Thus, the ER poise is tuned by a reciprocal control of GSH import into the ER and Ero1 activation. Such a reciprocal control is aimed at preventing a "short circuit" between the ER oxidative and reductive pathways, which would lead to Ero1 chronic cycling, cellular GSH consumption and cell death.

S.3.3.A-002
Protein import and redox regulation converge in the mitochondrial intermembrane

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Protein import into mitochondria is fundamental for their biogenesis and therefore critical for cell survival. Protein biogenesis in the intermembrane space relies on the Mia40 pathway that orchestrates oxidative folding of proteins in this mitochondrial compartment. The key components Mia40 and Erv1 of this pathway are known but regulation of this process and its putative links to redox homeostasis and redox signalling are still elusive. We find that (i) the Mia40 pathway is influenced by oxidative stress (ii) a fraction of cytosolic redox active proteins like thioredoxin and the thiol peroxidase Orp1/Gpx3 are targeted to the intermembrane space. The presence of these antioxidant machineries in the IMS ensures protection against oxidative stress damage in a compartment-specific manner. Import of these antioxidants portiens is unkown and apparently engages unconvineitnal import routes. The ramifications of these unexpected protein targeting and protein interaction pawthays for cell physiology, apoptosis and survival will be discussed.

ShT.3.3.A-001
Subunit specific actions of H_2S on NMDA receptors in rat hippocampus

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Hydrogen sulfide (H_2S) is an endogenous gasotransmitter with neuroprotective properties that participates in the regulation of transmitter release and neuronal excitability in various brain structures. It was shown that in adult hippocampal slices sodium hydrosulfide (NaHS) -donor of H_2S potentiated NMDA-mediated responses and facilitates the induction of long-term potentiation. However in neonatal hippocampus NaHS induced a biphasic effect on neuronal activity with initial increase followed by inhibition of giant depolarizing potentials and decrease of NMDA mediated currents. It was suggested that differences in the subunit composition of NMDA-receptors are responsible for the effects of NaHS in neonatal and adult animals. The aim of our study was to investigate the age-specific effects of NaHS on NMDA receptors. Experiments were performed on slices of Wistar rats of different ages (P3-7 and P18-26, where P0 – birth day) and on GFP-positive HEK293 cells expressing recombinant GluN1/2A or GluN1/2B NMDA receptors using patch-clamp technique in whole cell configuration and focal puff application of NMDA receptors agonists. Superfusion with NaHS increased NMDA receptor mediated currents in neurons of mature slices, at the same time focal application NMDA in the presence of NaHS induced a reduction of NMDA responses in slices of newborn animals. At the same time NaHS significantly enhanced the NMDA-induced currents in GluN1/2A expressed in HEK293 cells and reduced the time of desensitization. In HEK293 cells expressing GluN1/2B receptors H2S induced the reduction of NMDA–mediated currents without changing the time of desensitization. Reducing agent – dithiothreitol abolished NaHS potentiation in GluN1/2A but not in GluN1/2B NMDA receptors. These observations suggest that the age-dependent effects of H_2S in rat hippocampus regulated by redox modulation of NMDA-

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receptor subunits. The work was supported by Russian Science Foundation [grant No. 14-15-00618].

ShT.3.3.A-002
Structure and functional modification of proteins by ROS: application to cancer drug development

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Proteins in a living cell are mainly controlled by their three-dimensional structures in communicating genetic information and oncogenic signaling. A number of studies have shown reactive oxygen species (ROS) generated by cold plasma on the wound healing, blood coagulation, skin regeneration and cancer treatment. It has been proposed that non-thermal plasma could be applied to cancer cells and tissues for clinical purpose. However, a detailed structural mechanism on oncogenic proteins has uncovered yet. Wnt/b-catenin pathway is aberrantly activated in most human colorectal cancers (CRCs) and many proteins interact with other molecules cooperatively in tumor promotion. Destabilization of both b-catenin and Ras signaling via targeting axin is a potential therapeutic strategy for treatment of CRC and other type cancers activated Wnt/b-catenin pathways. We identified axin as a direct target through in vitro binding studies, and uncovered details of the interaction between a novel drug compound and regulators of the G-protein signaling (RGS) domain of axin using NMR spectroscopy and X-ray crystallography. In this presentation, we show that structural biology would be a powerful technique to uncover ROS effect generated by non-termal plasma irradiation on oncogenic proteins during cellular signaling. Data suggest that plasma irradiation induces structural fluctuation as well a potent cancer drug, which are important aspects in understanding molecular basis of oncogenic proteins modified by ROS.

Tuesday 12 September
9:00–11:00, Teddy Hall

Molecular Neuroscience A

S.3.4-005
Rewiring molecular networks underlying neural plasticity in down syndrome

M. Dierssen
Cellular and Systems Neurobiology, Systems Biology Program, Center for Genomic Regulation, Barcelona, Spain

Intellectual disability (ID) disorders present neurodevelopmental delays in motor, sensory, and cognitive processes. Regardless of their molecular cause, most IDs are characterized by neural plasticity disruption, that therefore is a natural target for therapeutic purposes opening a window of opportunity for a novel therapeutic concept: therapies that improve and stabilize experience-dependent plasticity by targeting specific molecular substrates with drugs, in combination with non-pharmacological interventions. The flavonoid epigallocatechin-3-gallate (EGCG), the most abundant polyphenol from green tea, is effective to restore cognitive function in mouse models of Alzheimer's disease, Down syndrome (DS) and Fragile X syndrome (FXS). In DS mouse models EGCG recovers cognitive and neural plasticity, a result that was replicated in a pilot clinical trial in humans. EGCG also significantly improves memory, executive functions and adaptive behavior and increases functional connectivity in specific brain regions of DS adults (Phase I NCT01394796 and Phase II NCT01699711 clinical trials). We discovered that EGCG was most effective when paired with cognitive stimulation, suggesting that it acts in favor of physiological activity-dependent plasticity. A pilot clinical trial showed also efficacy of EGCG treatment in FXS individuals. This is a crucial first step in DS therapy that has opened new important questions. EGCG has a plethora of different effects that make it difficult to fully identify and understand the underlying molecular therapeutic mechanisms. Among others, it has anti-oxidant and anti-inflammatory effects and is able to regulate the kinase activity of the dual-specificity tyrosine (Y)-phosphorylation kinase 1A (DYRK1A), a strong candidate for DS. DYRK1A regulates fundamental cellular functions such as cell proliferation, survival, differentiation and motility and is a major candidate to explain DS phenotypic abnormalities. Mass spectrometry-based proteomics suggest that it possibly acts by rewiring the molecular network responsible for activity-dependent plasticity, but also revealed that DYRK1A presents a pleiotropic behavior as a result of the phosphorylation of a myriad of diverse protein substrates making it difficult to interpret the obtained results and to select the most relevant pathways. I will discuss our conclusions that make EGCG a good candidate for the treatment of ID.

S.3.4-002
Tubulin polyglutamylation – a novel player in neurodegeneration

C. Janke
Institut Curie, Orsay, France

The microtubule cytoskeleton is playing essential and divergent roles in all eukaryotic cells. Microtubule functions are regulated by a multitude of factors and pathways. An emerging mechanism that directly modulates the microtubule lattice are posttranslational modifications of tubulin. While the modifications of tubulin have been discovered decades ago, functional insight is only recently emerging. We have studied the role of tubulin polyglutamylation, a modification prevalent in neurons, and found that deregulation of this posttranslational modification leads to neurodegeneration. To demonstrate the specificity of this mechanism, we have generated a series of combinatorial knockout mice in which we can specifically induce or avoid degeneration of certain neuron populations in the nervous system. We have further investigated the potential mechanisms of the hyperglutamylation-induced degeneration and found a strong impact on axonal transport. Our data suggest that alternations in polyglutamylation could be a biochemical mechanism linked to a range of neurodegenerative disorders in human.

S.3.4-001
Extracellular proteolysis in control of diseased mind

L. Kaczmarek
Nencki Institute, Warsaw, Poland

Matrix metalloproteinase-9, MMP-9 is an extracellularly operating enzyme that has been demonstrated as important regulatory molecule in control of synaptic plasticity, learning and memory. Either genetic or pharmacological inhibition of MMP-9 impairs late phase of long-term potentiation at various pathways, as well as appetitive and spatial memory formation, although aversive learning remains apparently intact in MMP-9 KO mice. Thus, MMP-9 plays a role in a healthy mind. Extrasynaptic MMP-9 is

required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Animal studies have implicated MMP-9 in such neuropsychiatric conditions of diseased mind, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, and depression. In humans, MMP-9 appears to contribute to mind disorders, such as epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders.

ShT.3.4-001
Thermogenetic neurostimulation with single-cell resolution

Y. Ermakova[1], A. Lanin[2,3,4], I. Fedotov[2,3,4,5], M. Roshchin[6], P. Balaban[6], A. Zheltikov[2,3,4,5], V. Belousov[1]
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Optogenetic methods allow specific neuronal subsets to be operated by light with high precision in space and time, and are important tools helping to understand mechanisms of functioning of the brain and peripheral neural system. Optogenetics is not free from serious limitations such as low penetration depths for the activating light, causing difficulties with activation light delivery, and also low conductance of most ChRs necessitates high channel expression levels and high activation light intensities, which are very likely to induce rarely considered phototoxic effects. Thermogenetics is a promising innovative stimulation technique, which enables robust activation of neurons using thermosensitive transient receptor potential (TRP) cation channels. The TRP channels are three orders of magnitude more conductive than ChRs and can be activated by heat induced with infrared light or magnetic particles in a strong magnetic field. In this work, the snake TRPA1 channels are used for thermogenetic activation of mouse neurons and induction of zebrafish larvae escape behavioral response. Neurons expressing TRPA1 were activated in our experiments using IR-laser-controlled heating with a fiber-optic probe integrated with a quantum sensor used for *in situ* high-resolution thermometry, thus enabling an exceptionally mild, precisely controlled heating of the tissue. With this combination of methods, we carried out a systematic, in-depth characterization of the performance of TRPA1 channels in neurons, including accurate measurements of the pertinent Ca2 + dynamics and electrophysiological analysis of the responses, providing, for the first time, a complete framework for a thermogenetic manipulation of individual neurons using IR light. Our study demonstrates that the snake TRPA channels are ideally suited as a tool for thermogenetics, providing a carefully validated kit of molecular and optical instruments as a route toward mature thermogenetic technologies for neuroscience.

ShT.3.4-004
Robust design of biological timers shapes neuroblast lineage formation in the drosophila embryo

I. Averbukh[1], S. Lai[2], B. Shilo[1], C. Doe[3], N. Barkai[1]
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Development often utilizes gene regulatory networks operating as biological timers to induce temporal cascades of events. Such timers must operate robustly despite variability between cells and individuals. An example for such a robust timer is temporal patterning of *Drosophila* embryonic neuroblast (NB) lineages, which is controlled by sequentially expressed transcription factors. This TF sequence is a cell autonomous timer whose robustness is poorly understood. We define two general timing strategies which are both simultaneously implemented in the NB timer: degradation-based and relay timers. While the first employs the declining concentration of a repressor for timing, the second depends on both the accumulation and decay of all genes. Our mathematical model showed clear robustness advantages to the degradation timer and predicted it is dominant in the NBs. We then developed a methodology which allows us to infer the relative importance of the two strategies in the NB timer *in vivo* and test our predictions on the embryonic NB7-1 lineage. Our results indicate that repressors, rather than activators, have crucial roles in temporal regulation of the NB network, thus supporting a mainly degradation-based timing strategy, as predicted. The robustness advantages of degradation-based timers are general and can be utilized in many other biological timers.

Tuesday 12 September 9:00–11:00, Oranim Hall 3

Special Session on New Molecular Structures

(All talks selected from submitted abstracts)

S.3.5-001
Near-atomic resolution cryoEM structure of the 30-fold homooligomeric SpoIIIAG channel essential to endospore formation

N. Zeytuni[1], C. Hong[2], K. Flanagan[3], L. Worrall[1], K. Theiltges[1], M. Vuckovic[1], R. Huang[2], S. Massoni[3], A. Camp[3], Z. Yu[2], N. Strynadka[1]
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Endospore formation in the Gram-positive bacterium Bacillus subtilis involves a series of morphological changes that result in a mature dormant spore. In response to nutrient depletion or other extreme conditions these bacterial cells can undergo an asymmetric cell division to a mother cell and a forespore. Following the hydrolysis of division septum peptidoglycan, the mother cell septal membrane engulfs the forespore and nurtures it to maturity. During this engulfment process an essential channel, named the feeding tube apparatus, is thought to cross both membranes to create a direct conduit between the cells. At least nine proteins

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are required to form this channel including SpoIIQ under forespore control and SpoIIIAA-AH under the mother cell control. Several of these proteins share similarity to components of Type-II, -III and -IV secretion systems as well as the flagellum from gram-negative bacteria. . Here, we present the near-atomic resolution structure of one of these proteins, SpoIIIAG, determined by single-particle cryo-EM. Three-dimensional reconstruction revealed that SpoIIIAG assembles into a large and stable 30-fold symmetric complex with a unique mushroom-like architecture. The complex is collectively composed of three distinctive circular structures; a 60-stranded vertical β-barrel that forms a large inner channel encircled by two concentric rings, one β-mediated and the other one formed by repeats of a ring-building motif (RBM) common to the architecture of a variety of dual membrane secretion systems of distinct function. Our near-atomic resolution structure clearly indicates SpoIIIAG exhibits a unique and dramatic adaptation of the RBM fold with a novel β-triangle insertion that assembles into the prominent channel, the dimensions of which suggest the potential passage of large macromolecules between mother and forespore during the feeding process.

S.3.5-002
New structure-activity paradigms for amyloids from pathogenic microbes

E. Tayeb-Fligelman, N. Salinas, S. Perov, O. Tabachnikov, **M. Landau**
Technion, Haifa, Israel

Microbial functional amyloids are structured protein aggregates serving specific and highly diverse functions, including biofilm structuring and interactions with the host. Despite their role as key virulence factors and antimicrobial drug targets, amyloids are mostly known for their involvement in fatal human aggregation diseases, and their structures have been mostly studied only in eukaryotes. To bridge this informational gap, we leverage methods developed for research of human amyloids to shed light on this unheeded aspect of microbial physiology. This allowed us to correlate specific structural features of various amyloid states to dedicated biological functions. We discovered unique amyloid-like structures, including, to our surprise, a structure of a full-length bacterial cross-alpha amyloid-like fibril which is unprecedented in >100 structures of eukaryotic cross-beta amyloids solved to date. The fibrils, of the PSMα3 peptide secreted by *Staphylococcus aureus,* are toxic to human cells, clarifying their involvement in pathogenicity (Tayeb-Fligelman *et. al., Science* 355(6327): 831–833; 2017). Given our results we predict that the structural and functional repertoire of amyloids is far more diverse than previously anticipated, providing a rich source of targets for antimicrobial drug discovery.

S.3.5-003
Structural and functional model of the core complex of the eukaryotic Fe/S cluster synthesis machinery

M. Cygler[1], M. Boniecki[1], S. Freibert[2], U. Mühlenhoff[2], R. Lill[2]
[1]*University of Saskatchewan, Saskatoon, Canada,* [2]*Philipps-Universität, Marburg, Germany*

Iron-sulfur (Fe/S) clusters are essential protein cofactors with crucial functions in electron transfer, catalysis, sensing, and sulfur donation. The mutations in the machinery that assembles the Fe/S cluster and transfers them to apoproteins lead to a variety of genetic diseases, the best know being the Friedreich ataxia. The core proteins of the synthetic machinery of Fe/S clusters is highly conserved from bacteria to humans and include a cysteine desulfurase (IscS in bacteria, NFS1 in mitochondria) as a sulfur donor, a scaffold protein (IscU/ISCU) for the assembly of the cluster, ferredoxin for reduction of the sulfur to sulfide, and CyaY/frataxin, presumably for iron supply. Despite these similarities, there are significant differences between the bacterial and mitochondrial machineries. While bacteria continue to make Fe/S clusters in the absence of CyaY and the cells continue to grow, the knockout of frataxin leads to a Fe/S cluster deficit and fatal Friedreich ataxia. Another difference is that in contrast to bacterial desulfurase IscS, the mitochondrial NFS1 desulfurase additionally binds the helper protein ISD11 and, as recently discovered, also the acyl carrier protein (ACP). ISD11 is essential for Fe/S biosynthesis in mitochondria, however, despite much effort its molecular mode of action is unknown. Cellular assays showed that the absence of ACP destabilizes the Fe/S cluster synthesis complex but the precise mechanistic role of ACP is also unclear. We will report structural and functional data on the various complexes of the mitochondrial Fe/S cluster synthesis machinery and present a model for the entire core assembly complex. Our results shed light on the function of ISD11 and ACP in Fe/S cluster formation and the dynamics of the assembly machinery and provide structural basis for the deleterious effects of the several known mutations.

ShT.3.5-001
Structural basis for the membrane fusion step in Hantavirus entry

W. Shmuel[1], H. Bar-Rogovsky[1], E. A. Bignon[2], N. D. Tischler[2], Y. Modis[3], **M. Dessau**[4]
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Hantaviruses are important emerging human pathogens and are the causative agents of serious diseases in humans with high mortality rates. Like other members in the *Bunyaviridae* family their M segment encodes two glycoproteins, G_N and G_C, which are responsible for the early events of the viral infection. Hantaviruses deliver their tripartite genome into the cytoplasm by fusion of the viral and endosomal membranes in response to the reduced pH of the endosome. Unlike phleboviruses (e.g. Rift valley fever virus), that have an icosahedral glycoprotein envelope, hantaviruses display a pleomorphic virion morphology as G_N and G_C assemble into spikes with apparent four-fold symmetry organized in a grid-like pattern on the viral membrane. We determined the crystal structure of glycoprotein C (G_C) from Puumala virus (PUUV), a representative member of the *Hantavirus* genus. The crystal structure shows G_C as the membrane fusion effector of PUUV and it presents a class II membrane fusion protein fold. Furthermore, G_C was crystallized in its post-fusion trimeric conformation that until now had been observed only in *Flavi-* and *Togaviridae* family members. The PUUV G_C structure together with our functional data provides new mechanistic insights into class II membrane fusion proteins and reveals new targets for membrane fusion inhibitors against these important pathogens. Both similarities and differences to other class II membrane fusion proteins implies a revise paradigm for the evolution of these unique proteins.

ShT.3.5-002
The phytohormone ligand repertoire of plant PR-10 proteins includes melatonin

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[1]*A. Mickiewicz University, Poznan, Poland,* [2]*Institute of Bioorganic Chemistry, PAS, Poznan, Poland*

Melatonin has a prominent place as an animal hormone but its significance in the plant kingdom has emerged only recently. The biological role of melatonin as a phytohormone is still under study and its protein binding partners are largely unknown. We studied plant Pathogenesis-Related proteins of class 10 (PR-10) as potential melatonin binders. PR-10 proteins with their canonical fold featuring a large cavity formed between a β-sheet and an α-helix, have gained increasing recognition as versatile phytohormone binders. We demonstrate, using crystallographic methods, melatonin binding by two PR-10 proteins: Hyp-1 from St John's wort and LlPR-10.2B from yellow lupine. Hyp-1 binds melatonin in three unusual binding sites, two of which are internal chambers, while the third one is formed as an invagination of the protein surface. The electron density in one of the internal sites does not allow unambiguous modeling of a melatonin molecule but suggests a melatonin degradation product. Among a number of potential natural mediators tested (including the cytokinin phytohormone *trans*-zeatin), melatonin was the only one to form a crystalline complex with Hyp-1. On incubation with melatonin, LlPR-10.2B also bound three ligand molecules. Two of them are found within the internal cavity while a well defined electron density near the cavity entrance suggests an unknown molecule, probably a product of melatonin transformation. In a cocrystallization experiment with a 1:1 mixture of melatonin and *trans*-zeatin, LlPR-10.2B formed a complex in which one of the melatonin binding sites is occupied by *trans*-zeatin, while the binding of melatonin at the second site and binding of the unknown ligand are unchanged. This unusual complex, when compared with the previously described PR-10/*trans*-zeatin complexes, provides insight into the involvement of PR-10 proteins in phytohormone binding in plants and implicates the PR-10 proteins as low-affinity melatonin binders.

Tuesday 12 September
15:00–17:00, Oranim Hall 3

Protein Folding and Misfolding

S.3.1.B-003
Kinetics of protein aggregation

T. Knowles
University of Cambridge, Cambridge, United Kingdom

Filamentous protein aggregation underlies a number of functional and pathological processes in nature. This talk focuses on the formation of amyloid fibrils, a class of beta-sheet rich protein filament. Such structures were initially discovered in the context of disease states where their uncontrolled formation impedes normal cellular function, but are now known to also possess numerous beneficial roles in organisms ranging from bacteria to humans. The formation of these structures commonly occurs through supra-molecular polymerisation following an initial primary nucleation step. In recent years it has become apparent that in addition to primary nucleation, secondary nucleation events which are catalysed the the presence of existing aggregates can play a significant role in the dynamics of such systems. This talk describes our efforts to understand the nature of the nucleation processes in protein aggregation as well as the dynamics of such systems and how these features connect to the biological roles that these structures can have in both health and disease. A particular focus will be on the development of new microfluidics-based approaches to study heterogeneous protein self-assembly and their application to explore the molecular determinants of amyloid formation from peptides and proteins.

S.3.1.B-002

Withdrawn

S.3.1.B-001
Exploiting protein-ligand interactions to ameliorate amyloid disease

S. Radford
The University of Leeds, Leeds, United Kingdom

Understanding how different proteins assemble into the ordered, insoluble aggregates associated with amyloid disease is a formidable challenge. Whilst it is generally accepted that protein misfolding is required for the formation of amyloid fibrils, the point at which the folding and aggregation free energy landscapes diverge, and the role of different amino acid residues in determining folding *versus* aggregation, remain obscure. Even more challenging is the identification of early aggregation-prone monomers and oligomeric species and their structural characterisation, since such species are aggregation-prone, short-lived and rapidly equilibrating. In this seminar I will describe how different biophysical methods are being used to reveal the mechanism by which normally soluble proteins convert into amyloidogenic conformations, how bimolecular collisions between protein variants can result in very different outcomes of assembly, and how we have used small molecules to modulate the aggregation process.

ShT.3.1.B-001
Studying of aggregation of nuclear export protein (NEP) Influenza virus A

A. O. Golovko[1], O. N. Koroleva[2], E. V. Dubrovin[3], V. A. Radyukhin[4], V. L. Drutsa[4]
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Nuclear export protein of influenza virus A (NEP) is involved in a number of important phases in the virus life cycle. NEP is prone to aggregation, while expressed in our earlier designed bacterial expression system. Preventing this undesirable process requires an understanding the basic mechanisms of this protein self-assembly. The objective of this study was to investigate the

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peculiarities of NEP aggregation and factors affecting this process. To this end, NEP variants with either C- or N-terminal $(His)_6$-tags (NEP-C and NEP-N) were expressed in *E.coli* cells, thus obtaining highly purified homogenous solutions after purification on Ni-NTA-agarose column. The formation of oligomers in protein solutions using variable parameters (temperature, pH, concentration, additives, potential disaggregating agents), was analyzed with different techniques: dynamic light scattering (DLS), chemical cross-linking with bifunctional reagent (glutaric aldehyde), gel-filtration chromatography, atomic force microscopy (AFM). DLS measurements have shown the presence of polymeric particles both in NEP-C and NEP-N solutions, with hydrodynamic radius being in the range of 50–500 nm. AFM studies have revealed diverse morphology of the aggregates. Spherical particles (diameter is about 12–14 nm) are mainly presented in NEP-N solution, while fibrillar amyloid-like aggregates (height is 3.5–4.0 nm and length is up to 500 nm) are predominated in case of NEP-C protein. Structures of the N- and C- termini were proposed to affect the propensity of NEP to aggregation. Analysis of structural peculiarities of NEP, viz. amphipathicity of some secondary structure elements accompanied with the concerted combination of outer exposed hydrophobic, aromatic and charged basic amino acid residues, allows suggesting involvement these specific domains in spontaneous aggregation process through multiple hydrophobic and cation/dipole/π-π interacions. The work was supported by RFBR, grant No. 16-04-00563.

ShT.3.1.B-002
Ultrafast protein folding under mechanical force: experiments and simulations

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Single molecule force spectroscopy (smFS) has been used extensively to probe the dynamics of biomolecules, resulting in much enriched insights on the protein folding process. However, smFS has typically been applied to proteins that upon the application of force, follow a traditional two or three state mechanism, confirming previous findings from bulk. Here we focus in the case of an ultrafast folding protein, gpW, in the low force regime (5 pN) carried out with an atomic force microscope (AFM). GpW has been characterized as an ultrafast folding protein from both equilibrium and kinetic experiments and simulations. Using smFS we observe a characteristic hopping pattern between two distinct states, suggesting that the external mechanical force induces a barrier in the free energy surface. Surprisingly, there is a 10,000-fold slowdown in the apparent folding/unfolding rate relative to the bulk. Using molecular simulations we rationalize the experimental results. Much of the slowdown in the observed dynamics is determined by the tethering of the molecule to the cantilever, as proposed in recent theory. The resulting picture of barrier-limited folding with dynamics damped by instrumental effects is likely general for ultrafast biomolecules studied with smFS.

Tuesday 12 September
15:00–17:00, Oranim Hall 1

Chromatin Structure and Epigenetic Modifications B

S.3.2-003
Functional insights into chromatin topology and gene expression during embryonic development

E. Furlong[1], G. Cavalheiro[2], T. Pollex[2], Y. Ghavi-Helm[2], A. Jankowski[2]
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Embryonic development requires the coordinated expression of genes in both a space and time dependent manner. This complex regulation is controlled through the binding of transcription factors to enhancer elements, sometimes located at great distances from their target gene. Chromatin conformation studies have shown that gene activation by remote enhancers is generally associated with the establishment of a chromatin 'loop' to the promoter element. Our previous work indicates that enhancer-promoter loops are often formed hours prior to transcription during embryonic development, suggesting that chromatin loops are necessary, but not sufficient, for gene activation. To better understand the relationship between chromatin organization and transcriptional regulation, we are currently examining when enhancer-promoter proximity is first established during embryogenesis. Going back to earlier and earlier stages in development, we have measured the distances of enhancer-promoter interactions in individual nuclei using quantitative FISH at multiple loci, including distances at TAD border and intra-TAD regions. Our results indicate major changes at the Maternal to Zygotic Transition (MZT). Although the analysis is still ongoing, I will present our results using MZT mutants to uncover the relationship between chromatin topology and transcriptional regulation. In addition to examining the function of different *trans* factors, we are also using CRISPR deletion, as well as more global rearrangements, combined with Capture C to dissect the functional relationship between genome organization and gene expression in *cis*. This is yielding interesting results, which will be presented in detail.

S.3.2-001
Chromatin regulators in cell differentiation and cancer

L. Di Croce
Centre for Genomic Regulation, Barcelona, Spain

Polycomb group of proteins are transcriptional repressors and play essential roles in regulating genes required for differentiation and embryonic development. Moreover, alteration in the expression of Polycomb group proteins have long been linked to the occurrence of different types of human diseases. Mechanistically, Polycomb proteins form at least two distinct complexes: the Polycomb-repressive complexes 1 and 2 (PRC1 and PRC2). It is especially critical for stem cells that their potential to self-renewal and to differentiate is tightly controlled and properly orchestrated. Misregulation of the levels of Polycomb protein often leads to either a block or an unscheduled activation of developmental pathways, and thus to an alteration in the cell cycle control. The consequences of this misregulation have been linked to the establishment of cancer stem cells, which can produce tumors through the

combination of an increase in self-renewal with a lack of complete cellular differentiation. I will discuss how Polycomb proteins impact on cancer, and their role in stem cell biology.

S.3.2-004
Single cell epigenomics

A. Tanay
Weizmann institute, Rehovot, Israel

Genomics and epigenomics are providing global and comprehensive approaches to study genetic variation and epigenome regulation. Nevertheless, understanding genome function through bulk genomics was so far difficult since it generated average genomic profiles over populations of millions of cells, and since these failed to represent variability and dynamics in the tissues or cell populations under study. Single cell transcriptional and Single cell epigenomis are recently being used to bridge this gap and apply powerful high throughput sequencing methodologies at a microscopic cellular resolution. We will describe research using single cell transcriptional analysis, single cell DNA methylation analysis and single cell Hi-C to understand genome regulation and epigenetic memory. Some experimental and computational challenges will be presented, and their impact on the design and balance among the different single cell genomics techniques will be discussed. In particular, studies characterizing epigenetic memory in embryonic and differentiated cells will be described.

ShT.3.2-003
CpG Traffic Lights: functional positions that are involved in regulation in humans

A. V. Lioznova[1], A. M. Khamis[2], A. V. Artemov[1,3,4], V. Ramensky[5,6,7], V. B. Bajic[2], Y. A. Medvedeva[1,5,8]
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DNA methylation is probably the most investigated mechanism of expression regulation. Current technologies allow one to study DNA methylation with single base resolution, nevertheless usually it is averaged over several dozens adjacent CpGs during downstream analysis. Yet, experimental evidence show that the methylation levels of single CpG dinucleotides can affect the expression, for example in case of ESR1 gene. In this work we analyzed 40 different human cell types for which both WGBS and RNA-seq were available. CpG positions that have significant correlation between methylation levels and neighboring gene expression across all the samples were called CpG traffic lights (TL). We observe that the average methylation of promoter less frequently demonstrates significant correlation with gene expression than the methylation levels of CpG TL, even after proper correction for multiplicity testing. Analyzing CpG TL, we noticed that they often demonstrate intermediate methylation levels compared to background CpG dinucleotides. Hydroxymethylcytosine (5hmC) is also more frequently observed in such positions, supporting the dynamic demethylation of CpG TL. Also the causality analysis shows that positions where methylation is determined by expression are enriched in 5hmC, suggesting that there is a positive feedback loop: transcription activates DNA demethylation. We also observe that CpG TL are enriched in conserved positions both in mammalians and primates and depleted of SNPs. We show that CpG TL are overrepresented in promoters of all known types (especially bivalent/poised) and in corresponding chromatin states, spiking at exact transcription start sites, measured by CAGE. We also observe a strong enrichment in enhancers determined both by CAGE and by chromatin modifications. To support our claims we expand our analysis to variation across different humans using TCGA data. We believe that our data provides a new insight to the functional role of CpG TL methylations.

ShT.3.2-004
CENP-B protects centromere chromatin integrity by facilitating histone deposition via the H3.3-specific chaperone Daxx

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The Daxx chaperone deposits transcription-associated histone H3.3 at centromeres, but mechanism of centromere-specific Daxx targeting remains unclear. We identified unexpected function of the constitutive centromeric protein CENP-B that serves as a "beacon" for H3.3 incorporation. CENP-B depletion reduces Daxx association and H3.3 incorporation at centromeres. Daxx/CENP-B interaction and Daxx centromeric association are SUMO-dependent and requires SIM's of Daxx. Depletion of SUMO-2, but not SUMO-1, decreases Daxx/CENP-B interaction and reduces centromeric accumulation of Daxx and H3.3, demonstrating distinct functions of SUMO paralogs in H3.3 chaperoning. Finally, disruption of CENP-B/Daxx-dependent H3.3 pathway deregulates heterochromatin marks H3K9me3, ATRX, and HP1α at centromeres and elevates chromosome instability. The demonstrated roles of CENP-B and SUMO-2 in H3.3 loading reveal a novel mechanism controlling chromatin maintenance and genomic integrity. Given that CENP-B is the only centromere protein that binds centromere-specific DNA elements, our study provides a missing link between centromere DNA and unique epigenetic landscape of centromere chromatin.

Tuesday 12 September
15:00–17:00, Oranim Hall 2

Systems Biology

S.3.3.B-001
Feedbacks in signalling – robustness and drug resistance

N. Bluthgen
Charite, Berlin, Germany

Conceptually, we think of signalling pathways as direct cascades between receptor and (nuclear) targets. However, signalling pathways are complex feedback-controlled information processing systems. In this talk, we discuss the consequences of feedback regulation for robustness of signalling and for designing targeted therapies to block these pathways. In EGFR/MAPK signalling, we find that variation in concentration of kinases is fully buffered by negative feedback control, which also leads to drug resistance.

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S.3.3.B-003
Chromatin dynamics During DNA replication
N. Barkai
Weizmann institute, Rehovot, Israel

Genome replication introduces a step-like increase in the DNA template available for transcription. Genes replicated early in S-phase experience this increase before late replicating genes, raising the question of how relative expression levels are affected by DNA replication. I will describe our studies showing that in the budding yeast, the time in which the gene is replicated does not influence its level of expression and new results pointing to the molecular mechanisms providing this buffering capacity.

S.3.3.B-002
Evolutionary tradeoffs and the geometry of phenotype space
U. Alon
Weizmann Institute of Science, Rehovot, Israel

Organisms, tissues and molecules often need to perform multiple tasks. But usually no phenotype can be optimal at all tasks at once. This leads to a fundamental tradeoff. We study this using the concept of Pareto optimality from engineering and economics. Tradeoffs lead to an unexpected simplicity in the range of optimal phenotypes- they fall on low dimensional shapes in trait space such as lines, triangles and tetrahedrons. At the vertices of these polygons are phenotypes that specialize at a single task. We demonstrate this using data from animal and fossil morphology, bacterial gene expression and other biological systems.

ShT.3.3.B-001
Heterosis as a consequence of regulatory incompatibility
D. Bar-Zvi[1,2], R. Herbst[1,2,3,4], S. Reikhav[1], I. Soifer[1], M. Breker[1], G. Jona[5], E. Shimoni[6], M. Schuldiner[1], A. Levy[2], N. Barkai[1]
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The merging of genomes in inter-specific hybrids can result in novel phenotypes, including increased growth rate and biomass yield, a phenomenon known as heterosis. Heterosis is typically viewed as the opposite of hybrid incompatibility. In this view, the superior performance of the hybrid is attributed to heterozygote combinations that compensate for deleterious mutations accumulating in each individual genome, or lead to new, overdominating interactions with improved performance. Still, only fragmented knowledge is available on genes and processes contributing to heterosis. We describe a budding yeast hybrid that grows faster than both its parents under different environments. Phenotypically, the hybrid progresses more rapidly through cell cycle checkpoints, relieves the repression of respiration in fast growing conditions, does not slow down its growth when presented with ethanol stress, and shows increasing signs of DNA damage. A systematic genetic screen identified hundreds of *S. cerevisiae* alleles whose deletion reduced growth of the hybrid. These growth-affecting alleles were condition-dependent, and differed greatly between from alleles that reduced growth of the *S. cerevisiae* parent. Our results define a budding yeast hybrid that is perturbed in multiple regulatory processes but still shows a clear growth heterosis. We propose that heterosis results from incompatibilities that perturb regulatory mechanisms, which evolved to protect cells against damage or prepare them for future challenges by limiting cell growth.

ShT.3.3.B-002
Evolthon: the first experimental evolution community challenge
S. Kaminski, D. Schirman, O. Dahan, Y. Pilpel
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Evolution is a key process in biology since organisms must survive in constantly changing environments. In order to study evolution in the lab, various techniques of lab evolution are used. Although much work was done looking at individual evolution strategies there is no comprehensive study aiming to compare the effect of different evolution strategies. Thus, we have conducted for the first time a world-wide lab-evolution challenge aiming to find new and creative strategies of microorganisms' adaptation toward a given challenge in a laboratory setting. Each of the participants got a genomically labeled microorganism (*E. coli* or *S. cerevisiae*) and evolved it toward an environmental challenge, a cold shock. Together, we obtained 30 different strains, covering a wide range of evolution strategies such as changing the mutation rate, mating in yeast, directed and non-directed engineering and more. We determined the fitness of the strains by individual growth curves, as well as in a pooled competition manner to assign a fitness value based on the frequency of each strain. The fittest *S. cerevisiae* strain used a sexual mating strategy for evolution while the fittest *E. coli* strain featured a gradual decline of the mutation rate during the evolution. Beyond just characterizing each strategy in the designated challenge we examined each strain's fitness in additional environments, to examine a potential trade-off between capacity to specialize in a given condition or to generalize an adaptive solution in other challenges. Interestingly, the fittest *S. cerevisiae* in the cold environment had the highest fitness in all tested environments, indicating that the sexual mating strategy did not suffer from a sever trade-off across conditions. This project is a community effort to study an important aspect of evolution, by comparing different routines of evolution and as such, we are aiming for the involvements of the community in further aspects of this study.

Tuesday 12 September
15:00–17:00, Teddy Hall

Molecular Neuroscience B

S.3.4-003
Nanoscopic organisation and use-dependent plasticity of synaptic microenvironment
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Memory trace formation in the brain is thought to involve structural remodelling of synaptic connections. This is likely to engage ultrathin astroglial processes that often occur in the immediate proximity of excitatory synapses. While astroglia have emerged as an important regulator of synaptic circuits, the causal relationships between activity-triggered synaptic restructuring and the changes in nearby astroglia remain poorly understood. We therefore combined electrophysiology with two-photon excitation microscopy, photolytic uncaging, super-resolution techniques, and 3D electron microscopy, to monitor fine astroglial morphology during the induction of synaptic long-term potentiation (LTP). We document the NMDA receptor dependent-withdrawal of astroglial processes from the vicinity of synapses following LTP induction, both at the level of synaptic populations and at the level of individually monitored potentiated synapses. The reduction in synaptic astroglial coverage boosts the escape of synaptically released glutamate thus facilitating the NMDA receptor-mediated signal exchange among neighbouring excitatory connections. The cellular mechanisms underlying astroglial restructuring involve local Ca^{2+} elevations but they do not rely on metabotropic glutamate receptors, IP_3-receptor signalling, aquaporins, or Ephrin-associated synaptic morphogenesis. They do, however, require the ion exchanger NCCK1, thus pointing to the importance of the ion and water homeostasis machinery. Experiments are under way to understand activity-dependent changes in the 3D nano-organisation of perisynaptically expressed signalling proteins using dSTORM imaging.

S.3.4-004
Mechanisms of neuronal remodeling

O. Schuldiner
Weizmann institute, Rehovot, Israel

In our laboratory, we study the molecular mechanisms that regulate, control and execute developmental neuronal remodeling in *Drosophila melanogaster*. Remodeling refines neural circuits by a combination of degenerative processes, such as axon and synapse pruning, as well as regenerative processes, such as regrowth to form adult specific connections. Neuronal remodeling is essential for sculpting the mature nervous systems of both vertebrates and invertebrates during development. Our knowledge of the mechanisms that regulate remodeling are far from being complete. We believe that understanding the mechanisms of developmental remodeling should shed light on the mechanisms that regulate axon degeneration and growth during development, injury and disease. In addition, defects in neuronal remodeling has been linked to human diseases such as schizophrenia and autism. The *Drosophila* mushroom body (MB), a central nervous system (CNS) structure involved in learning and memory, is comprised of three types of neurons (γ, α'/β', and α/β), sequentially born from identical neuroblasts. MB γ neurons undergo stereotypic remodeling such that their larval connections undergo pruning during early metamorphosis and later regrow to adult specific lobes. The genetic power of *Drosophila*, and especially the ability to perform detailed mosaic analyses, together with the stereotypy of the MB development make this a unique system to study the mechanisms of remodeling. Finally, in recent years we began to combine genomics and genetics in pursuit of a systematic understanding of neuronal remodeling. I will present our unpublished experiments that reveal a complex transcriptional regulation of MB remodeling.

S.3.4-006
Dynamic regulation of excitatory-inhibitory ratio in neural circuits by experience-induced secreted molecules

I. Spiegel
Department of Neurobiology Weizmann Institute of Science, Rehovot, Israel

The maintenance of the proper ratio between excitation and inhibition (E/I ratio) is critically important for the normal function of neural circuits, but the molecular mechanisms that dynamically regulate E/I-ratio in the face of ever-changing experiences and altering patterns of neural activity are poorly understood. Our studies in the cortex reveal that different types of neurons respond to sensory experience by expressing distinct sets of secreted molecules, suggesting that these molecules modulate specific synapses in a cell-autonomous and local manner. Our findings on the insulin-like growth factor 1 (IGF1) are consistent with this idea: IGF1 is expressed and experience-induced in the cortex selectively in VIP- (Vasoactive Intestinal Protein) expressing GABAergic neurons and VIP neuron-derived IGF1 promotes inhibitory inputs onto VIP neurons in a manner that is necessary for the experience-dependent development of the visual cortex. I will discuss these findings in the context of previous literature and propose a model whereby cortical neurons maintain their E/I-ratio by inducing the expression of secreted factors that modulate a neuron's synaptic connectivity in a cell-autonomous and local manner according to a circuit-wide homeostatic logic.

ShT.3.4-002
The MAGUK protein MPP2 is a novel synaptic scaffold that links SynCAM cell adhesion molecules to AMPA receptor complexes

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Membrane-associated guanylate kinases (MAGUKs) are a family of multi-domain scaffolding proteins, several of which have been implicated in neurodevelopmental disorders. They are characterised by their PDZ-SH3-GK (PSG) core module. These and other domains allow for multiple protein-protein interactions and account for the important role of MAGUK proteins in the assembly of membrane-associated scaffolds in neurons and in other cell types. The MPP ('membrane protein palmitoylated') subfamily of MAGUK scaffolds is well known to date for its function in epithelial cells in the assembly of cell polarity complexes and tight junctions. There is now growing evidence that MPP proteins may also play a critical role synapse development and function; importantly, they have been purified from synaptic glutamate receptor complexes and also found to bind other receptors and channels that are relevant for neuron function. We have demonstrated that MPP2 is a member of AMPA receptor associated protein complexes; moreover, we show that it is expressed in primary cultured rat hippocampal neurons and enriched in dendritic spines, where it co-localises with the postsynaptic markers PSD-95 and Homer1. In line with a role in synaptogenesis and synapse maturation we have found MPP2 to be developmentally up-regulated in cultured hippocampal neurons and brain lysates. Through co-immunoprecipitation experiments and a parallel yeast-2-hybrid screen, we were able to identify several new interaction partners of MPP2, including the established synaptic scaffold proteins PSD-95 and GKAP, as well as the transmembrane synaptic cell adhesion protein SynCAM1. Taken together, our results suggest that MPP2 is a novel postsynaptic

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scaffold molecule of significant importance, and we are currently investigating the impact of knock-down and overexpression of MPP2 in order to understand its function in neurons.

ShT.3.4-003
Distribution and dynamics of synaptic proteins in presynaptic boutons induced on micropatterned host substrate

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Maintaining of synaptic transmission requires segregation of exo- and endocytic zones. In order to visualize functionally distinct nanodomains by high resolution microscopy we grew hippocampal neurons on microstructured glass coverslips, functionalized with synaptic cell adhesion proteins. Formation of purely presynaptic sites is triggered on this microstructured host substrate, which we call "xenapses". We found that xenapses contain several active zones facing the coverslip and hudreds of synaptic vesicles. Our conditions facilitate growth of both vGlut- and vGAT-positive synapses. Experiments with calcium sensors, FM dyes and endogenously expressed pHluorin constracts have shown, that xenapses respond to stimulation and are functionally normal. Xenapses allow to reconstruct separate nanodomains with high localization percision using single molecule localizational microscopy. Xenapses are also useful for studing the molecular dynamics of mebrane proteins in presynaptic membrane. TIRF-dSTORM and sptPALM of various CAZ (cytomatrix of active zone) and SNARE proteins allow to investigate distribution and interaction of proteins during presynapse formation and function. Xenapses form active zones, positive for Bassoon, whereas clathrin predominantly localizes in periactive zones. Syntaxin clusters are associated with active zones, but not co-localized with Bassoon. We suggest a new model of the presynaptic membrane compartmentalization: Bassoon forms the core of the active zone surrounded by synaptic vesicle fusion machineries; active zones are separatd by endocytic zones. Another interesting finding is a spatial segregstion of Syntaxin-1A and SNAP-25B enriched domains in presynaptic membranes, which leads us to conclude, that these SNARE-nanodomains work as buffers, preventing uncontroled synaptic vesicle fussion.

Wednesday 13 September
9:00–11:00, Teddy Hall

Cancer Biology A

S.4.1-006
Imaging and targeting pre-metastatic niches in melanoma

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Cutaneous melanoma is the deadliest form of skin cancer. Although the presence of tumor cells in lymph nodes is a poor prognosis indicator, the specific contribution of lymphangiogenesis to the mortality associated with melanoma metastasis remains unclear. This is mainly due to the lack of tumor markers and experimental models for non-invasive imaging of lymphangiogenesis in vivo. To address molecular mechanism(s) regulating metastatic spread of melanoma, we developed a series of genetically engineered mouse strains derived from a "lymphoreporter model" in which a luciferase cassette is expressed as a reflection of the endogenous Flt4 (VEGFR3) promoter. These "lymphoreporter" melanoma models allowed tracing of metastatic dissemination from very early stages of melanoma development. In particular, we have identified distinct patterns of melanoma-driven neo-lymphangiogenesis activated at distal sites before the actual colonization by tumor cells. Proteomic analysis of the melanoma secretome revealed new pro-lymphangiogenic factors that promote tumor metastasis. Here we will present the prognostic value of these metastatic genes and discuss the use of the lymphoreporter mice for the screening of anti-metastatic agents. Together, these results support the VEGFR3-luc mouse melanoma models as a cost-effective strategy for gene discovery in the context of otherwise aggressive and intrinsically metastatic tumors.

S.4.1-003
Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress

E. Gottlieb
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Reactive oxygen species (ROS) are increasingly recognized as important regulators of biological processes through the oxidative modification of protein cysteine residues. Comprehensive identification of redox-regulated proteins and cellular pathways are crucial to understand ROS-mediated events. We developed a new stable isotope cysteine labelling with iodoacetamide (SICyLIA) mass-spectrometry-based proteomic workflow to assess protein cysteine oxidation in diverse cellular models and primary tissues. This approach informs on all possible cysteine oxidative modifications and achieves proteome-wide sensitivity with unprecedented depth without using enrichment steps. Our results demonstrate that acute and chronic oxidative stress result in specific oxidation of metabolic and mitochondrial proteins despite their relatively lower cysteine content, indicating that metabolic adaptation is an essential response to redox stress. Obtaining accurate peptide oxidation profiles from a complex organ using SICyLIA holds promise for future application to patient-derived samples in studies of human disease.

S.4.1-005
Widespread parainflammation in human cancer

A. Lasry[1], D. Aran[2], A. Zinger[1], A. Hellman[1], E. Pikarsky[1], A. Butte[2], **Y. Ben-Neriah**[3]
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Chronic inflammation has been recognized as one of the hallmarks of cancer. Parainflammation is a unique variant of inflammation, characterized by epithelial-autonomous activation of inflammatory response. Parainflammation has been shown to

strongly promote mouse gut tumorigenesis upon p53 loss. In a recent study, we explored the prevalence of parainflammation in human cancer and determined its relationship to certain molecular and clinical parameters affecting treatment and prognosis. Parainflammation can be identified from a 40-gene signature, and is found in both carcinoma cell lines and a variety of primary tumors, independently of tumor microenvironment. We will discuss the implications of our findings in analyses of tumor microenvironment, suggesting that as tumor cell gene expression may often mimic immune and inflammatory infiltration, caution should be applied when interpreting tumor expression data. We will also address the connection between parainflammation and prevalence of p53 mutations in specific types of tumors, and cancer prevention by regular usage of NSAIDs. We suggest that parainflammation may serve as a novel biomarker for screening patients who may particularly benefit from NSAID treatment.

ShT.4.1-001
Obligate haploinsufficiency in cancer: Expression levels of PTPROt determine its role as a tumor promoter or suppressor in chronic lymphocytic leukemia

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The Weizmann Institute of Science, Rehovot, Israel

The roles of proteins in cancer can be context-dependent; we describe how a single protein can both promote and suppress the same tumor. The hematopoietic tyrosine phosphatase PTPROt is a putative tumor suppressor in B cell chronic lymphocytic leukemia (CLL), where its expression is reduced. In order to causally link PTPROt and CLL we targeted expression of PTPROt in mice and induced CLL in them. Unexpectedly, complete loss of PTPROt delayed disease detection and lengthened survival, indicating that PTPROt fulfills a novel tumor-promoting role in CLL. Tumor cells lacking PTPROt exhibited reduced B-cell receptor (BCR) and Src family kinase (SFK) signaling, both known positive prognostic factors in CLL, due to lack of Lyn activation by the absent PTPROt. Reduced BCR/SFK signaling increased apoptosis and autophagy in tumor cells, reducing disease severity. In contrast, loss of a single *Ptpro* allele induced the opposite phenotype – earlier detection of CLL and reduced survival, consistent with a tumor suppressing role for PTPROt. BCR/SFK signaling and cell death were normal in these tumor cells. Transcriptomic analysis of tumor cells lacking one or both *Ptprot* alleles revealed increased IL-10 expression and signaling, which are negative prognostic factors in CLL. We conclude that loss of one *Ptpro* allele increases IL-10 signaling and causes a severe CLL phenotype. Loss of the second allele uncovers another function of PTPROt by reducing BCR/SFK signaling and increasing cell death, overshadowing the tumor-promoting effects of IL-10 and reversing the CLL phenotype. PTPROt thus acts in CLL as an obligate haploinsufficient tumor suppressor, a new class of proteins whose expression levels switch between their functions as tumor promoters or tumor suppressors. Partial loss of PTPROt generates the strongest CLL phenotype, suggesting that its intermediate expression in CLL is selected for *in vivo* and that further inhibition of PTPROt could be useful in treating CLL patients.

ShT.4.1-002
eIF6 phosphorylation on Ser235 has a critical role in mammals development and in T-cell tumor progression

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eIF6 is an oncogenic translation factor with two roles: it is necessary for ribosome synthesis and maturation in the nucleus and it regulates 60S availability in the cytoplasm, controlling active 80S complex formation. eIF6 levels are rate-limiting for cell growth, cell transformation and tumor progression. eIF6 activation occurs through mTORc1-independent phosphorylation, driven by RACK1-PKCβ axis on Ser235. The overexpression and the hyperphosphorylation of eIF6 protein are reported in several cancer cell lines and human tumors. For instance, high levels of PKC and eIF6 are found in T-cell lineage and in T-cell lymphoma. Accordingly, human CD4 + T-cells require eIF6 for T-cells activation. Here we combine multidisciplinary studies on murine and cellular models to define: i) the relevance of eIF6 activation in mammals development and in particular in T-cell development, ii) the role of eIF6 phosphorylation in T-cell lymphomagenesis. Overall, we demonstrate for the first time that signaling cascades converge to eIF6 to regulate rate-limiting events in tumorigenesis and development. We used a conditional $eIF6^{S235A}$KI mouse model which we crossed with both CMV-Cre and CD4-Cre mice. CMV-$eIF6^{S235A/S235A}$ homozygosity causes embryonic lethality after gastrulation and homozygous blastocysts show a delayed *in vitro* development. Similarly, *in vitro* generated $eIF6^{S235A/S235A}$ MEFs have a markedly reduced proliferation and a senescence-like phenotype. Viability of $eIF6^{S235A/S235A}$ MEFs is rescued by transducing wild-type but not S235A eIF6. In contrast, CD4-$eIF6^{S235A/S235A}$ mice are viable with a slight defect in thymic T-cell maturation. Next, we used the NPM-ALK-driven T-cell lymphoma mouse model. Here, eIF6 is hyperactivated and its depletion increases mice survival. Since $eIF6^{S235A}$ mutation reduces oncogene-induced transformation in MEFs, we are analyzing if this mutation cause resistance to lymphomagenesis. This work confirms the therapeutic relevance of eIF6 inhibition.

Wednesday 13 September 9:00–11:00, Oranim Hall 1

Translational Control and mRNA Localization A

S.4.2-004
Dynamic control of dendritic mRNA expression by CNOT7 regulates synaptic efficacy and higher cognitive function

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Translation of mRNAs in dendrites mediates synaptic plasticity, the probable cellular basis of learning and memory. Coordination of translational inhibitory and stimulatory mechanisms as well as dendritic transport of mRNA is necessary to ensure proper control of this local translation. Here, we find that the deadenylase CNOT7 is a central factor in dynamically regulating dendritic

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mRNA translation and transport as well as synaptic plasticity and higher cognitive function. Synaptic stimulation induces a rapid decrease in CNOT7 which in the short-term results in poly (A) tail lengthening of target mRNAs. However, at later times following stimulation, decreased poly(A) and dendritic localization of mRNA is observed, similar to what is observed when CNOT7 is depleted over several days. In vivo, CNOT7 is essential for hippocampal-dependent learning and memory. This study identifies CNOT7 as a central regulator of RNA transport and translation in dendrites as well as higher cognitive function.

S.4.2-006
The role of the RNA-binding protein Staufen2 in dendritic mRNA localization and memory

M. Kiebler
BMC, LMU, Munich, Germany

Targeting of mRNAs to synapses and the subsequent regulation of local synaptic translation are essential for hippocampal synaptic plasticity and for learning and memory. A set of RNA-binding proteins (RBPs), amongst others the brain-specific double-stranded RBP Staufen2 (Stau2) critically contributes to these processes, however, the precise machinery and the underlying mechanisms involved in dendritic mRNA localization remain poorly understood. In my talk, I would like to present several independent lines of investigation that we are currently exploring to elucidate the role of Stau2 at the molecular, the cellular and at the organismic level. On the molecular level, we benefit of having recently identified physiologically relevant target mRNAs for Stau2 (Heraud-Farlow et al., 2013, *Cell Reports*). Amongst them is a set of transcripts including (1) *Rgs4* (regulator of small G protein signaling) whose protein products all cluster in the signaling pathway of G-protein coupled receptors and (2) an intron-containing isoform of Calm3 (calmodulin 3) in its 3′-UTR. To delineate the *in vivo* role of Stau2, we generated – in collaboration with Dusan Bartsch (Mannheim) a transgenic rat model, in which Stau2 expression is conditionally silenced by Cre-inducible expression of a miRNA targeting Stau2 mRNA in adult forebrain neurons. Interestingly, this transgenic rat revealed synaptic strengthening upon stimulation together with deficits in hippocampal spatial working memory, spatial novelty detection and in associative learning for operant rewards. Together, our findings provide new insight into how Stau2 contributes to learning and memory.

S.4.2-001
Identification of the interactome of a localized mRNA by mRNA proximity biotinylation

R. Jansen, J. Mukherjee
Tuebingen University, Tuebingen, Germany

Localized mRNA translation is found in diverse organisms throughout phylogeny and contributes to processes like cell polarization, neuronal function, and embryonic development. Proteins associated with a localized mRNA mainly regulate its function, including its transport and translation. Thus, characterizing these trans-regulatory factors will help us to unlock the molecular mechanisms of localized translation. Using mouse embryonic fibroblasts ß-actin as model mRNA, we introduce a new tool to identify the proteome of localized mRNAs (mRNA proximity ligation). A biotin ligase (BirA*) is targeted to MS2-tagged ß-actin mRNA via MS2 coat protein (MCP), which allows specific biotinylation of proteins associated with or in the close neighbourhood of the tagged mRNA. We demonstrate that this method allows identification of proteins stably or transiently interacting with the zipcode of the ß-actin mRNA. We show that the interacting proteome changes upon serum-induced localization of ß-actin mRNA. Along with the known ß-actin binding proteins ZBP1 (*Igf2 bp1*), ZBP2 (*Fubp2*), and an already described myosin II motor protein, we will report on additional candidate interactors. Our new capturing method will allow the identification of the interactome of any mRNA.

ShT.4.2-001
Crystal structures of fungal eIF4G and 4E-BPs bound to eIF4E provide insight into the regulation of translation initiation

S. Grüner, M. Chung, E. Valkov, C. Igreja, E. Izaurralde
Department of Biochemistry, Max Planck Institute for Developmental Biology, Tübingen, Germany

The eukaryotic initiation factor 4F (eIF4F) complex recruits the 43S preinitiation complex to capped mRNAs. eIF4F comprises the cap-binding protein eIF4E, the RNA helicase eIF4A and the scaffolding protein eIF4G, which binds to both eIF4E and eIF4A. Assembly of eIF4F is regulated by eIF4E-binding proteins (4E-BPs), which repress translation by competing with eIF4G for binding to eIF4E. Metazoan eIF4G and 4E-BPs interact with eIF4E using a conserved bipartite binding mode involving canonical and non-canonical eIF4E-binding motifs that bind to the dorsal and lateral surface of eIF4E, respectively. In *S. cerevisiae* (*Sc*), the two 4E-BPs p20 and Eap1p regulate cap-dependent translation. However, structural information on the assembly of yeast eIF4E-containing complexes is limited to the complex with eIF4G. Upon binding to eIF4E, *Sc* eIF4G forms a bracelet-like structure remarkably different from the bipartite binding mode seen in metazoan eIF4E–eIF4G complexes. This finding suggested that distinct interaction modes are used in yeast eIF4E-complexes, raising the question of how 4E-BPs function in translation control in fungi. Here, we present crystal structures of *Sc* eIF4E bound to p20 or Eap1p and the structure of the eIF4E–eIF4G complex from *C. thermophilum*. In contrast to prior studies, these structures show that in all eIF4E complexes, fungal eIF4E partners share conserved, core eIF4E-binding elements present in metazoans and contain previously unknown non-canonical motifs that engage the lateral surface of eIF4E. Moreover, fungal eIF4E partners exhibit molecular adaptations that stabilize binding to eIF4E: p20 possesses a high-affinity canonical motif; Eap1p harbors a unique sequence stabilizing the interaction with the eIF4E dorsal surface; and eIF4G uses a fungi-specific bracelet-like structure to enhance binding to eIF4E. Our structural and biophysical studies uncover conserved principles governing the assembly of the eIF4F complex and its regulation by 4E-BPs.

ShT.4.2-002
Global mRNA polarization regulates translation efficiency in the intestinal epithelium

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Asymmetric mRNA localization facilitates efficient translation in cells such as neurons and fibroblasts. However, the extent and significance of mRNA polarization in epithelial tissues has not been established. Here, using single molecule transcript imaging and subcellular transcriptomics, we uncover global apical-basal intracellular polarization of mRNA in the mouse intestinal epithelium. We find that the localization of mRNAs does not

generally overlap protein localization. Rather, ribosomes are more abundant on the apical sides, and apical transcripts are consequently more efficiently translated. Refeeding of fasted mice elicits a rapid translocation of mRNAs encoding ribosomal proteins to the apical sides, yielding a specific boost in their translation. This leads to increased protein production, required for efficient nutrient absorption. Our work exposes a post-transcriptional regulatory mechanism involving dynamic polarization of mRNA and polarized translation. Such regulation could be relevant to other polarized tissues that need to rapidly respond to external stimuli.

Wednesday 13 September 9:00–11:00, Oranim Hall 2

Protein Degradation

S.4.3.A-001
Ubiquitin signaling in physiology and diseases

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Cellular ubiquitination, i.e. the covalent attachment of the small modifier ubiquitin to target proteins, is one of the most prevalent and elaborated post-translational modifications developed by eukaryotic cells. The importance of ubiquitin in physiology and in medicine has amazingly increased during the past two decades due to the accumulation of evidences supporting a paramount role of this protein in the regulation of various pathological phenomena. The therapeutic potential of targeting the ubiquitin system was demonstrated in cancer by the approval of the reversible proteasome inhibitor Valcade (bortezomib) for the treatment of multiple myeloma by the FDA. Better than proteasome, E3 ligases appear to be promising targets for drug discovery as they represent the last step of the enzymatic cascade, capable of conferring a high degree of specificity and selectivity towards target substrates in cells. Our laboratory studies HECT E3 Nedd4 ligases that recognize and directly catalyze ubiquitination to their protein substrates. These enzymes are K63 specific and thus their targets are not directly directed to degradation. We have recently defined molecular details of NEDD4 catalytic activity and regulatory mechanism by solving few structures of the enzyme and by studying its activation via phosphorylation. We are interested in understanding the specific role of the various members of the family in the regulation of fundamental processes such as endocytosis, cell migration, and autophagy in normal and pathological conditions. Current studies will be presented.

S.4.3.A-003
Team-tagging ubiquitin ligation pathways

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A major focus of the department is to understand regulation of and by the largest E3 family, consisting of the multisubunit Cullin-RING ligases (CRLs). CRL-mediated ubiquitination dynamically modulates target protein properties including half-life, subcellular localization, enzymatic activity, conformation, and intermolecular interactions, to regulate the cell cycle, transcription, DNA repair, stress responses, signaling, immunity, plant growth, embryogenesis, circadian rhythms, and a plethora of other pathways. We and others have found that cullin-RING E3 activities are modulated through a variety of protein-protein interactions influencing enzyme conformations and activities. In my presentation, I will discuss our latest data investigating dynamic mechanisms underlying regulation of and by cullin-RING E3 ligases.

S.4.3.A-002
Degrading mitochondria by mitophagy – a new ubiquitin playground

D. Komander
MRC LMB, Cambridge, United Kingdom

Ubiquitin signals come in many flavours, forming a complex 'ubiquitin code', in which eight chain types mix with other PTMs such as phosphorylation and acetylation, leading to a mind-boggling potential architectural complexity. One area of immense interest focuses on mitochondrial maintenance, and in particular mitophagy has emerged as an exciting area for ubiquitin research. This process involves unstudied ubiquitin Lys6-linked chains, which are assembled by the RBR E3 ligase Parkin, and disassembled by the Lys6-selective deubiquitinase USP30. Using new affinity tools for Lys6-linked chains, we recently uncovered new E3 ligases for this chain type, which may constitute a fast-track proteasomal degradation signal. Mitophagy induction also relies on PINK1, the first described ubiquitin kinase, which phosphorylates ubiquitin Ser65 specifically, and we recently learned how this signal is distributed across mitochondria. In my talk, I will discuss our latest insights into the mechanisms of mitophagy and of the proteins involved.

ShT.4.3.A-001
"Ubiquitin or Substrate" – What determines Proteasome processivity?

I. Sahu[1], S. M. Mali[2], A. Brik[2], O. Kliefeld[1], M. H. Glickman[1]
[1]*Faculty of Biology, Technion—Israel Institute of Technology, Haifa, Israel,* [2]*Schulich Faculty of Chemistry, Technion—Israel Institute of Technology, Haifa, Israel*

The Ubiquitin Proteasome System (USP) is the major machinery for breaking down unused, redundant, old, or damage proteins in eukaryotes. Proteins targeted for degradation are either ubiquitinated or non-ubiquitinated; however, in either case they are processed in the 20S proteasome core, cleaved into small peptides and released. Some peptides escape further hydrolysis and are loaded onto MHC class I as a "self" signal to CD8-T-cells. Therefore, the cleavage specificity by proteasomes consequently determines efficiency of the immune response. However, it is still unclear how ubiquitination affects substrate processivity by different specifies of proteasomes which was the main focus of this study. By choosing an unstructured substrate, we could compare proteolytic outcome of highly homogenous synthetic non-ubiquitinated and ubiquitinated conjugates. We executed enzymatic reactions for different human proteasome species (20S and 26S) with the above substrates. We found that a non-ubiquitinated substrate was degraded more efficiently by 20S than by 26S proteasome. In contrast, ubiquitination slowed down the rate of degradation of the same substrate by 20S while enhanced degradation by 26S. Moreover, from MS/MS analysis of the proteasome-generated products, we elucidated the contribution of ubiquitination to cleavage specificity, peptide sequences, and distribution of product size. Furthermore, we are trying to recapitulate this phenomenon in cellular model systems that mimic pathological conditions. In conclusion we demonstrated parameters that could influence cleavage specificity by proteasome and their implications in the production of antigenic peptides; however, its direct implementation to immune response by *in vivo* experimentation would be a breakthrough for disease intervention.

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ShT.4.3.A-002
Regulation mechanisms for writing, editing and reading the ubiquitin code

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Ubiquitylation regulates essentially all cellular pathways in eukaryotes and is therefore involved in numerous pathologies. High redundancy of the ubiquitylation cascades, high efficiency of deubiquitylation enzymes and rapid proteasomal / lysosomal degradation severely restrict our knowledge. To circumvent these limitations we developed *E. coli* based selection and purification systems for ubiquitylated proteins. Expression of functional ubiquitylation cascades in bacteria that lack deubiquitylase facilitate the purification of milligram quantities of ubiquitylated targets. Similarly, we genetically linked ubiquitylation and bacterial survival, a system that facilitates screening for ubiquitylation targets and small molecule modulators. The systems' application to identify and characterize the structure of ubiquitylated-ubiquitin receptors, as well as novel regulation mechanism of HECT E3-ligases that control myriad of ubiquitylation targets, will be presented.

Wednesday 13 September
9:00–11:00, Oranim Hall 3

Structural Computational Biology

S.4.4.A-001
Integrative modelling of macromolecular machines: 3D-EM and more

M. Topf
Institute of Structural and Molecular Biology, Birkbeck/UCL, London, United Kingdom

The integration of data derived from a variety of biophysical techniques at multiple levels of resolution is becoming increasingly common in structural determination of large macromolecular machines, with 3D electron microscopy (3D-EM) being one of the key players. In recent years, there has been a substantial rise in the number of 3D-EM reconstructions and a major fraction of these are still at the low-to-intermediate resolution range. Currently, there is a great need to develop automated methods for fitting of atomic structures into 3D-EM maps, also including information from other experiments (e.g., cross-linking mass spectrometry). Such methods can be challenging, and their success depends on the resolution, size and shape of the map, and conformational differences. Additionally, assessing the quality of the resulting atomic models is not trivial. Here, I will show our recent approaches to density fitting and model validation at different resolutions.

S.4.4.A-002
Structure determination of genomes and genomic domains by satisfaction of spatial restraints

M. A. Marti-Renom
CNAG-CRG, Barcelona, Spain

The genome three-dimensional (3D) organization plays important, yet poorly understood roles in gene regulation. Chromosomes assume multiple distinct conformations in relation to the expression status of resident genes and undergo dramatic alterations in higher order structure through the cell cycle. Despite advances in microscopy, a general technique to determine the 3D conformation of chromatin has been lacking. We developed a new method for the determination of the 3D conformation of chromatin domains in the interphase nucleus, which combines 3C-based experiments with the computational Integrative Modeling Platform (IMP). The general approach of our method opens the field for comprehensive studies of the 3D conformation of chromosomal domains and contributes to a more complete characterization of genome regulation. During the seminar, I will introduce our recent work on assessing the accuracy of the 3D models and applying our approach to modelling the genome of Mycoplasma.

S.4.4.A-003
A network biology approach to novel therapeutic strategies

P. Aloy
IRB Barcelona, Barcelona, Spain

Network and systems biology offer a novel way of approaching drug discovery by developing models that consider the global physiological environment of protein targets, and the effects of modifying them, without losing the key molecular details. Here we review some recent advances in network and systems biology applied to human health, and discus how they can have a big impact on some of the most interesting areas of drug discovery. In particular, we claim that network biology will play a central part in the development of novel polypharmacology strategies to fight complex multifactorial diseases, where efficacious therapies will need to center on altering entire pathways rather than single proteins. We briefly present new developments in the two areas where we believe network and system biology strategies are more likely to have an immediate contribution: combinatorial therapies in cancer and drug repurposing.

ShT.4.4.A-002
New statistical potentials for probing protein binding affinity at the interactome scale

F. Pucci, Q. Hou, J. M. Kwasigroch, M. Rooman
Université Libre de Bruxelles, Bruxelles, Belgium

Since their introduction about 30 years ago, statistical potentials represent one of the powerful methods in structural bioinformatics to analyze protein biophysical characteristics. Despite the approximations on which their construction is based, they are widely used in many applications for their accuracy and their computational efficiency. These two characteristics make them a perfect instrument especially for large-scale, structuromic, investigations. Here we present some recent developments involving the definition of a new version of these potentials, which is appropriate for the analysis of protein-protein interactions. These interface potentials are mean force potentials derived from 3D structure datasets of dimer interfaces, and are based on a coarse-grained representation in which the side chains are simplified by average side chain centroids. Denoting by s a sequence element and by c a structure motif, the energetic contribution associated to the configuration (c, s) is obtained using the inverse Boltzmann law from the probabilities of occurrences of (c,s), (c) or (s), which are in turn approximated in terms of the number of occurrences in the dataset of experimental structures. These interface potentials allow analyzing the relevance of some specific interactions, such as salt bridges and cation-pi interactions, in the modulation of protein binding affinity. We show how these potentials can be fruitfully applied for protein design and present an improved version of our BeatMuSiC tool for the prediction of protein-protein binding affinity change upon mutations. Finally

we present preliminary results about its application to the analysis of the interactome's stability.

ShT.4.4.A-001
Computational design of a large network of specific and multi-specific protein-protein interactions

R. Netzer, S. J. Fleishman
Weizmann institute, Rehovot, Israel

Signaling in multicellular organisms depends on highly orchestrated interactions among proteins, and networks of both specific and multispecific interactions play important roles. These networks are built of homologous receptor-ligand complexes that are highly similar in structure but differ in the conformation and amino acid sequence at their binding site. To date, however, computational specificity design has not demonstrated an ability to design interaction networks, largely because of the challenges of designing new backbone conformations at protein binding sites. We introduce a design method that controls all the molecular elements observed in natural high-specificity networks, including the proteins' conformation. We applied the method to the bacterial colicin toxin/anti-toxin family, generating hundreds of unique designed pairs, from which we selected 58 for experiments. 19 designs formed the desired complexes *in vivo* and *in vitro* and had high expression levels. The designs showed diverse specificity patterns: some were highly specific and bound their cognate partner 3–5 orders of magnitude more tightly than other designs. Others showed multi-specific binding to several designed partners. Overall, these cognate and non-cognate interactions span all the biologically relevant interaction affinities, from sub-nanomolar to millimolar dissociation constants. A crystal structure of a highly specific pair showed atomic accuracy in backbone conformation, orientation, and the conformations of most of the designed side chains. Computational analysis showed that the specific designs were rich in polar interactions and had rigid interfacial loops. By contrast, the multispecific designs had flexible loops at their interaction surfaces that promoted multispecific binding. Our study therefore exposes rules for specificity and multispecificity in protein interaction networks, opening the way to engineering of insulated or cross-reactive interaction networks, as desired.

Wednesday 13 September 15:00–17:00, Teddy Hall

Cancer Biology B

S.4.1-001
Systematic genetic perturbations to reveal therapeutic melanoma vulnerabilities

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Netherlands Cancer Institute, Amsterdam, Netherlands

For a long time, advanced-stage melanomas were refractory to the available therapeutic options, but recent developments have begun offering better perspectives for patients. The small molecule inhibitor vemurafenib, specifically targeting the mutant $BRAF^{V600E}$ kinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually most melanomas become resistant and progress on treatment. This occurs by the acquisition of additional mutations or other alterations, most of which reactivate the mitogen-activated protein kinase (MAPK) pathway. Although further suppression of BRAF-MAPK signaling by the inclusion of MEK inhibitor delays resistance, eventually most patients relapse. The clinical outcome of late-stage melanoma patients has also greatly improved thanks to the recent availability of T cell checkpoint modulation, primarily by CTLA-4 and PD-1/PD-L1 blockade. But still, large patient groups fail to (durably) benefit from these treatments, underscoring the continuing need for developing novel therapeutic modalities. Therefore, in spite of these new perspectives, there is a dire need to identify additional targets amenable to therapeutic intervention, possibly to be used in combination settings with tumor inhibitors alongside immune activators. We are studying (lack of) sensitivity to both tumor and immune cell treatment using patient biopsies, patient-derived xenografts (PDX) and low-passage cell lines. These systems are used for systematic function-based genetic screens to identify melanoma and immune cell factors representing pharmacologically tractable therapeutic targets. The results from these and related studies will be discussed.

S.4.1-002
Bearing the cross of old age: How aging ECM guides melanoma progression

A. Weeraratna
The Wistar Institute, Philadelphia, United States

We have recently shown that the aged microenvironment promotes melanoma progression,via secreted changes in the aged fibroblast secretome that affect Wnt signaling, and response to reactive oxygen species. The aged secretome further reveals that proteins involved in the cross-linking of the extracellular matrix (ECM) are decreased. This led us to propose that age-related physical changes in the skin ECM could promote the metastasis of melanoma cells. Using a combination of mathematical modeling, fibroblast activity assays and in vivo experiments, we show that age-related changes in ECM stiffness can have profound effects on tumor cell metastasis. Further, these changes can affect the invasion of immune cells into the tumor site, as immune cells are known to use collagen fibres as "tracks" along which to migrate. Overall, our data reveal that age-related changes in the microenvironment affect ECM integrity and ultimately both tumor and immune cell motility.

S.4.1-004
Coordinated regulation of gut microbiota and immune checkpoint activity by the ubiquitin ligase RNF5 controls melanoma growth

Y. Li[1], R. Tinoco[1], L. Elmén[1], Y. Fujita[1], I. Segota[1], A. Sahu[2], R. Zarecki[3], Y. Feng[1], A. Khateb[4], H. Kim[1], S. Yooseph[5], M. A. Tam[6], T. Zhang[7], K. Brown[7], E. Ruppin[2,3], T. Long[1], S. N. Peterson[1], L. M. Bradley[1], **Z. Ronai**[1,4]
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The success of immune checkpoint inhibitors in cancer therapy has been limited to a few targets and tumor types. We identified a coordinated regulation of gut microbiota and immune checkpoint by the RNF5 ubiquitin ligase. Growth of mouse melanoma

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cells *in vivo* is attenuated, while tumor infiltration of $CD4^+$/$CD8^+$ T cells and dendritic cells (DCs) is increased in $Rnf5^{-/-}$ mice, resembling changes seen upon immune checkpoint therapy. This phenotype was immune system intrinsic, reflected in elevated inflammatory cytokines and TLR signaling. Notably, co-housing of $Rnf5^{-/-}$ and WT mice largely abolished these phenotypes. Intestinal epithelial cells and organoids derived from $Rnf5^{-/-}$ mice exhibited increased ER stress and cell death. Increased infiltration of DCs to the intestinal epithelium and to proximal Peyer's patches, reduced villi length, and altered glycosylation of mucin2, also signified $Rnf5^{-/-}$ mice. Microbiota analyses, using a novel analysis procedure identified 42 bacterial phylotypes that distinguished the gut microbiota of $Rnf5^{-/-}$ mice. Computer simulations identified altered uptake of select metabolites enriched in $Rnf5^{-/-}$ mice. Pre-biotics known to enrich these metabolites that were administered to WT mice phenocopied $Rnf5^{-/-}$ mice, exhibiting increased tumor infiltration of immune cells and reduced tumor growth. Our findings demonstrate a coordinated gut microbiome-immune checkpoint anti-tumor activity by the ubiquitin ligase RNF5 and select metabolites, which can mimic RNF5 deficiency.

ShT.4.1-003
Identification of tumor-suppressive and oncogenic regulatory elements in the human genome using CRISPR-based functional genomic screens

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Acquirement of somatic mutations (SMs) in the genome is the driving force of cancer. To date, most research on the functional significance of SMs was focused on the discovery of cancer-driving alterations in protein-coding sequences. However, the protein-coding exome comprises less than 3% of the human genome. Emerging evidence indicate a role for cancer-driving noncoding SMs that alter the activity of regulatory elements, controlling the expression of genes that affect tumor development. The maturation of the CRISPR-Cas9 genome-editing technology revolutionizes our ability to study the function of noncoding elements. Recently, we performed the first CRISPR-based functional genomic screen that targets enhancer regions in the human genome on a genomic scale. In these proof-of-principle screens, we focused on two transcription factors (TFs), p53 and ERα, which play key roles in cancer initiation and progression, and identified enhancers that are critical for the function of these regulators. We are now significantly expanding the scope of these initial screens, and, based on various epigenomic techniques and the CRISPR-Cas9 tool, systematically identify and target regulatory elements that are bound by multiple cancer-related TFs. We discovered novel enhancer regions that mediate oncogenic and tumor-suppressive effects, and mapped the target genes that are regulated by these elements. As exome-sequencing becomes a central tool for precision oncology, our results pinpoint critical genomic elements that go beyond the exome and should be prioritized too for sequencing, to improve our understanding of the mutational processes that drive each tumor and thus potentially guide a better treatment.

ShT.4.1-004
Structural basis of PD-1/PD-L1 checkpoint inhibition

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Targeting the immunologic checkpoints is a promising strategy for new anticancer therapies. One of the important checkpoints comprises of PD-1 and PD-L1 proteins. PD-1 (Programmed cell death protein 1) is expressed on the surface of activated T cells and plays a critical role in modulation of the host's immune response. One of its ligands, PD-L1 (Programmed cell death 1 ligand 1), is overexpressed in cancer cells and binds to PD-1, thus silencing the immune response against the tumor. This immunologic checkpoint was shown to be relevant in cancer and recent developments have shown that targeting it with monoclonal antibodies has provided breakthrough progress in the treatment of melanoma, non-small cell lung cancer and bladder cancer. Lack of detailed structural information concerning PD-1/PD-L1 interaction postponed development of small molecule drugs which however changes in the recent years. Here we report the crystal structure of a low molecular weight PD-1/PD-L1 checkpoint inhibitor in complex with its target PD protein. By revealing the molecular details of the interaction we facilitate the rational optimization and design of novel small molecule inhibitors based on the defined hot spots.

Wednesday 13 September 15:00–17:00, Oranim Hall 1

Translational Control and mRNA Localization B

S.4.2-007
The role of post-transcriptional regulation in neurogenesis

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A simple view of gene regulation during *Drosophila* and vertebrate brain development is that regulatory protein levels are largely determined by the rate of respective mRNA production. To test the generality of this principle in intact *Drosophila* larval brains, we have compared mRNA and protein expression distributions using single molecule FISH on protein-trap lines that tag endogenous proteins with YFP. We find a surprising number of cases of discordance between RNA and protein distributions across the brain, relating to mRNAs that encode proteins of diverse functions. These results suggest that post-transcriptional mechanisms of regulating gene expression are very common. We will present two prominent examples of such discordance, involving mRNAs encoding the conserved transcription factor Prospero and the mRNA-binding protein Syncrip/hnRNPQ. We show that a long *prospero* mRNA isoform containing a 15 kb 3′ UTR is stabilised in vivo by binding to Syncrip. Both the long *prospero* isoform and Syncrip are required to trigger the cessation of neural stem cell division in pupae that terminates neurogenesis. Our results demonstrate an unexpected function for mRNA stability in limiting neuroblast proliferation as required for normal brain development. We argue that the post-transcriptional mechanisms

we have uncovered involving Prospero/Prox1 and Syncrip/hnRNPQ are likely to be conserved in mammals.

S.4.2-002
Imaging the life and death of mRNAs in single cells

J. Chao
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Gene expression requires a precisely orchestrated series of events that is regulated temporally and spatially within the cell. The life of an mRNA begins when it emerges from the transcription complex and is recognized by factors that are responsible for its capping, splicing and polyadenylation. The mature transcript is then exported to the cytoplasm where it can be transported, translated and, eventually, degraded. While some of the factors that associate with a transcript may remain bound from birth to death, others can be remodeled as the transcript passes through different stages of its life. The dynamic and heterogeneous composition of mRNPs remains a challenge for understanding the mechanisms that control gene expression in the cytoplasm. While the list of factors involved in gene expression has expanded rapidly over the past few years, our understanding of how these macromolecular complexes are put together and function within their cellular context has not kept pace. The aim of our research is to characterize post-transcriptional regulation of gene expression from the perspective of individual mRNAs. My group takes a multidisciplinary approach by combining quantitative single-molecule fluorescent imaging of mRNAs in living cells with structural and biochemical studies of mRNPs in order to understand how these complexes assemble and function within cells. Recently, we have developed methodologies that enable the translation and degradation of single mRNA molecules to be monitored in real-time in living cells. We are using these tools to characterize the lives of mRNAs during normal cell growth and stress.

S.4.2-003
Regulating neuronal RNA granules in space and time

B. Florence
Institut de Biologie Valrose, Nice, France

Eukaryotic cells contain large cytoplasmic RNP granules that contain RNAs and associated regulatory proteins and are involved in the spatio-temporal regulation of transcript expression. In neurons, so-called neuronal granules have been implicated in the long-distance transport of mRNAs to axons or dendrites, and in their local translation in response to external cues. Although it has become clear that the properties of these complexes are modulated in response to developmental and environmental cues as well as aging, how such changes are achieved at the molecular and cellular levels is currently poorly understood. Using the fruitfly as a model organism, we have identified the conserved RNA binding protein Imp as a component of neuronal RNP granules and have shown that its transport to axons is tightly regulated during brain maturation. Furthermore, Imp function is essential for axonal remodeling (Medioni et al., 2014). Our current work aims at understanding how neuronal RNP granules are assembled, and how their properties and transport are regulated during development. Assembling and maintaining such large complexes represents a challenge for cells, as RNP granule components must first undergo a demixing reaction that segregates them from the soluble cytoplasmic fraction, and then establish extremely dynamic interactions with each others. While defective demixing will prevent the formation of RNA granules, alterations in RNA granule dynamics will generate static pathological inclusions. To identify factors controlling RNA granule assembly and turnover, we are combining different approaches including structure/function analyses, purification of RNP granules, and high throughput microscopy-based RNAi screen. In particular, we have recently uncovered the importance of prion-like domains in the regulation of RNA granules using FRAP and in vitro phase transition assays, and have revealed their requirement for axonal transport and remodeling.

S.4.2-005
mRNAs meet cytoplasmic stress granules

Y. Shav-Tal
Bar Ilan University, Ramat Gan, Israel

Cytoplasmic stress granules are non-membranous bodies that form in response to various types of stress that can lead to translational arrest. These structures contain mRNAs and proteins, many of which are RNA-binding proteins. It has been suggested that stress granules sequester translationally inhibited mRNAs. We have performed single molecule RNA FISH to examine the localization of endogenous mRNA molecules to stress granules in relation to the mRNA population that does not enter these structures. Using a technique we devised, which allows to differentiate between mRNAs transcribed from different alleles of the same gene, we could also examine the distribution of allele specific variants within and outside stress granules and search for allele-specific response to stress. Altogether, we analyze the distribution of mRNAs in the cytoplasm and in stress granules before stress, during stress, and after relief of the stress.

ShT.4.2-003
LARP1: gatekeeper of ribosome production

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The eukaryotic ribosome is composed by 4 ribosomal RNAs (rRNAs) and 80 distinct ribosomal proteins (RPs). The production of a functional ribosome unit requires that each individual ribosomal protein be produced in a synchronous manner and in its correct stoichiometry. Higher eukaryotes engendered a simple mechanism for the synchronized production of RPs at their correct stoichiometry: mRNAs encoding for ribosomal proteins share a common *cis*-acting mRNA regulatory motif – a stretch of 4–15 pyrimidines positioned immediately downstream of the N7-methyl-guanosine 5'-5' triphosphate (m7Gppp) mRNA cap; this stretch of pyrimidines is known as the 5'terminal oligopyrimidine (TOP) motif and allows for the simultaneous translation of mRNAs encoding for every ribosomal protein. Mammalian target of rapamycin complex 1 (mTORC1) has long been known to regulate the translation of TOP mRNAs but the mechanism by which this kinase complex regulates this process has remained elusive for over 20 years. Here, we present data that elucidates a molecular mechanism by which mTORC1 controls TOP mRNA translation/ribosome production. Our data show that La-related protein 1 (LARP1) is a novel downstream target of mTORC1 and that it represses the translation of TOP mRNAs. LARP1 binds the 5'TOP motif and the upstream m7Gppp cap of TOP mRNAs (Lahr et al., 2017). Upon binding to the 5'TOP motif, LARP1 blocks the binding of the eIF4F complex to TOP

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mRNAs effectively preventing their translation. Collectively, these data indicate that LARP1 functions as a gatekeeper of ribosome production downstream of mTORC1.

Wednesday 13 September 15:00–17:00, Oranim Hall 2

Autophagy

S.4.3.B-001
Autophagy and ubiquitylation, friends or foes?

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Recent studies have revealed that the autophagy upstream regulator AMBRA1 can coordinate a cell response to starvation or other stresses by integrated functions that include translocation of the autophagosome core complex to the ER, regulative ubiquitylation and stabilization of the kinase ULK1, selective mitochondria removal and cell cycle down-regulation. On the other side, AMBRA1 itself appears to be targeted by a number of regulations, such as Cullin-dependent degradation, caspase cleavage and several modifications, ranging from phosphorylation to ubiquitylation. The complex interplay among autophagy, the ubiquitin-proteasome system and novel regulatory functions of E3 ubiquitin ligases will be discussed in depth, together with its implication in cancer biology and therapy.

S.4.3.B-003
Expanding the functions of autophagy regulators to the nucleus

C. Behrends
Munich Cluster for Systems Neurology (SyNergy) Ludwig-Maximilians-University, Munich, Germany

Autophagy is an intracellular recycling and degradation pathway that depends on membrane trafficking. Rab GTPases are central for autophagy but their regulation especially through the activity of Rab GEFs remains largely elusive. We employed a RNAi screen simultaneously monitoring different populations of autophagosomes and identified 34 out of 186 Rab GTPase, GAP and GEF family members as potential autophagy regulators, amongst them SMCR8. SMCR8 uses overlapping binding regions to associate with C9ORF72 or with a C9ORF72-ULK1 kinase complex holo-assembly, which function in maturation and formation of autophagosomes, respectively. While focusing on the role of SMCR8 during autophagy initiation, we found that kinase activity and gene expression of ULK1 are increased upon SMCR8 depletion. The latter phenotype involved association of SMCR8 with the ULK1 gene locus. Global mRNA expression analysis revealed that SMCR8 regulates transcription of several other autophagy genes including WIPI2. Collectively, we established SMCR8 as multifaceted negative autophagy regulator.

S.4.3.B-002
Regulation of autophagy by lipid-binding proteins

A. Simonsen
University of Oslo, Oslo, Norway

Autophagy is a catabolic mechanism that allows recycling of cytoplasmic organelles and macromolecules through their sequestration into double-membrane vesicles (autophagosomes) which fuse with lysosomes, leading to degradation of the sequestered cargo. Long considered a non-selective process induced in response to cellular starvation, autophagy is now emerging as a highly selective quality control mechanism whose basal levels are important to allow cells to rapidly eliminate superfluous or damaged organelles, as well as unwanted structures as invading pathogens. Recently, our understanding of the hierarchy of autophagy-related proteins have increased significantly, but the membrane remodeling and trafficking events involved in autophagy are still poorly understood. We are investigating the interplay between lipids and lipid-binding proteins in the regulation and execution of autophagy. We recently carried out an imaging based siRNA screen targeting human FYVE and PX proteins where we identified the PX-BAR protein SNX18 as a positive regulator of autophagosome formation and the actin-binding PX protein HS1BP3 as a negative regulator of autophagy. We found that SNX18 facilitates recruitment of Atg16L1- and LC3-positive recycling endosome membranes to autophagosome precursors and that the pro-autophagic activity of SNX18 depends on its membrane binding and tubulation capacity. HS1BP3 is recruited to ATG16L1 and LC3 positive membranes and inhibits autophagy through binding to phosphatidic acid (PA) and inhibition of the activity of the PA-producing enzyme PLD1. I will also discuss how the phosphatidylinositol 3-phosphate (PtdIns3P)-binding protein ALFY facilitates selective degradation of protein aggregates by autophagy.

ShT.4.3.B-001
A novel phosphodegron-dependent mechanism regulates intramitochondrial selectivity in stationary phase mitophagy

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[1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*University of Fribourg, Fribourg, Switzerland*

Eukaryotic cells can accumulate mitochondrial damage due to oxidative damage and spontaneous mutation. Mitophagy, or the autophagic degradation of mitochondria, is an important housekeeping function of eukaryotic cells that prevents the accumulation of defective mitochondria. Mitophagy-dependent clearance of defective mitochondria has been suggested to delay the onset of aging symptoms, and defects in mitophagy have been linked to late onset hereditary disorders such as Parkinson's disease and type II diabetes. We previously showed that different mitochondrial matrix proteins undergo mitophagy at different rates. An attractive hypothesis, supported by the existing data, is that mitophagy is preceded by segregation of defective mitochondrial components from undamaged ones, in a 'distillation' mechanism that leads to the selective turnover of defective compartments. We now demonstrate that dynamic mitochondrial matrix protein phosphorylation and dephosphorylation generate a segregation principle that could couple with mitochondrial fission and fusion to generate such a distillation process. The data indicate that structural determinants on a mitochondrial matrix protein can determine its mitophagic fate, independent of overall mitophagic flux, and that posttranslational modifications such as phosphorylation modify the function of these determinants. The results are consistent with a model wherein differences in protein-protein interactions between differentially phosphorylated proteins of the same species can drive a microscopic phase separation which, coupled with fusion-fission dynamics, could account for the observed selectivity.

ShT.4.3.B-002
The Hedgehog receptor PTCH1 regulates autophagy through its interaction with Atg101

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The Hedgehog (Hh) proteins bind to and *inhibit* their receptor Patched1 (PTCH1), a well-established tumour suppressor. Loss-of-function mutations of PTCH1, heterozygosity, or suppression of its activity by upregulation of Hh proteins are frequent in malignancies. Although the best-known function of PTCH1 is to repress Smoothened (SMO) and prevent activation of the Gli transcription factors in the absence of Hh ligands, we and others have reported that PTCH1 has additional SMO-independent functions regulated through its C-terminal domain (CTD). We performed a yeast–two-hybrid screening of the CTD and identified a novel physical interaction with Atg101, a subunit of the Ulk complex that controls initiation of autophagy in mammals. This interaction was confirmed in normal and cancer cells (HEK 293 and HeLa) by co-immunoprecipitation. Immunoprecipitates of PTCH1 contained the entire Ulk complex, which was lost by Atg101 siRNA, suggesting that Atg101 links PTCH1 to the complex. PTCH1 expression inhibited homeostatic and nutrient starvation-induced autophagy in an Atg101-dependent manner, which was reverted upon addition of Shh. PTCH1 expression reduced the formation and/or acidification of autolysosomes, assessed with a RFP-GFP-LC3B reporter by confocal microscopy, by around 75%, supporting the notion that PTCH1 impairs the completion of autophagy. Comparing mouse embryonic fibroblasts of different genotypes as well as HEK293 and HeLa cells in the presence and absence of a SMO inhibitor, we concluded that PTCH1 reduces the autophagy flux by both SMO-dependent and SMO-independent pathways. We predict that loss of this homeostatic function of PTCH1 contributes to tumour growth and survival of Hh-dependent cancers that are refractory to SMO inhibitors currently used in the oncology clinic. Further studies of the mechanistic basis will be necessary to identify specific markers for diagnosis and generate novel therapeutic options.

Wednesday 13 September 15:00–17:00, Oranim Hall 3

The Structural Organization of the Cell

S.4.4.B-002
Protein trafficking at the crossroads to mitochondria

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We aim to understand cellular consequences of defects in the mitochondrial protein import. Two main arms of the cellular response to protein import dysfunction include the inhibition of cytosolic translation and activation of the major protein degradation machinery, the proteasome. The stimulation of the proteasome is driven by its more efficient assembly as a direct response to the amount of mistargeted proteins. To understand translational inhibition, we performed a global, quantitative, and site-specific redox proteomic analysis to delineate the yeast redoxome up to a depth of more than 4,300 unique cysteine residues in over 2,200 proteins. Increased levels of intracellular reactive oxygen species (ROS) caused by the mitochondria serve as a signal to attenuate global protein synthesis. Mapping of redox-active thiols in proteins revealed ROS-sensitive sites in several components of the translation apparatus. We demonstrate that increased levels of intracellular ROS caused by dysfunctional mitochondria serve as a signal to attenuate global protein synthesis. Hence, we propose a novel mechanism that controls protein synthesis by inducing oxidative changes in the translation machinery.

S.4.4.B-001
Unravelling the structure of toxic protein aggregates in situ

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Max Planck Institute of Biochemistry, Martinsried, Germany

Protein aggregation is a hallmark of many neurodegenerative diseases, including Huntington's, Parkinson's and amyotrophic lateral sclerosis. However, the mechanisms linking aggregation to neurotoxicity remain poorly understood, partly because only limited information is available on the native structure of protein aggregates inside cells. We are addressing this challenge utilizing the latest developments in cryo-electron tomography (cryo-ET). We prepare thin lamellas of vitrified cells containing protein aggregates using cryo-focused ion beam, and subsequently image them in three dimensions by cryo-ET. This allows us to analyse aggregate structure within pristinely preserved cellular environments and at molecular resolution. Here I will discuss how our latest results shed new light into the cellular mechanisms of neurodegeneration.

S.4.4.B-003
Peroxisomal membrane contact sites

A. Aksit, Y. W. Yuan Wei, H. Hu, A. M. Krikken, R. de Boer, **I. Van der Klei**
University of Groningen, Groningen, Netherlands

Peroxisomes are ubiquitous cell organelles that occur in almost all eukaryotic cells. It is still debated whether peroxisomes are semi-autonomous organelles or represent a branch of the endomembrane system. Also, it is not yet known whether peroxisomal membrane lipids are transported to this organelle via non-vesicular or vesicular transport. We explored the possible role of peroxisomal membrane contact sites (MCSs) in non-vesicular lipid transport. Electron microscopy studies, using the yeast *Hansenula polymorpha,* revealed that the single small peroxisome that is present in glucose-grown cells (peroxisome repressing growth conditions) is invariably associated with the endoplasmic reticulum, whereas at conditions that induce massive peroxisome proliferation (methanol) large MCSs with vacuoles are formed as well. In addition, we established that several peroxins localize to ER-peroxisome contact sites (EPCONS). However, the absence of these proteins only results in mild peroxisomal phenotypes. Interestingly, deletion of genes encoding proteins of the mitochondrial-vacuolar MCS, vCLAMP, also resulted in weak peroxisomal phenotypes. The peroxisomal phenotype was much more severe in the absence of components of both EPCONS and vCLAMP. These cells contained small peroxisomes that appeared to be unable to expand. This phenotype could be suppressed by the introduction of an artificial ER-peroxisome tethering protein, suggesting that EPCONS plays a role in growth of the peroxisomal membrane. Based on our observations, we speculate that EPCONS may play a redundant role together with vacuole-peroxisome MCS in lipid transport to the peroxisomal membrane.

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ShT.4.4.B-001
Molecular determinants of a lipid droplet subpopulation at the contact site between the nucleus and vacuole

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Lipid droplets (LDs), the lipid reservoirs of the cell, perform key functions in bioenergetic homeostasis and membrane biosynthesis. In recent years, it is becoming apparent that not all LDs are the same. Instead, different sub-populations of LDs exist within the cell that can be differentiated by their coating proteins. Little is known about the biogenesis and function of LD subpopulations. In this study we identified a specialized LD subpopulation equipped with a unique set of surface proteins that is localized in close proximity to the nucleus vacuole junction (NVJ). One protein that resides in this LD subpopulation is the phosphatidylinositol transfer protein Pdr16. We used high-throughput screening in yeast to identify factors required for correct localization of Pdr16. We discovered that Pdr16 fails to be targeted to LDs in the absence of Ldo45 (lipid droplet organization protein of 45 kDa) which is the product of a unique splicing event connecting two adjacent open reading frames (*YMR147W* and *YMR148W*). Overexpression of *LDO45* as well as its encompassed gene *LDO16* (*YMR148W*/*OSW5*) induces clustering of bulk cellular LDs at the NVJ and Ldo16 is required for physiological LD accumulation in proximity to the NVJ in response to nutrient depletion. Ldo16 and Ldo45 are linked to the conserved LD biogenesis factor seipin. We conclude that Ldo proteins determine LD identity both by mediating LD positioning in a unique cellular niche and by surface protein targeting. Our studies suggest a mechanism to establish functional diversity of organelles, opening the door to better understanding of metabolic decisions in cells.

ShT.4.4.B-002
Quantification of scattering signals in cryo-scanning transmission electron tomography

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Weizmann institute, Rehovot, Israel

Cryo-scanning transmission electron tomography (CSTET) can be used to obtain high-resolution three dimensional reconstructions of cellular ultrastructure in intact unstained vitrified cells. Compared to traditional cryo-electron tomography (CET), CSTET offers advantages in sample thickness, resistance to radiation damage and quantifiable unipolar contrast. In CSTET, a focused electron beam is scanned across the sample and scattering signals can be simultaneously acquired on separate bright-field (BF) and dark-field (DF) detectors. Images formed from these signals can be used to construct separate BF and DF tomograms. The incoherent nature of signal collections allows for the pixel intensities within tomograms to be related directly to the scattering cross-sections of specific elements, based on the defined angular collection ranges from the respective detectors. In this manner, information about the elemental composition can be extracted and related to 3-dimensional cellular ultrastructure. Quantification of the scattering can be demonstrated in model systems within cultured eukaryotic cells. Supramolecular assemblies of expressed Ferritin-YFP and the intracellular uptake of a modified rhodamine dye containing heavy metal atoms are used here as models to show elemental sensitivity in CSTET.

Thursday 14 September
9:00–11:00, Oranim Hall 1

Intrinsically Disordered Proteins

S.5.1-001
How order and disorder orchestrate the molecular ballet of transcription and replication in paramyxoviruses

S. Longhi
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In the course of the structural characterization of the nucleoprotein (N) and phosphoprotein (P) from measles, Nipah and Hendra viruses we discovered that they contain long disordered regions. The N and P proteins from these viruses thus provide an excellent model system to study the functional impact of disordered motifs. The non-segmented, single-stranded RNA genome of these paramyxoviruses is encapsidated by the nucleoprotein (N) within a helical nucleocapsid. Transcription and replication are carried out onto this ribonucleoproteic complex by the viral RNA dependent RNA polymerase that consists of a complex between the large protein (L) and the phosphoprotein (P). The P protein serves as an essential polymerase co-factor as it allows recruitment of L onto the nucleocapsid template. Tethering of L relies on the interaction between the C-terminal X domain (P_{XD}) of the P protein and the C-terminal, intrinsically disordered domain (N_{TAIL}) of N. This latter is disordered not only in isolation but also in the context of the nucleocapsid, being partly exposed at the surface of this latter. Within N_{TAIL}, a short motif, serving as molecular recognition element, has been identified and the mechanisms of its interaction with P_{XD} thoroughly investigated. Binding to P_{XD} triggers α-helical folding of this motif, while the majority of N_{TAIL} remains "fuzzy". In my talk, I will focus on measles virus and will summarize the main results we obtained so far. In particular, I will focus on the mechanistic and functional aspects of the N_{TAIL}- P_{XD} interaction and will highlight the functional implications of disorder for viral transcription and replication.

S.5.1-002
Decoding molecular plasticity in the dark proteome

E. Lemke
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The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and yet highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function, where nuclear transport receptors (NTRs) move through the NPC by binding disordered phenylalanine-glycine-rich nucleoporins (FG-Nups). I will present a combined single molecule, ensemble spectroscopy, solvation approach that paired with atomic simulations revealed that a rapidly fluctuating FG-Nup populates an ensemble of conformations that are prone to bind NTRs with diffusion-limited on-rates. This is achieved using multiple, minimalistic, low affinity binding motifs that are in rapid exchange when engaging with the NTR, allowing the FG-Nup to maintain an unexpectedly high plasticity in its bound state. Since site-specific labeling of proteins with small but highly photostable fluorescent dyes inside cells remains the major bottleneck for directly performing such high resolution

studies in the interior of the cell, I will also demonstrate an approach how to overcome this limitation. We have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell. Expressed proteins only differ from their natural counterparts by very few atoms, constituting a ring-strained cyclooctyne or cyclooctene functional group. This allowed rapid, specific "click" labeling and even multi-color studies of living cells and subsequent super resolution microscopy of the permeability barrier of the nuclear pore complex in situ.

S.5.1-003
Fuzzy assemblies: towards a stochastic structure-function relationship for proteins

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Fuzziness defines the disorder-to-disorder transitions in proteins, which provide an adaptive response to the cellular environment. Fuzzy assemblies are characterized by structural multiplicity or dynamic disorder in the bound state. This results in variations/ambiguities in the interaction pattern between the partners, which in turn impact the regulated formation and function of the assembly. Intrinsic fuzziness is exemplified in all higher-order protein organizations, such as amyloids and prions, various kinds of signalosomes, nuclear and cytoplasmic granules and is a key factor to determine the dynamics of higher-order states. Molecular mechanisms of how conformational multiplicity or disorder influences the activity of protein assemblies, have been described using the Fuzzy Complexes Database (FuzDB, http://protdyn-database.org). Based on increasing experimental evidence a fuzzy protein theory emerges that introduces a stochastic structure-function paradigm. The FuzPred algorithm has been developed to predict the dynamic state of proteins within a cellular context. Regions that fold or undergo adaptive disorder-to-disorder transitions upon partner interactions could be identified from the primary sequence. FuzPred can also locate context-dependent motifs, structural characteristics of which are modulated by post-translational modifications. We demonstrate that altering the degree of fuzziness can influence signalling pathways as well as cell-differentiation processes. All these results illustrate that the fuzzy protein theory can be employed to explain the structural basis of and modify protein functions within cellular conditions. Wu, H, Fuxreiter M (2016) The Structure and Dynamics of Higher-Order Assemblies: Amyloids, Signalosomes, and Granules. *Cell* 165, 1055–1066. Miskei, Cs. Antal, M. Fuxreiter (2017) FuzDB: database of fuzzy complexes, a tool to develop stochastic structure-function relationships for protein complexes and higher-order assemblies. Nucleic Acids Res. 45, D228–235.

ShT.5.1-001
Single-molecule spectroscopy of the Myc-Max-Mad transcription factor network

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Weizmann Institute of Science, Rehovot, Israel

Intrinsically disordered proteins (IDPs) are ubiquitously found in eukaryotic systems. Their lack of a well-defined structure suggests that their broad conformational ensemble is functionally advantageous. A particularly important system is the IDP-network formed by the basic helix-loop-helix leucine zipper (bHLH-LZ) domains of c-Myc, Max, and Mad, which are major regulators of transcriptome dynamics. Here, we use single-molecule fluorescence resonance energy transfer to investigate the link between the polymer properties of the bHLH-LZ domains of c-Myc, Max, and Mad and the process of coupled binding and folding that leads to functional complexes. In contrast to archetypal IDPs, all three proteins form densely collapsed ensembles under physiological conditions that are dominated by strong attractive electrostatic interactions as quantified using polymer theory. Importantly, the ionic strength sensitivity of the disordered ensembles has pronounced consequences for their functional interactions within the network since salt modulates the binding affinity by almost three orders of magnitude. Our results suggest that the properties of the disordered ensemble and the stability of the functional complexes are strongly correlated.

ShT.5.1-002
Structural and functional insights from the conformational ensemble of intrinsically disordered WIP

A. Halle-Bikovski, E. Rozentur-Shkop, H. Elazari-Shalom, H. Shaked, **J. H. Chill**
Bar Ilan University, Ramat Gan, Israel

The intrinsically disordered WASP-Interacting Protein (WIP) is a multifunctional key participant in mediating actin-related cytoskeletal changes in human T cells. Two critical interactions involved are with actin via a double actin-binding domain at the N-terminus and with WASP at the C-terminus. The pivotal role of the latter in the immune response is demonstrated by the fact that WASP mutants unable to bind WIP cause hereditary immunodeficiencies Wiskott-Aldrich syndrome and X-linked thrombocytopenia, and uncontrolled WASp expression is involved in hematopoietic malignancies. As an IDP, WIP is best described as an ensemble of multiple conformations contributing to its transient structural properties as well as its function. Using solution nuclear magnetic resonance (NMR) we demonstrate the ability to follow subtle ensemble shifts induced by environmental factors, such as temperature, denaturant or crowding agents, providing new insights into the biophysics governing the behavior of these proteins in the cellular environment. We also investigated the larger conformational changes effected by WIP binding partners actin and WASP, and determined the structure of the WIP-WASP complex. We established that transient structural propensities in both WIP interaction domains echo their bound conformations, suggesting a conformational selection mechanism for binding. In addition, we determined the contribution of various WIP epitopes to complex affinity, gaining insight into how WIP protects WASP from degradation. Taken together our results provide a comprehensive map of WIP structure and dynamics and how these affect its interaction with T cell binding partners, and highlight the potential impact of high-resolution NMR upon the field of biologically active unstructured proteins.

Thursday 14 September
9:00–11:00, Oranim Hall 3

Medicinal Chemistry

S.5.2-002
Druggability of lectins – mission possible?

B. Ernst, D. Eris, S. Rabbani, P. Zihlmann, P. Dätwyler, X. Jiang, O. Schwardt
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Urinary tract infections (UTI) belong to the most common infections worldwide and are predominantly caused by uropathogenic

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E. coli (UPEC). UPEC initiate the infection cycle by adhering to high-mannose glycoproteins on urothelial cells through their lectin FimH. Preventing the initial adhesion by blocking FimH offers a promising alternative to the conventional antibiotic treatment, which has become increasingly inefficacious due to antibiotic resistance. The FimH-mannose interaction is shear stress dependent, meaning that FimH mediates weaker binding at low shear stress and stronger binding at high shear stress. This type of bond is called catch-bond and results from the ability of FimH to adopt two distinct bound conformations, a medium- and a high-affinity conformation. In the urinary tract, the shear stress-induced switch from medium- to the high-affinity becomes an effective tool for UPEC to evade clearance by the bulk flow of urine. So far, efforts in developing FimH antagonists have been predominantly focused on the high-affinity conformation. Clinical FimH variants, however, are typically characterized by a catch-bond behavior. By studying bacterial binding in a cell motility assay, we were able to show that these variants were highly mobile on a mannosylated surface, allowing optimal colonization in the bladder. However, by switching to the high-affinity conformation, they can resist upcoming shear stress and are thus protected from being washed out through urination. In contrast, a variant locked in the high-affinity state, which is often used for *in vitro* and *in vivo* studies, formed long-lived interactions with mannose also in the absence of shear stress, rendering bacteria immobile. It becomes evident that dynamic FimH variants are pathophysiologically favored and represent the dominant therapeutic target. To further elucidate this conformational issue, antagonists with high affinities to both affinity states were synthesized and broadly characterized.

S.5.2-001
Synthetic lethality triggered by combining olaparib with BRCA2/Rad51 disruptors

A. Cavalli
Italian Institute of Technology, Genova, Italy

Olaparib is the first PARP inhibitor approved by FDA in 2014 for BRCA mutation-positive ovarian cancer, and represents a successful example of the concept of synthetic lethality applied to the development of innovative anticancer agents.[1–3] Despite the undeniable benefit in BRCAness tumors, PARP inhibitors suffer from lack of efficacy in tumors with active homologous recombination (HR). Rad51 is a key recombinase of HR process. Rad51 repairs DNA double-strand breaks (DSBs), and it is recruited to DSBs site through interaction with tumor suppressor protein BRCA2.[4] Disrupting the protein-protein interaction between Rad51 and BRCA2 could have a major impact on the survival of a cancer cell. In the present talk, we discuss the possibility to achieve *fully-small-molecule-induced synthetic lethality* by combining Olaparib with a BRCA2-Rad51 disruptor identified through a lead discovery campaign. We investigated whether the new molecule could block the repair of DNA damage caused by Cisplatin in BxPC3 pancreas adenocarcinoma cell lines. Further tests were then undertaken to demonstrate the ability of the new compound to increase the response to Olaparib in cells expressing a functional BRCA2/Rad51 signaling pathway (BxPC-3), while no synergic effects were observed in Capan-1, pancreas adenocarcinoma cells lacking a functional BRCA2 protein.

S.5.2-003
Structural, thermodynamic and kinetic aspects of molecular recognition in protein-ligand complexes in Medicinal Chemistry

G. Klebe
Philipps Univ. Inst Pharm. Chemistry, Marburg, Germany

Small-molecule drug discovery involves the optimization of various physicochemical properties of a ligand, particularly the binding affinity for its target receptor(s). In recent years, there has been growing interest in using thermodynamic profiling of ligand–receptor interactions in order to select and optimize those ligands that look most promising to become a drug candidate with desirable physicochemical properties. The thermodynamics of binding are influenced by multiple factors, including hydrogen bonding and hydrophobic interactions, desolvation, residual mobility, dynamics and the local water structure. How well do we understand these properties on the molecular level? Is it only important that a sufficient number of hydrogen bonds are formed and the shape of the molecules fit perfectly together? How much contributes the burial of hydrophobic surface portion and how important are changes of the degrees of freedom upon complex formation? Both binding partners are conformationally flexible species and will adapt to one another upon complex formation. However, who is going to pay for these adaptations? All biological processes occur in water, thus the ubiquitously present water molecules take an important impact on protein-ligand binding, and even rearrangements of water molecules on the surface of a formed protein-ligand complex modulate the affinity and the binding kinetics of complex formation. By closely linking the results of high resolution X-ray and neutron diffraction, microcalorimetry, binding kinetics and computer simulations we want to characterize the determining influence of water on the efficacy of ligand binding.

ShT.5.2-001
The novel pyrimidine-based total inhibitors of the monocyte rolling

T. Getter[1], P. Bradfield[2], A. V. Kumar[3], S. Kahremany[1], L. Levy[1], E. Blum[1], H. Senderowitz[1], T. Matthes[3], B. Imhof[2], **A. Gruzman**[1]
[1]Bar-Ilan University, Ramat-Gan, Israel, [2]Department of Pathology and Immunology, Geneva University, Geneva, Switzerland, [3]Hematology Service, Geneva University Hospital, Geneva, Switzerland

Monocyte rolling and recruiting is one of the most important events in physiological tissue immune response. However, overactivation of the immune system might lead to damage of healthy tissues. Thus, the effective monocyte migration inhibitors considered as a very promising potential therapeutic agents against inflammatory and autoimmune diseases. In addition, inhibition of the homing of B-lymphocytes to lymphoid organs might be recruiting as a new therapeutic strategy reducing lymphoma B-cell proliferation and their capacity to reach supportive lymphoid microenvironments. Junctional adhesion molecules (JAM) that belong to the immunoglobulin superfamily, localize in inter endothelial surface and regulate monocytes transmigration. Based on a pharmacophore model derived from the JAM-C and integrins' interaction sites, new molecules with modified pyrimidine scaffold were designed in silico and synthetized. Human endothelial cells and monocytes were used for in vitro evaluations of the effect of test compounds on the monocyte rolling. Three compounds were active in nM concertation range. It is Important to mention, that one of the compounds (GT-73) completely blocked

monocytes rolling, without damaging monocytes or endothelial cells. So far, even pan antibody blockers of the beta-1 and -2 integrins were not able to block monocyte transmigration. GT-73 was also active (85% reduction of lymphocytes in the peritoneal liquid) in animal inflammatory model. Moreover, GT-73 also decreased the amount of activated macrophages. A possible effect on the rolling of lymphocytes was tested in the model of B-lymphoma. Homing assay was used. GT-73 was injected together with human B-lymphoma cells to the peritoneum of NOD mice. GT-73 significantly reduced the amount of cancer cells in spleen and liver. Based on the observed results, GT-73 might represent a novel class of monocyte rolling blocking agents with broad therapeutic potential against autoimmune diseases and B-lymphomas.

ShT.5.2-002
Magic bullets to fight resistance: uncovering how peptide-antibiotics break down the bacterial cell envelope

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[1]NMR Spectroscopy Group, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, Netherlands, [2]Membrane Biochemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, Netherlands

The rapid rise of resistant bacteria urgently calls for novel antibiotics that are robust to resistance development. Ideal templates could be peptide-antibiotics that destroy the bacterial cell wall by binding to its membrane-anchored precursor lipid II at irreplaceable phosphate groups. Indeed, these drugs can kill the most refractory bacteria without detectable resistance. However, due to the challenge of studying antibiotic-receptor complexes in membranes, structural information on these highly promising drugs is extremely small and physiological binding modes could never be visualized. This lack of knowledge critically limits the development and application of lipid II attacking drugs. Here we present extensive high-resolution data of lipid II peptide-antibiotics in physiological conditions. These studies were enabled by the use of cutting-edge ^{1}H-detected solid-state NMR experiments acquired at 950 MHz and very fast sample spinning. We first show comphrehensive structural data of the nisin-lipid II pore, acquired in liposomes and directly in native bacterial cell membranes. Nisin is the most prominent lipid II binding peptide and kills bacteria by a unique mechanism called targeted pore formation. Despite of extensive research, structural models of the nisin-lipid II pore have been lacking hithero. Furthermore, we present progress towards a solid-state NMR structure of the plectasin-lipid II complex. Plectasin is a defensin with strong potential to kill multi-resistant superbugs such as MRSA. Our data, acquired in native-like membranes, reveal the drug-binding interface and show that plectasin exhibits several binding modes in physiological conditions. Moreover, we analyze the dynamics of bound and unbound plectasin, which is of great value to optimize antibiotic binding. We expect our data to critically improve our understanding of lipid II binding peptides, which is an important step towards the medical use of these powerful antibiotics.

Thursday 14 September
9:00–11:00, Oranim Hall 2

The Human Microbiome

S.5.3-003
Antibiotics in early life: dysbiosis and the damage done

O. Koren
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Antibiotics are the most common type of medication prescribed to children, including infants, in the Western world. While use of antibiotics has transformed previously lethal infections into relatively minor diseases, overuse and misuse of antibiotic treatments can have negative consequences, as well. It has been shown in adults, children and animal models that antibiotics cause dysbiosis in the healthy gut microbiota, altering the microbial populations residing within the gut. Since the gut microbiota has crucial roles in immunity, metabolism, and endocrinology, the effects of antibiotics on the microbiota may lead to further complications. The talk will summarize our latest findings regarding the short and long term effects of antibiotic treatment during pregnancy and infancy on the microbiome in children and the influence on behavior.

S.5.3-001
Dysbiosis – a cause or consequence of preterm delivery

H. Kumar[1], A. Endo[2], S. Rautava[1], R. Luoto[1], P. P. Kantola[1], T. Ishige[3], E. Isolauri[1], S. Salminen[1]
[1]Turku University, Turku, Finland, [2]Tokyo University of Agriculture, Hokkaido, Japan, Hokaido, Japan, [3]NODAI Genome Research Center, Tokyo University of Agriculture, Tokyo, Japan, Tokyo, Japan

Preterm delivery is one of the important causes of fetal health problems worldwide. The mechanisms for spontaneous preterm are not fully understood and intrauterine infection has been suggested to account for 25–40% of preterm births. In our earlier studies we have shown that gut microbiota shifts towards more pro-inflammatory type at third trimester of pregnancy in healthy women. This study was designed to determine whether maternal gut microbiota during pregnancy is associated with risk of spontaneous preterm delivery. Pregnant women who showed symptoms of preterm delivery (premature contractions, cervical changes or premature rupture of membranes) and before pregnancy weeks 35 were included. The stool sample was obtained at the time when the first signs of possible preterm delivery occurred. The gut microbiota was assessed by 16S rRNA gene sequencing using Illumina MiSeq. The sequence analysis was carried out using QIIME using standard parameters. Sequencing results revealed significant differences in microbial phylotypes among women who delivered preterm (PT, n = 21) compared to women who delivered full term (FT, n = 15). Proteobacteria was found to be significantly higher in PT group compared to FT group. Interestingly, subgrouping preterm women in very preterm (n = 8, less than 32 weeks) and pre-term (n = 13, 32–36 weeks) showed that Proteobacteria was also higher in the women delivering very preterm. On the other hand, Actinobacteria was higher in FT group when compared to PT group. These results suggest that the pregnant women who delivered preterm have more inflammatory microbiota which could have potential role in induction of preterm labour. Given the fact that the gut microbiota becomes aberrant during the course of pregnancy, it

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is important to note that more dysbiotic/inflammatory microbiota may act as a signature for preterm delivery.

S.5.3-002
Perinatal microbiota modification- long-term safety and efficacy

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University of Turku, Turku, Finland

A growing number of clinical conditions, ranging from allergic diseases to obesity, are linked to aberrant gut microbiota. The compositional development of the child′s gut microbiota, again, is initiated during the perinatal period and early infancy, when the regulatory systems are sensitive to the environmental exposures. Preventing dysbiosis and promoting age-appropriate intestinal microecosystem by probiotics or prebiotics may be taken as a target to attain prophylactic or therapeutic effects in non-communicable diseases. Recent evidence from experimental studies suggest that gut microbiota is also involved in the control of body weight and energy metabolism, affecting the key causes of obesity: energy acquisition and storage and the chronic low-grade inflammatory state. Also clinical evidence has accumulated that a microbiota profile in favour of a higher number of bifidobacteria in infancy provides protection against allergic disease and obesity development later in life. A series of clinical intervention studies by the NAMI (Neonatal exposures, Adverse outcomes, Mucosal immunology and Intestinal microbiota) research group included a long-term follow-up, to assess the safety and efficacy of perinatal probiotic supplementation in infants with high risk of abnormal early microbe contact. The safety of the probiotic approach during pregnancy was attested by normal duration of pregnancies with no adverse events in mothers or children, and no significant deviations in prenatal or postnatal growth rates. In children, perinatal intervention with specific probiotic strains was well tolerated and succeeded in reducing the risk of atopic eczema and early excessive weight gain. To judge from these pioneer studies, pre-, peri- and postnatal probiotic intervention may have a long-term programming effect on child health.

ShT.5.3-001
Using imaging mass spectrometry to study bacterial sociality: the role of secondary metabolites in spatial community structures

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Quorum sensing (QS) describes the ability of a population of unicellular bacteria to synchronize their gene expression in a cell-density-dependent manner, through the secretion and recognition of small diffusible molecular signals. QS relies on the increase in physical proximity of bacterial cells to one another and the resulting local increase in concentration of signaling molecules, and therefore is especially relevant to understanding bacterial sociality and the emergence of spatial structure in mixed bacterial communities. Such structure has variously been linked to genetic drift, growth rates, nutrient limitation, and other phenomena. We are interested in the role of secondary metabolites, especially siderophores, signaling molecules, and other 'common goods', in governing the organization and interactions within a community. Using *Pseudomonas aeruginosa*, an opportunistic human pathogen, we study the development of spatial structure and metabolite distribution in mixed communities of wild-type bacteria together with strains impaired in the production of various common goods due to knockouts of relevant QS signaling pathways . Such mutants are dependent on their neighbors in stress conditions in which these secondary metabolites become critical for survival. To this end, we use novel imaging mass spectrometry (IMS) techniques, specifically matrix-assisted laser desorption/ionization (MALDI) imaging spectrometry. MALDI-IMS has been utilized to provide a macro-scale view of bacterial information flow by directly imaging metabolite secretion from colonies grown on agar, resulting in heat maps of metabolite distribution over a wide range of masses. We are currently using this technique to track the production and diffusion of various relevant secondary metabolites between the wild-type and mutant strains, and to address the question of how such metabolite flow can shape the spatial structure of a mixed community in a complex environment such as the human body.

Thursday 14 September
9:00–11:00, Teddy Hall

Metabolism and Signaling

S.5.4-001
Fructose-1,6-bisphosphate activation of Ras and nutrient transceptor – eIF2B/eIF2 interaction as novel mechanisms in yeast nutrient signaling

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The Ras-cAMP-PKA pathway in yeast mediates nutrient regulation of physiology, growth and development. Glucose addition to deprived cells causes a rapid spike in cAMP, which triggers activation of PKA. The cAMP spike is induced by combined action of the glucose-sensing GPCR, Gpr1, and activation of Ras by glycolytic breakdown of glucose, the mechanism of which has remained unclear. Addition of another essential nutrient, like nitrogen, phosphate, sulfate or a metal ion, to growth-arrested cells deprived for such nutrient, triggers rapid activation of the PKA pathway concomitant with the upstart of growth. This activation is not mediated by an increase in the cAMP level, and although ample evidence has been obtained that starvation-induced high-affinity transporters function as transporter-receptors in this nutrient activation process, the underlying molecular mechanism has remained unclear. We show that glycolytic activation of Ras is mediated by fructose-1,6-bisphosphate, acting as metabolic messenger for coupling glycolytic flux to PKA. It is likely mediated by a conserved domain in yeast Cdc25 and Sos, its mammalian homolog. Glucose addition to mammalian cells also triggers rapid activation of Ras. Investigation of a possible role of protein synthesis initiation in PKA activation during nutrient-induced upstart of growth revealed that the nutrient transceptors, Gap1, Mep2 and Pho84, physically bind in vitro to eIF2B and eIF2 subunits. Bimolecular fluorescence complementation experiments with half-citrin tagged constructs confirm the interaction in vivo, which apparently occurs at multiple membranes suggesting the new concept of 'startosome'. Nutrient transceptor-eIF2B/eIF2 interaction allows to design the first molecular model explaining how cells can detect with a common mechanism the absence of any single essential nutrient among the presence of all other essential nutrients, so as to downregulate protein synthesis and arrest cell cycle progression.

S.5.4-003

Withdrawn

S.5.4-003
Growth sensors in the Start network to set cell size

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Coordination of cell growth and DNA replication ensures size adaptation and homeostasis. Cells are able to adapt their size to growth rate both at population and single-cell levels, which suggests that growth is intimately linked to the molecular mechanisms that govern the cell cycle. Budding yeast cells, as most eukaryotic cells, exert this coordination essentially during G1, where a critical size must be attained before cells trigger Start. The most upstream activator of cell cycle entry in budding yeast is Cln3, a cyclin that critically depends on molecular chaperones to accumulate in the nucleus in late G1. On the other hand, chaperones are massively involved in key growth processes, and we have investigated the possibility that coordination between proliferation and growth relies on the competition for limiting stocks of shared chaperones. As deduced from mathematical modelling, a molecular competition device would be able to (1) accumulate Cln3 in the nucleus in a growth-rate dependent manner during G1, and (2) trigger Start at a cell size that is proportional to growth rate. We have used different experimental approaches to test predictions of the model, and found that availability of chaperones is negatively correlated with growth rate. More important, chaperone availability increases during G1 as cells grow, thus emerging as a key player for adjusting the critical size to the individual growth potential.

S.5.4-002
A CDK-independent metabolic oscillator orchestrates the budding yeast cell cycle

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Eukaryotic cell division is known to be controlled by the cyclin/CDK machinery. However, eukaryotes have evolved prior to CDKs, and cells can divide in the absence of major cyclin/CDK components. We hypothesized that an autonomous metabolic oscillator provides dynamic triggers for cell cycle initiation and progression. Using microfluidics, cell cycle reporters, and single-cell metabolite measurements, we found that metabolism of budding yeast is a CDK-independent oscillator that oscillates across different growth conditions, both in synchrony with and also in the absence of the cell cycle. Using environmental perturbations and dynamic single-protein depletion experiments, we found that the metabolic oscillator and the cell cycle form a system of coupled oscillators, with the metabolic oscillator separately coordinating both the early and late cell cycle. Establishing metabolism as a dynamic cell cycle regulator opens new avenues for cell cycle research and therapeutic interventions for proliferative disorders.

ShT.5.4-001
TORC1 organised in inhibited domains (TOROIDs) regulate TORC1 activity

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The Target of Rapamycin complex 1 (TORC1) protein kinase is a master regulator of eukaryotic growth and metabolism and its dysregulation in humans has been implicated in cancer and metabolic syndrome. GTPases, responding to signals generated by abiotic stressors, nutrients, and, in metazoans, growth factors, play an important, but poorly understood role in TORC1 regulation. We found out that, in budding yeast, glucose withdrawal, which leads to an acute loss of TORC1 kinase activity, triggers a similarly rapid Rag GTPase-dependent reorganisation of TORC1 in the cell. We could identify, both by super-resolution optical microscopy and 3D reconstructions of cryo-electron micrograph (cryo-EM) images, that inhibited TORC1 molecules organise in a novel structure which we name a TOROID (TORC1 Organised in Inhibited Domain). Guided by this new structure, we could describe the first molecular mechanism of TORC1 inhibition in yeast.

ShT.5.4-002
17ß-estradiol reduces mitochondrial cAMP content and cytochrome oxidase activity in a phosphodiesterase 2 dependent manner

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Objectives: Energy metabolism and mitochondrial adaptation is regulated differently in pressure overloaded male and female hearts. Estrogen plays an essential role in regulation of mitochondrial function and may contribute to these sex differences. Estrogen regulates cytosolic cAMP content. We therefore aimed to test whether 17ß-estradiol (E2) may affect mitochondrial function via mitochondrial cAMP signaling, that may activate protein kinase A (PKA) dependent phosphorylation of several subunits of the complexes I and IV of the mitochondrial electron transport chain and thereby control mitochondrial function. Methods: Expression of main mitochondrial matrix proteins involved in the cAMP signaling (western blot), mitochondrial cAMP content and activity of mitochondrial soluble adenylyl cyclase (FRET-based live imaging, mass spectrometry), cytochrome c oxidase (COX) activity (photometry) were analysed in H9C2 and C2C12 cells with and without estrogen treatment. Results: Treatment with E2 or PPT (specific estrogen receptor alpha agonist) significantly reduced the basal level of mitochondrial cAMP. Inhibition of PDE2 blocked this effect, suggesting that it occurred in a phosphodiesterase 2 dependent manner. Since PDE2 is cGMP-activated PDE, we tested the potential role of nitric oxide (NO)-signaling in the E2 effects.

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Treatment of cells with E2 or NO donor increased cellular cGMP level and reproduced effects of E2 on mitochondrial cAMP. Furthermore, the expression of the catalytic subunit ß of soluble guanylyl cyclase (sGC) was shown in mitochondrial matrix. The E2-induced suppression of mitochondrial cAMP level in H9C2 cells was accompanied by reduction of COX activity, which was prevented by PDE2 inhibition. Conclusion: In cardiac and muscle cells, E2 reduced the basal level of mitochondrial cAMP content and COX activity in a phosphodiesterase 2 dependent manner. The role of E2-induced NO-signaling activation in regulation of mitochondrial cAMP content is suggested.

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