2019 LOUIS-JEANTET SYMPOSIUM

15 October 2019
Centre Medical Universitaire (CMU), Geneva
Auditorium 250

Jonathan Weissman

Howard Hughes Medical Institute, San Francisco, USA

Monitoring protein synthesis in space and time with ribosome profiling

The translation of mRNA into protein and the folding of the resulting protein into an active form are prerequisites for virtually every cellular process and represent the single largest investment of energy by cells. These are also the most likely steps to fail. We are broadly interested in how cells ensure the integrity of protein production, and cotranslational targeting and folding. We have a particular focus on the following questions: How do cells maximize the efficient production of proteins by ensuring proper trafficking of ribosomes on mRNAs? How do cells dispose of failed translation products? How do cells ensure that the appropriate amount of each protein is produced in the right place and time?
A key tool we developed to address these questions is ribosome profiling which has transformed our ability to globally monitor protein synthesis in vivo. I will discuss our recent applications of ribosome profiling including: the identification of novel protein coding regions, monitoring localized protein translation, and the discovery and characterization of the ER Membrane Complex (EMC).
I will also present our work on the ribosome quality control (RQC) complex which is responsible for degrading nascent chains from failed translation reactions. This will include our discovery of a remarkable mechanism for tagging such nascent chains with carboxy-terminal alanine and threonine extensions (CAT tails) through a noncanonical translation reaction as well as our very recent novel discovery of a novel branch of the RQC that translationally silences faulty mRNAs by blocking ribosome initiation.

Biography

Jonathan Weissman, Ph.D., studies how cells ensure proteins fold properly, as well as the role of protein misfolding in disease and normal physiology. He is also widely recognized for building innovative tools for broadly exploring organizational principles of biological systems. These include ribosome profiling, which globally monitors protein translation, and CRIPSRi/a for controlling the expression of human genes.
Jonathan Weissman is a Howard Hughes Medical Institute Investigator and Professor of Cellular and Molecular Pharmacology at UCSF. He is a member of the National Academy of Sciences and has received numerous awards including the 2015 NAS Award for Scientific Discovery.

https://weissmanlab.ucsf.edu/

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Ramanujan S. Hegde

MRC Laboratory of Molecular Biology, Cambridge, UK

Mechanisms of Quality and Quantity Control during mRNA Translation

A major goal of my group's research efforts is to understand how cells identify failures during protein maturation and dispose of aberrant products to maintain cellular homeostasis. Failures can occur at any step on the pathway to a functional protein, including translation, folding, localization, modifications, and assembly. This talk will focus on the mechanisms cell use to monitor the translation of mRNAs by ribosomes and make crucial decisions about whether to degrade the mRNA and nascent protein. During quality control, cells have mechanisms to selectively detect when mRNAs are damaged or incorrectly processed. This is accomplished by recognizing stalled ribosomes, an indicator of a defective mRNA, and recruiting factors that recycle some components and degrade others. During quantity control, cells respond to an excess of particularly critical proteins such as tubulins and histones by selectively targeting their respective mRNAs for degradation. This is accomplished by poorly understood mechanisms by which excess proteins communicate privately to the ribosomes synthesizing more copies of that protein and degrade the associated mRNA. I will discuss our ongoing efforts to delineate the molecular machinery and mechanisms underlying quality and quantity control occurring on translating ribosomes.

Biography

Ramanujan Hegde earned his MD and PhD from UCSF, then established his laboratory at the US National Institutes of Health. After eleven years at the NIH, he moved to the MRC Laboratory of Molecular Biology in Cambridge, where he is currently a Programme Leader. The Hegde lab investigates the mechanistic basis of protein biosynthesis and how cells deal with inevitable inefficiencies and errors in these protein maturation pathways. Hegde's research has been recognized by several prizes and by his election as a member of EMBO and as a Fellow of the Royal Society.

https://www2.mrc-lmb.cam.ac.uk/groups/hegde/

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Oliver Mühlemann

University of Bern, Switzerland.

Exploring the link between translation termination and nonsense-mediated mRNA decay

Nonsense-mediated mRNA decay (NMD) is best known for its role in cellular mRNA quality control by recognizing and degrading aberrant mRNAs with prematurely truncated reading frames. However, many physiological mRNAs have meanwhile been found to be targeted by NMD, strongly suggesting that NMD represents yet another mechanism contributing to post-transcriptional gene regulation. NMD is essential in vertebrates and appears to be mechanistically linked with translation termination. To gain more insight into the connection between NMD and translation termination, we conducted ribosome profiling in cells depleted of the core NMD factor UPF1 or the ribosome recycling factor ABCE1. While UPF1 knockdown did not cause any significant changes in the ribosome profiles neither on NMD sensitive nor on NMD insensitive transcripts, ABCE1 knockdown unexpectedly resulted in an upregulation of many but not all endogenous NMD-sensitive mRNAs. Notably, the suppression of NMD on these mRNAs occurs at a step prior to their SMG6-mediated endonucleolytic cleavage. Ribosome profiling revealed that ABCE1 depletion results in ribosome stalling at stop codons and increased ribosome occupancy in 3' UTRs, indicative of enhanced stop codon readthrough or re-initiation. Using reporter genes, we further demonstrate that the absence of ABCE1 indeed increases the rate of readthrough, which would explain the observed NMD inhibition, since enhanced readthrough has been previously shown to render NMD-sensitive transcripts resistant to NMD by displacing NMD triggering factors like UPF1 and exon junction complexes (EJCs) from the 3' UTR. Collectively, our datashow that improper ribosome disassembly interferes with proper NMD activation.

Biography

Oliver Mühlemann is professor of biochemistry at the University of Bern and the director of the NCCR RNA & Disease. After studies in biology at the University of Bern, Mühlemann conducted his PhD research at the Karolinska Institute in Stockholm and at Uppsala University. Following postdoctoral work with Melissa Moore at Brandeis University in Boston, U.S.A., he returned to the University of Bern as a junior group leader in 2000. He was awarded an ERC starting grant in 2007 and appointed as full professor in 2010. Since 2018, he is also a member of the SNF research council.

http://muehlemann.dcb.unibe.ch/

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Narry Kim

Center for RNA Research, Institute for Basic Science, Seoul 151-742, Republic of Korea.
School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea.

Mixed tailing in viral infection

TENT4 enzymes generate “mixed tails” on mRNAs by adding adenosines with intermittent non-adenosine residues, which protect mRNAs from deadenylation. Here we discover the extensive mixed tailing in the transcripts of two distinct DNA viruses, hepatitis B virus (HBV) and human cytomegalovirus (HCMV), and their striking similarity in the mechanism to exploit the TENT4 complex. TAIL-seq analyses of HBV and HCMV RNAs revealed that TENT4A and TENT4B are responsible for the mixed tailing and protection of viral poly(A) tails. By fCLIP-seq on HBV-producing cells, we found that the HBV posttranscriptional regulatory element (PRE) is the primary site of the TENT4 interactome. Unexpectedly, HCMV also utilizes a similar element, an interesting example of convergent evolution. We further discovered that the RNA is recognized by the ZCCHC14 protein which recruits TENT4 to stabilize viral transcripts. Altogether, our study reveals the action mechanism of PRE that has been widely used to enhance gene expression and introduces the TENT4-ZCCHC14 complex as the key host factor and a potential target for antiviral therapeutics.

Biography

Narry Kim currently serves as the Director of RNA Research Center at Institute for Basic Science and a Professor of Biological Sciences at Seoul National University. She received her Ph.D. in 1998 from Oxford University, UK, where she studied the functions of retroviral proteins in the construction of gene transfer vectors in the Kingsman lab. She then carried out her postdoctoral research on mRNA surveillance in the laboratory of Gideon Dreyfuss at the University of Pennsylvania, USA. She set up her own research group at Seoul National University in 2001. Narry Kim received L’Oreal-UNESCO Women in Science Award (2008), the Ho-Am Prize in Medicine (2009), the S-Oil Fellowship (2013), the Korea S&T Award (2013), Chen Award (2017) and Asan Awards in Medicine (2019), and was elected as Foreign Associate of European Molecular Biology Organization (EMBO, 2013), Foreign Associate of National Academy of Science (NAS, 2014), and Member of Korean Academy of Science and Technology (KAST, 2014).

http://www.narrykim.org/en/

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Stefan Ameres

IMBA - Institute of Molecular Biotechnology, Vienna, Austria

Time-resolved small RNA sequencing unravels the molecular principles of microRNA homeostasis

Argonaute-bound microRNAs silence mRNA expression in a dynamic and regulated manner to control organismal development, physiology and disease. We employed metabolic small RNA sequencing for a comprehensive view on intracellular microRNA kinetics in Drosophila. Based on absolute biogenesis- and decay-rates, microRNAs rank among the fastest produced and longest-lived cellular transcripts, disposing up to 105 copies per cell at steady-state. Mature microRNAs are produced within minutes, revealing tight intracellular coupling of biogenesis that is selectively disrupted by pre-miRNA-uridylation. Control over Argonaute protein homeostasis generates a kinetic bottleneck that cooperates with non-coding RNA surveillance to ensure faithful microRNA loading. Finally, regulated small RNA decay enables the selective rapid turnover of Ago1-bound microRNAs but not of Ago2-bound siRNAs, reflecting key differences in the robustness of small RNA silencing pathways. Time-resolved small RNA sequencing opens new experimental avenues to deconvolute the timescales, molecular features, and regulation of small RNA silencing pathways in living cells.

Biography

Stefan L. Ameres earned his Master's degree in Biology at the Friedrich-Alexander University Erlangen-Nuremberg (Germany), and his PhD degree at the University of Vienna (Austria). In his PhD thesis, he reported the first in-depth enzymatic characterization of the human RNA interference effector complex. During his postdoctoral studies at UMass Medical School (USA), he discovered a novel pathway for the sequence-specific destruction of microRNAs in flies and mammals, establishing a potential therapeutic approach for microRNA-related diseases. In 2012 he joined IMBA (Vienna, Austria) as a group leader, where his lab developed innovative transcriptomics approaches (i.e. SLAMseq or Mime-seq) to study fundamental biological mechanisms of gene regulation.

https://www.imba.oeaw.ac.at/research/stefan-ameres/

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Elena Conti

Max Planck Institute of Biochemistry, Martinsried, Germany

The exosome – ribosome connection: coupling the RNA degradation and translation machineries

To date, biochemical and structural studies on the macromolecular complexes that synthesize or degrade eukaryotic RNAs and proteins have investigated these machines individually to understand how they execute different steps in the gene expression process. Although the individual complexes catalyze their reactions independently of each other in vitro, increasing evidence suggests that they function in a highly coordinated manner in vivo. The molecular basis for how these macromolecular machines are linked and coordinated is largely unknown.
Over the years, we have used biochemical and structural approaches to understand the molecular mechanisms of the RNA exosome, a conserved RNA-degrading machine that mediates the processing and decay of a wide variety of transcripts. The exosome has constructive functions (e.g. in the maturation of structured RNAs in the nucleus) but mostly destructive functions (e.g. in the degradation of nuclear and cytoplasmic RNAs). We are studying how the cytoplasmic exosome is structured and functions. After working on yeast as a model organism, we have now characterized the human complex and its cytoplasmic cofactors, particularly in the context of cytoplasmic ribosomes. Overall, the results highlight how the RNA exosome cofactors and ribosome may work together in a transient fashion as a single structural and functional unit.

Biography

Elena Conti was born in 1967 in Varese, Italy. She studied chemistry at the University of Pavia and in 1996 received her PhD from the Faculty of Physical Sciences at the Imperial College of Science, Technology and Medicine in London (UK). Conti carried out her post-doctoral work at the Rockefeller University in New York (USA). She returned to Europe in 1999 to set up her own research group at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany). In 2006, she was appointed Director and Scientific Member at the Max Planck Institute of Biochemistry in Munich (Germany), where she heads the Department for Structural Cell Biology.

https://www.biochem.mpg.de/en/rd/conti

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Michaela Frye

Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany

RNA methylation in the regulation of gene expression

Many of the hundreds of known chemical modifications in RNA were discovered over forty years ago but then forgotten because suitable, sensitive tools to detect the modifications at high resolution were lacking. Through the development of novel biochemical, functional and genomics tools we are only now beginning to understand the whole breadth and extensive functional roles of RNA modifications in higher organisms. I will present some mechanistic examples how RNA modifications help to shape normal tissue homeostasis, and how aberrant formation of RNA modifications contributes to human diseases. I will focus on the functional roles of modification in transfer RNA (tRNA) in regulating gene expression and present and discuss novel and emerging molecular functions of RNA modifications in non-coding RNAs. Together, our work demonstrates that by understanding the role of RNA modifications in physiology and pathology, novel and powerful therapeutic drug targets for human diseases and can potentially be identified and further optimized for clinical studies

Biography

Michaela Frye completed her PhD in Frankfurt/Main in Germany in 2000 studying the role of epithelial defensins in Cystic Fibrosis. In 2001, she joined Cancer Research UK (CR-UK) in London as a Postdoctoral Fellow, where she developed her fascination for the question how stem cells form and maintain adult tissues. In 2007, Michaela started her independent research group at the Wellcome Trust – Medical Research Council Cambridge Stem Cell Institute. She received a CR-UK Career Development Fellowship in 2007 and a CR-UK Senior Fellowship and an ERC Consolidator Grant in 2013 to study how dysregulation of stem cell function contributes to human diseases and cancer. In 2019, she accepted a Professorship at the DKFZ in Heidelberg Germany.

https://www.dkfz.de/en/regulatorische-mechanismen-genexpression/index.php

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Danny D. Nedialkova

Max Planck Institute for Biochemistry, Martinsried, Germany

A need for speed: mechanisms coordinating mRNA translation and protein folding

Proteins begin to fold during mRNA translation, and the rate at which ribosomes elongate a nascent chain is a key determinant of successful structure acquisition. Elongation rates along mRNAs are not uniform and are controlled by diverse factors, chief among which is the balance between codon usage and tRNA supply. Accordingly, synonymous mutations or tRNA defects that alter decoding rates can both trigger protein misfolding. To understand the rules behind this dimension of the protein folding code, we study how the codon-tRNA balance promotes proteome integrity in different cellular contexts. Quantitation of cellular tRNA pools is a long-standing challenge, because the substantial sequence similarity among tRNAs and their abundant chemical modifications make them refractory to standard high-throughput approaches. We have developed a workflow that overcomes these hurdles to accurately capture the abundance and key functional features of eukaryotic tRNAs, shedding light on previously intractable aspects of their biology.

Biography

Danny Nedialkova studied biotechnology at the University of Perugia (Italy) and received a PhD in molecular virology from Leiden University (The Netherlands) in 2010. She then joined the MPI for Molecular Biomedicine (Münster, Germany) as an EMBO postdoctoral fellow in the group of Dr. Sebastian Leidel. In 2017, she took up a position as a Max Planck Research Group Leader at the MPI of Biochemistry (Martinsried, Germany). She holds a joint appointment as an assistant professor at the Department of Chemistry in the Technical University of Munich.

https://www.biochem.mpg.de/nedialkova

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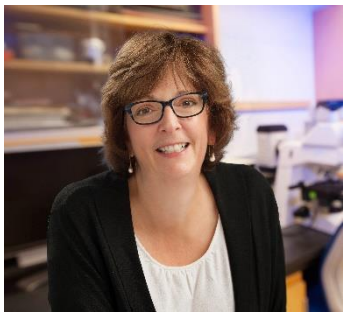

Susan Ackerman

Howard Hughes Medical Institute University of California San Diego, La Jolla, USA

Translational modulation of neuronal homeostasis

Growing evidence indicates that neurons are particularly reliant on the spatial and temporal regulation of mRNA translation for their function and survival. In agreement, mutations in numerous components of the translational machinery have been linked to neurological disorders. Using a phenotype-driven approach in mice, we have identified several novel translational pathways that when disrupted can lead to loss of neuronal homeostasis. Recently, we found that loss of function of a novel ribosome rescue factor combined with a hypomorphic mutation in a brain-specific tRNA leads to neurodegeneration. Here I will present evidence that loss of other ribosome rescue factors or of tRNA function induces alterations of neuronal function that are accompanied by widespread reprogramming of translation. Our work highlights the exquisite sensitivity of the nervous system to even subtle disruption of cellular homeostasis, and demonstrates that the regulation of tRNAs and other factors involved in translation elongation play a critical role in complex neuronal processes.

Biography

Dr. Ackerman is the Steven W. Kuffler Chair of Biology and a professor in the Neurobiology Section in the Division of Biological Sciences at the University of California, San Diego; she is also a Professor in the Department of Cellular and Molecular Medicine in the School of Medicine and an Investigator of the Howard Hughes Medical Institute. Dr. Ackerman focuses on the mechanisms involved in the maintenance of neuronal homeostasis during aging. Her studies combine mouse genetic screens, genomics, cell biology, and biochemistry to identify novel molecular pathways in this process, including those involved in mRNA translation and RNA homeostasis.

https://biology.ucsd.edu/research/faculty/sackerman

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Erin Schuman

Max Planck Institute for Brain Research, Frankfurt

Protein Synthesis at Neuronal Synapses

The complex morphology of neurons, with synapses located 100's of microns from the cell body, necessitates the localization of important cell biological machines and processes within dendrites and axons. Using expansion microscopy together with metabolic labeling we have discovered that both postsynaptic spines and presynaptic terminals exhibit rapid translation, which exhibits differential sensitivity to different neurotransmitters and neuromodulators. These data suggest that selective translation of mRNAs in response to different extracellular cues can give rise to plasticity phenotypes at both sides of the synapse. In addition, we have explored the unique mechanisms neurons use to meet protein demands at synapses.

Biography

Erin Schuman was born in California. She received B.A. in Psychology from the University of Southern California and her Ph.D. in Neuroscience from Princeton. She conducted postdoctoral studies in Molecular and Cellular Physiology at Stanford. She joined the Biology Faculty at Caltech in 1993. During that time, Erin was an HHMI Investigator. In 2009, she moved to Frankfurt to found a new Max Planck Institute for Brain Research. Schuman's lab studies the cell biology of synapses. She has received several awards and grants, including the Pew Scholars Award and 2 advanced ERCs. She is a member of EMBO and the German Academy of Sciences Leopoldina. In 2018 she was awarded the Society for Neuroscience's Salpeter Lifetime Achievement Award.

https://brain.mpg.de/research/schuman-department.html

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Jeffrey Chao

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Imaging the life and death of single mRNAs in living cells

After transcription, an mRNA's fate is determined by an orchestrated series of events (processing, export, localization, translation and degradation) that is regulated both temporally and spatially within the cell. In order to more completely understand these processes and how they are coupled, it is necessary to be able to observe these events as they occur on single molecules of mRNA in real-time in living cells. To expand the scope of questions that can be addressed by RNA imaging, we are developing multi-color RNA biosensors that allow that status of a single mRNA molecules (e.g. translation or degradation) to be directly visualized and quantified.
In order to image the first round of translation, we have developed TRICK (translating RNA imaging by coat protein knock-off) which relies on the detection of two fluorescent signals that are placed within the coding sequence and the 3′UTR. In this approach, an untranslated mRNA is dual labeled and the fluorescent label in the coding sequence is displaced by the ribosome during the first round of translation resulting in translated mRNAs being singly labeled. A conceptually similar approach was used for single-molecule imaging of mRNA decay, where dual-colored mRNAs identify intact transcripts, while a single-colored stabilized decay intermediate marked degraded transcripts (TREAT, 3′ RNA end accumulation during turnover). We are using these tools to characterize localized translation and degradation during normal cell growth and stress.

Biography

Jeffrey Chao obtained his PhD from The Scripps Research Institute in La Jolla, CA where he worked with James Williamson on the structure and function of RNA-protein complexes. His postdoctoral studies with Robert Singer at Albert Einstein College of Medicine in Bronx, NY focused on understanding RNA localization and developing fluorescent microscopy techniques for imaging single mRNAs. In 2013, he established his own group at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland. His group combines biochemistry, structural biology and single-molecule imaging to investigate the mechanisms that control post-transcriptional regulation in the cytoplasm.

https://www.fmi.ch/research/groupleader/?group=132

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Roy Parker

Department of Biochemistry, University of Colorado Boulder and Howard Hughes Medical Institute (HHMI), Boulder, CO, USA

RNP Condensates in Health and Disease

Eukaryotic cells contain multiple assemblies of RNA and protein referred to as RNP granules, or RNP condensates. In the cytosol, ubiquitous RNP granules include stress granules, which form when translation initation is limited, and P-bodies, which are constitutive RNP granules containing mRNAs and the RNA decay machinery. Both stress granules and P-bodies contain complex proteomes and transcriptomes and their assembly/disassembly are regulated by diverse RNP remodeling complexes.

Focusing on stress granules, we have provided evidence that stress granule, and presumably other RNP condensate, assembly occurs in part through intermolecular RNA-RNA interactions. However, based on in vitro studies, we demonstrate that RNA condensation should be expected to be a thermodynamically favored process in cells. This argues that cells must contain mechanisms to limit RNA-driven condensation. We have demonstrated that abundant RNA helicases reduce RNA recruitment to RNA condensates in vitro and in cells, as well as limiting stress granule formation. This defines a new function for abundant RNA helicases to limit thermodynamically favored intermolecular RNA-RNA interactions in cells as “RNA decondenases”, thereby allowing proper RNP function.

Biography

Roy Parker is an Investigator with the Howard Hughes Medical Institute, Cech-Leinwand Endowed Chair of Biochemistry and Distinguished Professor at the University of Colorado Boulder. He has a joint appointment with the Department of Molecular, Cellular and Developmental Biology. He received his Ph.D. from the University of California, San Francisco and completed his Postdoctoral work at the University of Massachusetts, Worcester. His research focuses on the biogenesis, translation, and degradation of eukaryotic mRNA and how cells regulate different steps in this process to modulate gene expression. He has served on, and chaired, the NIH CDF-1 study section, and co-organized the Nucleic Acids Gordon Conference (1997), the RNA Processing Meeting at CSHL (2001), and the 2004 FASEB Conference on Post-Transcriptional Control (2004). He is, or has been, on the editorial boards of MCB, Science, Cell, RNA, Nucleic Acids Research, and is an editor of the Journal of Cell Biology and eLife. He was the President of the RNA Society (2010). He is an elected Fellow of the American Academy of Arts & Sciences (2010) and Member of the National Academy of Sciences (2012).

https://www.colorado.edu/lab/parkergroup/