Protein Folding on the Ribosome

or

The Nascent Chains Meeting

Cleveland, OH, USA

December, 12-15, 2026



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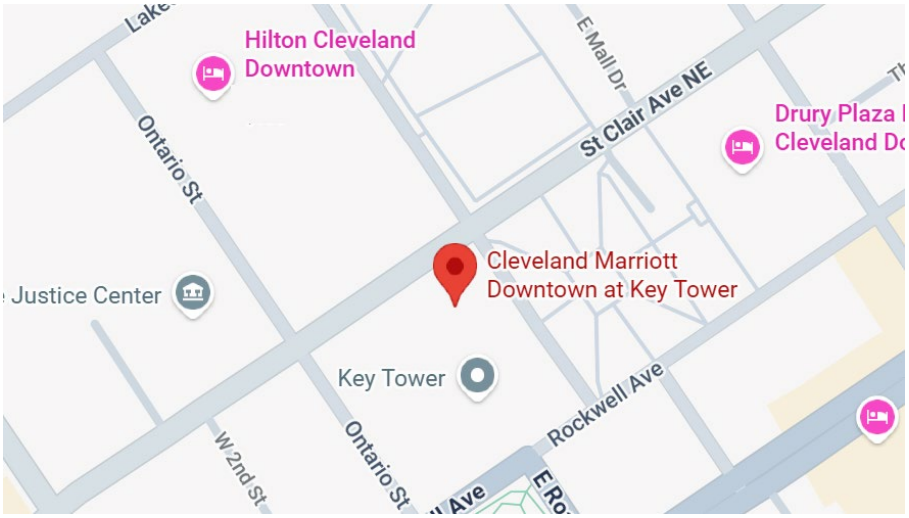
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How to get to Cleveland Marriott Downtown at Key Tower

Cleveland Marriott Downtown at Key Tower is a modern hotel in downtown Cleveland, Ohio. Address: 1360 W Mall Dr, Cleveland, OH 44114.





Start at Cleveland Hopkins airport CLE (20-30 min)

The distance between Cleveland Airport (CLE) and Cleveland Marriott Downtown at Key Tower is 12 miles (~19 km). An Uber from CLE to downtown Cleveland typically takes about 20 minutes and costs between $30 and $40 for a standard UberX, depending on traffic and demand.

RTA's Red Line provides regular service between Cleveland Hopkins International Airport and downtown Cleveland via the Tower City Station (walking distance from the hotel). The trip takes less than 30 minutes and trains depart every 15 minutes for the majority of the day.

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RACHAEL UHLIK | SENIOR EVENT MANAGER

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Marina Rodnina, Max Planck Institute for Multidisciplinary Sciences, Germany

Ayala Shiber, Technion - Israel Institute of Technology, Israel

Anton A. Komar, Cleveland State University, USA

Program

Focus talk ≈ 30 min + 10 min discussion
Talk ≈ 20 min + 5/10 min discussion
Short talk ≈ 15 min + 5 min discussion

Saturday, December 12

4:30 PM – 6:20 PM **Registration:** Marriott North Foyer

6:20 PM – 6:30 PM **Welcome:** Anton A. Komar

Session 1: Translation: where folding is born
Chair: Marina Rodnina

6:30 PM – 7:30 PM KEYNOTE: **Rachel Green**
Ribosome-mediated signaling activation on the colliding ribosome

7:30 PM – 7:55 PM TALK: **Hila Ben Arie Zilberman**
Ribosome-associated ISGylation reshapes translation during interferon-driven stress and recovery

8:00 PM – 10:00 PM Dinner (buffet)

10:00 PM – 11:00 PM **Informal discussions**/ Cocktails (at own expenses)
East Foyer

Sunday, December 13

7:30 AM – 9:00 AM Breakfast (buffet)

Session 2: Translation and co-translational chaperoning at the ribosome
Chair: Johannes Buchner

9:00 AM – 9:40 AM Focus TALK: **Judith Frydman**
Ribosome stalling causes proteotoxic stress by overwhelming cotranslational proteostasis

9:40 AM – 10:10 AM TALK: **David Balchin**
Molecular logic of a generalist chaperone at the ribosome

10:10 AM – 10:50 AM Focus TALK: **Elke Deuerling**
Co-translational regulation of protein biogenesis

10:50 AM – 11:00 AM Break (coffee, pastries)

Session 3: Translational, chaperones and folding
Chair: Judith Frydman

11:00 AM – 11:40 AM Focus TALK: **Johannes Buchner**
Chaperoning of eEF2 links translation to protein homeostasis

11:40 AM – 12:10 AM TALK: **Shu-ou Shan**
Defining the mammalian cotranslational Hsp70 cycle

12:10 AM – 12:30 PM Short TALK: **Gyan Prakash**
Cotranslational chaperones coordinate heme-dependent maturation of mitochondrial membrane proteins

12:30 AM – 1:00 PM TALK: **Christian Kaiser**
Dissecting co-translational chaperone interactions with single-molecule spectroscopy

1:00 PM – 2:00 PM LUNCH (buffet)

Session 4: Co-translational assembly of protein complexes
Chair: Ayala Shiber

2:00 PM – 2:30 PM TALK: **Bernd Bukau**
Mechanisms of co-translational assembly of protein complexes

2:30 PM – 3:00 PM TALK: **Emmanuel Levy**
Structure dictates co-translational assembly, evolution follows

3:00 PM – 3:30 PM TALK: **Martin Bushell**
DDX6 is required to regulate ribosome traffic to control co-translational assembly of protein complexes

3:30 PM – 3:50 PM Break (coffee, pastries)

Chair: Emmanuel Levy

3:50 PM – 4:20 PM TALK: **Ayala Shiber**
Metastable Intermediates anchor co-translational assembly interactions during synthesis of misfolding-prone subunits

4:20 PM – 4:50 PM TALK: **Sander Tans**
Cotranslational protein assembly at the single-molecule level

4:50 PM – 5:10 PM Break (coffee, pastries)

Session 5: Misfolding, aggregation, quality control, and disease

Chair: John Christodoulou

5:10 PM – 5:40 PM TALK: **Lisa Cabrita**
A model for protein misfolding and oligomerisation that begins on translating ribosomes

5:40 PM – 6:00 PM Short TALK: **Niko Dalheimer**
Widespread mis-splicing upon TRiC dysfunction - a link to neurodevelopmental TRiCopathies

6:00 PM – 6:30 PM TALK: **Sina Ghaemmaghami**
Structure and dynamics of the nascent ubiquitinome

6:30 PM – 7:00 PM TALK: **Joost Schymkowitz**
The hidden vulnerability of nascent proteins: folding, protection and aggregation during translation

7:00 PM – 8:00 PM Dinner (buffet)

8:00 PM – 11:00 PM **POSTER SESSION 1** (coffee, pastries)

Monday, December 14

7:30 AM – **9:00 AM** Breakfast (buffet)

Session 6: Watching co-translational folding: real-time and kinetic mechanisms

Chair: Anton Komar

9:00 AM – **9:40 AM** Focus TALK: **Marina Rodnina**
Real-time analysis of cotranslational folding of a bacterial membrane protein

9:40 AM – **10:00 AM** Short TALK: **Justas Kvietkauskas**
Conformational checkpoints during cotranslational membrane protein biogenesis

10:00 AM – **10:40 AM** Focus TALK: **Susan Marqusee**
A molecular picture of protein folding during active translation

10:40 AM – **11:00 AM** Break (coffee, pastries)

Session 7: Structure, Folding, Energetics and the Exit Tunnel

Chair: Susan Marqusee

11:00 AM – **11:30 AM** TALK: **Eugene Shakhnovich**
Protein evolution with and without chaperones

11:40 AM – **12:10 AM** TALK: **Stephen Fried**
Kinetically stable proteins depend on cotranslational folding for efficient biogenesis

12:10 AM – **12:30 PM** Short TALK: **Morgana Kellogg**
CryoEM of a variant of the SDD1 arrest peptide reveals the mechanism of enhanced arrest

12:30 AM – 1:00 PM TALK: **Olivia S. Rissland**
Investigating the fitness landscapes of 2A peptides

1:00 PM – 2:00 PM LUNCH (buffet)

Session 8: Synonymous codons, translation kinetics, and folding
Chair: Marina Rodnina

2:00 PM – 2:30 PM TALK: **Patricia Clark**
A new view for synonymous codon usage

2:30 PM – 3:00 PM TALK: **Chava Kimchi-Sarfaty**
Beyond the genetic code: position-specific codon usage regulates translation and co-translational protein folding

3:00 PM – 3:30 PM TALK: **Edward P. O'Brien**
A recently discovered class of protein misfolding links synonymous mutation effects on structure and function to aging

4:00 PM – 4:30 PM TALK: **Anton A. Komar**
Folding of the oncolytic virus (OV) proteome in cancer cells: the impact of endogenous chaperones and codon optimization

4:30 PM – 4:50 PM Break (coffee, pastries)

Session 9: The ribosome: decoding, recording, and the exit tunnel
Chair: Rachel Green

4:50 PM – 5:20 PM TALK: **Joseph D. Puglisi**
Origins and impacts of translational errors

5:20 PM – 5:50 PM TALK: **Jonathan P. Schlebach**
Nascent chains tune translational decoding and recoding

5:50 PM – 6:10 PM Short TALK: **Ahmad Jomaa**
A Z-shaped nascent chain in the ribosome exit tunnel blocks translation termination

6:10 PM – 6:30 PM Short TALK: **Maxim Svetlov**
Ribosome-protecting ABCF proteins extract the nascent peptide to displace tunnel-bound antibiotics and confer resistance

6:30 PM – 7:00 PM TALK: **Alanna Schepartz**
New molecules from nature's most sophisticated catalyst

7:00 PM – 8:00 PM Dinner (buffet)

8:00 PM – 9:00 PM **Piano concert presented by Dmitry Tyurin**

For Dmitry Tyurin's bio, please see page 70

(The concert is partially supported by Svetlana Stolyarova)

Scherzo No. 2 in B-Flat minor, Op. 31	Frédéric Chopin (1810–1849)
Paraphrase de concert sur *Rigoletto*, S. 434 (after Verdi)	Franz Liszt (1811–1886)
Mephisto Waltz No. 1, S. 514	Franz Liszt
From *The Nutcracker*, Op. 71 Dance of the Sugar-Plum Fairy Andante maestoso	Pyotr Tchaikovsky (1840–1893) arr. Mikhail Pletnev
Variations on a Theme from *Carmen* (after Bizet)	Vladimir Horowitz (1903–1989)

9:00 PM – 11:00 PM **Informal Discussions; POSTER SESSION 2** (coffee, pastries)

Tuesday, December 15

7:30 AM – 9:00 AM Breakfast (buffet)

Session 10: The molecular origami of proteins, how we study it, and relevance to disease

Chair: Stephen Fried

9:00 AM – 9:30 AM TALK: **Julian O. Streit**
Structures and energetics of protein folding intermediates on the ribosome

9:30 AM – 10:00 AM TALK: **Ivana Bukvin**
Structural and mechanistic insights into huntingtin biosynthesis

10:00 AM – 10:30 AM TALK: **Ning Zhao**
Live-cell co-translational folding tracking reveals bidirectional coupling between translation and protein folding

10:30 AM – 10:50 AM Break (coffee, pastries)

10:50 AM – 11:45 AM KEYNOTE: **Gabriel Rocklin**
Large-scale discovery of protein energy landscapes

11:45 AM – 12:00 AM **CLOSING REMARKS**

12:00 PM – 2:00 PM LUNCH (buffet)

Speaker Abstracts

The presenter's name is underlined

Saturday, December 12

Session 1: Translation: where folding is born

1

Ribosome-mediated signaling activation on the colliding ribosome

Rachel Green

Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Ribosomes are critical constituents of the cell both for protein expression and for driving cell fate signaling decisions. We use genetic, proteomic, biochemical and ribosome profiling approaches in mammals to identify and characterize critical factors involved in recognizing ribosome dysfunction and implementing cell fate outcomes. Central to many of these events is a collided ribosome that serves as a hub to integrate interactions with the QC machinery as well as cellular signaling pathways. Key kinases that directly bind to ribosome collisions to signal downstream responses include GCN2 and the MAP3K ZAK. I will present our recent efforts to define the molecular details of activation of both GCN2 and ZAK.

2

Ribosome-associated ISGylation reshapes translation during interferon-driven stress and recovery

Hila Ben Arie Zilberman[1], Rawad Hanna[2], Qamar Shhade[1], Eden Ehrenkranz[1], Fabian Glaser[1], Ayla Shiber[1]

[1] Technion – Israel Institute of Technology, Haifa, Israel,
[2] Goethe University Frankfurt, Frankfurt am Main, Germany

The association of translation initiation and elongation factors, molecular chaperones, and protein quality-control machinery with translating ribosomes

coordinates protein synthesis with nascent-chain folding and maturation pathways. How this machinery is remodeled during the innate immune response and translational recovery remains poorly understood. ISG15, an Interferon Stimulated Gene, is a ubiquitin-like modifier that plays a key role in the innate immune response via its conjugation to a variety of target proteins (ISGylation).

Its major E3 ligase, HERC5, associates with translating ribosomes, positioning this pathway to influence newly synthesized proteins as they emerge from the ribosome. Yet the functional consequences of ribosome-associated ISGylation remain unresolved: Does it regulate translation initiation or elongation, alter decoding fidelity, or influence which transcripts are translated? How does it affect ribosome composition and the factors that act on nascent chains? And how do these activities contribute to recovery following interferon-induced stress? Here, we investigate how ISG15 shapes translation, ribosome-associated initiation factors, chaperones, and protein quality control during the IFN-I response.

TRIM25, a second E3 ligase for ubiquitin and ISG15, also associates with translating ribosomes. We hypothesized that the ISG15-TRIM25-HERC5 axis constitutes a ribosome-associated surveillance and regulatory module that couples translation dynamics to co-translational quality control during the IFN-I response and recovery.

We combined polysome profiling with proteomic analysis of monosome and polysome fractions from wild-type (WT) and ISG15-knockout HeLa cells, with and without IFN-I treatment for increasing durations. ISG15-knockout cells exhibited lower translation than WT cells at all time points, while WT cells showed the expected reduction in translation following IFN-I treatment. Compared with WT cells, polysome fractions from knockout cells contained lower levels of translation initiation and elongation factors, co-translational chaperones, mRNA-modifying enzymes, and tRNA synthetases, but higher levels of E3 ubiquitin ligases. These findings suggest that ISG15 influences which factors associate with translating ribosomes, including machinery involved in nascent-chain folding and maturation.

Mass spectrometry of the polysome fractions also identified candidate ISGylation sites: diglycine (GG) remnants on lysines of five ribosomal proteins, detected in at least two WT replicates and in none of the knockout replicates. These proteins lie near the interface between the 40S and 60S subunits, a region central to subunit joining and translation-factor engagement.

Together, our findings highlight a role for ISG15 in remodeling the ribosome-associated machinery during the IFN-I response. Our results suggest that ISGylation influences ribosome composition and the recruitment of factors involved in

translation and co-translational protein quality control, potentially contributing to the regulation of translational recovery.

Sunday, December 13

Session 2: Translation and co-translational chaperoning at the ribosome

3

Ribosome stalling causes proteotoxic stress by overwhelming cotranslational proteostasis

Natália M Barbosa[1], Saswata Ganguly[2], Pavel Simakin[3], Jae Ho Lee[4], Felix Boos[5], Markus Räschle[3], Zacharias Campbell[2], Sebastian Filbeck[2], Colin C C Wu[6], Cleslei F Zanelli[7], Johannes M. Herrmann[3], Stefan Pfeffer[2], Judith Frydman[1*]

[1] Department of Biology, Stanford University, Stanford, CA, USA
[2] Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 345, Heidelberg, Germany
[3] Cell Biology, University of Kaiserslautern, RPTU, Kaiserslautern, Germany
[4] Department of Biochemistry and Cell Biology, Stony Brook University, NY, USA
[5] Department of Genetics, Stanford University, Stanford, CA, USA
[6] Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD, USA
[7] School of Pharmaceutical Sciences, São Paulo State University, Araraquara, SP, Brazil

The link between translation elongation rate and protein fate are incompletely understood. We here examine the consequences of globally slowing elongation. eIF5A depletion leads to pervasive, severe stalling, including collided disomes visualized *in situ*, but rarely aborts translation. Most stalls resolve and protein output is preserved regardless of stalling strength or disome formation. Surprisingly, the major impact of severely slowed and collided ribosomes is impaired cotranslational proteostasis. Those polypeptides depleted from the proteome combine multiple strong stalls with a cotranslational requirement of chaperones Hsp70/Ssb or TRiC/CCT. Exacerbated pausing at sites where these chaperones normally bind doubles cotranslational Hsp70/Ssb binding. The overload of cotranslational proteostasis is compounded by selective depletion of

cotranslational chaperones, causing synthetic sickness with Δssb1/2 and widespread aggregation. The ensuing proteotoxic damage is buffered by transcriptional stress responses required for survival. Ribosome slowdowns and stalling thus primarily affects proteome quality by overwhelming cotranslational proteostasis rather than aborting translation.

4

Molecular logic of a generalist chaperone at the ribosome

David Balchin

Protein Biogenesis laboratory, The Francis Crick Institute, London, UK

Molecular chaperones promote protein folding by forming temporary complexes with their clients. Many chaperones have broad remit, binding 100s-1000s of different clients, yet select specific folding intermediates based on their conformation. This is exemplified by ribosome-bound chaperones which cater to a large fraction of the nascent proteome. How do such general-purpose chaperones recognize and influence the folding of nascent polypeptides with diverse sequences and native structures? We find that the ribosome-associated chaperone Trigger factor (TF) uses a large composite binding surface to form “fuzzy complexes” with nascent chains (NCs). Different sites on TF support enthalpically- or entropically-driven binding, and cryo-EM shows that TF dramatically adjusts its orientation to accommodate diverse NCs. By quantifying local NC folding equilibria, we show that TF binding tunes the kinetic and thermodynamic cooperativity of cotranslational folding. We also directly visualize NCs that escape TF, showing that these form stable near-native folds that plug the ribosome exit port or bury the TF-binding site on the ribosome. Overall, these data reveal the molecular origins of TF promiscuity, and show how the ribosome and a cotranslational chaperone cooperate to shape protein biogenesis pathways**.**

5

Co-translational Regulation of Protein Biogenesis

Elke Deuerling

University of Konstanz, Konstanz, Germany

The biogenesis of newly synthesized proteins is tightly regulated by specific biogenesis factors that interact with the ribosome exit site. Accurate selection of nascent polypeptide chains by the appropriate protein biogenesis factors—such as those mediating N-terminal modifications—is essential for ensuring correct protein folding, localization, and function. Inappropriate or unsolicited binding of these processing factors to translating ribosomes can result in protein misfolding and mislocalization, compromising cellular function and viability. Despite its importance, the mechanism that governs the selective access of biogenesis factors to ribosomes has remained elusive. This presentation will highlight recent advances in our understanding of how these interactions are regulated and consider their potential relevance to disease-related processes.

Session 3: Translation, Chaperones and Folding

6
Chaperoning of eEF2 links translation to protein homeostasis

Johannes Buchner and Jordyn Markle

Department Bioscience, Technical University of Munich, Germany

The Hsp90 chaperone machinery plays an essential role in protein homeostasis through interactions with specific co-chaperones. In yeast, we identified Cns1 as an essential regulator of translation elongation that promotes the folding and solubility of eukaryotic elongation factor 2 (eEF2). Structural and NMR studies revealed key features of Cns1, while functional analyses demonstrated its interaction with Hgh1, Hsp90, and eEF2, forming a quaternary complex essential for yeast viability. Furthermore, we uncovered a connection between eEF2 chaperoning and diphthamide modification, a conserved post-translational modification required for translational fidelity. The Hsp90 co-chaperones Hgh1 and Cpr7 are specifically required for efficient eEF2 diphthamide modification, suggesting that chaperone-mediated maintenance of specific eEF2 conformations facilitates its modification.

An important question is whether this mechanism is conserved in humans. The identification of TTC4 as the human homologue of yeast Cns1, together with the evolutionary conservation of eEF2, diphthamide biosynthesis, and the Hsp90 chaperone machinery, provides a strong rationale for investigating this possibility. Whether TTC4 and other human Hsp90 co-chaperones participate in coordinating eEF2 folding and diphthamide modification remains to be established. Exploring this conserved chaperone network may reveal fundamental mechanisms linking protein homeostasis to translational fidelity in human cells.

7

Defining the mammalian cotranslational Hsp70 cyclehaperoning of eEF2 links translation to protein homeostasis

Ziyi Fan, Radoslaw Gora, HaoHsuan Hsieh, Shu-ou Shan

California Institute of Technology, Pasadena, CA, USA

The Hsp70 family of molecular chaperones, together with the J-domain protein (JDP) cochaperones, comprise a central chaperone hub in the cell that interacts with numerous newly synthesized proteins and use JDP-activated ATPase cycles to assist *de novo* protein folding. In yeast cells, the ribosome-associated Hsp70 homolog Ssb is specifically activated by the ribosome-associated complex (RAC) to regulate the cotranslational folding of nascent proteins as they emerge from the ribosome. Mammalian cells do not contain an Ssb homolog. Instead, mammalian RAC (mRAC) must work with cytosolic Hsp70s to enable an Hsp70 cycle on the ribosome; the molecular mechanism underlying this process is poorly understood. To address this question, we studied the chaperone cycle of mRAC using biochemical and biophysical measurements. We find that the ribosome strongly promotes RAC-dependent ATPase activation of the major cytosolic Hsp70s, HSPA8 and HSPA1, via a combination of induced proximity and allosteric mechanisms, thus enabling localized activation of Hsp70 near the polypeptide exit tunnel (PET) of the ribosome. On ribosomes bearing a correct nascent protein substrate, mRAC-mediated activation leads to high affinity and site-specific loading of Hsp70 on the nascent chain at a distance from the PET, followed by rapid displacement of mRAC from the ribosome. The mRAC/Hsp70 system and the other major eukaryotic cotranslational chaperone, nascent polypeptide associated complex (NAC), recognize distinct site(s) on the nascent polypeptide chain. Our work defines a

mechanistic framework for the cotranslational RAC/Hsp70 cycle in mammalian cells.

8

Cotranslational chaperones coordinate heme-dependent maturation of mitochondrial membrane proteins

Gyan Prakash[1,*], Dawafuti Sherpa[2,*], Mary T. Couvillion[1], Sichen Shao[2,3,#], L. Stirling Churchman[1,#]

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[3] Howard Hughes Medical Institute, Chevy Chase, MD, USA.
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Membrane proteins begin folding and transmembrane domain (TMD) insertion before their synthesis is complete. For mitochondrial-encoded respiratory subunits, these events must be coordinated with cofactor acquisition and assembly with imported partners. Through genetic screens and selective mitoribosome profiling, we resolved an ordered recruitment of the OXA1L insertase and substrate-specific chaperones during COX1 and cytochrome b (CYB) synthesis. Cotranslational chaperone recruitment patterns are consistent with pairwise TMD insertion by OXA1L and their distinct roles in protein folding and complex assembly. Cryo-electron microscopy revealed the CYB-specific UQCC chaperones docked beside OXA1L at the mitoribosomal exit, where UQCC1–UQCC2 engages nascent CYB regions that conformationally rearrange upon heme acquisition. Heme depletion reduced assembled respiratory complexes while chaperone-associated subcomplexes persisted, and preferentially reduced relative CYB ribosome occupancy at sites of UQCC engagement. These effects were restored by hemin supplementation. Our findings show how cotranslational chaperones protect nascent respiratory subunits to coordinate cofactor-regulated membrane protein maturation.

9

Dissecting co-translational chaperone interactions with single-molecule spectroscopy

Nandakumar Rajasekaran[1], Dmitri Toptygin[1], Ting-Wei Liao[2], Vincent J. Hilser[1], Taekjip Ha[2,3,4], Christian M. Kaiser[1,5,*]

[1] Department of Biology, Johns Hopkins University, Baltimore, MD, USA
[2] T.C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD, USA
[3] Program in Cellular and Molecular Medicine and Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA, USA
[4] Department of Pediatrics, Harvard Medical School, Boston, MA, USA
[5] Bijvoet Centre for Biomolecular Research, Department of Chemistry, Utrecht University, The Netherlands

Early co-translational folding of nascent proteins is guided by dedicated molecular chaperones. The ribosome-associated bacterial chaperone trigger factor binds to emerging nascent chains, but its dynamic interactions with client proteins remain poorly defined. We have used single-molecule fluorescence spectroscopy to directly observe and characterize trigger factor interactions with a ribosome-bound client protein at increasing nascent-chain lengths. We found that trigger factor dwell times on ribosome-nascent chain complexes increase non-monotonically with nascent chain length. Binding to nascent proteins is best described by multiple weak, dynamic interactions that are established after the chaperone docks onto the ribosome and evolve during polypeptide elongation. Experiments combining fluorescence detection with single-molecule manipulation using optical tweezers support this multivalent interaction model. This binding mode may help to stabilize nascent proteins against misfolding while allowing them to dynamically sample conformational space in search of their native structures.

Session 4: Co-translational assembly of protein complexes

10
Mechanisms of co-translational assembly of protein complexes

Jaime Santos, Jaro Schmitt, Guenter Kramer, Bernd Bukau

Center for Molecular Biology of Heidelberg University (ZMBH), DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, Heidelberg, Germany

The assembly of newly synthesized proteins into functionally active oligomers has long been regarded as a post-translational process driven by random collision of subunits. However, growing evidence indicates that, for many proteins, assembly occurs co-translationally, tightly coupling synthesis, folding, and subunit assembly. This fundamentally different mechanism enables the spatial and temporal coordination of assembly, promotes the hierarchical formation of multi-subunit assemblies and enhances the stability of involved subunits. Here we report results of our investigation of mechanistic aspects of co-translational assembly using ribosome profiling technology combined with biophysical approaches.
We show that the coupling of assembly with translation expands the range of possible protein architectures, especially the formation of domain-swapped assemblies and coiled-coils. We further investigated the role of chaperones and quality control factors in co-translational protein assembly, revealing an intricate relationship between productive assembly and quality control at the level of nascent chains.

11

Structure dictates co-translational assembly, evolution follows

Emmanuel Levy

University of Geneva, Geneva, Switzerland

Proteins often assemble into complexes while still being synthesized by the ribosome. I will describe the structural determinants of this process, which typically involves intertwined subunits that appear unstable in isolation. Leveraging these structural signatures and AlphaFold2 models, we predicted co-translational assembly and partner identities at proteome scale, and validated these predictions with several experimental approaches. Finally, I will discuss how co-translational assembly influences the evolutionary fate of genes after duplication.

12

DDX6 is required to regulate ribosome traffic to control co-translational assembly of protein complexes

Joseph Waldron[1], James Ettles[1], Pauline Herviou[1], David Gay[1], Tobias Schmidt[1], Sarah Gillen[1], Jonathan Bohlen[2], Ania Wilczynska[1], Martin Bushell[1]

[1] Cancer Research UK Scotland Institute, Glasgow, UK
[2] Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität München, Germany

The precise spatiotemporal regulation of gene expression is critical for cellular function, ensuring proteins are properly synthesised, folded, and assembled. While the role of translation initiation in regulating protein yield is well-established, how translation elongation dynamics actively coordinate the co-translational assembly (CoTA) of multiprotein complexes remains a critical gap in our understanding of proteome architecture.
Here, we demonstrate that the highly conserved DEAD-box RNA helicase DDX6, traditionally regarded as a general translational repressor and RNA decay factor, actively regulates ribosome traffic during the elongation phase to orchestrate CoTA. Utilising Translation Complex Profiling (TCP-seq), disome-profiling, and DDX6 selective ribosome-profiling (Sel-RiboSeq) in human cells, we mapped the precise positional dynamics of DDX6 recruitment.
We reveal that DDX6 associates selectively with elongating ribosomes translating mRNAs enriched in AU3 codons, particularly the non-optimal GAA codon, within broader purine-rich contexts. Structural and biochemical analyses indicate that DDX6 binds to the ribosome at the mRNA entry channel in a POST state, directly clamping onto the downstream mRNA sequence. This interaction acts as a targeted brake, locally slowing ribosome movement to enforce prolonged pauses at discrete critical junctures. These physiological pauses manifest as controlled ribosome collisions that are tolerated by the cell. Crucially, the loss of DDX6-mediated traffic control leads to dysregulated ribosome movement, resulting in aberrant collisions that trigger a ZAKα-dependent ribotoxic stress response.
By sequencing footprints from multisomes engaged in nascent chain interactions, we established a striking spatial and temporal synchrony between DDX6 recruitment and CoTA engagement. For multiprotein complexes such as POLE3 and POLE4, DDX6-mediated pausing provides the essential temporal window for nascent protein subunits to structurally engage with one another during their

synthesis. Depletion of DDX6 disrupts this precisely timed ribosome traffic, causing a failure of proper co-translational subunit engagement. Ultimately, our findings reveal that CoTA is not solely dictated by the intrinsic biophysical properties of nascent polypeptides, but is an actively choreographed regulatory process. By uncovering a hidden regulatory code embedded within the mRNA sequence, this study reframes the active ribosome as a dynamically managed assembly hub, representing a major paradigm shift in our understanding of how post-transcriptional dynamics physically choreograph protein fate.

13

Metastable intermediates anchor co-translational assembly interactions during synthesis of misfolding-prone subunits

Fabian Glaser[1*], Johannes Venezian[2*], Hagit Bar-Yosef[2], Juan Fernández-Recio[3&], Ayala Shiber[2&]
*Equal contribution, & Co-corresponding

[1] Technion Center for Structural Biology - Computational Section, Technion Human Health Initiative, Technion - Israel Institute of Technology, Haifa, Israel
[2] Faculty of Biology, Technion – Israel Institute of Technology, Haifa, Israel
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The folding of proteins into their native states is a major challenge in the crowded cellular environment. Co-translational folding pathways have emerged as key mechanisms promoting the efficient acquisition of native conformations while limiting misfolding and kinetic trapping. Although most cellular proteins function within complexes, how transient conformations sampled during synthesis influence interface formation, and how co-translational assembly protects the growing chain from misfolding remain poorly understood. Here, we investigate the interplay between co-translational folding, misfolding, and assembly pathways in the structurally similar but functionally divergent complexes of the N-terminal acetyltransferase family, NatA and NatB. We combine molecular dynamics simulations and BioEmu AI-based conformational sampling with in vivo mutagenesis, degradation, and aggregation assays to characterize successive N-terminal fragments representing intermediates of vectorial protein synthesis. Our

conformational analyses predict early metastable conformations that can support assembly interactions before unstable regions emerge from the ribosome exit tunnel. In vivo analyses validated the predicted misfolding propensity and stability of these fragments, identifying the onset of co-translational assembly as a strong predictor of nascent-chain stability. Specifically, at approximately 110 aa length, the nascent chain of NatB catalytic subunit samples a distinct, metastable conformation, sufficiently folded to engage its auxiliary subunit. In contrast, the predicted conformational ensembles of shorter and longer fragments indicate instability and steric clashes with the auxiliary subunit that would disrupt assembly, defining a narrow assembly-competent window during synthesis. Early partner engagement stabilizes the growing chain as subsequent, unstable regions emerge. Analyses of additional complexes reveal a similar pattern: N-terminal fragments corresponding to assembly onset are comparatively stable, whereas neighboring fragments, both shorter and longer, exhibit greater conformational instability. Together, these findings reveal how transient folding intermediates enable early assembly and establish an experimentally validated, fragment-based computational framework for predicting co-translational assembly onset and mapping nascent-chain conformational stability at near-residue resolution.

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Cotranslational protein assembly at the single-molecule level

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Increasing evidence indicates that many protein complexes already form during translation. These findings stand to profoundly impact our understanding of protein folding at the ribosome, as different nascent chains must then productively interact before translation and folding is completed. However, the folding implications and functional purpose of this assembly route remain unclear, as the employed biochemical and ribosome profiling approaches cannot address them. These technical challenges also limit mechanistic insight into potentially major

consequences for related processes, including chaperone guidance and translation regulation.
We developed single-molecule approaches to begin addressing this gap. Specifically, a novel optical tweezers approach allowed us to probe two interacting nascent chains. We have recently used it to study intertwined protein complexes, which suggests that domain-swapping or other forms of structural remodelling is needed for assembly. We focussed on BTB domains, which underlie the dimerization of many proteins such as in the E3 ubiquitin ligase Cullin complex (unpublished). It revealed a 'temporal control' mechanism to directly form intertwined dimers without requiring domain-swapping. First we show that the BTB domains of KEAP1, KLHL12, and PATZ1 form stable closed states as monomers, thus impeding domain-swapping assembly routes. By contrast, the sequentially ordered emergence of nascent chain segments during translation enables alternative folding-assembly pathways that bypass the closed monomeric state. Analysis indicates that this mechanism works in concert with dimerization quality control by the E3 ligase SCF-FBXL17, and is relevant across the BTB domain family. These findings suggest that ribosome cooperation expands the range of possible protein architectures.
If time allows, I will also discuss how our single-molecule methods can provide insight into the reciprocal self-chaperoning of two nascent chains (Wruch, Nat. Comm. 2025), the cotranslational formation of hetero-dimers (Schmitt, PNAS 2026), and the cotranslational folding guidance of the bacterial chaperone trigger factor (Till, PNAS 2025), and the human chaperone NAC (Santos, Mol. Cell 2026, and unpublished).

Session 5: Misfolding, aggregation, quality control, and disease

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A model for protein misfolding and oligomerisation that begins on translating ribosomes

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Protein misfolding is hazardous to cell and is a process that is liked to an ever-increasing range of human conformational diseases: neurodegeneration, respiratory diseases, and cancer are linked to proteins which readily form ordered or amorphous structures that are toxic to cells. Our understanding of protein folding is that it begins co-translationally on ribosomes and is a process that is supported by molecular chaperones and other forms of quality control. Despite these measures, protein folding remains an imperfect process which intrinsically competes against misfolding. Additionally, although there is an emerging understanding of co-translational folding, far less is understood of this process when it goes awry.

Our research thus aims to uncover the earliest origins of protein misfolding and aggregation in disease by tracing these events back to their earliest point: during biosynthesis on the ribosome. We show with alpha-1-antitrypsin, an archetype of conformational disease, that translating ribosomes pause to form co-translational folding intermediates, which are important for directing the protein's post-translational folding fate. By applying a set of biochemical, cell-based and structural studies, we observe the earliest stages of aberrant protein oligomerisation that begins on translating ribosomes, in a mechanism that we call "co-post mis-assembly" (2). Our data thus reveal that protein misfolding involves parallel co-translational and post-translational processes with implications for conformational disease aetiology.

(1) Nascent chains can form co-translational folding intermediates that promote post-translational folding outcomes in a disease-causing protein. *Plessa E et al, Nat Communications (2021)*

(2) Visualisation of translating ribosomes reveal the earliest steps of co-translational protein misfolding and oligomerisation in a human disease model. *Plessa E et al, Accepted Nat. Comm (2026)*

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Widespread mis-splicing upon TRiC dysfunction - a link to neurodevelopmental TRiCopathies

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Proteome integrity in cells relies on a network of molecular chaperones that guide nascent and conformationally unstable polypeptides toward their native conformations and prevent toxic aggregation. The essential hetero-oligomeric chaperonin TRiC is a central player in this network, assisting the folding of ~10% of the cytosolic proteome, including numerous obligate substrates. Mutations in TRiC subunits have recently been linked to severe neurodevelopmental disorders with brain malformations and epilepsy, termed TRiCopathies. However, the molecular mechanisms connecting chaperonin dysfunction to disease remain poorly understood. To acutely and reversibly perturb the essential TRiC machinery while avoiding long-term adaptive responses, we employed HaloPROTAC-mediated degradation of the CCT4 subunit in human U2OS cells. Loss of CCT4 results in the formation of a remodeled holocomplex in which the remaining subunits structurally compensate for the loss of CCT4, giving rise to altered client specificity. Most classical TRiC substrates fail to associate with this complex, while a subset remains bound but cannot be productively folded, and novel interaction partners are gained. Strikingly, this neomorphic TRiC complex fails to support the folding of key spliceosomal components, leading to widespread splicing defects characterized by prominent exon skipping. These splicing defects are accompanied by a pronounced attenuation of translation, which we also observe in TRiCopathy patient-derived fibroblasts. We hypothesize that aberrant splicing triggers this reduction in translation, which may in turn serve as a compensatory response to TRiC dysfunction by reducing the influx of newly synthesized polypeptides and thus

the folding demand on the compromised TRiC system. Additionally, these transcriptome-wide alterations are accompanied by impaired mitochondrial biogenesis and lysosomal dysfunction, resembling cellular phenotypes observed in TRiCopathy patients.
Our findings reveal how disruption of the essential chaperonin complex rewires its client network and links TRiC dysfunction to aberrant RNA splicing, translational attenuation, and impaired organellar homeostasis in neurodevelopmental disease. Together with the finding that TRiC interacts with approximately 40% of annotated splicing factors, these results establish an extensive connection between chaperone-mediated protein folding and RNA processing.

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Structure and dynamics of the nascent ubiquitinome

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A substantial fraction of newly synthesized proteins is ubiquitinated during or shortly after translation. This ubiquitination clears nascent chains from stalled ribosomes as well as polypeptides that fail to fold during active synthesis. However, our current understanding of the prevalence, substrates, and mechanisms of nascent protein ubiquitination derives largely from studies of a few model proteins or bulk protein populations. To survey global patterns of nascent protein ubiquitination, we have developed a suite of dynamic and structural mass spectrometry-based proteomic methodologies. These include proteome birthdating, which uses sequential labeling with neutron-encoded amino acids to partition the proteome into discrete age cohorts, and proteome-wide measurements of methionine oxidation rates that report on the conformational states of ubiquitinated proteins. Combined with deep-coverage data-independent acquisition, these approaches allow the age, modification sites, folding state, and proteasomal flux of ubiquitinated proteins to be measured and integrated on a proteome-wide scale. Our analyses of nascent ubiquitinomes have identified the modification sites associated with nascent protein clearance, as well as the structural and functional properties of proteins that predict rapid proteasomal degradation. We find that newly synthesized proteins constitute the majority of

proteasomal flux in cells, and that these nascent substrates are misfolded and preferentially ubiquitinated at lysines normally buried within native structures, often near their N termini. Substrates of this pathway are enriched in hydrophobic proteins, nuclear-encoded mitochondrial proteins, and proteins harboring native non-covalent lasso entanglements. Our comparative analyses across species further indicate that nascent ubiquitination patterns are evolutionarily conserved. Collectively, our studies provide a quantitative, proteome-wide census of ubiquitinated nascent proteins and their structural features.

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The hidden vulnerability of nascent proteins: folding, protection and aggregation during translation

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Protein folding begins before synthesis is complete, as the nascent polypeptide progressively emerges from the ribosome. This vectorial process creates a fundamental challenge: sequences that will eventually be buried within the native structure may remain exposed for extended periods while their interaction partners have yet to be synthesized. During this interval, the nascent chain must navigate competing opportunities for folding, chaperone recognition and intermolecular interactions. We investigate how protein sequence and topology determine these transient vulnerabilities and how they influence protein fate during synthesis. Using structure-based analyses, we show that the timing of native contact formation creates distinct windows of exposure, frequently involving aggregation-prone regions that are normally protected within the mature protein. These vulnerable regions are preferentially recognized by co-translational chaperones, suggesting that protein folding and quality control are closely coupled through the progressive emergence of the nascent chain. An important consequence of this view is that the same molecular surfaces required for productive folding may also support inappropriate interactions before the native structure is established. We explore this possibility experimentally using ribosome-associated nascent chains, examining how exposed aggregation-prone sequences influence interactions between translating complexes and the organization of

ribosomes. Together, our findings suggest that protein synthesis creates a succession of transient molecular opportunities whose outcomes depend on the interplay between sequence, structural topology, translation and the cellular environment. Understanding how these opportunities are resolved may help explain why some proteins are particularly vulnerable to misfolding and aggregation during their synthesis.

Monday, December 14

Session 6: Watching co-translational folding: real-time and kinetic mechanisms

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Real-time analysis of cotranslational folding of a bacterial membrane protein

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Membrane protein folding begins during translation and is influenced by interactions of the nascent chain with the ribosome and the SecYEG translocon. We investigate the early stages of membrane protein biogenesis using a bacterial translation system. By combining time-resolved translation with FRET measurements between different positions within the nascent protein, we monitor the emergence of structure in real time and explore how translation, membrane insertion, and protein folding are coordinated.

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Conformational checkpoints during cotranslational membrane protein biogenesis

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Membrane protein biogenesis requires the coordinated action of translating ribosomes and the SecYEG translocon, yet how nascent membrane proteins influence the conformational dynamics of this machinery remains poorly understood. Using a fluorescence correlation spectroscopy (FCS)-based approach, we directly monitor nascent chain length-dependent changes in the conformational landscape of the ribosome-SecYEG complex during cotranslational membrane protein insertion.
We find that ribosome-bound translocons dynamically interconvert between loosely and tightly associated conformational states and that the equilibrium between these states is remodeled at defined stages of nascent chain synthesis. We identify a common conformational change that occurs when the nascent chain becomes long enough for the first transmembrane helix to engage the translocon pore, revealing an early checkpoint in membrane protein biogenesis. Beyond this point, the conformational landscape becomes strongly dependent on the properties of the client protein. Specifically, the length, topology, and charge of inter-transmembrane loops govern subsequent rearrangements of the ribosome–translocon complex. Strikingly, long cytoplasmic loops impose distinct dynamic signatures that are highly sensitive to loop charge, revealing a surprising role of nascent-chain electrostatics in shaping the dynamics of the ribosome-SecYEG complex.
These findings establish that the ribosome–SecYEG complex is a highly dynamic molecular machine whose conformational state adjusts to its client protein. This link between membrane protein structure and ribosome–translocon dynamics provides a mechanistic framework for understanding how structurally diverse membrane proteins are accommodated by a common insertion apparatus.

21
A molecular picture of protein folding during active translation

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In vivo, all proteins have the opportunity to begin folding during their biosynthesis on the ribosome. Investigations of stalled ribosome nascent chains can reveal how being tethered to the ribosome can affect the equilibrium folding process of such ribosome-bound nascent chains. These studies, however, cannot capture the non-equilibrium nature and coupling between elongation and folding. These important dynamics can be obtained by following an order parameter such as FRET or ligand binding during active translation. We have developed an exciting methodology based on pulse-labeling hydrogen exchange, to follow co-translational folding during active translation at the level of amide hydrogen protection across all regions of a protein simultaneously. Using this approach, we observe distinct strategies that promote robust folding on the ribosome. For two proteins, we find that folding occurs hierarchically on the ribosome as regions are translated—a process that may facilitate *in vivo* folding for complex folds. For a third protein, however, folding is *delayed* by the ribosome and avoids formation of an aggregation-prone intermediate. Critically, these different mechanisms rely on tight kinetic coupling between folding and the process of elongation.

Session 7: Structure, folding, energetics and the exit tunnel

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Protein evolution with and without chaperones

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I will present recent theoretical and experimental developments aimed at understanding the two-way link between protein biophysics and protein evolution. The novel multiscale models integrate the molecular effects of mutations on physical properties of proteins, most notably their stability, into physically intuitive yet detailed genotype-phenotype relationship (GPR) assumptions. One of the key predictions from biophysics-based evolutionary models is that maintaining protein stability represents a fundamental constraint on evolutionary dynamics in many scenarios such as viral escape and antibiotic resistance. Foldase chaperones can relax this constraint opening sequence space to exploration of more diverse evolutionary pathways.

Next, I will describe experimental efforts to test these predictions in evolutionary dynamics of antibiotic resistance under the stress of antibiotic Trimethoprim (TMP) which inhibits essential *E coli* enzyme DHFR. Comparing strains evolving under endogenous conditions and under overexpression of key chaperone GroEL we found that many resistance mutations under GroEL+ were upstream of the gene leading to overexpression of DHFR whose detrimental effects were buffered by GroEL. In agreement with theoretical predictions, many missense resistant variants under GroEL+ were indeed more MG-like than under endogenous GroEL. However, in variance with all theoretical predictions, one GroEL+ resistant variant M20I was more stable and bound TMP more strongly than WT. Careful analysis of the mechanism of resistance of this variant lead us to the discovery of a new mechanism of GroEL action whereby the chaperone recognizes enhanced dynamics of the protein. Further I will discuss our recent results on the proteome level effect of Trigger factor on protein abundance and its surprising role in formation of cell flagellum.

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Kinetically stable proteins depend on cotranslational folding for efficient biogenesis

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Many proteins cannot refold after being denatured. We propose that this is possible because the ribosome gives nascent chains a special opportunity to fold correctly. If a protein reaches its native state during synthesis and then unfolds only very slowly on cellular timescales, there is little selective pressure for it to refold reversibly. Such proteins can instead be stabilized kinetically, by high barriers to unfolding, rather than thermodynamically. To test this idea, we turned to the thermophilic bacterium *Thermus thermophilus*. Proteome-wide refolding measurements show that most Thermus proteins do not refold spontaneously after chemical denaturation. Biophysical assays show that several Thermus enzymes unfold very slowly despite only modest thermodynamic stability, which suggests their stability is largely kinetic. We find that proteins whose native states are protected by high kinetic barriers rely disproportionately on cotranslational folding

to fold successfully. Finally, we identify interpretable sequence and structural features that distinguish thermodynamic from kinetic stability across many protein families. This provides a unifying molecular basis for when cotranslational folding is essential.

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CryoEM of a Variant of the SDD1 Arrest Peptide Reveals the Mechanism of Enhanced Arrest

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When a nascent polypeptide begins to fold into its three-dimensional structure while still being synthesized by the ribosome, this process is referred to as cotranslational protein folding. Traditional methods that are used to study protein folding like nuclear magnetic resonance (NMR) and X-ray crystallography fail to adequately capture intermediates of this dynamic process. The lack of effective tools to study cotranslational folding in vivo led to the development of Force Profile Analysis (FPA), which utilizes short tracts of amino residues that stall translation, called arrest peptides (APs). APs have been found and optimized in both E. coli (SecM) and human cells (XBP1u), and work as sensors which are sensitive to the amount of force exerted on the nascent chain due to protein folding or membrane insertion.
Despite its tractability as a model eukaryote, *Saccharomyces cerevisiae* has lacked a characterized arrest peptide suited to force profile analysis. This changed with the recent identification of SDD1, a previously uncharacterized yeast protein whose arrest peptide triggers ribosome quality control (RQC), the pathway that rescues stalled or collided ribosomes in eukaryotes. Because SDD1-mediated arrest engages RQC and impairs growth, it offers a readout of stalling within the genetic toolkit of yeast, complementing SecM and XBP1u, which operate in bacterial and human cells, respectively.

This discovery presents an opportunity to define the region of SDD1 that promotes arrest and to generate variants spanning a dynamic range of arrest strengths as a tool for studying cotranslational protein folding. Here, we show that single amino acid substitutions identified an arrest-enhanced variant. Structure-function analysis of this variant leads us to propose that additional π-stacking between the variant and the ribosome tunnel wall prevents activation of the peptidyl-transferase center, resulting in enhanced stalling and growth defects.

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Investigating the fitness landscapes of 2A peptides

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2As are short peptides, consisting of 18-22 amino acids, which interact with the ribosome exit tunnel to modulate the co-translational hydrolysis of the peptidyl-tRNA bond. While recent work from our lab and others has employed in silico homology-based searches to expand the number of 2A peptides by an order of magnitude, we do not yet understand the molecular underpinnings of their activity. We have found that 2A peptides, prior to hydrolysis, are resistant to ribosome catalysis, as measured by puromycin reactivity and ability to mediate termination. We next interrogated the sequence drivers of these biochemical properties and quantified the effect of tens of thousands of single and double amino acid mutations on examples from the two major 2A-peptide classes. Both classes of 2A peptides exhibit a high level of sequence plasticity, with only six positions constrained within the broader element. For instance, we identified the -3 position in both classes as a key driver of this plasticity. Excitingly, we observe a strong correlation between impaired termination and co-translational hydrolysis suggesting that these aberrant translation events are functionally related. Taken together these results suggest that the activity landscapes of 2A peptides, like that of proteins and catalytic RNAs, are continuous and thereby evolvable via natural selection. These findings may have broad implications for our understanding of the evolution and abundance of nascent chain mediated translation control.

Session 8: Synonymous codons, translation kinetics, and folding

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A new view for synonymous codon usage

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Historically, substitutions between synonymous codons were considered “silent” because such substitutions do not alter the encoded amino acid sequence. Yet paradoxically, the standard advice for enhancing heterologous gene expression is to re-code a gene using the most common codons of the host organism (“codon optimization”), to exploit the well-known correlation between codon usage frequency and ribosome elongation rate. Over the past decade, a partial resolution of this paradox has emerged as it has become increasingly clear that synonymous codon substitutions can significantly impact essentially all mechanisms related to gene expression, including DNA plasmid copy number, mRNA export from the nucleus, splicing efficiency, translational efficiency and co-translational protein folding yield. While the resulting emerging “new view” of synonymous codon usage has begun to resolve the paradox above, new challenges have emerged. In particular, the impact of synonymous substitutions is typically recorded as a single downstream effect (e.g., changes to GFP fluorescence intensity), which can mask the specific effects on diverse mechanisms. My talk will cover recent advances in my lab and others to develop model systems capable of deconvoluting the varied impact of synonymous codon substitutions, as a step towards developing a predictive set of rules governing the impact of synonymous codon usage on gene expression, including effects on co-translational protein folding.

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Beyond the genetic code: position-specific codon usage regulates translation and co-translational protein folding

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The genetic code is degenerate, yet synonymous codons are increasingly recognized as important determinants of protein biogenesis. Rather than simply encoding the same amino acid, synonymous codons and codon pairs can alter translation kinetics, ribosome pausing, and the temporal environment in which a nascent polypeptide folds. We investigated this concept using complementary computational and experimental approaches, focusing on therapeutically relevant proteins, including ADAMTS13.

Analysis of homologous coding sequences revealed that codon and codon-pair usage is not uniform throughout a transcript. Instead, distinct sequence positions and functionally important regions exhibit conserved, position-specific codon and codon-pair biases. Notably, codon and codon-pair usage showed strong associations at individual sequence positions despite weak or absent relationships when analyzed across entire genes. These findings suggest that evolutionary conservation of synonymous codons may encode positional information relevant to translation and potentially co-translational folding.

We next experimentally tested whether altering this information affects protein biogenesis. Synonymous recoding of human ADAMTS13 using either codon optimization or codon-pair optimization, without changing its amino acid sequence, produced markedly different translation kinetics and protein phenotypes. Codon-pair optimized ADAMTS13 retained translational pauses present in the wild-type sequence, whereas conventional codon optimization eliminated many of these features. In cell-free translation, recoded variants displayed substantially different translation rates and yields. In mammalian cells, synonymous recoding altered extracellular expression, protein stability, folding dynamics, substrate interactions, post-translational modification patterns, and cellular bioenergetics. Increased interaction with the ER chaperone BiP and altered ER-stress responses further suggested that the cellular machinery responds differently to proteins produced from synonymous sequences.

Together, these findings support a model in which synonymous sequence information can influence the trajectory of protein folding by modulating the kinetics and context of translation. They also challenge the conventional assumption that maximizing codon usage or translation speed necessarily improves recombinant protein production. Instead, optimal gene design may require

preservation or reconstruction of position-specific translational patterns that allow the nascent protein to fold appropriately as it emerges from the ribosome. This framework has important implications for understanding co-translational protein folding and for designing therapeutic proteins and gene-based medicines.

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A recently discovered class of protein misfolding links synonymous mutation effects on structure and function to aging

Edward P. O'Brien

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Non-covalent lasso entanglement (NCLE) protein misfolding was discovered by my lab in 2021. It involves the formation of soluble, near-native, non-functional states that can, in some cases, be as long-lived as the native ensemble. Because of their native-like structure, these misfolded states do not necessarily form aggregates. This class of misfolding—which involves the incorrect intertwining of protein backbone segments—is likely to be widespread, as it arises from geometric constraints inherent to linear polymers. Some evidence suggests that up to half of globular proteins may have subpopulations in these states. Here, by synthesizing diverse datasets—including Ubq-MS, LiP-MS, and biochemical data—and using statistical methods and computer simulations, I will provide evidence that (1) synonymous codon mutations alter the population of these misfolded states co- and post-translationally; (2) proteins more likely to misfold in this manner follow divergent fates, with some rapidly degraded and others appearing to bypass ubiquitin–proteasome degradation; and (3) these misfolded states are more likely to occur in proteins that exhibit structural changes as organisms age. Thus, we have linked NCLE misfolding to the fundamental subcellular process of protein degradation and the emergent property of subcellular aging. Future efforts will focus on identifying protein drug targets to prevent this class of misfolding altogether.

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Folding of the oncolytic virus (OV) proteome in cancer cells: the impact of endogenous chaperones and codon optimization

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Oncolytic viruses (OVs) have emerged as promising cancer therapeutics because they combine direct tumor cell lysis with the induction of systemic antitumor immunity. In contrast to conventional therapies (chemotherapy and radiotherapy) and rapidly advancing checkpoint inhibitor–based immunotherapies, which are often associated with significant adverse effects, OVs generally produce milder side effects and offer less toxic treatment options. Both natural and genetically engineered oncolytic viruses from various viral families (e.g., *Adenoviridae*, *Herpesviridae*, *Paramyxoviridae*, *Picornaviridae*, and *Reoviridae*) are currently being evaluated in clinical trials. However, OV development has been hampered by several limitations, including: (i) modest clinical efficacy; approximately 20% of patients respond to treatment; (ii) rapid development of antiviral adaptive immunity, which limits repeated dosing and systemic delivery; and (iii) potential systemic toxicity. For example, some engineered viruses expressing potent cytokines such as GM-CSF are not suitable for systemic (e.g., intravenous) administration due to high toxicity. Enhancing OV selectivity, improving our ability to predict their efficacy against individual tumors, and accelerating their capacity to destroy cancer cells represent major frontiers in precision cancer immunotherapy. As such, current efforts focus on engineering viruses that selectively target, replicate within, and lyse tumor cells while sparing healthy tissues, thereby reducing toxicity and improving therapeutic outcomes. This work is based on the following premises: (i) Folding of the viral proteome is a co-translational process that relies heavily on the host cell's chaperone machinery, primarily involving members of the Hsp70 and Hsp90 families; (ii) Rapidly

proliferating cancer cells generally exhibit elevated levels of molecular chaperones; (iii) Viral genomes often exhibit codon usage patterns that differ significantly from, or are distant relative to the codon usage bias of their host cells; (iv) tRNA repertoire and codon usage patterns of normal cells differ from those of rapidly proliferating cells, such as cancer cells. We found that endogenous chaperone levels in individual cancer cells can serve as predictors of their susceptibility to oncolytic viral killing. In malignancies such as mesothelioma, an aggressive cancer arising from the pleura and peritoneum, chaperone levels may serve as a companion diagnostic to identify patients most likely to benefit from oncolytic virotherapy, thereby helping avoid unnecessary treatment and potentially improving clinical outcomes. We also demonstrate that codon optimization can enhance cancer-selective viral replication by exploiting differences in tRNA repertoires and codon usage between normal and rapidly proliferating cells. This finding provides a novel strategy for engineering oncolytic viruses with improved tumor selectivity and reduced toxicity.

Session 9: The ribosome: decoding, recording, and the exit tunnel

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Origins and impacts of translational errors

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Translational fidelity is established through specific aminoacylation of tRNAs and subsequent decoding on the ribosome. Errors in protein synthesis are associated to protein misfolding and misfunction, and lower levels correlate with longer lifespan. We have measured proteome-wide misincorporation frequencies in two experimentally tractable eukaryotic systems, yeast and mouse, using mass spectrometry. We find translation fidelity is organism and organ specific. The measured error frequencies correlate with codon:anticodon pairing thermodynamics and tRNA pool composition, which can have synergistic or opposing effects on fidelity depending on organism or tissue type. Most identified protein errors carry a negative fitness burden. Aging experiments in our two systems reveal that, with age, error frequencies increase in post-mitotic cells, but

not so in mitotic ones. These changes correlate with remodeling of the tRNA pool, which we identify as a crucial regulator of translation fidelity during lifespan.

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Nascent chains tune translational decoding and recoding

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Efficient protein synthesis hinges upon the processivity of translational elongation. Certain RNA structures can promote translational stalls that trigger cotranslational shifts in the ribosomal reading frame and the subsequent termination of translation. Our lab recently found that ribosomal frameshifting (RF) can also be stimulated by structural features within the nascent chain. Using biochemical, cellular, and computational approaches, we have found that the coupling between nascent TMDs and repetitive “slippery” transcript sequences is sufficient to promote RF. Through a mechanistically-informed computational search, we have unambiguously identified 32 predicted RF peptides within a single public proteomic data set. To broaden our understanding of this feedback, we explored additional nascent chain features associated with viral RF motifs. An investigation of the HIV RF motif shows that the cotranslational folding and metalation of its Ncp7 zinc finger within the exit tunnel also enhances RF. A subsequent informatic search for comparable sequence motifs identifies thousands of similar sites in the human transcriptome, and proteomic searches identify several frameshifted peptides derived from these motifs within the same proteomic data. Contextual cues and exploratory experiments suggest this feedback can occur in response to cotranslational misfolding and/ or aberrant splicing. Interestingly, we find that transcripts encoding these RF motifs are more likely to undergo mRNA decay. However, we also identify several transcripts that appear to encode distinct protein domains encoded in alternative reading frames. Together, these findings suggest

transitions in the nascent chain can promote translational recoding events that can tune transcript stability and/ or promote the production of alternative proteoforms.

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A Z-shaped nascent chain in the ribosome exit tunnel blocks translation termination

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Arrest peptides can hold a growing polypeptide on the ribosome at a defined length, making it possible to study folding, processing, and targeting as the chain emerges from the exit tunnel [1,2]. Ribosome profiling revealed a striking example of naturally occurring arrest downstream of the annotated stop codon of human AMD1, which encodes an enzyme in polyamine biosynthesis. A subset of ribosomes read through this stop codon and synthesize a conserved C-terminal extension. When the ribosome reaches the next in-frame stop codon, the extension blocks termination. To determine how the extension prevents peptide release, we used cryo-electron microscopy to determine the structure of the stalled ribosome with eukaryotic release factor 1 (eRF1) and ABCE1 bound [3]. Inside the exit tunnel, the arrest peptide doubles back into a compact Z shape, bringing three conserved residue together clamping a single rRNA nucleotide. The resulting clamp anchors the peptide in the tunnel and positions an arginine side chain in the peptidyl transferase center acting as a hook. There, the arginine hook prevents the catalytic GGQ motif of eRF1 from reaching the ester bond linking the peptide to its tRNA. Mutating the clamp residues reduced ribosome stalling, and replacing the arginine that projects into the active site promoted peptide release. These findings explain

how the Z-shaped AMD1 arrest peptide blocks its own release even after eRF1 has bound the ribosome. Analysis of aggregated ribosome profiling data identified several other genes with a similar pattern of stop codon readthrough followed by a discrete downstream pause, raising the possibility that read-through generates arrest peptides beyond AMD1.

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33

Ribosome-protecting ABCF proteins extract the nascent peptide to displace tunnel-bound antibiotics and confer resistance

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To counter translational roadblocks, cells have evolved dedicated auxiliary translation factors. In bacteria, antibiotic-resistance ATP-binding cassette proteins of the F type (ABCFs) resolve translational arrests induced by antibiotics that target the ribosomal peptidyl-transferase center or the nascent peptide exit tunnel. The prevailing model holds that ABCFs restore translation by displacing the antibiotic from its binding site on the ribosome. However, how ABCFs accomplish this remains unclear, especially for antibiotics that bind deep within the tunnel, which is occupied by a nascent peptide. To gain insight into the molecular mechanism of

ABCF action, we use selective ribosome profiling to globally map where MsrE, an ABCF protein that protects bacteria against tunnel-targeting macrolide antibiotics, engages translating ribosomes in cells challenged with macrolides. Our data reveal that, in contrast to the prevailing model, MsrE does not associate with ribosomes already stalled at well-defined macrolide arrest sites. Instead, MsrE preferentially engages antibiotic-bound ribosomes during the initial rounds of translation, before they reach the problematic sites. Our cryo-EM structural analysis of an MsrE-bound translating ribosome suggests that, upon binding, the ABCF protein first extracts a short nascent peptide linked to the P-site tRNA from the exit tunnel. Only then can MsrE reach the tunnel-bound macrolide and displace the drug. The tunnel-bound antibiotic likely facilitates MsrE-induced extraction of the nascent peptide by disrupting a network of hydrogen bonds that anchor the C-terminal segment of the peptide to 23S rRNA nucleotides in the tunnel. Thus, the ribosome, the nascent peptide, and even the bound macrolide all contribute to the ability of ABCFs to rescue cellular translation.

34

New molecules from nature's most sophisticated catalyst

Alanna Schepartz

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As far as we know, the translational apparatus has evolved for billions of years to perform a single chemical reaction: formation of an amide bond between two α-amino acids brought into proximity by tRNAs within the ribosomal PTC. In cells, the chemistry possible within the PTC of natural ribosomes includes reactions of more than 200 different non-proteinogenic α-amino- and hydroxy acids and a small number of β-amino and hydroxy acids. But there is a big chemical world beyond β-amino and hydroxy acids. One can imagine two different strategies to expand the chemistry of translation further to generate diverse new-to-nature drug-like molecules and materials with novel backbone architectures. These materials offer opportunities for expanded function, predictable structure, tunable stability, and orthogonal reactivity. They also could be useful for probing protein folding of ribosomal nascent chains. This lecture will describe recent progress towards these goals.

Tuesday, December 15

Session 6: The molecular origami of proteins, how we study it, and relevance to disease

35

Structures and energetics of protein folding intermediates on the ribosome

Julian O. Streit, Sammy H. S. Chan, Tomasz Włodarski, Alkistis N. Mitropoulou, Anaïs M. E. Cassaignau, Ivana V. Bukvin, Christopher A. Waudby, Lisa D. Cabrita, John Christodoulou

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Most proteins begin folding during biosynthesis on the ribosome and a hallmark of the co-translational folding (coTF) process are highly stable folding intermediates. These partially folded intermediates are typically not observed in isolation and persist for long times during translation. CoTF intermediates are a common feature for many proteins and important for folding fidelity and post-translational protein function, yet their structures have remained inaccessible to traditional structural biology techniques. Here, we uncover the structures and energetics of coTF intermediates of an immunoglobulin-like domain, combining high-resolution experimental data with state-of-the-art computational structural biology. We developed an integrative framework that unites 19F NMR observables—including chemical shifts and paramagnetic relaxation enhancement (PRE) data—with AI-guided protein engineering and extensive all-atom molecular dynamics simulations. While experimental measurements report on distances and contacts within the protein, computational modelling was essential to resolve these data into atomic-level structural ensembles, enabling visualisation of co-translational folding intermediates. Our results reveal two long-lived, highly stable intermediates that arise uniquely during biosynthesis, highlighting the ribosome as an active modulator of the folding energy landscape. These intermediates adopt conserved structural motifs across the domain family and, strikingly, permit distinct binding to a molecular chaperone and suggest parallel folding pathways. More broadly, our work establishes a generalisable strategy exploiting AI-guided

experimental design, physics-based simulations and integrative structural biology for discovering previously inaccessible conformational states in their native cellular context.

36

Structural and mechanistic insights into huntingtin biosynthesis

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The earliest decision between folding and misfolding for most proteins is made during their biosynthesis on the ribosome. During this process, the ribosome can slow down or pause at specific positions, transiently altering which regions of the nascent chain are able to sample conformations, thereby influencing the fate of the newly synthesised protein. However, how the information encoded in the mRNA and protein sequences act together to guide biosynthesis remains poorly understood, especially for disease-associated proteins. Here, we explore the structural and mechanistic aspects of huntingtin biosynthesis on the ribosome, a protein implicated in Huntington's disease.
Using an integrative approach combining ribosome profiling, toe-printing and DMS-seq, together with single-particle cryo-EM and all atom MD simulations, we provide structural and mechanistic insight into how specific features of huntingtin mRNA and nascent chain jointly shape its biosynthesis on the ribosome.

37

Live-cell co-translational folding tracking reveals bidirectional coupling between translation and protein folding

Rhiannon M. Sears†, Luis U. Aguilera†, Tristan M. Bunting, Ning Zhao*

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†These authors contributed equally to this work.

Translation elongation and protein folding have long been proposed to coordinate during co-translational folding, yet the lack of technologies capable of simultaneously tracking both processes in live cells has hindered mechanistic understanding of this relationship. Here, we developed co-translational folding tracking (coTFT), a live-cell imaging platform that directly and simultaneously tracks translation and protein folding from individual mRNAs. Using reporters with distinct folding kinetics, we found that differences in protein folding kinetics were accompanied by corresponding changes in translation elongation rates despite nearly identical nucleotide and amino acid sequences. Conversely, perturbing translation elongation markedly altered co-translational folding outcomes. Combining coTFT with mathematical modeling enabled estimation of reporter folding times on translating ribosomes in live cells, confirming their distinct folding kinetics. Together, our results establish coTFT as a quantitative imaging platform for studying protein co-translational folding in live cells and provide the first direct experimental evidence that translation elongation and protein folding are bidirectionally coupled during co-translational folding.

38

New molecules from nature's most sophisticated catalyst

Gabriel Rocklin

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Proteins with similar structures can often vary widely in their folding stability and in the energies of conformational fluctuations occurring throughout the structure. These biophysical properties influence protein function, cellular abundance, aggregation propensity, and immunogenicity, but remain unknown and impossible to predict for most proteins. We developed new high-throughput approaches to

quantify folding stability and conformational fluctuations for thousands to millions of proteins in parallel, creating unprecedented large-scale data linking sequence to biophysical properties. Using these data, we are developing new models to predict protein folding stability and conformational fluctuations for applications in biology, medicine, and protein design.

Poster Abstracts

Posters should generally fit within a space of 48 inches x 96 inches (121.92 cm x 243.84 cm). Supplies (poster pins) for hanging posters will be available.

All posters will be presented during both Sunday and Monday Poster Sessions 1 and 2.

1

Live-cell single-molecule tracking reveals local codon optimality as a key regulator of co-translational folding

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Protein folding can begin as nascent chains emerge from the ribosome, a process known as co-translational folding. The fidelity of this process depends on the tight coordination between translation and folding kinetics. Deoptimized codons have long been implicated in facilitating co-translational folding by slowing translation elongation. However, our live-cell single-mRNA translation tracking data revealed that global codon deoptimization does not substantially alter overall translation elongation rates, despite significantly reducing co-translational folding outcomes. These findings suggest that codon optimality regulates co-translational folding through a more complex and previously unrecognized mechanism. Despite its fundamental importance, how codon optimality regulates co-translational folding in live cells has remains poorly understood, due to the lack of technologies capable of directly visualizing folding events on translating ribosomes. To overcome this limitation, we developed a live-cell single mRNA co-translational folding tracking technology that enables direct and simultaneous tracking and quantification of translation and folding at individual translation sites. Leveraging this technology, we demonstrate that the spatial distribution of deoptimized codons, rather than global codon deoptimization, is a key determinant of co-translational folding outcomes. Our study establishes a mechanistic framework for understanding how local codon optimality modulates translation kinetics to shape co-translational folding, thereby advancing fundamental understanding of the principles that maintain co-translational folding homeostasis.

2

Nascent chain dynamics slow during elongation on the ribosome

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During translation, many single domain proteins cannot fold unless almost the complete domain has exited the ribosome tunnel, but it is not known if the nascent chain is completely disordered and free to reconfigure during elongation. Previous measurements of intramolecular diffusion of unfolded proteins have shown that dynamics can span 3 orders of magnitude, with intrinsically disordered proteins having fast diffusion and unfolded proteins under folding conditions having extremely slow diffusion. In this work we have measured intramolecular diffusion of free and ribosome-associated nascent chains of the B1 domain of protein G (GB1) using two different optical techniques and found that the nascent chain traverses this dynamic range during elongation. For intermediate lengths of the nascent chain, we find that free chains are slower than the shortest length measured but faster than the ribosome-associated chain, suggesting that dynamics are further slowed by ribosome interactions. These types of measurements can be coupled with measurements of structure formation to illustrate a more nuanced free energy landscape of co-translational folding.

3

How non-native interactions and Trigger factor shape the cotranslational folding of HemK NTD

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Proteins fold cotranslationally through the formation of compact intermediates both inside and outside the ribosomal peptide exit tunnel. At the same time, nascent polypeptides can interact with molecular chaperones and biogenesis factors that modulate their folding trajectories. However, the structural and biophysical properties of these folding intermediates, as well as the contribution of chaperones to cotranslational folding, remain largely unknown. In this work, we combined all-atom Monte Carlo simulations with photoelectron transfer–fluorescence correlation spectroscopy (PET-FCS) to probe nascent-chain (NC) dynamics during cotranslational folding. We show that cotranslational folding of the HemK NTD proceeds through non-native intermediates stabilized by interactions involving residues that do not contribute to the final folded structure. Disruption of these interactions destabilizes the intermediates and shifts the folding pathway toward the misfolded state.
In cells, molecular chaperones help prevent protein misfolding. We found that trigger factor (TF) exerts additional tension to the nascent peptide, destabilizing both native-like and potentially misfolded premature structures and thereby delaying final folding. This unfoldase mechanism keeps the nascent chain flexible and capable of adopting its correct native conformation once the complete domain has formed. However, TF cannot overcome the folding defect caused by disruption of the hydrophobic core.
Our findings reveal an unexpected role for surface-exposed residues in shaping cotranslational folding and demonstrate how the chaperone TF can mitigate cotranslational misfolding and provide insights into strategies for improving protein-folding prediction and protein design.

4

Quasi-continuous cotranslational compaction and folding of a multidomain protein

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Most proteins start to fold cotranslationally as they come off the ribosome. So far, studies of cotranslational folding have focused mainly on small, single-domain proteins. Here, we have used Force Profile Analysis to study the cotranslational folding of Firefly Luciferase, a complex 550-residue protein composed of an N-terminal domain (NTD) encompassing two split Rossmann folds (RF-1, RF-2) and a beta-roll, and a flexibly attached C-terminal domain (CTD). The folding process is characterized by a quasi-continuous series of compaction/folding steps that generate intermediate-size pulling forces on the nascent chain, punctuated by a prominent high-force event that represents the folding of the RF 2 domain, and a few low-force instances that likely indicate the formation of distinct folding intermediates. Trigger Factor interacts extensively with the nascent chain when the central part of RF-2 and the early parts of the CTD are synthesized. Our analysis uncovers a cotranslational compaction/folding process that is rich in detail and not just a simple succession of a few distinct, cooperative folding transitions.

5

A Ribosome-binding GTPase sustains protein synthesis across domains of life

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Productive protein synthesis requires ribosomes to navigate diverse mRNA sequences while coordinating nascent-chain folding, targeting, and maturation. Although pathways that recognize and eliminate defective translation products are well established, how cells sustain productive synthesis before committing to

quality control and how cells distinguish physiological ribosomal pauses versus pathological stalls remains poorly understood. We identify a conserved function of Obg/Rbg/Drg ribosome-binding GTPases that connects translation activity with nascent-chain handling and cellular protein homeostasis.

Cross-species complementation demonstrates that bacterial ObgE rescues growth and translation-associated defects in yeast lacking Rbg proteins and human cells lacking Drg1. Rescue requires GTPase activity, supporting conservation of a catalytic function across domains of life. These findings reveal a shared functional capacity despite substantial evolutionary divergence in protein architecture and cellular context. To define the translational landscape associated with this pathway, we combined selective ribosome profiling, transcript analysis, and interaction proteomics. Rbg1 preferentially associates with late elongation ribosomes, with distinct ribosome-occupancy patterns among mRNAs encoding cytosolic, nuclear, mitochondrial, and endoplasmic-reticulum proteins. Its interactome includes nascent-chain chaperones and protein-biogenesis factors, including the ribosome-associated complex, Hsp70 proteins, and the nascent polypeptide-associated complex. These associations place Rbg1 at an interface between productive translation and cotranslational protein maturation.

Structural studies reveal how Rbg/Drg complexes engage translating ribosomes. Our earlier structure established contacts between the GTPase, the large-subunit GTPase-associated region, and A-site tRNA, with its partner protein contributing interactions with the head-shoulder gap in the small subunit. I will also report our recent work in progress on new structures of human Drg1/Dfrp1 bound to ribosomes in the GTP or GDP-Pi state, which provide a framework for examining how GTPase conformational changes are coupled to ribosome engagement and continued translation. Mechanistically, ObgE enhances puromycin incorporation on stalled ribosomes in a GTP-hydrolysis-dependent manner, supporting restoration of peptidyl-transfer competence. Together, cross-species complementation, biochemical measurements, and structural observations support a model in which Obg/Rbg/Drg GTPases protect and help ribosomes overcome translation impediments and sustain nascent-chain synthesis before terminal quality-control pathways intervene.

Our findings support hat Obg/Rbg/Drg GTPases act at a translational decision point, preserving productive protein synthesis and providing a foundation for understanding how elongation dynamics are coordinated with cotranslational folding across domains of life.

6

Developmentally controlled co-translational assembly of the synaptonemal complex

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Co-translational assembly ensures that subunits of multiprotein complexes reach correct stoichiometry and avoid the premature aggregation of orphaned subunits. Yet how RNA regulation, translation, and assembly are coordinated in space and time, especially during developmental transitions, remains poorly understood. Here, we use the C. elegans synaptonemal complex (SC), a zipper-like scaffold comprising SYP-1 through SYP-5 and SKR-1 that assembles at meiotic entry, as a tractable system to dissect this coordination.

Biochemical reconstitution identifies two stable SC subcomplexes, SYP-1/SYP-2/SYP-5 and SYP-3/SYP-4/SKR-1, that fail to associate when produced separately and then mixed, suggesting that productive assembly requires co-translational engagement. Single-molecule imaging in intact gonads reveals that syp-3 and syp-4 mRNAs specifically colocalize in the cytoplasm, peaking immediately before meiotic entry. This colocalization diminishes upon translation inhibition, and nascent SYP-4 protein is detected at mRNA foci, directly linking mRNA organization to ongoing translation. Systematic RNA immunoprecipitation defines an ordered co-translational pathway: SYP-3 and SYP-4 form an early core that recruits additional subunits before engaging nascent SYP-5 as the final step, a directionality matching known structural interfaces. Consistent with a requirement for coordinated assembly, loss of individual SC subunits destabilizes their orphaned partners, which are cleared by the proteasome. Notably, most mRNAs of SC subunits are bound by PUF RNA-binding proteins that are downregulated at meiotic entry, relieving translational repression and enabling synchronous translation, thereby coupling developmental timing with co-translational assembly.

Together, our findings support a model in which SC biogenesis is coordinated by developmentally timed mRNA organization and ordered co-translational assembly. More broadly, we propose that developmental RNA regulons can coordinate the

timing and spatial organization of functionally related transcripts to enable rapid, accurate complex formation when cellular transitions occur.

7

Diverse nascent chains adopt a common extended conformation within the ribosome exit tunnel

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Protein folding can begin during translation, while the nascent chain (NC) is still confined within the ribosome exit tunnel. Previous studies have shown that some NCs can form compact states in the tunnel, and suggested that the tunnel environment favours α-helix folding. Here, we used cryo-electron microscopy to systematically assess the conformational landscape of NCs in the exit tunnel. We determined 75 structures of ribosome-nascent chain complexes (RNCs) spanning diverse natural and de novo designed protein sequences. By analysing NCs at successive lengths, differing by only a few amino acids, we determine how the same sequences change conformation as they vectorially emerge from the ribosomal exit tunnel.
Despite differences in sequence and native fold, NCs predominantly adopt extended conformations within the tunnel, with β-strand-like and polyproline-II-like geometries that differ from conformational preferences predicted from amino acid sequence alone. Across diverse sequences, we identify recurrent sites of interaction between NCs and the ribosome. These interaction hotspots are associated with local differences in NC trajectories and accommodate variations in NC charge, hydrophobicity and bulk. In contrast to the predominantly extended conformations, well-resolved α-helices are rare and occur primarily in cases where favourable electrostatic interactions stabilise secondary structure. As NCs emerge from the tunnel, additional intrachain contacts and interactions with the ribosomal surface can stabilise structures that are present in the native fold.

Together, these findings show that the ribosomal exit tunnel generally favours extended conformations of the nascent polypeptide, while local interactions, primarily determined by NC charge, influence their trajectories and secondary structure formation. Our structures provide a systematic description of how ribosomal interactions influence the earliest stages of protein folding during vectorial emergence.

8

Translocon remodelling modulates ribosomal frameshifting and the maturation of the sindbis virus structural polyprotein

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Like other enveloped RNA viruses, alphaviruses synthesize and assemble their envelope glycoproteins at the endoplasmic reticulum (ER) membrane. There, protein-conducting channels called translocons provide nascent proteins access to the membrane and allow them to fold into their correct shapes. We previously showed that a hydrophobic segment in the Sindbis virus structural polyprotein forms cotranslational interactions with the translocon that enhance −1 programmed ribosomal frameshifting (−1PRF), a recoding event that regulates polyprotein biogenesis. Recent discoveries concerning translocon remodeling suggest this segment, which corresponds to the second transmembrane domain of the E2 protein, could serve as a signal that recruits the multipass translocon (MPT).

Here, we show that knocking out certain components of the MPT increases −1PRF efficiency. These differences in recoding coincide with changes in the membrane topology of the nascent polyprotein and in its downstream proteolytic processing in a manner that ultimately reduces viral fitness. Together, our results indicate that −1PRF and spike protein maturation in alphaviruses is tuned by the dynamic remodeling of the ER translocon. Such coupling could allow polyprotein biogenesis to adapt to different stages of viral replication and to distinct host or vector environments.

9

Co-translational folding and mechanical forces on stalled nascent chains control mRNA-free protein synthesis

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Faithful translation of mRNAs into proteins is crucial for cellular function and life. However, defective mRNAs or amino acid deficiency can cause ribosomes to stall and collide, generating incompletely synthesized polypeptides that impose proteotoxic stress. To address this, all domains of life have evolved the ribosome-associated quality control (RQC) pathway, which detects collided ribosomes and disassembles them into subunits. Remarkably, the large ribosomal subunit can then resume protein synthesis without an mRNA template or the small subunit, adding amino acids to the C-terminus of stalled polypeptides. These untemplated extensions, known as “CAT tails,” can be up to ~60 amino acids long and represent a central dogma-defying form of protein synthesis. Our research in yeast revealed that, in the absence of mRNA, mechanical pulling forces on stalled nascent chains regulate CAT tail structure and function. These forces can be generated by co-translational protein folding and/or by Cdc48 extracting the ubiquitylated nascent chain from the ribosome. High pulling force favors short, alanine-rich CAT tails, which promote release of the nascent chain from the ribosome and its subsequent

degradation. In contrast, low pulling force favors long, threonine-rich CAT tails that are prone to aggregation. We also discovered that CAT tailing is self-terminating: as CAT tails are synthesized, they generate pulling force on the nascent chain, which favors incorporation of alanine over threonine, that ultimately terminates CAT tailing. This work established a framework for understanding the paradoxical role of CAT tailing in both targeting stalled polypeptides for clearance and predisposing them to aggregation, a line of research that continues in my laboratory at Virginia Tech. Dysfunctional CAT tailing contributes to motor neuron disorders and neurodegeneration in humans, flies, and mice, underscoring the human health relevance of studying RQC and CAT tailing.

10

Structural basis for context-specific inhibition of the ribosome by macrolides

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The ribosome's peptidyl transferase center (PTC) catalyzes peptide bond formation during protein synthesis and is targeted by many antibiotic classes. Remarkably, macrolides that bind in the peptide exit tunnel some ~10Å away from the PTC also remotely inhibit PTC and cause translational arrest depending on the synthesized polypeptide sequence. The Arg/Lys-X-Arg/Lys (also known as +X+) motif is particularly susceptible to this inhibition, as peptidyl-tRNA carrying nascent peptide with penultimate arginine or lysine residue fails to react with aminoacyl-tRNA carrying the same amino acids in the presence of macrolides. While structural studies of macrolide-bound ribosomes have shed light on the context-specific nature of this inhibition, the precise roles of the drug, ribosome, and tRNA in modulating PTC activity remain unclear. In this study, we present a detailed structural analysis of ribosome-nascent chain complexes (RNCs) that represent either arrested or non-arrested states, containing various combinations of peptidyl- and aminoacyl-tRNAs, with or without macrolides. Our findings reveal a dynamic interaction between the ribosome-bound drug, the nascent peptide, and the incoming amino acid, which collectively modulates PTC function. This lays the

foundation for designing antibiotics that can overcome drug resistance by preventing the induction of inducible erm genes in pathogens.

11

The long reach of a small chaperone: how the flexible arms of NAC coordinate nascent protein processing at the ribosome

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The nascent polypeptide-associated complex (NAC) is a conserved ribosome-associated heterodimer that plays multiple roles in coordinating co-translational protein biogenesis. NAC contains a central globular domain, positioned near the polypeptide exit tunnel (PET) of the ribosome, from which four extended flexible arms present recruitment sites for ribosome-associated protein biogenesis factors (RPBs). For example, the C-terminal ubiquitin-associated (UBA) domain of NACα recruits N-terminal acetyltransferases (NATs) and signal recognition particle (SRP), and the C-terminal VPDLV motif of NACβ recruits methionine aminopeptidase 1 (MetAP1) and N-terminal myristoyltransferases (NMTs). How these recruitment events lead to substrate-specific activities of the recruited RPBs remains incompletely understood.

Here, single-molecule Förster resonance energy transfer (smFRET) on a total internal reflection fluorescence (TIRF) microscopy platform reveal extensive, RBP- and substrate-regulated conformational dynamics of NAC arms on translating ribosomes. The binding of NatA/D can induce the NACα C-terminal arm to adopt an extended conformation, in which its UBA (and associated NATs) is far from the ribosome, or a compact conformation, in which the enzymes dock near the PET opening. On NatA/D substrates, transition between the two conformations is dictated by MetAP-mediated excision of initiator methionine, ensuring sequential and sequence-specific modification of the nascent chain by these enzymes. Analogous conformational rearrangements of the NACα arm were observed with NatA on RNCs bearing an NMT substrate. However, binding of MetAP1 or NMT1 to NACβ prevents NatA from docking near the PET opening, thereby favoring N-terminal myristoylation over acetylation.

Our findings demonstrate that the NAC arms not only recruit diverse RPBs but also regulate their access to the correct nascent protein substrate with substantial inter-subunit crosstalk. In this way, the dynamics of the NAC arms form a coordinated molecular network for integrating factor recruitment with accurate co-translational nascent protein processing.

12
Towards proteome-wide profiling of cotranslational folding mechanisms

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Nascent polypeptides begin to fold during translation before synthesis is complete. Though we know relatively little about these processes, it is clear that proteins vary with respect to when and how they acquire structure on the ribosome. To survey cotranslational folding coordinates at the proteomic scale, we developed a genetic platform that leverages arrest peptides to compare length-dependent maturation of 446 E. coli proteins in parallel. Overall, this library contains 11,884 progressive N-terminal fragments of these proteins, with each protein sampled at two-residue resolution. To control for variations in cellular degradation we incorporated this collection into two distinct reporter constructs including a stalled WT SecM arrest peptide and a non-stalling P166A SecM variant. By comparing the signals of each fragment across these backgrounds, we can identify specific nascent chain lengths at which each protein begins to fold. Cells expressing these libraries exhibit distinct distributions of the reporter signal, with higher signal for the un-stalled P166A-SecM library than the stalled WT-SecM library. To quantitatively compare the readthrough generated by each fragment, we used FACS to fractionate cells expressing these libraries into quartiles based on the signal of their expressed reporter then applied Oxford Nanopore sequencing to track the signal of individual constructs. We are currently optimizing computational analyses to generate a

statistically robust scoring system. Once complete, our results will facilitate systematic analyses of the molecular features that give rise to cotranslational folding transitions across diverse proteins.

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Co-translational cycle of mammalian RAC-Hsp70 chaperone system

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Ribosome-associated complex (RAC), composed of DNAJC2 (J-domain protein) and HSPA14 (non-canonical Hsp70) in human, is a conserved eukaryotic chaperone which couples with Hsp70 proteins in mediating co-translational folding of nascent proteins. Little is known about mammalian RAC co-translational function cycles, and many mechanistic details are also unclear in yeast RAC studies. In this study, we show RAC activating Hsp70 ATPase activity upon ribosome binding and confirm independent ribosome interaction of RAC and Hsp70 which are both moderate. Both DNAJC2 J-domain/Hps70 and HSPA14-NBD/Hsp70-NBD interactions are essential for RAC-Hsp70 co-translational function cycle in terms of Hsp70 interactions and RAC-mediated Hsp70 ATP hydrolysis rate. Single-molecule and ensemble fluorescent resonance energy transfer (FRET) confirm that Hsp70 interaction on nascent polypeptides is RAC-mediated and sequence-specific, and high-affinity Hsp70-nascent polypeptide interactions are enabled by RAC-mediated ATPase cycle. RAC is displaced by nascent polypeptide-bound Hsp70 after catalyzing ATP hydrolysis, which is likely to recycle RAC for turnover. Overall, we provide the basis for understanding the co-translational function of RAC and Hsp70 in mammalian system.

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RIBOFRIEND: Mapping co-translational factors` interplay on individual transcripts

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Ribosome-associated proteins, including molecular chaperones, co-translational assembly factors, and quality-control machinery, coordinate nascent-chain folding and maturation in the crowded cytoplasm. Although many of these systems have been individually characterized, how multiple factors engage the same translating mRNA in vivo remains poorly understood. We developed RIBOFRIEND, a single-molecule approach that combines RNA proximity marking with long-read direct-RNA sequencing to map the engagement of multiple ribosome-associated factors across individual native transcripts.

We focused on two conserved chaperone systems in yeast: the Ribosome-Associated Complex-Ssb system (RAC–Ssb) and the Nascent Polypeptide-Associated Complex (NAC). NAC α-subunit was fused to the human ADAR2 deaminase domain and Ssb1 was fused to the N6-adenosine methyltransferase IME4. Short-term induction deposited distinguishable A-to-I editing events and m^6A modifications on mRNAs *in vivo*. Nanopore direct RNA sequencing enabled detection of both signatures on individual RNA molecules, linking factor-associated marks across the 5′ UTR, coding sequence, and 3′ UTR within the same read.

We identified more than 1,000 co-modified transcripts, revealing a shared transcript interaction landscape for Ssb1 and NAC. Coding-region modification patterns were consistent with interaction sites identified by selective ribosome profiling, while long-read analysis additionally revealed early coding-region marks and overlapping modification zones on individual molecules. Ssb1-associated signatures were strongly enriched in 5′ UTRs, raising the possibility of engagement near translation initiation. Co-modified transcripts were enriched for those encoding cytosolic, mitochondrial, and ER-targeted proteins, motivating investigation of how chaperone engagement relates to nascent-protein destination. We are testing the contributions of transcript regulatory elements using yeast strains with exchanged promoters, 5′ UTRs, or 3′ UTRs. Furthermore, we are developing complementary split-ADAR fusions, to determine cooperativity levels. In parallel, a computationally designed split-IME4 variant provides a route toward an endogenous, inducible dual-enzyme system.

Together, RIBOFRIEND connects the interactions of distinct ribosome-associated factors across coding and untranslated regions of individual mRNAs, providing a framework for dissecting chaperone coordination during co-translational protein biogenesis.

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A detailed molecular picture of protein folding during active translation

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All proteins can begin to fold during their synthesis on the ribosome, and many rely on co-translational folding to attain their native conformation and avoid detrimental misfolded states. Despite its critical importance, the molecular details underlying co-translational folding remain largely unknown, and are not accounted for by structure prediction algorithms. To uncover the time-resolved mechanisms of co-translational folding, we develop an amide hydrogen-deuterium labeling approach to reveal the timing and order of secondary structure formation across an actively translating nascent chain. We observe different behaviors across proteins governed by both nascent chain folding propensities and the kinetic coupling with elongation. Two proteins begin folding co-translationally via a hierarchical sequence of intermediates smaller than a domain, while a third protein does not have time to adopt native structure during active elongation, even though it is capable of doing so at equilibrium on stalled ribosomes. Instead, the actively translating protein populates a persistent, kinetically trapped state. This biases subsequent post-translational folding to avoid an aggregation-prone intermediate populated upon refolding from a denatured state. Together, these results reveal the detailed mechanisms by which the ribosome promotes robust protein folding, and how these are shaped by the non-equilibrium nature of translation.

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Elucidating how dysregulated translation couples cag expansion to early huntingtin aggregation

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Biogenesis on the ribosome is a critical decision time in the life of a protein. As they emerge from the ribosomal exit tunnel, nascent chains (NCs) begin (mis)folding and interact with other NCs and chaperones. Many homo-oligomeric complexes assemble co-translationally via co-co or co-post assembly. Huntington's Disease (HD) is a neurodegenerative disorder caused by abnormal CAG repeat expansion (above 35 repeats) in exon 1 of the HTT gene, encoding a polyglutamine (polyQ) tract in Huntingtin protein (Htt). The direct correlation between uninterrupted CAG repeat length, increased aggregation propensity and earlier disease onset supports a causal link between Htt aggregation and neurotoxicity. Htt contains an N17 domain that promotes self-association and a polyQ tract that is prone to misfolding and aggregation. These regions are exposed during early biogenesis stage of the Htt. The CAG-expansion-induced ribosome collisions likely kinetically favor these events by pausing and providing proximity enhancing premature N17 self-association, lowering the energy barrier to amyloid nucleation. Our studies investigate whether expanded polyQ enables aberrant co-co/co-post interactions between Htt NCs, seeding early Htt oligomeric species.

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Cotranslational folding of the integral membrane protein GlpG into an *de novo* protein WRAP

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Most membrane proteins are cotranslationally integrated into the membrane with the assistance of translocons such as bacterial SecYEG and YidC. Disentangling

these processes in vivo is challenging, as they occur simultaneously and involve multiple chaperones and translocon complexes. In contrast, *in vitro* experiments provide a controlled environment that, combined with *in vivo* studies, offers mechanistic insights into membrane protein biogenesis. Designed proteins, such as water-soluble RFdiffused amphipathic proteins (WRAP), can be tailored to solubilize specific membrane proteins, enabling their study in solution. WRAP-GlpG, a fusion of WRAP and GlpG, preserves GlpG assembly and function while enhancing thermostability in solution [1]. The *E. coli* protease GlpG is widely used for membrane protein folding studies to dissect the mechanisms of insertion and folding within the membrane. In vivo force profile analysis (FPA) of GlpG, identified the different insertion and folding steps during biogenesis in the membrane [2]. The aim of this work was to study the folding of the WRAP-GlpG fusion in vitro, focusing on the interaction between the transmembrane domain (TMD) of GlpG and the hydrophobic surface provided by WRAP, comparing this behavior with cotranslational folding in the membrane. We achieved this by FPA using arrested peptides (AP) as sensors. We employed this methodology by expressing the WRAP-GlpG design protein in vitro using AP sensors of different strengths: the *E. coli* (Ec) SecM AP and a hybrid between the *E. coli* and the AP from *Mannheimia succiniproducens* SecM (EcMs). The pattern of pulling force oscillations in the force profile is consistent with an interaction between TMD1 of GlpG and the hydrophobic region of WRAP. In vitro FPA of GlpG in the absence of the WRAP region shows a first peak similar to that observed for GlpG in vivo, although with a slight shift. This result suggests that the amplitude of the first pulling force peak is also contributed by the interaction between GlpG TMD1 and the ribosome in both assays, as this is the only common factor between the different experimental conditions.

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Caloric restriction reprograms circadian rhythms in global and selective protein translation, thereby modulating proteostasis

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Precise regulation of protein turnover is essential for metabolic homeostasis and is perturbed in metabolic diseases and aging. Protein translation and folding are tightly interconnected processes that are central to proteostasis, as translational fidelity and the rate of protein synthesis influence the burden placed on cellular protein-folding and quality-control systems. In organs with high rates of protein synthesis, precise translational control is particularly important for preventing the accumulation of misfolded proteins and their associated cytotoxicity. An important feature of protein biosynthesis *in vivo* is its circadian regulation. Caloric restriction (CR), a well-established anti-aging intervention, reprograms circadian rhythms in protein translation. We found that, rather than simply suppressing protein synthesis, CR alters circadian rhythms in global translation in association with coordinated patterns of mTOR signaling and the less extensively studied *in vivo* phosphorylation of eIF2α in mouse liver in the context of CR and aging. By comparing the transcriptome and translatome (mRNAs associated with heavy polysomal fractions), we found that circadian rhythms in the expression of enzymes involved in glycogen and fatty acid metabolism are regulated transcriptionally. In contrast, mRNAs encoding ribosomal proteins and harboring 5' TOP elements, as well as mRNAs harboring TISU elements, showed no corresponding changes in transcript abundance but appeared to be rhythmically regulated at the translational level. Similar translational regulation was observed for nuclearly encoded mitochondrial proteins, including those involved in Fe–S cluster biogenesis. These mRNAs exhibited nocturnal translation rhythms, had short 3' untranslated regions (3' UTRs), and showed food-anticipatory rhythms under CR. In addition, CR induced translational rhythms in a subset of mRNAs, most of which exhibited diurnal translation peaks when global protein synthesis was suppressed. These mRNAs, previously reported to associate with stress granules, are

characterized by long coding sequences and long 3' UTRs and encode translational regulators, large structural and trafficking proteins, chromatin remodelers, and ubiquitin ligases. Together, our findings reveal previously unrecognized mechanisms linking translational control to CR-induced changes in translational rhythms and proteostasis, providing further insight into the relationship between aging and circadian regulation of protein synthesis. These findings highlight the effects of CR on both global translation rhythms and translational selectivity *in vivo.*

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Investigating the role of Sirt6 in protein translation and proteostasis during aging

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Loss of proteostasis is a primary hallmark of aging. Therefore, maintaining the proteostasis network through tightly regulated protein synthesis, folding, and degradation is essential for cellular function and longevity. Interventions that lower protein synthesis rates are associated with increased lifespan across multiple species, possibly by alleviating the burden on proteostasis machinery. However, the molecular mechanism underlying this association remains unclear. Sirt6 is a conserved pro-longevity gene with NAD^+- dependent histone deacetylase and ADP-ribosylase activities implicated in transcriptional regulation and DNA repair. Previous studies report that Sirt6 overexpression (OE) reduces protein translation rates. However, the molecular mechanisms linking Sirt6-mediated translational regulation to longevity remain poorly understood. Here, we used Sirt6 deletion (Sirt6-/-) and Sirt6 OE Drosophila models to determine how Sirt6 regulates protein synthesis, proteostasis, and stress response during aging. In contrast to our previous findings in Sirt6 OE flies, Sirt6-/- flies exhibited shortened lifespan, greatly increased levels of acetylated H3K9, and increased expression of ribosome biogenesis genes and Myc target genes. Meta-analysis of mammalian transcriptomic datasets further revealed increased expression of ribosomal genes

with loss of Sirt6, highlighting its role as a conserved regulator of ribosomal genes. Surprisingly, SUnSET assay showed reduced protein synthesis in both Sirt6-/- flies and Sirt6 OE flies, suggesting that Sirt6 regulates translation through distinct molecular mechanisms. Sirt6 OE flies showed reduced S6K activation, a key regulator of protein synthesis. Interestingly, under basal conditions, Sirt6-/- flies exhibited elevated phosphorylation of eIF2α (eIF2α-P), a central regulator of the integrated stress response (ISR). Upon various stress stimuli, including protein misfolding, amino acid deprivation, and ribosome collisions, ISR activation inhibits global translation, while allowing for selective translation of stress-responsive genes. To investigate the physiological implications of Sirt6 deficiency, we examined proteostasis and stress resistance. Sirt6-/- flies exhibited increased accumulation of protein aggregate markers (Ref(2)P and polyubiquitin). Sirt6-/- flies also exhibited significantly impaired resistance to heat stress, consistent with disrupted proteostasis. Using Sirt6 separation-of-function mutants, we found that deacetylase activity is necessary and sufficient to suppress protein synthesis in Sirt6 OE flies and to rescue impaired heat stress resistance. This suggests that Sirt6 regulates protein synthesis through distinct translational control pathways. Together, these findings reveal a novel role of Sirt6 in coordinating translation, proteostasis, and ISR during aging.

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rRNA 2’-O-methylation supports ribosome biogenesis, translational selectivity, and cellular stress responses

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Ribosomal RNA (rRNA) is extensively modified by 2’-O-methylation, yet how these modifications contribute to ribosome assembly and translation remains poorly understood. We integrate cryo-electron microscopy, yeast genetics, ribosome

profiling, translation assays, and proteomics to investigate how rRNA 2′-O-methylation supports ribosome biogenesis, translational selectivity, and cellular stress responses.
Cryo-EM structures of yeast ribosomes lacking native 2′-O-methylation capture intermediates in the chaperone-mediated incorporation of uL16, the final large-subunit protein, revealing how its integration completes the peptidyl transferase center. Structural and genetic analyses demonstrate that chaperone-mediated handoff of uL16 requires both rRNA 2′-O-methylation and an intact GTPase-associated center. These findings establish a framework for understanding how rRNA modifications facilitate the incorporation of intrinsically disordered ribosomal protein segments during assembly.
Beyond biogenesis, loss of rRNA 2′-O-methylation disproportionately affects the translation of ribosomal protein transcripts through changes associated with codon usage and recognition of structured RNA. This translational reprogramming accompanies reduced ribosome thermostability and altered ribosomal structures and conformations. Under stress, hypomethylated ribosomes exhibit selective depletion of proteins whose production is downregulated, together with assembly defects.
Together, these results reveal complementary roles for rRNA 2′-O-methylation in chaperone-mediated ribosome assembly and selective translation, providing structural and mechanistic insights into how rRNA modifications support ribosome integrity and cellular adaptation to stress.

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Structural proteomics of the human ubiquitinome

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The proteasome maintains the integrity of eukaryotic proteomes by selectively degrading ubiquitinated protein substrates. Ubiquitination targets a wide range of substrates for degradation, including translationally stalled nascent chains, misfolded proteins, and properly folded but short-lived proteins destined for regulatory degradation. Distinct structural features and ubiquitination patterns across these classes of substrates remain largely undefined. In this study, we combine structural proteomics and time-resolved isotopic labeling to profile the modification sites, dynamics, and conformational properties of the human ubiquitinome. We show that proteins undergoing rapid proteasomal degradation are ubiquitinated at lysine residues that are normally buried within structured regions of their native conformations. We provide proteome-wide evidence that this high-flux subset of the ubiquitinome is enriched in newly synthesized proteins that have non-native conformations. Together, our findings demonstrate how the lack of structural integrity of misfolded nascent proteins influences their ubiquitination patterns and leads to rapid proteasomal degradation.

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Structural and mechanistic insights into huntingtin biosynthesis

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The earliest decision between folding and misfolding for most proteins is made during their biosynthesis on the ribosome. During this process, the ribosome can slow down or pause at specific positions, transiently altering which regions of the nascent chain are able to sample conformations, thereby influencing the fate of the newly synthesised protein.

However, how the information encoded in the mRNA and protein sequences act together to guide biosynthesis remains poorly understood, especially for disease-associated proteins. Here, we explore the structural and mechanistic aspects of huntingtin biosynthesis on the ribosome, a protein implicated in Huntington's disease. Using an integrative approach combining ribosome profiling, toe-printing and DMS-seq, together with single-particle cryo-EM and all atom MD simulations, we provide structural and mechanistic insight into how specific features of huntingtin mRNA and nascent chain jointly shape its biosynthesis on the ribosome.

Dmitry Tyurin



Born in Nizhny Novgorod, Russia, Dmitry Tyurin began playing the piano at the age of five. His first teacher was his mother, Svetlana Bakhmatkova, at the V. Yu. Villuan Music School, named after a prominent 19th-century musician and founder of the Nizhny Novgorod branch of the Russian Musical Society. In 2010, he was admitted to the famous Balakirev Music College (founded in 1873), where he studied with Bella Alterman. In 2012, he moved to the United States to continue his studies at the Cleveland Institute of Music under the guidance of Sergei Babayan, earning his Bachelor of Music degree in 2016. From 2016 to 2018, he studied with Alexander Toradze and Ketevan Badridze at the Ernestine M. Raclin School of the Arts at Indiana University South Bend, where he earned his Master of Music degree.

Since giving his first recital at the age of nine, Tyurin has performed extensively as both a soloist and a collaborator with numerous symphony orchestras. He won First Prize in 2003 and the Grand Prix in 2009 at the Villuan International Young Pianists Competition. In 2018, he won the IUSB Philharmonic Concerto Competition. He is also a laureate of several international competitions, including the Nikolaeva Competition (2004), the Artobolevskaya Competition (2005), the Rotary Competition (2008), and the Cranbrook Music Guild Emerging Artists Competition (2019), where he also received the People's Choice Award.

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