Friday, March 22, Session III

L8

STRUCTURE-FUNCTION STUDIES OF POLYGLUTAMYLASE TTLL-11

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Microtubules (MTs) undergo various post-translational modifications, including polyglutamylation, which is the primary modification at unstructured C-terminal tubulin tails catalyzed by members of the tubulin tyrosine ligase-like (TTLL) protein family. In this report, we present the structural and functional characterization of TTLL11, the least studied member of the TTLL family. The cryoEM structure of the TTLL11/MT complex reveals a unique bipartite pattern of microtubule recognition, where the microtubule-binding and catalytic TTLL11 domains engage adjacent MT protofilaments. Biochemical experiments revealed a previously unknown glutamylation pattern, in which the polyglutamate tail directly extends the main chains of either - or -tubulin subunits. Finally, we have identified an intricate interplay between the enzymatic activities of TTLL11, TTL, and glutamate carboxypeptidases that governs selective modifications at individual tubulin protomers. Our work uncovers a novel type of tubulin polyglutamylation that expands the repertoire of known modifications comprising the 'tubulin code'. This code plays a major role in differentiating microtubules for distinct functions within cells and tissues.

L9

RNA POLYMERASE RECYCLING BY MYCOBACTERIAL HELD: AN ALTERNATIVE, HelD-PROTECTED PATHWAY OF TRANSCRIPTION INITIATION

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Mycobacterial HelD is a transcription factor that binds stalled RNA polymerase (RNAP), dissociates it from nucleic acids and, if present, the antibiotic rifampicin. The rescued RNAP, however, must disengage from HelD to participate in subsequent rounds of transcription. The mechanism of release is unknown. We show that HelD from *Mycobacterium smegmatis* forms a complex with RNAP associated with the primary sigma factor ^{A} and transcription factor RbpA but not CarD. We solved a series of RNAP- ^{A}-RbpA-HelD structures with or without promoter DNA. These snapshots capture HelD during transcription initiation, describing mechanistic aspects of HelD release from RNAP and its protective effect against rifampicin. Biochemical evidence supports these findings, defines the role of ATP binding and hydrolysis by HelD in the process, and confirms the rifampicin-protective effect of HelD. Taken together, HelD mediates an alternative pathway of transcription initiation where this process is protected from rifampicin until the last possible moment.

L10

THE ROLE OF SEMI-DISORDERED SCAFFOLD PROTEINS IN PHAGE LUZ19 PROCAPSID ASSEMBLY

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Pseudomonas phage LUZ19 exhibits broad infectivity across clinically relevant *P. aeruginosa* strains. Phage assembly starts from a dodecameric portal complex attached to the host cell membrane. The construction of the capsid shell around the portal is facilitated by scaffold proteins which create a mesh structure located inside the completed immature procapsid. The scaffolding is cleaved and discarded during the genome filling and the accompanying procapsid expansion. To understand how the scaffold protein of LUZ19, with roughly half of its residues predicted in disordered linkers, establishes the icosahedral shape of the phage capsid and mediates the symmetry mismatch between the capsid and the portal vertex, we conducted a cryo-EM study of the immature LUZ19 phage particle. We show that the immature procapsid is 16% smaller and exhibits a rougher surface compared to the expanded capsid. The particle contains scaffold and inner core proteins alongside portal and capsid proteins. The inner core complex forms a tower structure inside the capsid, composed of three types of proteins stacked over the portal complex. Interestingly, the portal complex is not in direct contact with capsid proteins; instead, C-terminal -helical domains of scaffold proteins bridge the interaction. Linkers connecting these domains to the central helical bundles of scaffold proteins intercalate asymmetrically between portal subunits. Preliminary findings suggest that central domains and long N-terminal arms of scaffold proteins interlock to assemble the inner support mesh. Our ongoing research aims to elucidate the intricate interactions between scaffold proteins within the mesh, with particular focus on the functional role of their long, disordered N-termini.

L11

ASSEMBLY OF TORPEDO TERMINATION COMPLEXES IN THERMOPHILIC AND MESOPHILIC ORGANISMS

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Transcription termination is essential for delineating the genetic information stored in DNA, as it establishes the boundaries of transcriptional units [1]. In yeast, there are two model pathways how termination of mRNA coding genes is organized: allosteric and torpedo models. The torpedo model considers that the unprotected free 5'-end of the mRNA transcript is digested by nuclease until it collides with RNA polymerase II (RNAPII), leading to dissociation from the template. Studies in the yeasts showed that exposed free 5'-end of RNA serves as an entry point for Rat1, a 5'-3' exonuclease. Stimulated by its cofactor Rai1, the Rai1/Rat1 (RR) complex greatly stimulates spontaneous termination [2]. However, the exact mechanism of how the RR complex is recruited to the site of transcription and how RNAPII is released from the DNA is unknown. Findings also suggest that the torpedo complex is recruited by Rtt103, recognizing Ser2 [3] and or Thr4 phosphorylation marks of RNAPII. This allows us to hypothesize that Rtt103 helps to recruit the 5'-3' RNA termination machinery to the site of transcription.

Due to the variations in Rai1/Rat1/Rtt103 complexes in mesophilic (yeast) and thermophilic organisms, we set out to investigate whether and how these complexes assemble in yeast *Saccharomyces cerevisiae* and fungi *Chaetomium thermophilum*. Using a combination of structure biology techniques including cryoEM, small-angle X-ray scattering (SAXS) and cross-linking mass spectrometry (XLMS) we reveal differences of how the two torpedo complexes assemble in mesophilic and thermophilic yeasts. Our observations suggest that thermophilic organisms have adapted protein-protein interfaces to favor the presence of highly structured elements, whereas mesophilic organisms prefer the utilization of unstructured elements that fold upon binding to their interaction partners.

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3. Nagarajan, V. K., Jones, C. I., Newbury, S. F. & Green, P. J. XRN 5 f ¨3 f exoribonucleases: Structure, mechanisms and functions. Biochim Biophys Acta 1829, 590 (2013).

L12

ASSESSMENT OF SAMPLE QUALITY WITH EVERY MEASUREMENT FIDA 1 FUNDAMENTALS

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Protein quality control is the most crucial checkpoint of any protein production and characterization process. All too many experiments end up unsuccessfully due to poor quality protein. Not only do you risk that useful data is not generated, but significant amounts of time can be lost on troubleshooting assay design without reliable insight to the protein itself. It is therefore highly valuable to have in-depth confirmation that the protein you work with behaves as in its native state. Typically, a set of different techniques needs to be employed prior to running an assay to gain insights into the state and condition of a given protein sample. Flow induced dispersion analysis (FIDA) is an immobilization-free ligand binding methodology employing Taylor dispersion analysis for measuring the hydrodynamic radius (size) of biomolecular complexes. The Fida 1 instrument offers great advantage for deciphering a wide array of quality control (QC) parameters. With Fida 1, these parameters do not have to be measured in a separate assay in advance of your experiments but are integrated in every measurement you perform. Combining these QC parameters with Fida 1 assays being tolerant to any type of buffer composition and consuming only nanoliter (nL) sample volume, is resulting in an assay robustness that cannot be achieved with any other technology. Flow induced dispersion analysis can measure the absolute in-solution size of your molecule in nanometers, quantifying sample aggregation and viscosity, identifying sample stickiness, defining the heterogeneity of a sample via the polydispersity index, and identifying free versus conjugated fluorophores in labelled samples.