Saturday, March 23, Session VIII

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DISTINCT 30S SUBUNIT DIMERIZATION ARCHITECTURE FACILITATED BY A NOVEL RIBOSOME DIMERIZATION FACTOR IN ARCHAEA

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Protein synthesis utilizes a significant portion of the cell's available resources. In the face of unfavourable conditions, specialized mechanisms come into play to reduce the overall costly protein synthesis. Several ribosome-associated factors play a role in this regulation in bacteria. Some induce an inactive, hibernating state in the ribosome, forming 70S monomers (such as RaiA) [1] or 100S dimers (RMF and HPF) [2-4]. Other factors hinder translation at various stages in the translation cycle acting as anti-association factors not allowing the formation of 70S ribosome (such as RsfS) [5]. Therefore, ribosome dimerization and anti-association are important regulatory events to inactivate the protein synthesis in bacteria and enable their survival under various stress conditions.

While the hibernation and anti-association mechanisms have been extensively studied in various bacterial species, the ribosomal response to adverse conditions causing growth arrest is not well understood in archaea and eukaryotes.

Here, we describe the first single particle cryo-electron microscopy structures of archaeal 30S dimers bound to a novel archaeal ribosome dimerization factor (aRDF) [6]. The overall arrangement of the 30S-30S dimer exhibits a head-to-body orientation connected by two homodimers of aRDF. aRDF forms a direct interaction with the L41e ribosomal protein, a key player in the establishment of a ribosomal bridge during subunit association. Therefore, the binding mode of aRDF illustrates its anti-association capability, preventing the formation of archaeal 70S ribosomes. Thus, the comprehensive structural architecture of aRDF-mediated 30S subunit dimerization provides unprecedented insights into the mechanism of ribosome shutdown in archaea.

1. Vila-Sanjurjo, A., Schuwirth, BS., Hau, CW., et al. Structural basis for the control of translation initiation during stress. *Nat Struct Mol Biol.* **11**(11), 1054-9 (2004).
2. Beckert, B., Turk, M., Czech, A., *et al.* Structure of a hibernating 100S ribosome reveals an inactive conformation of the ribosomal protein S1. *Nat Microbiol.* **3**, 1115–1121 (2018).
3. Matzov, D., Aibara, S., Basu, A., *et al*. The cryo-EM structure of hibernating 100S ribosome dimer from pathogenic *Staphylococcus aureus*. *Nat Commun*. **8**(1), 723 (2017).
4. Khusainov, I., Vicens, Q., Ayupov, R., *et al*. Structures and dynamics of hibernating ribosomes from *Staphylococcus aureus* mediated by intermolecular interactions of HPF. *EMBO J*. **36**(14), 2073-2087 (2017).
5. Khusainov, I., Fatkhullin, B., Pellegrino, S., *et al*. Mechanism of ribosome shutdown by RsfS in Staphylococcus aureus revealed by integrative structural biology approach. *Nat Commun*. **11**(1), 1656 (2020).
6. Chiaki, Y., Natsumi, M., Sonoko, I., *et al*. A novel ribosome-dimerization protein found in the hyperthermophilic archaeon Pyrococcus furiosus using ribosome-associated proteomics. *Biochem Biophys Res Comm.* **593**, 116-121 (2022).

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L28

ASYMMETRIC RECONSTRUCTIONS OF IMMATURE TICK-BORNE ENCEPHALITIS VIRUS PARTICLES REVEAL ASSEMBLY MECHANISM OF FLAVIVIRUSES

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Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*, which causes severe disease of central nervous system in humans. The smooth virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in the virus lipid bilayer. The immature, non-infectious virus goes through a maturation phase during the viral life cycle. Proteolytic cleavage of prM and a significant rearrangement of the envelope proteins on the viral surface are two aspects of this process.

We isolated immature TBEV particles from infected tissue culture cells and visualized them using cryo-electron microscopy in order to determine their structure. The E-protein-prM-protein complex forms the "spiky" surface of the immature particles. Weused cryo-electron tomography and single-particle analysis to show that the TBEV immature particles are asymmetric. Defects induced during immature particle assembly frequently disturb the symmetric, icosahedral structure of the E-protein-prM-protein spikes on the particle surface. However, these irregularities do not hinder the subsequent maturation process and produce mature particles with vacant patches in the "herring bone" structure of the mature viral surface

The findings shed additional light on the viral assembly and maturation process, which may be the subject of future antiviral medication development.

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HIGH-RESOLUTION *IN SITU* STRUCTURES OF HIV-1 MATRIX PROTEIN BY SINGLE PARTICLE ANALYSIS

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The N-terminal matrix domain (MA) of the HIV-1 Gag polyprotein targets and recruits Gag to the plasma membrane of virus producing cells. After virus budding, Gag is cleaved by the viral protease into its individual domains, triggering the morphological transformation of the non-infectious immature virus into its infectious mature form. We have shown recently that upon maturation MA rearranges its lattice and recruits a co-factor into a side binding pocket in the MA domain. However, the relatively low resolution of the reconstructions prevented a precise description of MA-MA interactions and of the binding pocket. Here we present high-resolution *in situ* structures of the wild-type mature MA lattice and of an immature-like MA lattice formed by a MA point mutant. These structures provide novel insights into how the MA domain binds and clusters lipids during Gag assembly and maturation. We also present experimental data towards determining how MA maturation is controlled and triggered.

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STRUCTURE AND REPLICATION CYCLE OF A VIRUS INFECTING CLIMATE-MODULATING ALGA *EMILIANIA HUXLEYI*

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The globally distributed marine alga *Emiliania huxleyi has* cooling effect on the Earth's climate. The population density of *E. huxleyi* is restricted by Nucleocytoviricota viruses, including *E. huxleyi* virus 201 (EhV-201). Despite the impact of *E. huxleyi* viruses on the climate, there is limited information about their structure and replication. Here we show that the dsDNA genome inside the EhV-201 virion is protected by an inner membrane, capsid, and outer membrane. EhV-201 virions infect *E. huxleyi* by using their fivefold vertices to bind to and fuse the virus' inner membrane with the cell plasma membrane. Progeny virions assemble in the cytoplasm at the surface of endoplasmic reticulum-derived membrane segments. Genome packaging initiates synchronously with the capsid assembly and completes through an aperture in the forming capsid. The genome-filled capsids acquire an outer membrane by budding into intracellular vesicles. EhV-201 infection induces a loss of surface protective layers from *E. huxleyi* cells, which enables the continuous release of virions by exocytosis.