Session 7: Structural Studies of Large Assemblies

Lectures

L7.1

Structure of the spliceosome

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The spliceosome is a dynamic RNA-protein complex involved in the removal of non-coding segments (introns) from the precursors of mRNAs (pre-mRNAs). Spliceosome assembles on pre-mRNA substrates by the sequential binding of five canonical subunits – small nuclear ribonucleoprotein particles (U1, U2, U4/U6 and U5 snRNPs) and numerous non-snRNP factors. Hierarchical assembly of the complex is initiated by the recognition of the 5'-splice site and branch site by U1 and U2 snRNPs. Subsequent recruitment of U4/U6.U5 tri-snRNP results in a fully assembled but catalytically inactive complex B. A series of complex structural and compositional rearrangements lead to the formation of a group II intron-like RNA catalytic core required for the two trans-esterification steps of splicing. The enormous complexity and highly dynamic nature of spliceosomes have limited high-resolution structural analysis by x-ray crystallography to individual proteins and smaller sub-complexes. Recent developments in the field of cryo-electron microscopy (cryo-EM) provided solutions to some of these limitations, as illustrated by numerous high-resolution reconstructions of spliceosomal complexes reported in the last three years. I will present our recent progress in the structural analysis of the spliceosomal complexes by cryo-EM.

L7.2

Eukaryotic replisome assembly and fork progression visualized by cryo-EM

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All proliferating cells must synthesize a copy of their genome before dividing. Errors in the mechanisms of DNA replication can lead to chromosomal instability, which is a hallmark of cancer cells. We use cryo-electron microscopy and biochemistry to understand the molecular mechanism of genome duplication in eukaryotic cells. Before chromosome duplication can start, replication origins are '*licensed*' by initiator factors that recruit a set of two MCM helicases onto DNA. Once activated, MCM unwinds the double helix, providing the single-stranded DNA template for the replicative polymerases. We have determined the structure of MCM before and after origin loading, explaining *licensing* at a molecular level. DNA unwinding depends on the recruitment of several factors including MCM activators, GINS and Cdc45 (together forming the CMG holo-helicase). We employed cryo-EM and single-molecule fluorescence to analyze CMG-DNA interactions and found that CMG unwinds DNA by translocating on single-stranded DNA. We are now studying helicase-polymerase coupling to understand replication-fork progression. With our research we seek to establish a molecular framework that explains how eukaryotes respond to DNA damage and how cell proliferation is regulated to avoid tumorigenesis.

L7.3

Structure and function of macromolecular machines

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In mammalian cells, genetic information is stored in two locations: in the nucleus and in mitochondria. DNA in mitochondria, just like in the nucleus must be faithfully copied and mistakes i.e. mutations due to exogenous and endogenous DNA damaging agents lead to formation of DNA lesions. Persistence of these DNA lesions leads to genomic instability and human diseases like cardiovascular, skeletal muscular and neurological disorders, cancer as well as normal aging process. Although great progress towards understanding mitochondrial DNA metabolism has been made, relatively little is known about human mitochondrial DNA replication and DNA repair pathways. While there are a number of DNA repair enzymes shared by the nucleus and mitochondria, only human mitochondrial DNA polymerase γ (Polγ) and 5'-exo/endonuclease (EXOG) are mitochondria-specific and critical for both DNA replication and repair in human mitochondria. Interestingly, EXOG, Polγ, APE1 and Lig3 fare components of a large complex called mitochondrial repairsome, a macromolecular machine, proposed to coordinate DNA repair process in human mitochondria. We recently solved replicating, ternary structures of Polγ in the complex with DNA and incoming nucleotide and illuminated the mechanism of allosteric regulation of this multi-subunit DNA polymerase. Concurrently, we solved atomic resolution structures of EXOG in the complex with DNA and provided structural basis for its action in human mitochondrial DNA repair. Now, using a combination of structural biology (x-ray crystallography and electron microscopy), biochemistry and biophysics, we tackle the organization, structure and function of human mitochondrial DNA repairosome.

Oral presentations

O7.1

Towards the near-atomic resolution structure of light-dependent protochlorophyllide oxidoreductase

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Light-dependent protochlorophyllide oxidoreductase (POR) is a plant enzyme that catalyzes the penultimate reaction of the chlorophyll biosynthetic pathway. Interestingly, the enzyme has light-harvesting properties and its activity is triggered by light. Namely, in the dark the enzyme binds its two substrates – NADPH and protochlorophyllide (Pchlide) – however, the reaction is not initiated until the Pchlide molecule absorbs light. Some data suggested that POR forms oligomers and that the absorbed light energy can be transfer within subunits.
Recently we have shown that plant lipids interact with POR oligomers, affecting the spectral properties of the enzyme:substrates complex. In the present work we have successfully determined the structure of the photoactive POR complexes bound to lipid membranes using cryo-electron microscopy (cryo-EM) to currently ~7 Å resolution. Our data show that the enzyme forms a two start helical filament that wraps around a highly-curved lipid bilayer. The subunits are highly ordered and strictly light-dependent, as exposure to light causes filament disassembly.
Our results suggest that POR forms unique, long tubular filaments that can effectively absorb and transfer light energy. The data shed a new light on the role of the enzyme in plant physiology and will help to better understand the last steps of chloroplast maturation.

Posters

P7.1

The intrinsically disordered F domain of ecdysteroid receptor from *Aedes aegypti* possesses multiple Zn^{2+} and Cu^{2+} binding sites

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Intrinsically disordered regions (IDRs) do not possess a stable 3D structure under physiological conditions. Their elasticity enables them taking part in the protein-protein or protein-ligand interactions. *Aedes aegypti* mosquitoes are the main vectors of the world's most devastating human diseases, such as dengue, Zika, chikungunya and yellow fever. Their reproduction is controlled by the ecdysteroid receptor (AaEcR). Like other nuclear receptors (NRs), EcR possesses a canonical structure consisting of the N-terminal domain, DNA binding domain, hinge region and ligand binding domain (LBD). However, the amino acid sequence after helix 12 from LBD forms an additional domain called region F (AaFEcR). The presence of F domains is evolutionarily not well preserved in the superfamily of NRs. The structure-function relationship of EcRs' F domains in NRs is obscure and enigmatic, especially in arthropods.Isolated AaFEcR exhibits characteristics of IDRs and is likely to undergo posttranslational modifications, such as phosphorylation and glycosylation, which may be crucial for proper functioning, structure forming and AaFEcR-ligand interactions. Extensive *in silico* analyses of AaFEcR sequence were also performed and indicated the presence of the histidine and proline ion-binding repeats. ESI-TOF MS of AaFEcR in the presence of Zn^{2+} and Cu^{2+} revealed the stoichiometry of emerging complexes. AaFEcR is able to bind up to two Zn^{2+} and up to 3 Cu^{2+}, which suggests existing of multiple ion-binding sites in AaFEcR sequence. This multiple ion-binding propensity demonstrated by the AaFEcR domain may be a part of the ecdysteroid receptor's mechanism for regulating the expression of target genes.

P7.2

N-terminal domain of *Helicoverpa armigera* Ultraspiracle controls functionality of DNA-binding domain and ligand-binding domain

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Ultraspiracle (Usp) is a member of the nuclear receptors (NRs) family. Like all other NRs it possesses typical for them molecular structure, consisting of two highly conserved well-folded domains – DNA-binding domain (DBD) and ligand-binding domain (LBD). Beside these two globular domains we can distinguish also some intrinsically disordered regions (IDRs) among which the most puzzling is N-terminal domain (NTD). However, despite many years of investigation of NRs the knowledge regarding NTDs is still very small. We decided to investigate the influence of NTD on *Helicoverpa armigera* Usp (HaUsp) functionality. We chose to test the potential role of the NTD in dimer formation and in the interaction with specific DNA sequence. Dimerization of full-length HaUsp and HaUsp lacking NTD (HaUsp_DNTD) was analyzed by SEC-MALS. Obtained results suggested that both proteins can rapidly self-associate, but for full-length HaUsp additional peaks corresponding to dimers was observed. This may suggest that NTD influences LBD which possesses strong dimerization surface. Analysis of the interaction of both proteins with DNA sequences revealed that presence of NTD significantly changes the pattern of this interaction. In summary, both experiments showed that disordered NTD modulates the functionality of globular domains of HaUsp. Nonetheless, more experiments are necessary for understanding of this phenomena and explanation of far-reaching consequences of such modulation.

P7.3

Interaction of replication initiation proteins with ssDNA of AT-rich regions of replication origins

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Initiation of DNA replication is triggered by binding of initiator proteins to specific sequences within the double-stranded DNA (dsDNA) origin region, what results in melting of helix in the region named DUE (DNA unwinding element). The formed single-stranded DNA (ssDNA) becomes the site where the replisome assembly occurs. The bacterial replication initiator DnaA, via its DBD domain (DNA binding domain) binds to DnaA-boxes and forms the right-handed helical filament on the dsDNA. Next, via its AAA+ (ATPases Associated with diverse cellular Activities) domain, DnaA forms the oligomeric complex in the ssDNA DUE region. Plasmid replication initiation proteins (Rep) do not possess the DBD and AAA+ domains. The binding of specific sequences (iterons) within the dsDNA by Reps is achieved through the Winged Helix (WH) domains. The WH domains are also responsible for dsDNA binding by eukaryotic and archaeal proteins. Plasmid Reps, although lacking DBD and AAA+ domain, do also bind ssDNA of DUE. For Archaeal Orc proteins this interaction was not known. Here we show, due to obtained crystals, the complex of plasmid F replication initiator, RepE protein, with ssDNA of DUE region and we indicate the amino acid residues important for this interaction. We also show that Orc1 protein from archaeon *Aeropyrum pernix* binds specific sequence of AT-rich region of replication origin and that in this interaction both the WH and the AAA+ domains are engaged.

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