MS78 STRUCTUROMES – STRUCTURES OF RIBOSOMES NUCLEOSOMES AND OTHER -OMES
Chairpersons: Anders Liljas, Wayne Hendrickson

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Nucleosome Core and Compact Nucleosome Array Structures
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The fundamental building block of chromatin is the nucleosome comprising 157-240 bp of DNA, two each of the four core histone proteins, and a single linker histone H1/H5. The nucleosome core is the greater part of the nucleosome and contains 147 base pairs of DNA wrapped in 1.67 left-handed superhelical turns around the histone octamer. Arrays of nucleosome in their most compact form constitute the "30 nm" chromatin fiber.

The crystal structure of the nucleosome core particle refined to 1.9 Å resolution reveals the details of DNA conformation as well as all the direct and water-mediated histone-DNA contacts. The acute DNA bending induced by the histone proteins results in an alteration of the form of the double helix every five base pairs along its superhelical path. Sequence-dependent DNA conformations are apparent.

Two nucleosome core particle structures containing different 146 base pair DNA sequences contain distinct regions in which the DNA is relatively over-twisted and stretched. These regions represent trapped-intermediates relevant to the "twist-defect diffusion" mechanism for nucleosome sliding and provide a means of buffering DNA linker length variation in the chromatin fiber.

The crystal structure of a tetranucleosome determined at 9 Å resolution comprises two stacks of two nucleosomes with three segments of linker DNA running between them. This structure is compatible with a two-start helix, but not with a one-start helix. A continuous fiber model built by stacking tetranucleosomes results in a nucleosome higher structure that is nearly fully compact.
Keywords: DNA, nucleosome, chromatin fiber

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Structural View of the Clamp-loading Mechanism onto DNA
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DNA replication is a highly coordinated process, which involves numerous proteins in nuclei. To promote the integrated system, proteins involved in this event constitute several kinds of molecular machinery. Archaeal systems generally exhibit attractive properties to study DNA metabolism; their DNA binding proteins are very similar to those from eukarya both functionally and structurally, irrespective of their morphological difference from eukaryotic ones, and hence they are good model systems for understanding eukaryotic DNA processing. We have been working on several DNA processing proteins from a hyperthermophilic archaeon, *Pyrococcus furiosus*.

Replicative DNA polymerase requires two essential protein factors, a sliding clamp and a clamp loader, for rapid and accurate DNA duplication. In eukarya and archaea, a homo-trimeric proliferating cell nuclear antigen (PCNA) and a hetero-pentameric replication factor C (RFC) function as the clamp and the clamp loader, respectively. The ATP-dependent clamp-loading mechanism is particularly intriguing, because it requires opening and resealing of the PCNA ring. We have determined the three-dimensional structure of an archaeal RFC–PCNA–DNA clamp-loading complex by electron microscopy-single particle reconstruction. Importantly, the structure of the complex presents the first direct view of a washer-like open conformation of the PCNA ring in contact with RFC. In combination with the two X-ray structural data reported previously, our EM model implies an intriguing clamp loading mechanism.
Keywords: DNA replication, clamp-loading complex, single-particle reconstruction

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X-ray Crystal Structures of the *E. coli* Ribosome
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We are using x-ray crystallography to probe the structural basis for the many aspects of protein biosynthesis that require the intact ribosome. Our goal is to make an atomic-resolution "movie" of a ribosome in the process of making a protein. We have obtained crystals of the entire *E. coli* ribosome that diffract x-rays to a resolution of 3.1-3.2 Å. Thus, we now have the means to determine the first atomic-resolution structure of the intact ribosome, the first frame of the movie. Moreover, we are using these crystals to probe in atomic detail the effects of antibiotics on the full ribosome and mutations in the ribosome that lead to antibiotic resistance or perturb key steps in translation. We are presently refining ribosome structural models at a resolution of 3.5 Å, the results of which will be presented.
Keywords: ribosome structure, antibacterials, protein synthesis

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Structural Analysis of the L7/12 Ribosomal Stalk
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The L7/12 stalk of the large ribosomal subunit encompasses protein L10 and multiple copies of L7/12 and is involved in translation factor related functions. We have determined crystal structures of *Thermotoga maritima* L10 in complex with L7/12 N-terminal domains and of an archaeal L10 N-terminal domain *in situ* on the 50S subunit. A mobile C-terminal α-helix of L10 harbors three consecutive binding sites for L7/12 dimers in *T. maritima* and two in *E. coli*, where the helix is shorter. The N-terminal domain of L10 recognizes the overall fold of the thiostrepton loop of 23S rRNA and interacts with L11. Together with structures of isolated L7/12, we devised a complete atomic model of the stalk and reinterpreted the morphology and dynamics of the stalk region as seen in electron microscopic reconstructions of ribosomes. Flexible hinges in both L10 and L7/12 lead to a high freedom of motion for the L7/12 C-terminal domains. Our structural data and analysis of L7/12 mutants by fast kinetics reveal that the L7/12 C-terminal domains can reach far out into solution to bind translation factors. They thereby promote factor recruitment to the ribosome. The L7/12 C-termini can then reach back towards ribosome-bound factors to stimulate GTP hydrolysis by stabilization of the factors' active GTPase conformation.
Keywords: ribosome structure, translation factor recruitment, GTPase activation

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Ribosomal Crystallography Reveals Co-Translational Trafficking by Eubacterial Trigger Factor
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The correct folding of newly synthesized proteins is a vital process in all kingdoms of life. It is coordinated and concerted by a set of molecular chaperones that direct the folding of nascent proteins towards their final functional state. Nascent chains emerging out of the ribosomal tunnel are highly prone to aggregation and degradation. Consequently, chaperone activity is initiated during translation.

Trigger factor (TF), the first chaperone in bacteria to encounter the