2649-Symp News on ABC and CBS

Bert Poolman

University of Groningen, Groningen, The Netherlands.

Cystathionine-β-synthase (CBS) domains are found in over 10,000 proteins in species from all kingdoms of life, yet their functions are largely unknown. Tandem CBS domains are associated with membrane transport proteins, most notably members of the ATP-binding cassette (ABC) superfamily, voltage-gated chloride channels and transporters, cation efflux systems, and various enzymes, transcription factors, and proteins of unknown function[1]. We have shows that tandem CBS domains in the osmoregulatory ABC transporter OpuA are sensors for ionic strength that control the transport activity through an electrostatic switching mechanism. The on/off state of the transporter is determined by the surface charge of the membrane and the internal ionic strength which is sensed by the CBS domains[1,2]. By engineering of the CBS domains, we have been able to construct OpuA molecules that are no longer salt-dependent but also transporters with an increased ionic threshold for activation[2,3]. Recent progress on the functional and structural analysis of the ABC transporter OpuA and the regulatory role of the CBS domains will be presented.

References

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2650-Symp Structural Dyanmics of the ABC Transporter MsbA

Hassane S. Mchaourab, Ping Zou, Kavitha Surendhran, Marco Bortolus

Vanderbilt University, Nashville, TN, USA.

ABC transporters harness the free energy of ATP hydrolysis to power the thermodynamically unfavorable trafficking of a wide spectrum of substrates in and out of the cell. In Gram-negative bacteria, the transport of lipid A from its site of synthesis across the inner membrane is critically dependent on the expression of the ABC transporter MsbA. Orthologs of MsbA, including human P-glycoprotein, bind and transport cytotoxic molecules and are associated with multidrug resistance phenotypes. We used site-directed spin labeling and EPR spectroscopy to determine the conformational motion that couple energy expenditure to substrate translocation in MsbA. Spin labels were systematically introduced along 5 transmembrane helices and the adjacent extracellular and intracellular regions and at selected locations in the nucleotide binding domains. Distances between symmetry related spin labels in the dimer were used to monitor the relative separation of the transmembrane domains, the dimer interface, and the packing of the NBDs during the ATPase cycle. Spin labels accessibility to paramagnetic reagents reported on water accessibility in the putative substrate binding chamber. We find that ATP hydrolysis fuels a relative motion of the NBDs close to 30 Å. The movement of the NBDs is coupled to reorientation of the substrate binding chamber from cytoplasmic-facing to extracellular-facing through large amplitude motion on either sides of the transporters. The closing of the chamber on the intracellular side leads to a significant restriction in NiEDDA and by inference water accessibility to the substrate binding chamber. These results are not consistent with current models deduced from studies of substrate-specific ABC importers that envision the two NBD in contact throughout the ATP hydrolysis cycle.

Symposium 21: Nucleic Acid-based Motors

2651-Symp Single Molecule Studies of Transcriptional Termination Reveal a Mechanism

Matthew H. Larson[1], William J. Greenleaf[1], Robert Landick[2], Steven M. Block[1]

[1] *Stanford University, Stanford, CA, USA,*
[2] *University of Wisconsin-Madison, Madison, WI, USA.*

Transcription termination by prokaryotic RNA polymerase occurs at sequences that code for a G:C-rich hairpin in the RNA immediately followed by a 9-nt, U-rich tract. We used single-molecule optical trapping techniques to investigate the mechanism by which elements from three representative terminators (his, t500, and tR2) destabilize the transcription elongation complex. In wild-type (WT) terminators, loads exerted via the DNA did not affect the termination efficiency. However, the force-dependent kinetics for the release of transcripts carrying the t500 terminator sequence and the force-dependent termination efficiency of a t500 mutant imply a forward translocation mechanism for this particular terminator. Tension applied to isolated U-tract sequences (minus hairpins) induced transcript release in a manner consistent with a mechanism involving shearing of the RNA:DNA hybrid within the enzyme. We deduce that closure of the final 2–3bp at the base of the hairpin stem supplies energy sufficient to destabilize the hybrid, and we propose a quantitative model for termination based on both hairpin and hybrid energetics. The model successfully predicts the termination efficiency over a wide range of values, and also how this quantity varies with load for all three WT and several mutant terminators.

2652-Symp Exploring Chromatin Remodeling With Three-dimensional Electron Microscopy

Andres Leschziner

Harvard University, Cambridge, MA, USA.

The extreme packaging of DNA into chromatin constitutes a physical barrier to all DNA transactions. A variety of mechanisms have evolved that take advantage of this packaging and make chromatin a highly dynamic and regulated entity. The ATP-dependent chromatin remodeling complexes are large (often $>$ 1 MDa), multi-subunit assemblies that utilize the energy from ATP hydrolysis to non-covalently alter the structure of nucleosomes. Despite a significant body of biochemical and genetic data we do not understand how these complexes remodel chromatin.

We are taking a structural approach to elucidating the mechanism of ATP-dependent chromatin remodeling and use as our main techniques three-dimensional electron microscopy (3D EM) and single-particle methods. Our model system is RSC, a remodeling complex from the yeast S.cerevisiae.

We obtained an initial low-resolution reconstruction of RSC using a novel reconstruction method we developed that addresses some of the difficulties encountered with the more traditional methods when attempting to obtain ab initio structures from a heterogeneous sample (as is the case with a flexible macromolecule). The structure of RSC suggests a potential binding pocket for its substrate, the nucleosome. Our structural analysis also revealed a significant degree of conformational flexibility in one of the large domains in the complex.

We are currently working towards obtaining a reconstruction of RSC bound to a nucleosome and are starting to map the location of a number of the RSC subunits as a first step in understanding the roles they play within the complex. We are also beginning to collect the large data sets required to move towards our goal of obtaining high-resolution structures.

2653-Symp Under the Hood of the Replisome: A Single-Molecule View of DNA Replication

Antoine M. van Oijen

Harvard Medical School, Boston, MA, USA.

Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual enzymes and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these enzymes function in concert with other enzymes in multiprotein complexes, so an important future direction will be the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies. I will discuss results of single-molecule experiments on the replisome, the molecular machinery that is responsible for replication of DNA. We stretch individual DNA molecules and use their elastic properties to obtain dynamic information on the proteins that unwind the double helix and copy its genetic information. Furthermore, we use fluorescence microscopy to obtain detailed information about the spatial distribution of eukaryotic replication origins along DNA.

Minisymposium 4: The Physics of Protein Folding/ Unfolding

2654-Minisymp Mutations as Trapdoors: The Rop-dimer with two Competing Native Conformations

Alexander Schug, Jose' N. Onuchic

UCSD, San Diego, CA, USA.

Conformational transitions are the molecular basis of protein function. Structure-based models, based on the funneled energy landscape, typically accommodate a single basin for the native state. However, it is possible to accommodate multiple folding basins to represent different structures of the investigated protein. The presented study focuses on the homodimer Rop, which was the subject of extensive studies investigating the effect of mutations in the hydrophobic core. The mutants show strong changes in folding rates and Rop's ability to bind RNA. We investigate the possibility of two competing conformations representing a parallel (P) and the WT anti-parallel (AP) arrangement of the monomers. Given equivalent energetic bias towards each of the two states, P has a lower free-energy barrier and is therefore more accessible than AP. We suggest that the mutations trigger a trapdoor on the energy landscape. They undo the evolutionary bias towards the RNA-binding state AP, resulting in the competition of P and AP. This competition of two states with distinct kinetic behavior leads to the experimentally observed strong changes in kinetics. Apart from explaining Rop's mutational behavior, the general concept of competing states which are triggered by external factors might be applicable to explain allosteric control or signaling, for example.

2655-Minisymp Single Molecule Atomic Force Microscopy Reveals Kinetic Partitioning of the Mechanical Unfolding Pathway of T4-lysozyme

Qing Peng, Hongbin Li

University of British Columbia, Vancouver, BC, Canada.

Kinetic partitioning is believed to be a general mechanism for proteins to fold into their well-defined native three-dimensional structure from unfolded states following multiple folding pathways. Here we use single molecule atomic force microscopy (AFM) to present direct experimental evidence of the kinetic partitioning of the mechanical unfolding pathway of T4-lysozyme, a small protein composed of two sub-domains. We observed that upon stretching from its N- and C-termini, T4-lysozyme unfolds via multiple distinct unfolding pathways: the majority of T4-lysozymes unfold in an all-or-none fashion, and a small fraction of T4-lysozymes unfold in three-state fashion involving unfolding intermediate