small-but-measurable net unwinding (~7 deg/protein). A torsional free energy model couples this unwinding to observed limits in cooperative cluster size. These results predict that AGT will partition in favor of torsionally-relaxed, relatively protein-free DNA structures like those near replication forks. AGT binds O^6-methylG-C and O^6-methylG-T lesions with a specificity ratio (K_s/K_a) far too low for efficient lesion search. This suggests that other factors are needed to direct AGT to lesion sites. Recently we have found that AGT binds the human MutSbeta homologue and PCNA proteins. We propose that these proteins target AGT to sites of mismatch repair (including those containing O^6-methylG-T pairs) and to sites near the replication fork, where O^6-methylG lesions may be repaired before their potential for mutagenesis can take effect. This work was supported by NIH grant GM070662 to MGF.

944-Symp  
DNA Supercoiling Enhances Cooperativity and Efficiency of an Epigenetic Switch  
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Upon infection by bacteriophage λ the λ repressor protein, CI, interacts with the λ operator DNA, thereby regulating protein expression and deciding between the lysogenic and lytic state. This λ switch is a model on the basis of which epigenetic switch regulation is understood. In order to study the interaction between naturally supercoiled DNA and the DNA associating protein CI, we invented a novel assay where supercoiled circular DNA plasmids were individually tethered by peptide nucleic acid (PNA) handles [1]. We used this tethered plasmid assay for a single molecule investigation of the dynamics of supercoiled DNA and studied both the dynamics of the molecule itself and its interactions with the regulatory CI protein. The dynamics of the construct was analyzed by tracking the tethered bead. This revealed that compared with relaxed DNA, the presence of supercoils greatly enhances juxtaposition probability [2]. When CI was added to the supercoiled assay, the protein would attach to the operator sites thereby loopinng DNA. Our studies reveal that the efficiency and cooperativity of the epigenetic λ switch are significantly increased in the supercoiled system compared with a linear assay, thus increasing the Hill coefficient [2,3]. In contrast to other single molecule assays, the current methodology allows for studying DNA dynamics and DNA-protein interactions of DNA in its naturally supercoiled state.


Symposium: Mechanisms of Actin Filament Nucleation and Mechatranstduction

945-Symp  
Mechanosensitivity of Formin-Actin Interactions  
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Cells are able to sense and adapt to their physical environment, translating mechanical inputs into chemical outcomes. The molecular basis for this fascinating property, referred to as “mechanotransduction”, remains largely unexplored. The actin cytoskeleton, which generates and transmits forces throughout the cell, is certainly a key player in this process. Here, I will focus on the situation where tension is applied to actin filaments that are interacting with formins at their barbed ends. In cells, formins are typically anchored to a substrate and thus undergo a mechanical force as the filament is put under tension. We have studied this situation in vitro, by manipulating individual filaments with a microfluidic flow, as they are nucleated and elongated by formin mDia1 anchored to the bottom of the microchamber. I will show how filament tension in the piconewton range modulates the elongation rate of the actin filament, the processivity of the formin and its competition with other end-binding proteins. Quantitatively probing these mechanical properties also provides an interesting angle to test molecular models of formin-assisted filament elongation.

946-Symp  
WISH/DIP/SPIN90 Proteins Activate Arp2/3 Complex to Create Linear ActinFilaments that Seed Assembly of Branched Actin Networks  
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Arp2/3 complex nucleates branched actin filaments responsible for powering diverse cellular processes, including cellular motility and endocytosis. WASP-mediated activation of Arp2/3 complex requires preformed filaments, enabling that nucleation and branching are coupled, but leading to the biochemistry of emergence that branched networks assemble cannot be initiated without a preformed seed filament. We recently discovered a class of Arp2/3 complex activators, the WISH/DIP/SPIN90 (WDS) proteins, that activate Arp2/3 complex without preformed filaments. WDS proteins have several sequence features distinct from WASP proteins, making their mechanism of activation unclear. In addition it is unknown if linear filaments produced by WDS-activated Arp2/3 complex can seed assembly of branched actin networks. Here we use single molecule TIRF microscopy to determine the mechanism of Dip1, a WDS protein from fission yeast. We directly demonstrate that linear filaments nucleated by Dip1 and Arp2/3 complex are substrates for branching by WASP-bound Arp2/3 complex. Dip1 remains bound to Arp2/3 complex at the pointed ends of the linear filaments it nucleates for hundreds of seconds, unlike WASP proteins, which are released before nucleation. Using engineered cysteine crosslinking, we show that a structural change in the complex that brings Arp2 and Arp3 into a filament-like (short pitch) conformation bypasses the need for Wsp1 but not actin filaments in activation. Therefore, we propose a model in which a step-wise set of conformational changes activate Arp2/3 complex. This model explains both how Dip1 bypasses the need for preformed actin filaments and why Wsp1 requires them, thereby providing important new insights into the molecular regulation of the assembly of actin filament networks.

947-Symp  
Two Types of Actin Nucleators, Three Ways to Make ActinFilaments?  
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Spire (Spi) and Cappuccino (Capu) are both actin nucleators, which are essential for Drosophila oogenesis. Despite similar biochemical activities, Spire and Capu are not redundant: mutations in either gene cause the same phenotypes, including loss of an actin mesh throughout the oocyte, oocyte localization of polarity determinants and female sterility. Why are two actin nucleators required? To address this question we study how Spire and Capu nucleate independently and together.

Capu is a formin. Like other formins, it uses conserved FH1 and FH2 domains for nucleation and processive elongation. Capu does not have a DAD domain. It nevertheless uses its similarly-positioned tail in nucleation and autoinhibition, like other formins. Further, the Capu-tail enhances processivity. Our work indicates that this is a conserved function of tail motifs. Spire is a WH2-nucleator. The last two WH2 domains (of four) and their linker are sufficient to nucleate (C3D). Interestingly, these WH2 domains are reversed (D3C) nucleation activity is lost. C3D binds two actin monomers with positive cooperativity. In contrast, D3C binds actin with negative cooperativity - it binds only one actin monomer. A similar construct with Linker 3 mutated (C1g3S3D) also binds only one actin monomer. Thus domain order and Linker 3 are necessary for cooperative binding.

Direct interaction between Spire and Capu is essential for actin mesh formation, subsequent oogenesis and fertility. New data indicate that actin binding but not necessarily nucleation by Spire is necessary during oogenesis. Combined, these point to a role for Spire as a Capu activator. Indeed, in bulk in vitro assays, Spire stimulates Capu’s actin assembly activity in the presence of capping protein and profilin, conditions under which Spire alone does not promote filament formation. We continue to study this interaction in vivo and in vitro.

948-Symp  
Molecular Mechanism of Actin Filament Nucleation by Leiomodin (Lmod)  
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Leiomodin (Lmod) is related to tropomodulin (Tmod), and the two proteins have similar localization in striated muscle, i.e. near the M-line, in association with the pointed end of the actin filaments. However, in vitro the two proteins have fundamentally different activities: Lmod is a powerful actin filament nucleator, whereas Tmod caps the pointed end. Originally, it was assumed...