helicase necessary for initiating homologous DNA recombination, and FtsK, a DNA pump involved in chromosome dimer resolution, actively disrupts nucleoprotein complexes, including RNA polymerase (RNAP) holoenzyme. RecBCD pushed and eventually displaces RNAP, Lac repressor, EcoRI(E111Q) and even nucleosomes. FtsK pushed RNAP but was able to either push or bypass EcoRI(E111Q). We conclude that RecBCD acts as a powerful stripase that overwhelms potential roadblocks. In contrast, FtsK is able to bypass some roadblocks, possibly by dissociation and reassembly ahead of the block.

3104-MiniSymp
Visualizing Transcription In Vivo at Nucleotide Resolution using Nascent Transcript Sequencing
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Elegant single molecule approaches have elucidated the mechanisms that cause RNA polymerase to pause during transcription. However, it is unclear whether these pausing events and their subsequent recovery occur within the cell as there are a large number of elongation factors that facilitate the progression of RNA polymerase (RNAP) through a chromatized genome. To explore the mechanisms of RNA polymerase elongation in vivo, we require experimental strategies that can observe transcription with the same resolution as can be obtained in vitro. Here we present an approach, native elongating transcript sequencing - NET-seq, that accomplishes this goal by exploiting the extraordinary stability of the DNA-RNA-RNAP ternary complex to capture nascent transcripts directly from live cells without crosslinking. The identity and abundance of the 3' end of purified transcripts are revealed by deep sequencing thus providing a quantitative measure of RNAP density with single nucleotide precision. Application of NET-seq in Saccharomyces cerevisiae reveals pervasive polymerase pausing and backtracking throughout the body of transcripts. Average pause density shows prominent peaks at each of the first four nucleosomes with the peak location occurring in good agreement with in vitro single molecule measurements. Thus nucleosome-induced pausing represents a major barrier to transcriptional elongation in vivo.

3105-MiniSymp
Modulation of the Translocation Properties of a Model Helicase by DNA Damage and Sequence Content within the Track
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In Bacillus subtilis, broken DNA ends are processed for repair by conversion to a 3′-ssDNA overhang terminated at a recombination hotspot (Chi) sequence. This reaction is catalysed by the AddAB helicase-nuclease that unwinds the DNA duplex and degrades the nascent single-strands in a Chi-regulated manner (Yeeles and Dillingham, 2007). Recombination hotspots regulate AddAB function by down-regulating nuclease activity on the 3′-strand beyond Chi and by preventing reannealing of nascent single strands via formation of a DNA loop (Yeeles et al., 2011). In this work, we have used Magnetic Tweezers to investigate the real-time dynamics of AddAB translocation on damaged or undamaged DNA and the effect of recombination hotspot recognition on this process. AddAB translocation traces showed a complex appearance with variable velocities between 200-400 bp/s at room temperature. DNA translocation by AddAB was slower and more prone to pausing in areas of high GC content which contained Chi sequences. Experiments using an AddAB mutant unable to recognize Chi showed no pauses but the same overall kinetic behavior along the track. On undamaged DNA, the pause duration followed a single exponential distribution with a decay time of 0.8 s. In contrast, very long stochastic pauses were observed on UV-damaged or nicked DNA substrates. Experiments to address the effect of recombination hotspot recognition on DNA translocation using bespoke Chi-containing substrates are ongoing and will also be discussed.
References
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3106-MiniSymp
The Nucleotide-Binding State of Microtubules Modulates Kinesin Processivity and Tau's Ability to Inhibit Kinesin Mediated Transport
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Tau's ability to act as a potent inhibitor of kinesin motility *in vitro* suggests it may actively participate in the regulation of axonal transport *in vivo*. However, it remains unclear how kinesin based transport could then proceed effectively in neurons, where tau is expressed at high levels. One potential explanation is that tau, a conformationally dynamic protein, has multiple modes of interaction with the microtubule, not all of which are inhibitory for kinesin motility. Thus, if tau can bind microtubules in distinct conformations or at unique binding sites that no longer inhibit kinesin, transport would proceed unhindered along the axon. Previous studies support the hypothesis that tau has at least two modes of interaction with microtubules, but the mechanisms by which tau adopts these different conformations and their functional consequences have not previously been investigated. In the present study we have used single molecule imaging techniques to demonstrate that tau inhibits kinesin motility in an isoform dependent manner on GDP microtubules stabilized with either paclitaxel or glycerol, but not GMPCPP-stabilized microtubules. Furthermore, the order of tau addition to microtubules before or after polymerization has no effect on tau's ability to modulate kinesin motility regardless of the stabilizing agent used. Finally, the processive run length of kinesin is reduced on GMPCPP microtubules relative to GDP-microtubules. These results shed new light on tau's potential role in the regulation of axonal transport, which is more complex than previously recognized.

3107-MiniSymp
Biophysical Studies Reveals the Specific Activities of Fidgetin, a Microtubule Severing AAA Enzyme
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Cell morphology, development, and differentiation rely on the spatio-temporal dynamics of microtubules. Microtubule dynamics and network remodeling are finely tuned in cells by the orchestrated activity of microtubule-associated proteins (MAPs). Reorganization of the microtubule network is performed by a novel class of MAPs called microtubule severing enzymes that are AAA+ (ATPases Associated with various cellular Activities) family of ATPases. The former member of this novel class of AAA+ enzymes is katanin p60, the catalytic subunit of katanin complex that regulates microtubule length and dynamics in cells during interphase and mitosis and targets to microtubule defects. The newest member of the severing enzyme family is fidgetin, which is involved in mammalian development. We have performed the first biophysical characterization of fidgetin in vitro. Interestingly, at a low concentration this enzyme removes tubulin dimers preferentially from the minus end of the microtubules, making microtubules appear to depolymerizing. At a higher concentration fidgetin severs microtubules. We find that fidgetin targets and severs GMPCPP microtubules better than taxol-stabilized microtubules. Further, fidgetin removes extended regions of protofilaments, in an activity we call "protofilament stripping". Our results indicate that fidgetin is a microtubule severing enzyme with new and specific biophysical abilities and targeting on microtubules.

Platform: Ligand-gated Channels

3108-Plat
Apo and $InsP_3$-Bound Crystal Structures of the Ligand-Binding Domain of an $InsP_3$ Receptor
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The ligand-binding domain (LBD) of inositol 1,4,5-trisphosphate ($InsP_3$) receptors ($InsP_3R$), which comprises the ~600 amino-terminal residues, is coupled to and thereby exerts allosteric control over the trans-membrane pore domain. Even when produced as an isolated construct, LBD binds $InsP_3$ with affinity and selectivity comparable to those of the whole $InsP_3R$ protein. The LBD sequence encodes the two β-trefoil folds, β-TF1 and β-TF2, followed by an armadillo repeat fold (ARF). A construct comprising only β-TF2 and ARF (termed $InsP_3$-binding core) binds $InsP_3$ with even higher affinity than