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allowed us to extract quantitative kinetic parameters that precisely describe these processes in living cells.
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3099-Symp
Into the CoSMoS: Single Molecule Analysis of Spliceosome Assembly and Activation
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Melissa J. Moore[5].
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Excision of introns from pre-mRNAs is mediated by the spliceosome, a large, dynamic complex consisting of five small ribonucleoprotein particles (snRNPs) and scores of associated proteins. Current understanding of spliceosome assembly is based largely on the procession of stable complexes that can be resolved from in vitro splicing reactions. Such ensemble experiments have suggested a highly ordered, linear assembly pathway in which initial binding of U1 snRNP to the 5' splice site is followed by stable U2 association with the branch site and subsequent U4/U5/U6 tri-snRNP and Nineteen Complex (NTC) addition to form the fully assembled spliceosome. Previously unknown, however, were the detailed forward and reverse kinetics of each assembly step, the extent to which branched and/or dead-end assembly pathways exist, and whether or not different introns utilize the same or alternate assembly pathway(s). We are now addressing these questions by combining yeast genetic engineering, chemical biology, and multi-wavelengthfluoresence microscopy to follow assembly of single spliceosomes in real time. Because no protein purification or reconstitution is required for such Colocalization Single Molecule Spectroscopy (CoSMoS), this experimental strategy should prove widely useful for mechanistic analysis of many other macromolecular machines in environments approaching the complexity of living cells.

3100-Symp
Chromatin Dynamics: At the Source of RNA Production
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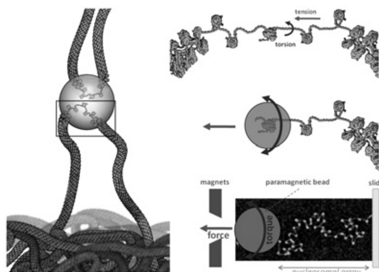
Through its local heterogeneities and transient structural changes resulting from chemical modifications and physical constraints imposed by numerous actors in vivo, chromatin dynamics influences (and is influenced by) DNA transcription, both at the initiation and elongation stages.
While transcription events often correlate with large chromatin movements, RNA polymerase access to its target sequence implies some nucleosome dynamics (potentially mediated by chromatin remodeling factors) and its subsequent tracking along the DNA template imposes some drastic topological and structural changes that propagate in the transcribed chromatin domain. Experiments and models aimed at deciphering the key features of chromatin dynamics and topology upon transcription will be presented.



In a second part of the talk, we will shift from eukaryotes to prokaryotes and show some recent data on ncRNA self-assembly, which role (and potential occurrence in eukaryotes) will be questioned.

3101-Symp
Biophysics of the Interaction of HIV with Mucus Barriers during Sexual Transmission
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During sexual transmission, HIV must overcome mucosal barriers to reach underlying target cells. The epithelial barrier function of the female reproductive tract is further enhanced by a protective layer of cervical mucus (CM). It is believed that antibodies associated with mucus barriers of the gut can have antimicrobial activity, and so we explored the possible role that antibodies which can bind to virions might alter particle transport through mucus. To characterize the effects of HIV-specific antibodies on viral diffusion in CM, we utilized two red and green fluorescently tagged HIV types: wildtype enveloped virus (WT-Gag-mCherry) and HIV devoid of envelope proteins, (ΔEnv-Gag-GFP) which allows for simultaneous visualization of both virus types in CM. For our assay, both virus types were mixed at equal concentrations and either added directly to CM or incubated with HIV-specific antibodies prior to being added to CM. Particle tracking software was used to determine particle position and measure the mean squared displacement (MSD), a standard measure of microscopic motion. We found that using anti-MHC class I antibodies, which bind epitopes found on WT and ΔEnv virus, decrease the movement of both virus types. In addition, neutralizing and non-neutralizing anti-gp41 and anti-gp120 antibodies specifically impaired the mobility of WT virus when compared to ΔEnv virus or when compared to virions that received no antibody treatment. In addition, we observed greater inhibition of HIV transport in CM with multimeric anti-Env antibodies when compared to monomeric antibodies. Our studies reveal that virus binding antibodies can slow the transport of HIV within the mucus coating of the female reproductive tract. This suggests that a vaccine that generates broadly binding antibodies could potentially prevent viral interactions with target cells thus offering protection against productive infection.

Minisymposium: Molecular Motors: Stopped or Slowed by Their Tracks

3102-MiniSymp
Single-Molecule Protein Unfolding and Translocation by the AAA+ Protease, ClpXP
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All cells employ ATP-fueled AAA+ proteases for protein-quality control and regulation. In the ClpXP protease, the ring-shaped AAA+ molecular motor ClpX first recognizes and mechanically unfolds specific protein substrates, then translocates the denatured polypeptide through a central axial pore into the barrel-shaped peptidase ClpP for degradation. Although the mechanisms of ATP utilization and substrate degradation have been extensively studied in solution and recently at the single-molecule level[1, 2], the molecular details underlying mechanical substrate unfolding and stepping along the polypeptide track remain unexamined.
Using a dual-laser optical trap, we probed how ClpXP unfolds and translocates a multi-domain protein substrate at the single-molecule level. In our assay, a ClpXP-substrate complex is tethered between two trapped polystyrene beads held in a dumbbell configuration [2]. Motility records of ClpXP along the polypeptide track show unique, fingerprint-like, substrate unfolding and translocation events. Following rapid and cooperative unfolding of individual domains, we find that ClpX translocates the polypeptide into ClpP, taking small steps of 5-8 amino acids. The nature of the polypeptide track affects ClpXP mechanochemical activity. ClpX step size does not depend on ClpP, though we observe substantial substrate refolding and slippage events when only ClpX is examined. Our results support a power-stroke model of denaturation in which successful unfolding requires mechanical pulling by the enzyme to coincide with transient stochastic protein destabilization.
[1] Shin, Y., Davis, J.H., Brau, R.R., Martin, A., Kenniston, J.A., Baker T.A., Sauer, R.T., Lang, M.J. Single-molecule denaturation and degradation of proteins by the AAA+ ClpXP protease. PNAS, 106, 19340 (2009)
[2] Aubin-Tam, M. E., Olivares, A. O., Sauer, R. T., Baker, T. A., Lang, M. J. Single-molecule protein unfolding and translocation by an ATP-fueled proteolytic machine. Cell, 145, 257-67 (2011)

3103-MiniSymp
Single-Molecule Imaging Reveals Mechanisms of Roadblock Clearance by DNA Motor Enzymes
Ilya J. Finkelstein[1], Ja Yil Lee[1], Estelle Crozat[2], David J. Sherratt[3],
Eric C. Greene[1].
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In the cell, nucleic acid motor proteins act on substrates occupied by other proteins, yet little is known regarding the inevitable collisions that must occur. Using nanofabricated curtains of DNA and real-time, multi-color single-molecule microscopy we visualized collisions between two model translocases and DNA-bound obstacles. We show that both RecBCD, a

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, EcoR-I(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.

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Yeeles, J. T., van Aelst, K., Dillingham, M. S., and Moreno-Herrero, F. (2011). Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Molecular Cell 42, 806-816.

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

Chun-Chi Lin, Kyuwon Baek, Zhe Lu.
Department of Physiology, Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA, USA.

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