Symposium 5: How Proteins Move on DNA

992-Symp
High-resolution Optical Trap Measurements Of A Ringed DNA Translocase
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Ringed ATPases are ubiquitous in the cell, involved in such diverse tasks as cell division, chromosome segregation, DNA recombination, strand separation, and conjugation. Despite their importance, the mechanism of coordination between the subunits of ringed ATPases remains poorly understood. Viral DNA packaging, a process driven by a ringed, homomeric DNA translocase, provides a model system to study this coordination. The *Bacillus subtilis* bacteriophage phi29 packaging motor has been proposed to operate by sequential action of its subunits, each packaging DNA in stepwise increments of 2 base pairs (bp) per ATP hydrolyzed. Here, we present the first direct observations of individual packaging steps of this motor with recently developed high-resolution optical tweezers. Surprisingly, we find that packaging occurs in increments of 10 bp, each consisting of four 2.5-bp substeps. The dwell time statistics between these 10-bp ''bursts'' indicate that the motor loads multiple ATPs prior to translocation, and suggest a two-phase mechanochemical cycle in which ATP binding and translocation are temporally segregated. These studies reveal a much more complex coordination between motor ATPases than previously anticipated that contrasts sharply with most published models. In addition, the observation of a non-integer step size demands new models for motor-DNA interactions. Our findings are not only relevant to other viral packaging systems, but may also provide insight into the mechanisms of the large class of ringed molecular motors that share many structural similarities.

993-Symp
High throughput assays for visualizing individual protein-DNA interactions
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994-Symp
Coupling of two motors: T7 helicase-primase and DNA polymerase
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T7 bacteriophage encodes all the proteins it needs to replicate its duplex DNA genome. The T7 replisome consists of the homo-hexameric T7 gp4 helicase-primase protein, T7 DNA polymerase (T7 gp5 complexed with E. coli Thioredoxin) and the T7 gene 2.5 protein (the single stranded DNA binding protein). The simplicity of the T7 replisome makes it a model enzyme complex to understand the mechanisms of DNA replication and the roles of the motor proteins in DNA replication. T7 helicase is a ring-shaped motor protein that binds ssDNA within its central channel and uses the energy of dTTP hydrolysis to move processively along ssDNA as well as to unwind the strands of duplex DNA. In this manner, the helicase motor unwinds the dsDNA genome and creates ssDNA templates for the DNA polymerase to copy the duplex DNA strands via leading and lagging strand synthesis. We will present here our transient-state ensemble and single molecule kinetic data that characterized the movement (speed and directionality) and energy coupling (bp translocated per NTP hydrolyzed) of T7 helicase as a function of duplex DNA stability, the type of nucleotide fueling movement, and how the helicase activity is influenced by the presence of the DNA polymerase and primase.

995-Symp
Translocation and Unwinding by DnaB
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DNA replication requires the action of a helicase, a motor protein that unwinds dsDNA into its single-stranded components. In E. coli, the replicative helicase is DnaB, a hexameric, ring-shaped protein that encircles and translocates along ssDNA, denaturing dsDNA in advance of its motion. Using multiplexed single-molecule measurements with a magnetic tweezer (Ribeck and Saleh, 2008), we have investigated the translocation and unwinding activities of DnaB, both alone and in complex with other replisome components. We discuss our findings in the context of both 'Brownian ratchet' theories of dsDNA denaturation and mechano-chemical theories of motor protein motion.

Symposium 6: Store-operated Calcium Channels in the Molecular Age

996-Symp
STIMulating Calcium Entry at ER-Plasma Membrane Junctions
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Store-operated calcium (SOC) entry is essential for maintaining endoplasmic reticulum (ER) functions and generating the sustained calcium signals crucial for gene expression, secretion, cell motility, and cell proliferation. To understand the molecular mechanism of how receptor-evoked ER calcium store depletion induces calcium entry through SOC channels at the plasma membrane, we screened a small interference RNA (siRNA) library targeting the human signaling proteome and identified several regulators of SOC entry including STIM1 and STIM2. We found that STIM1 initiates SOC signaling by sensing calcium levels in the ER using its luminal EF-hand motif. Using a fluorescence resonance energy transfer (FRET) approach, we showed that STIM1 rapidly forms oligomers after calcium store depletion. We further demonstrated that STIM1 subsequently translocates to ER-plasma membrane junctions using confocal and total internal reflection fluorescence (TIRF) microscopy. Moreover, we showed that STIM1 translocation to ER-plasma membrane junctions requires a C-terminal polybasic motif. We further conducted human siRNA screens for regulators of basal calcium homeostasis and identified STIM2 as a feedback regulator that stabilizes basal cytosolic calcium levels. We showed that STIM2, like STIM1, senses ER calcium levels by its EF-hand motif, translocates to ER-plasma membrane junctions, and activates Orai1 calcium channels. In contrast to STIM1, STIM2 is activated in response to smaller reductions in ER calcium levels. Our data support two conclusions: 1) STIM2 functions independently of STIM1 for basal ER calcium levels or weak receptor stimuli and 2) STIM2 acts synergistically with STIM1 when calcium stores are depleted by strong receptor stimuli. We are further investigating the plasma membrane targeting mechanism of STIM1/STIM2 and are characterizing other regulators of SOC entry that we have identified in the siRNA screens.

997-Symp
Calcium Signals In Lymphocyte Activation And Disease
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Calcium ions function as universal second messengers in virtually all eukaryotic cells including cells of the immune system where they are crucial for the function of T and B cells, mast cells and dendritic cells. The predominant mechanism regulating intracellular Ca2+ levels in cells of the adaptive immune system is store-operated Ca2+ influx through so-called Ca2+−release activated Ca2+ (CRAC) channels. We identified ORAI1 (also named CRACM1) as a pore subunit of the CRAC channel essential for the function of T cells and mast cells. Mutation of ORAI1 in humans is associated with severe combined immunodeficiency (SCID), increased susceptibility to infections and a failure to thrive. ORAI1/CRAC channels are activated when intracellular Ca2+ stores are depleted. The resulting decrease in the ER Ca2+ concentration is sensed by stromal interaction molecule 1 (STIM1) that is required for activation of ORAI1/CRAC channels. We showed that murine T cells lacking STIM1 exhibit severely impaired store-operated Ca2+ influx. T cells from mice lacking STIM1 or its paralogue STIM2 both showed significantly reduced cytokine production in vitro and a defect in regulatory T cell development as well as lympho- and myeloproliferation in vivo. T cells from mice transgenic for an R91W mutation in ORAI1 that abrogates CRAC channel function in T cells from human SCID patients also showed substantially impaired store-operated Ca2+ influx and cytokine gene expression. This important role of STIM1 and ORAI1 in T cell function in mice is also apparent in T cells from human patients with SCID, which lack expression of ORAI1 and STIM1, respectively. Taken together STIM1, STIM2 and ORAI1 are essential regulators of store-operated Ca2+ entry in cells of the immune system and other tissues.

998-Symp
Structural And Mechanistic Insights Into Stim1-mediated Initiation Of Store Operated Calcium Entry.
Mitsu Ikura.