Symposium 5: How Proteins Move on DNA

992-Symp
High-resolution Optical Trap Measurements Of A Ringed DNA Translocase
Yann R. Chemla[1,2], Jeffrey R. Moffitt[2], K. Aathavan[2], Shelley Grimes[3], Paul J. Jardine[3], Dwight L. Anderson[3], Carlos Bustamante[2,4].
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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.
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Stromal interaction molecule-1 (STIM1) activates store operated Ca2+ entry (SOCE) in response to diminished luminal Ca2+ levels. We have recently determined the solution structure of the Ca2+−sensing region of STIM1 consisting of the EF-hand and sterile α motif (SAM) domains (EF-SAM) (Stathopulos et al. Cell Oct 3rd issue, 2008). The canonical EF-hand is paired with a previously unidentified EF-hand. Together, the EF-hand pair mediates mutually indispensable hydrophobic interactions between the EF-hand and SAM domains. Structurally critical mutations in the canonical EF-hand, 'hidden' EF-hand or SAM domain disrupt Ca2+ sensitivity in oligomerization via destabilization of the entire EF-SAM entity. In mammalian cells, EF-SAM destabilization mutations within full-length STIM1 induce punctae formation and activate SOCE independent of luminal Ca2+. We provide atomic resolution insight into the molecular basis for STIM1-mediated SOCE initiation and show that the folded/unfolded state of the Ca2+ sensing region of STIM is crucial to SOCE regulation. (Supported by CIHR and CFI).

999-Symp
A Molecular Mechanism for CRAC Channel Activation
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The Ca^{2+} release-activated Ca^{2+} (CRAC) channel is the most intensively studied member of the class of store-operated channels, and is well known to play a critical role in lymphocyte activation during the immune response. The central mystery of store-operated Ca^{2+} entry (SOCE) has been how depletion of Ca^{2+} within the ER lumen triggers CRAC channel activation in the plasma membrane (PM). Recent breakthroughs in identifying the ER Ca^{2+} sensor, STIM1, and the pore-forming subunit of the CRAC channel, Orai1/CRACM1, have spurred rapid progress in defining a molecular mechanism. Store depletion triggers the oligomerization of STIM1 and its redistribution to ER-plasma membrane (ER-PM) junctions, where Orai1 accumulates in the plasma membrane and CRAC channels open. These dynamic structures, consisting of clusters of STIM1 and Orai1 separated by a 10-25 nm gap comprise the elementary units of SOCE. Using fusion proteins in which the Ca^{2+} sensing domains of STIM1 were replaced by FRB and FKBP12, we showed that rapamycin-induced oligomerization causes the proteins to accumulate at ER-PM junctions and activate CRAC channels without Ca^{2+} store depletion. Thus, STIM1 oligomerization itself acts as a master switch to trigger the self-organization and activation of the SOCE machinery. Oligomerization acts by enabling STIM1 to accumulate at ER-PM junctions and to bind to CRAC channels diffusing throughout the overlying PM. Orai1 is trapped and activated by binding to a cytosolic subregion of STIM1 that we call the CRAC activation domain, or CAD. *In vitro* studies with purified proteins show that CAD binds directly to purified Orai1 and crosslinks CRAC channels to form clusters. These studies support a molecular mechanism for SOCE by which the STIM1 CAD traps and activates CRAC channels at ER-PM junctions via direct binding to Orai1.

Platform L: Membrane Protein Structure

1000-Plat
High Throughput Coarse-Grained Simulations of the Insertion of Transmembrane Helices
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Transmembrane helices play multiple vital roles in cell function, including signalling processes, channel gating and active transport. As such, there exists a significant body of data on the biological function and structural properties of naturally occurring helices and their mutants, and on synthetic helices such as WALP and LS3 which have been used to better understand the roles of different residues in determining position and orientation of helices in a membrane. Coarse grained MD simulations are becoming an increasingly popular tool for understanding the properties of biological systems, overcoming canonical limits of atomistic simulations such as timescale or system size. Such techniques involve several manual steps, including system build, simulation set up and analysis. Here we present the Sidekick software, which enables automation of these processes, thus enabling high throughput simulations on the basis of a small set of input sequences, or of a single sequence and a scanning mutation. We demonstrate the use of this software to approach two problems; the ability of two commonly used coarse grain methods to predict insertion efficiencies of helices generated from sliding window across a larger sequence, and molecular signalling of TM2 of the methyl accepting chemoreceptor protein Tar. Our results demonstrate the value of such an HT simulation approach in the interpretation of a range of experimental data.

1001-Plat
Structural models of Alzheimer's Abeta channels
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Amyloid beta (Aβ) peptides involved in Alzheimer's disease form Ca^{2+} permeant ion channels. We have been developing models of these channels to be consistent with experimental findings. These models are refined and evaluated by molecular dynamics (MD) simulations. In our segment nomenclature, residues 1-14 are called S1, residues 15-28 S2, and residues 29-42 S3. The models we find most consistent with functional properties have a pore lining formed by 6-12 S1 segments in a β-barrel structure. The cation selectivity of the pore is due to negatively charged residues at positions 1,3,7, and 11 that extend into the pore. This category of models may differ by the number of strands, whether these strands are parallel or antiparallel, and the structures of the protein that surround the S1 β-barrels. Heavy metal ions and histidine-containing peptides block Aβ channels and inhibit Aβ-induced apoptosis. Zn^{2+} and Cu^{2+} binding sites are formed in our models by E11, H13, and H14 residues. Clustering of these residues at the entrance to the pore is more pronounced in the parallel models, and they are nearer the axis of the pore in the 6-stranded models. The 6-stranded β-barrel models are also more consistent with blockade of the channels by Tris cations. We have developed hexameric and dodecameric models in which S2 and S3 segments are helical, and dodecameric models in which S2 and S3 segments form a 24-stranded β-barrel that surrounds the pore-forming S3 β-barrel. However, the models most consistent with microscopy studies of the channels are composed of 36 subunits with only a fraction of the S1 segments forming the pore. These models are hexamers of hexamer in which S3 segments of each hexamer forms a 6-stranded β-barrel that is exceptionally stable during MD simulations.

1002-Plat
Pore Formation and Structure of the Twin Arginine Translocase Subunit TatA from B. subtilis
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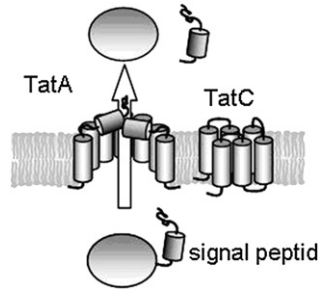
Cells have developed sophisticated transport machineries to allow proteins to cross membrane barriers. In bacteria, the twin arginine translocase (Tat) can translocate proteins even in the folded state. In *B. subtilis*, a membrane protein complex consisting of two subunits, TatA and TatC, is responsible for the Tat translocation process. TatA is believed to form a nanometer size pore trough which the protein is transported, whereas TatC is involved in recognition of the target protein signal peptide.
To get insight into the mechanism of the Tat translocation in *B. subtilis*, we studied the structure of the pore-forming subunit TatA and the pore assembly pursuing two complementary experimental approaches. The structure of individual TatA monomers in membranes or membrane-mimetic environments was characterized using solid state and solution NMR. The formation of oligomeric assemblies of TatA in the membrane, on the other hand, was investigated using in-plane neutron scattering of TatA reconstituted in aligned membranes. This way, we were able to both derive a detailed structural model of TatA, and to characterize pores formed by TatA.



1003-Plat
Lysophospholipid Micelles Sustain Diacylglycerol Kinase in Active and Stable Form for Biochemical and Structural Studies
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One of the challenges in the study of membrane proteins is that many methods require the proteins be soluble and stable in aqueous solution, which can be solved by the use of detergent micelles to provide an environment that mimics the hydrophobic environment of biological membranes. Yet, it remains to be determined which detergents are the best in sustaining the structural conformation and biological function of the proteins of interest. In this study, we used *Escherichia coli* DAGK, which is an integral membrane protein, to screen detergent conditions that can maintain the catalytic activity of the protein and are suitable for structural studies using NMR spectroscopy. We learned that