SYMPOSIUM 3: Superresolution in Biology: Applications in Important Biological Problems

82-Symp
Super Resolution Imaging of Dynamic Protein Complexes Provides New Insight into Bacterial Cell Cycle Regulatory Circuitry
Lucille Shapiro.
Stanford University Medical Center, Stanford, CA, USA.

83-Symp
Global Order and Self-Organization in the Escherichia Coli Cell Wall
Joshua W. Shaevitz.
Princeton University, Princeton, NJ, USA.
The ability of bacteria to grow into specific shapes depends on the accurate self-organization of the cell wall growth machinery. Absent a global roadmap, these enzymes must rely on local cues to guide the location and orientation of new material inserted into the peptidoglycan (PG) network. I will discuss our recent experimental and computational efforts in understanding the strategy used by Escherichia coli cells to grow into a rod shape. By combining 3D microscopy, particle tracking and physical simulations, we show that helical insertion of PG material guided by the actin homolog MreB leads to constant radius elongation and a robust global ordering of the glycan strands in a helical pattern relative to the cell long axis.

84-Symp
K-Shrec Analysis of Kinetochore Protein Archetecture at Nm Scale Accuracy
Edward (Ted) D. Salmon.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
Kinetochores link centromeric DNA to the plus ends of spindle microtubules (MTs). They contain more than 70 different proteins that function in chromatin anchorage, microtubule attachment, the spindle assembly checkpoint (SAC), force generation, and correction of MT attachment errors. Protein architecture within the kinetochore is poorly understood because the kinetochore core is only about 100 nm thick and it is has been very difficult to obtain molecular specificity at the resolution of electron microscopy. Based on the single molecule studies by Churchman and Spudich (P.N.A.S., 2005), the light microscopy methods that we developed called ''kinetochore-speckle high resolution co-localization'' (K-SHREC) allows us to determine the average separation between the centroids of two different color fluorescent probes that label different protein domains within kinetochores of human cells and budding yeast. Accuracy to less than 5 nm was achieved when we applied K-SHREC to metaphase cells where sister kinetochores were aligned in opposite directions. Using K-SHREC we have generated the first nm-scale maps of average protein position and orientation along the kinetochore inner-outer axis for 20 or more key protein complexes, and we have shown a correlation between changes in intrakinetochore separation of the Ndc80 complex from the periphery of the centromere and changes in SAC activity and phosphorylation activity within the kinetochore. (Joglekar et al., Curr. Biol., 2009; Wan, O'Quinn et al., Cell, 2010; Maresca et al., J. Cell Biol., 2009). We have also developed a fluorescence ratio method to measure protein copy number within kinetochores of budding yeast and chicken cells (Joglekar et al., Curr. Biol., 2006; Johnston et al, J. Cell Biol., 2010). Together these studies reveal high conservation in kinetochore protein architecture of the core MT attachment site between yeast and vertebrates. Supported by NIH GM 24364.

85-Symp
Real Time Imaging of Clathrin Coat Formation with Molecular-Scale Resolution
Tomas Kirchhausen.
Harvard Univ Med Sch, Boston, MA, USA.
This talk will focus on the use of cutting-edge high resolution structural visualization combined with dynamic live cell and single-molecule fluorescence imaging techniques to understand clathrin mediated endocytic processes involved in communication of cells with its environment, in pathogen invasion and viral infection, in cell growth control and cancer, and in the biogenesis of organelles. The goal is to generate molecular-resolution movies describing the function of machineries responsible for the control of these types of carefully choreographed interactions in cells. We will explore the regulation of the clathrin machinery engaged in classical endocytosis by presenting an integrated view based on recent data from snapshots (cryoEM tomography and 3-D single-particle reconstruction, x-ray crystallography) and from dynamic imaging (live cell and single molecule fluorescence microscopy) and show how they are used to inform cell, biochemical and mechanistic studies.

MINISYMPOSIUM 1: Apoptosis: The Killer Issue

86-MiniSymp
Mac and Bcl-2 family Proteins Conspire in a Deadly Plot
Kathleen W. Kinnally.
NYU CD, New York, NY, USA.
Bcl-2 family proteins regulate apoptosis by controlling formation of the mitochondrial apoptosis-induced channel, **MAC**. Assembly of this channel corresponds to the commitment step of apoptosis, as MAC provides the pathway across the outer membrane for the release of cytochrome c and other pro-apoptotic factors from mitochondria. While anti-apoptotic Bcl-2 suppresses MAC activity, oligomers of the pro-apoptotic members Bax and/or Bak are essential structural elements. Assembly of MAC from Bax or Bak was monitored in real time by directly patch-clamping mitochondria with micropipettes containing the sentinel tBid, which is a direct activator of Bax and Bak. Inducing MAC formation also caused outer membrane permeabilization, mitochondrial fragmentation, and a bystander effect in a Bax/Bak dependent manner. The approaches used for observing MAC opening were applied to characterize a series of high affinity inhibitors of MAC or iMACs. Our ability to pharmacologically open and close MAC may provide crucial clues in mechanistic studies of apoptosis and have potential therapeutic applications.

87-MiniSymp
Reconstitution of Proapoptotic Bak Function in Liposomes Reveals a Dual Role for Mitochondrial Lipids in the Bak-Driven Membrane Permeabilization Process
Olatz Landeta[1], Ane Landajuela[1], Itsasne Bustillo[1], David Gil[2], Carmelo DiPrimo[3], Mikel Valle[2], Vadim Frolov[1], **Gorka Basañez**[1].
[1]Unidad de Biofisica (CSIC-EHU), Leioa, Spain, [2]CIC Biogune Structural Biology Unit, Parque Tecnológico Zamudio, Spain, [3]Université Victor Segalen INSERM U869, Bordeaux, France.
BAK is a key effector of mitochondrial outer membrane permeabilization (MOMP) whose molecular mechanism of action remains to be fully dissected in intact cells, mainly due to the inherent complexity of the intracellular apoptotic machinery. Here, we show that core features of the BAK-driven MOMP pathway can be reproduced in a highly simplified in vitro system consisting of recombinant human BAK lacking the C-terminal hydrophobic domain (BAKΔC) and tBID in combination with liposomes bearing an appropriate lipid environment. Using this minimalist reconstituted system we established that tBID suffices to trigger BAKΔC membrane insertion, oligomerization and pore formation. Furthermore, we demonstrate that tBID-activated BAKΔC permeabilizes the membrane by forming structurally dynamic pores rather than a large proteinaceous channel of fixed size. We also identified two distinct roles played by mitochondrial lipids along the molecular pathway of BAKΔC-induced membrane permeabilization. First, using several independent approaches we showed that cardiolipin directly interacts with BAKΔC leading to a localized structural rearrangement at the N-terminal part of the protein which shares defined features with a conformational change observed in endogenous mitochondrial BAK early during apoptosis. Second, we provide evidence that selected curvature-inducing lipids present in mitochondrial membranes specifically modulate the energetic expenditure required to create the BAKΔC pore without directly interacting with the protein. Collectively, our results support the notion that BAK functions as a direct effector of MOMP akin to BAX, and also add significantly to the growing number of evidence indicating that mitochondrial membrane lipids are actively implicated in BCL-2 protein family function.

88-MiniSymp
Oligomeric Molecular Assemblies as Digital Switches in Apoptosis
Hao Wu.
Weill Cornell Medical College, Nw York, NY, USA.
Proteins of the death domain (DD) superfamily are central players in multiple cell death pathways. Previous structural studies have shown that DD proteins share a common six-helical bundle fold. However, how they mediate caspase activation was entirely unknown. Here we show that these proteins form highly oligomeric signaling complexes via elegant helical assembly modes, which presumably bring caspases into proximity for their interaction and activation. Structures of three oligomeric DD complexes will be presented, the 5: 7 PIDD: RAIDD complex in caspase-2 activation [1], the 5-7: 5 Fas: FADD complex in death receptor signaling [2] and the 6: 4: 4 MyD88: IRAK4: IRAK2 complex in Toll-like receptor signaling [3]. The highly oligomeric nature of the complexes dictates high cooperativity in their assembly, which may then control signaling in a digital manner, rather than in an analog manner. Recent single cell studies provide support that this digital control of signaling may be more common than previously known.