Symposium 18: DNA Processing in Genome Integrity and Maintenance

2843-Symp
Building A Replication Fork: Structural Synergy And Molecular Crosstalk Between Bacterial Initiators And Helicase Loaders
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The controlled initiation of DNA replication is critical to all cells. Dedicated initiator factors, which regulate this process, are members of the ATPases Associated with various cellular Activities (AAA+) superfamily, a broad grouping of evolutionarily related enzymes that remodel target macromolecules for numerous cellular transactions. In bacteria, ATP binding serves as the central event that allows the DnaA initiator to transition from a monomeric state into a large oligomeric complex that alters replication origins structure, triggers duplex melting, and facilitates replisome assembly. Using structural analyses, we show that ATP binding induces conformational changes in DnaA that permit the initiator to self-associate into an unanticipated, right-handed helical assembly. This quaternary arrangement actively wraps origin DNA into a positive supercoil about the DnaA oligomer, and frees ATPase catalytic motifs for interacting with other proteins at filament ends. Recent work on DnaC, the loading factor for the DnaB helicase, shows that this AAA+ protein is close structural homolog of DnaA that also assembles into a helical structure, and that engages the initiator in an ATP-dependent manner. Our findings provide a molecular framework for understanding how prokaryotic initiators transition between inactive monomer and functional multimer states, and implicate DnaC as an adaptor that plugs into an activated DnaA assembly to ensure the proper spatial deposition of replicative hexameric helicases onto a replication origin.

2844-Symp
Nucleosome Remodeling Complexes Increase Subnuclear Dynamics Of Chromatin In Yeast
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We have described the mobility of internal loci on yeast interphase chromosomes by live fluorescence microscopy with high precision. Based on single particle tracking and mathematical simulations of random walks in a confined volume, we could define the constraints exerted by the chromatin fiber. We find that local recruitment of the transcriptional activator VP16 and components of the Ino80 chromatin remodeling complex significantly increases diffusion rate, large rapid steps and the radius of constraint of a given locus. Importantly, the Ino80 induced mobility increase is largely dependent on its ATPase activity. Inhibition of transcription did not alter chromatin mobility and we find no correlation between transcriptional elongation and increased mobility. In analogy to experiments in mammalian cells, this indicates that transcription per se does not directly influence chromatin dynamics. These are the first mechanistic indications that chromatin remodelers can indeed alter mobility of the chromatin fiber - a process can facilitate chromatin contacts to distinct subnuclear regions. To examine the function of increased chromatin mobility, we have monitored the recombination rate of substrates to which Ino80 is targeted. We find that Ino80 enhances the spontaneous rate of gene conversion. This is not achieved by targeting the VP16 transactivation domain. The mode of Ino80 action at DSBs and at collapsed replication forks will be discussed both with respect to the physical characteristics of the chromatin fiber, and with respect to local nucleosome eviction.

2845-Symp
Visualization of Recombinational DNA Repair at the Single-Molecule Level
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We can visualize steps of recombinational DNA repair at the single-molecule level. Detection of first involves optical trapping of an individual dsDNA molecule that is attached to a polystyrene bead, and subsequent visualization by fluorescence microscopy. Protein, or protein action, is detected by attachment of an extrinsic fluorophore directly to our favorite protein. Using this approach, we have been visualizing the action of DNA motor proteins, such as RecBCD enzyme and Rad54 protein, and also of the DNA strand exchange proteins, RecA and Rad51 proteins. Visualization of individual RecA nucleoprotein filaments was achieved using a functional fluorescent RecA protein. Using this same approach, we have visualized assembly of human Rad51 nucleoprotein filaments. Although Rad51 nucleoprotein filament assembly occurs by a similar nucleation and growth mechanism, some characteristics differ: notably, the rates of nucleation and the extent of filament growth. Finally, we have examined how the a BRC repeat of BRCA2 protein affects Rad51 nucleoprotein filament dynamics. We discovered that one BRC repeat is sufficient to modulate RAD51-DNA interaction in two opposing, but functionally reinforcing ways: targeting RAD51 to ssDNA and prohibiting RAD51 nucleation onto dsDNA. The significance of these results in terms of control of Rad51 nucleoprotein assembly will be discussed.

2846-Symp
Replisome Dynamics And Polymerase Exchanges Enable Forks To Cope With Various Obsticals
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Replication only occurs once in the life of a cell, but the DNA genome is very large and many obstacles are encountered along the way. For example, RNA polymerases are slow and will sometimes be encountered by the replication fork. We find that the E. coli replisome successfully bypasses an in-line RNA polymerase by displacing it and using the transcript as a primer for continued leading strand synthesis. Encounter of the replisome with a head-on RNA polymerase transcribing the lagging strand will also be discussed. Encounter of the replisome with DNA lesions are often avoided during normal conditions though their efficient repair before the replication fork reaches them. But when damage to DNA is extensive, replisome encounters with DNA lesions are likely to be frequent, eventually leading to strand breaks and cell death. We find that the damage induced polymerases, Pol II and Pol IV, form ''alternative'' replisomes with DnaB helicase and the beta sliding clamp - even in the complete absence of Pol III. Pol II and Pol IV are much slower than Pol III, and we find that these polymerases control the DnaB helicase, coordinating the rate of unwinding to match their speed of synthesis (1-5 ntd/s). These forks are highly stabile, over 10 min, implying that these polymerases stabilize DnaB and preserve the fork, preventing it from collapse. We also find that Pol II, Pol III and Pol IV are highly fluid in their exchange among one another on the clamp at a moving replisome. Overall, these results imply that alternative polymerases may slow the fork during times of extensive DNA damage, thereby acting as a mechanical checkpoint to give more time for lesions to be repaired.

Symposium 19: Muscle: A Model System for Emergent Molecular Motor Properties

2847-Symp
Single Molecule Mechanics Of Myosin Motors Under Load
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Many types of cellular motility are driven by motor proteins of the myosin family. On the one hand the diversity of functions ranging from muscle contraction to cell locomotion, intracellular transport or even signal transduction in hearing is reflected in differences in structure, mechanics and regulation between different classes of myosins. On the other hand recent structural, kinetic and single molecule mechanical studies revealed basic mechanisms of chemo-mechanical energy transduction that seem to be shared amongst myosin motors, such as a working stroke in two phases coupled to the release of Pi and ADP or strain dependence of ADP release. Many details of the basic mechanism still remain unclear, including the effect of stall forces on the mechanics of a single motor head. Because of their large working stroke and relatively slow kinetics nonmuscle myosins including myosin V are well suited to investigate details of the basic mechanism. Using optical tweezers we have resolved load dependent conformational changes of single-headed myosin V constructs near stall and will discuss implications of these findings for the general mechanism of myosin motility and for processive movement of the native, dimeric myosin V motor. Supported by MRC and NIH.

2848-Symp
Structure-Function Studies of Myosin Motor Domains
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Domain insertions and replacement of functional modules in the myosin head fragment with synthetic sequences provide efficient means to manipulate key features of the myosin motor such as actin- and nucleotide-affinity, coupling between the actin- and nucleotide-binding sites, force production and even the direction of movement in a well defined manner. Additional, this approach facilitates the production of functional motor domains derived from a wide