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

range of members of the myosin family. In recent work, we have combined this approach with the use of small molecule effectors of myosin motor activity. We identified pentabromopseudilin (PBP) and related halogenated alkaloids as potent inhibitors of myosin-dependent processes such as isometric tension development, unloaded shortening velocity, and in vitro motility. Coupling between the actin and nucleotide binding sites is reduced in the presence of these inhibitors. PBP-induced changes in rate constants for ATP-binding, ATP-hydrolysis and ADP dissociation extend the time required per actin-activated myosin ATPase cycle. Additionally, the ratio of time spent per ATPase cycle in strong and weak binding states is shifted by PBP and related compounds in favor of non-force generating states. To elucidate the binding mode of these compounds, we crystallized their complexes formed with the Dictyostelium myosin-2 motor domain. In every case, the electron density for the small molecule inhibitor is unambiguous. All compounds bind to a novel allosteric site near actin-binding residues at the tip of the 50-kDa domain. The residues involved in the binding of this new class of inhibitors are only moderately conserved between the members of the different myosin classes. This is consistent with the observed differences in IC50 values. The results of molecular modeling studies show that these isoform-specific variations in the extent of inhibition can be predicted at least in trend for each of the new compounds.

2849-Symp
The collective mechanics of myosin in muscle
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2850-Symp
An Integrative Analysis of the Muscle Myosin Motor Using Genetic and Transgenic Tools
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We use *Drosophila melanogaster* to investigate the mechanisms by which myosin heavy chain isoforms contribute to muscle-specific cyto-architectures and contractile properties. We expressed various myosin isoforms in transgenic indirect flight muscles. Along with our collaborators, we examined 1) ATPase, *in vitro* actin sliding, step size and structure of the isolated myosin, 2) ultrastructure and mechanical properties of muscle fibers, and 3) locomotory abilities of the transgenic organisms. We found that isoform-specific differences in myosin cause relatively small structural variations in muscle assembly, but are critical to myofibril stability and function. ATPase, *in vitro* motility and fiber mechanical assays show that embryonic and indirect flight muscle isoforms represent slower and faster myosins, respectively. We have used a series of chimeric transgenes to study the function of the alternative domains that vary among the *Drosophila* myosins. We found that the converter is a key determinant of isoform-specific properties, and that some mechanisms of fine tuning myosin function differ from those of vertebrates.
Drosophila point mutants also help define the function of structural domains of myosin. We analyzed point mutations in the transducer domain near the nucleotide-binding pocket. While increased myosin ATPase and actin sliding ability of one transducer mutation induce dramatic degeneration of the indirect flight muscles, another mutation that reduces these properties does not affect myofibril stability. The effects of these mutations on the *Drosophila* heart are disparate as well, with increased function yielding restrictive cardiomyopathy and decreased function leading to dilated cardiomyopathy. These phenotypes parallel those arising from myosin mutations in humans. *Drosophila* may thus serve as a useful model for studying the molecular basis of cardiomyopathy, as well as mechanisms of its suppression.

Platform AX: Protein Dynamics II

2851-Plat
Slow Correlated Movement of Structural Elements in Hemoglobin and Myoglobin
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Structural fluctuations of proteins in solution are often reflections of functional movements and appear to follow low energy pathways that have evolved to accommodate conformational changes triggered, for instance, by binding to ligands or catalysis of reactions. The study of these fluctuations can provide substantial insight into protein function. The spatial extent and dynamics of intramolecular movements in proteins are highly dependent on the environment of the protein, being particularly sensitive to the concentration of proteins and other constituents in the solution. Consequently, studies as a function of protein concentration may provide further insight into the nature of these fluctuations than is possible when experiments are carried out at a single protein concentration. Wide-angle x-ray solution scattering (WAXS) and neutron spin echo spectroscopy (NSE) provide substantial information about the spatial extent and time scale, respectively, of structural fluctuations in solution. They are particularly well suited to the study of slow, correlated movements that are central to many protein functions, providing information complementary to that obtainable by x-ray crystallography and NMR. Using these approaches, we have characterized the spatial and temporal properties of hemoglobin and myoglobin across a wide range of environments. For these proteins, the spatial extent and dynamics of correlated movements both increase rapidly as protein concentration decreases below about 50 mg/ml. A combined analysis of WAXS and NSE data indicates that the slow correlated fluctuations in these molecules are dominated by movement of relatively rigid alpha helices within the subunits. These fluctuations are highly dependent on the ligation state of the molecules and are altered by mutations that impact the function of the molecules.

2852-Plat
Method to Characterize the Global Dynamics of Proteins by Temperature Dependent Phosphorescence Lifetime Measurements. Application for the Allosteric Effect in Hemoglobin
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At the glass transition temperature of proteins, large scale, anharmonic motions of the conformation become activated. It has been shown that in this temperature range the phosphorescence lifetime of Trp residues that is long (several s) at cryogenic temperatures become efficiently quenched. We report the elaboration of a systematic method based on this observation with the purpose to use this effect for characterizing the global conformational dynamics of proteins. In this method, special conditions were determined under which the onset temperature of phosphorescence quenching can be clearly separated from the slaving effect of fluctuations activated in the solvent, and the relevance of this temperature for the global dynamics of the protein has been verified by parallel experiments on human alpha-oxyFe-betaZn-Hemoglobin, w.t. horse heart Myoglobin and Trp mutant forms of a two-domain protein, yeast phosphoglycerate kinase. The method was used to characterize the role of global dynamics in the allosteric response of human hemoglobin to the binding of effectors as Cl, IHP, DPG, BZF. The quenching effect showed a two state behavior with a characteristic onset temperature sensitive to the presence and quality of allosteric effectors. The data points were fit by a simple two state model and the thermodynamic parameters - enthalpy and entropy changes characteristic for the quenching effect were discussed in terms of oxygen hopping models. The study on hemoglobin verifies the concept that in allosteric regulation, the global dynamics of the protein tetramer plays important role.
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2853-Plat
Computational Methods for Predicting Sites of Functionally Important Dynamics
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Understanding and controlling biological function of proteins at the atomic level is of great importance; allosteric mechanisms provide such an interface. Experimental and computational methods have been developed to search for residue mutations that produce changes in function by altering sites of correlated motion. These methods are often observational in that altered motions are achieved by random sampling without revealing the underlying mechanism(s). We present two deterministic methods founded on structure-function relationships that predict dynamic control sites (i.e. locations that experience correlated motions as a result of altered dynamics).
The first method (''static'') is based on a single structure conformation (e.g. the wild type (WT)) and utilizes a graph description of atomic connectivity. The local atomic interactions are used to compute the propagation of contact paths. This description of structure connectivity reveals flexible locations that are susceptible to altered dynamics.
The second method (''dynamic'') is a comparative analysis between the normal modes of a WT structure and a mutant structure. A mapping function is defined that quantifies the significance of the motions in one structure projected onto the motions of the other. Each mode is considered up- or down-regulated according to its change in relative significance. This description of altered dynamics is the basis for a motion correlation analysis, from which the dynamic control sites are readily identified.