1745-Pos Board B589

Mechanism of Cadmium-mediated Inhibition of Msh2-Msh6 in DNA Mismatch Repair

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The observation that Cadmium (Cd2+) inhibits DNA mismatch repair and suppresses the function of mismatch binding protein, Msh2-Msh6, has led to the proposal that this DNA repair system is a specific target of Cd2+ toxicity, which increases risk of carcinogenesis. Cd2+ effectively blocks both the DNA binding and ATP hydrolysis activities of Msh2-Msh6. The data support a mechanism whereby multiple Cd2+ ions bind to Msh2-Msh6 with high affinity, promoting changes in protein conformation and corresponding loss of function. The inhibitory effect involves cysteine sulfhydryl groups on Msh2-Msh6, and the high Cd2+:Msh2-Msh6 ratio at which it occurs suggests that chelating groups from other residues such as histidine, aspartate, and glutamate on the protein, and perhaps anions in solution, participate in the interaction. If Cd2+-mediated inhibition of Msh2-Msh6 activity occurs via such a generic mechanism, it follows that other proteins should be affected similarly by this metal ion; indeed, a survey of unrelated ATPases reveals that Cd2+ suppresses the activities of enzymes containing multiple cysteines in similar fashion. These findings raise the question whether Msh2-Msh6 and the mismatch repair system are specific targets of Cd2+ toxicity. It is more likely that Cd2+ interacts in this manner with numerous proteins in vivo, which is consistent with its marked broad-spectrum toxicity. Based on these findings, we propose that the presence of Cd2+ ligands on proteins, particularly thiols, and the propensity of proteins to unfold on Cd2+ binding to such nonspecific sites, are critical determinants of the susceptibility of biological systems to Cadmium toxicity.

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Kinetic Mechanism of Mismatch Recognition by *S. cerevisiae* DNA Mismatch Repair Protein, Msh2-Msh6

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Kinetic Mechanism of Mismatch Recognition by *S. cerevisiae* DNA Mismatch Repair Protein, Msh2-Msh6 Jie Zhai and Manju Hingorani MB&B, Wesleyan University.

DNA Mismatch Repair (MMR), a process conserved through evolution, corrects base-pair mismatches and small insertion/deletion errors generated during DNA replication and recombination. Msh2-Msh6 proteins initiate DNA mismatch repair by recognizing mismatches and insertion/deletion loops, and trigger a series of events that result in excision of the incorrect DNA strand and resynthesis. Mismatch binding and subsequent repair initiation by Msh2-Msh6 are driven by its ATP binding and hydrolysis activities.

My research project is aimed at understanding how *Saccharomyces cerevisiae* Msh2-Msh6 protein recognizes mismatched DNA and uses its ATPase activity to signal DNA repair. The primary approach is rapid kinetic analysis of Msh2-Msh6-DNA interactions and its ATPase activity using fluorescence-based stopped-flow methods. The measured kinetic and thermodynamic parameters are being used to develop a comprehensive model mechanism of Msh2-Msh6 actions in DNA repair.

Our data thus far reveal these interesting characteristics of Msh2-Msh6 function: a) Msh2-Msh6 binds distinct mismatch sites, such as G:T, with high affinity, and Watson-Crick base pair-like sites, such as 2Ap:T (2Ap= 2-Aminopurine), with at least 10-fold lower affinity; b) Msh2-Msh6 has a similar rapid association rate for both sites, but it dissociates at a >30-fold slower rate from G:T *versus* 2Ap:T; c) ATP binding to Msh2-Msh6 increases its rate of dissociation from both sites, but Msh2-Msh6-G:T complex still has a longer half life (~2 seconds) compared to Msh2-Msh6-2Ap:T complex (~0.3 seconds). Thus, Msh2-Msh6 appears to bind Watson-Crick base pairs in search of errors, and it distinguishes bona fide mismatches by forming stable, long-lived complexes specifically at these sites. ATP binding to Msh2-Msh6 appears to aid this discrimination process by further destabilizing false positive complexes.

1747-Pos Board B591

Kinetic Analysis of Arginine-Finger Motif Function in *S. cerevisiae* Clamp Loader

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Replication factor C (RFC) loads a circular clamp, proliferating cell nuclear antigen (PCNA), onto a primer-template junction at the replication fork during DNA replication. RFC consists of five subunits, A-E, each of which belongs to the AAA+ family member of ATPases. The subunits are arranged in an open ring form with four ATPase sites located at the interfaces of subunits A-B, B-C, C-D and D-E. In each ATPase site, an Arginine-finger motif (SRC), which is provided by the adjacent subunit, is implicated in sensing ATP binding and regulating ATP hydrolysis. According to a recent study of SRC→SAC point mutations, it appears that RFC subunits play distinct roles during clamp assembly (Johnson, et al. 2006). We analyzed these mutants by measuring their pre-steady state functions of DNA binding and ATPase activities. We found that C_{SAC} ($B_{inactive}$) and D_{SAC} ($C_{inactive}$) mutants have severe defects in DNA binding, while B_{SAC} ($A_{inactive}$) and E_{SAC} ($D_{inactive}$) mutants have severe defects in DNA dissociation. These disruptions in RFC interactions with DNA, and corresponding effects of RFC ATPase activities, elucidate further the distinct functions of individual RFC subunits in the clamp assembly reaction.

Reference: Johnson A. et al., (2006) J. Biol. Chem. 281, 35531-35543.

1748-Pos Board B592

Behavior Of The T4 Bacteriophage Primosome: Helicase Motion And Its Coupling With Primase

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In the T4 bacteriophage the primosome is formed by the helicase (gp41) and primase (gp61) complex. Here we use magnetic tweezers to manipulate a single tethered DNA hairpin. The substrate extension is a real-time reporter of the primosome-activity. We have investigated the action of the helicase in complex with primase or of it fusion protein on special DNA substrate having a small number of priming sites only in the lagging strand.

Different primosome behavior is observed depending on (1) rNTPs mix and concentration, and (2) whether the helicase and primase are fused or not. In absence of rNTPs the experimental signal show increases in the substrate extension associated with dsDNA unwinding, and decreases in the substrate extension corresponding to the ssDNA rehybridization following helicase translocation on ssDNA. In presence of CTP no change is observed in the unwinding activity of gp41; however, the rehybridization signal is strongly altered by RNA primers synthesized by the primase. Our results suggest that, in these conditions, primase dissociates from the helicase during priming. On the contrary, the activity of the gp41/gp61 fusion protein shows different unwinding signal revealing the formation of ssDNA loops. Results are consistent with a ''looping mechanism'', in which during priming helicase follows translocation and a ssDNA loop is formed and released after priming is over. Interestingly, in presence of all rNTPs looping can also be observed in the wild type primosome, even that much more unfrequently than in the gp41/gp61 fusion protein. Overall the results suggest that the primase processivity in the context of the primosome is very low. However, within the full replisome, the ssDNA looping might be the predominant mechanism which allows the helicase to continuously unwind the DNA without dissociate from the primase.

1749-Pos Board B593

The ATPase Reaction of *S. cerevisiae* RFC Reveals a Finely Choreographed Mechanism for Loading PCNA Clamps on DNA

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Circular clamp proteins enhance the processivity of DNA replication and participate in numerous other DNA metabolic and cellular processes. Replication factor C (RFC) loads PCNA clamps onto DNA, and our analysis of *S. cerevisiae* RFC ATPase kinetics reveals new information on the mechanism whereby energy from ATP binding and hydrolysis applied to this task. We find that ATP binding drives a slow, rate-limiting change in RFC conformation that is necessary for DNA binding, which in turn causes rapid ATP hydrolysis, DNA dissociation and catalytic turnover. PCNA accelerates this slow step, leading to formation of an RFC-ATP-PCNA(open) intermediate that allows DNA entry into the clamp. Thus, the conformational change serves as a checkpoint in favor of PCNA binding and opening, before DNA binds RFC and triggers ATP hydrolysis to end the reaction. The data showcase the remarkable choreography of the reaction components that results in circular clamps linked topologically to DNA.

1750-Pos Board B594

Quantitative Characterization of Interactions of the *Escherichia Coli* SOS DNA Damage Response Proteins UmuD and UmuD' with the Replicative DNA Polymerase

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The Y family of DNA polymerases is a group of DNA damage tolerance enzymes with the specialized ability to bypass DNA lesions by inserting nucleotides opposite damaged sites in DNA. Translesion synthesis (TLS) responsible for most of the mutagenesis induced by UV radiation requires the $UmuD'_2$ protein (the cleaved form of the $UmuD_2$), UmuC, and RecA. $UmuD'_2$ activates UmuC, the catalytic subunit of the Y family DNA polymerase V, for mutagenic DNA replication. $UmuD_2$ and $UmuD'_2$ make a remarkable number of specific protein-protein contacts to DNA polymerases. Despite the nearly identical primary structure of $UmuD_2$ and $UmuD'_2$, their interactions with the same partner can differ in affinity and functional significance. Analysis of the UmuD/UmuD'-pol III interactions by affinity chromatography indicated that UmuD has a lower affinity for alpha (α) subunit of *E. coli*'s replicative polymerase III than does UmuD'.

We aim to understand how binding of UmuD dimers is coordinated with the activity of α subunit. We are utilizing the biochemical and biophysical methods to look at the kinetics of α subunit activity and at the formation of α-$UmuD_2$ and α-$UmuD'_2$ complexes. We are characterizing the ability of the α subunit to copy both damaged and undamaged DNA in the presence of UmuD and UmuD'. In order to quantify the binding of UmuD and UmuD' to the α subunit, we are determining the K_d (equilibrium dissociation constant) for this interaction by measuring intrinsic tryptophan fluorescence of the α subunit.

1751-Pos Board B595

Single-Molecule Studies of Fork Dynamics in E. coli DNA Replication

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We present single-molecule studies of the Escherichia coli replication machinery. We visualize individual E. coli DNA polymerase III (Pol III) holoenzymes engaging in primer extension and leading-strand synthesis. When coupled to the replicative helicase DnaB, Pol III mediates leading-strand synthesis with a processivity of 10.5 kb, 8-fold higher than that of primer extension by Pol III alone. Addition of the primase DnaG causes a 3-fold reduction in the processivity of leading-strand synthesis, an effect dependent upon the DnaB-DnaG protein-protein interaction rather than primase activity. A single-molecule analysis of the replication kinetics with varying DnaG concentrations indicates that a cooperative binding of 2-3 DnaG monomers to DnaB halts synthesis. Modulation of DnaB helicase activity through the interaction with DnaG suggests a mechanism that prevents leading-strand synthesis from outpacing lagging-strand synthesis during slow primer synthesis on the lagging strand.

1752-Pos Board B596

Molecular Mechanism of the Acceleration of the Damaged Base Extrusion and its Recognition by Bacterial MutM DNA Glycosylase: Free Energy Simulation Studies

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8-Oxoguanine (8oxoG) is frequently generated endogenously through the attack of reactive oxygen species on the genome. In bacterial base excision repair system, the enzyme, 8-oxoguanine (8oxoG) DNA glycosylase (MutM), carries out the search of the damaged base 8oxoG in DNA. After having encountered the damaged base, the enzyme flips it out of DNA helix, and it enters into the active site where the catalytic cleavage occurs. The search of 8oxoG by MutM is a difficult task, because 8oxoG differs subtly from undamaged guanine (G), and compared to G, it is very rare under normal conditions in DNA (about 1 in 10^5). To determine the factors involved in the specific recognition function of MutM, free energy (potential of mean force) simulations and targeted molecular dynamics simulations are performed for a number of different systems. The simulations indicate that base extrusion and entrance into the active site is, essentially, a three-step process. We also analyzed the free energy contributions of different components, such as the effects of DNA-bending induced by the binding of MutM to DNA and several important residues, in the base extrusion of the damaged and undamaged base.

1753-Pos Board B597

Molecular Mechanisms in the Repair of the Cyclobutane Dimer

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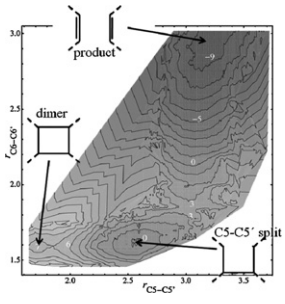
We investigate the molecular mechanism of the repair of the cyclobutane dimer radical anion in aqueous solution using ab initio MD simulations. Umbrella sampling is used to determine a two-dimensional free energy surface as a function of the C5-C5́ and C6-C6́ distances. The neutral dimer is unable to surmount a large free energy barrier for repair. Upon addition of an electron, the splitting of the C5-C5́ coordinate is virtually barrier less. Transition state theory predicts that the splitting of the C6-C6́ bond is complete on a ps timescale. The free energy surface suggests that the splitting of the two bonds is asynchronously concerted. Our work is the first to explicitly include the electronic degrees of freedom for both the cyclobutane dimer and the surrounding water pocket. The ab initio simulations show that at least 30% of the electron density is delocalized onto the surrounding solvent during the splitting process. Simulations on the neutral surface show that back electron transfer from the dimer is critical for the completion of splitting. To maximize splitting yield, the back electron transfer should occur beyond the transition state along the splitting coordinate.



1754-Pos Board B598

Regulation Of The DNA Damage Response By The DNA Polymerase Manager Protein UmuD

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Organisms experience DNA damage from environmental as well as endogenous sources. When bacterial cells experience DNA damage DNA and other stresses, the SOS response is induced, leading to upregulation of at least 50 genes in *E. coli*. Many of the genes whose expression is induced as part of the SOS response are responsible for DNA repair and cell cycle regulation. Another group of genes, specialized Y family DNA polymerases with the ability to replicate damaged DNA, play a role in tolerance to DNA damage at a potentially mutagenic cost. Multiple layers of regulation control the activity of these potentially mutagenic proteins. The function of Y family DNA polymerases is regulated by UmuD, a manager protein, and its cleaved form, UmuD'. The *umuD* gene products directly interact with both Y family polymerases as well as the beta processivity clamp. The goal of this study is to determine the conformation and dynamics of the *umuD* gene products in order to understand how they regulate the cellular response to DNA damage. We are using fluorescence resonance energy transfer (FRET) and hydrogen-deuterium exchange mass spectrometry (HXMS) to probe the conformations of UmuD and UmuD'. In HXMS experiments, backbone amide hydrogens that are solvent-accessible become labeled with deuterium over time, whereas those are are not accessible do not become labeled. Our HXMS results reveal that the N-terminal arm of UmuD, which is not present in the cleaved form UmuD', exhibits local partial unfolding. Residues that contact the N-terminal arm show large differences between UmuD and UmuD'. Additionally, there are substantial regions of stable conformation in both proteins. Complete characterization of UmuD and UmuD' dynamics is currently in progress.

1755-Pos Board B599

Mismatch Recognition Cycle in MutS and MSH2-MSH6 from Normal Mode Analysis and Simulations

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Post-replication DNA mismatch repair (MMR) is crucial in ensuring genetic fidelity in prokaryots and eukaryots. The initial step of MMR is recognition of defective DNA by MutS or its eukaryotic homologs. Binding of MutS to mismatched DNA, the subsequent initiation of repair, and eventual recovery to a mismatch scanning mode is coupled to ATPase activity in MutS. Crystal structures of MutS and the eukaryotic MSH2:MSH6 system place the ATPase domain far away from the DNA binding domains, implicating a complex allosteric mechanism.

Normal mode calculations and molecular dynamics simulations of MutS and MSH2:MSH6 structures were carried out to explore the coupling between DNA binding and ATPase activity. The mode analysis reveals conserved dynamics between the bacterial and eukaryotic complexes. Individual modes correlate ATPase activity with the probing of DNA kinking that is characteristic of mismatched DNA. Furthermore, differential ATPase activity between the MutS dimer moieties as observed experimentally is coupled to release of MutS from the mismatch during repair. Based on the calculations and consistent with available experimental