1745-Pos Board B589

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

F. Noah Biro.
Wesleyan University, Middletown, CT, USA.

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

Jie Zhai.
Wesleyan University, Middletown, CT, USA.

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.

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

Miho Sakato[1], Mike O'Donnell[2], Manju M. Hingorani[1].
[1]Wesleyan University, Middletown, CT, USA, [2]Rockefeller University, New York, NY, USA.

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.

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

Vincent Croquette[1], Maria Manosas[1], Michelle M. Spiering[2], Steve J. Benkovic[3].
[1]LPS-ENS-CNRS, Paris, France, [2]Department of Chemistry, Pennsylvania State University 104 Chemistry Building, University Park, PA 16802, Penn State, PA, USA, [3]2Department of Chemistry, Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802, Penn State, PA, USA.

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.

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

Yayan Zhou.
Wesleyan university, middletown, CT, USA.

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.

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

Jana Sefcikova, Jade Malcho, Kelly Foley, Penny Beuning.
Northeastern University, Boston, MA, USA.