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MutS sliding clamps on an uncertain track to DNA mismatch repair

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Mispairs in DNA are base pairs that violate Watson–Crick base-pairing rules or small insertions or deletions that affect only one strand. Most mispairs are DNA replication errors caused by incorporation of incorrect nucleotides or, more frequently, "slippage" of DNA polymerases on low-complexity sequences. Unrepaired mispairs alter RNA and protein sequences if the error affects the RNA polymerase template strand and cause heritable mutations when replicated. Defects in DNA mismatch repair (MMR) cause cancer predisposition syndromes in humans; inactivation of one copy of an MMR gene causes Lynch syndrome associated with increased incidence of many types of cancer, whereas inactivation of both copies causes constitutional mismatch repair deficiency associated with pediatric cancers.

Some bacteria and archaea use the NucS nuclease to mediate MMR; NucS cleaves DNA at mispairs, which likely initiates homologous recombination with the other daughter strand (1). In most organisms, however, MMR directs resynthesis of the newly synthesized DNA strand around the mispair either following strand excision or potentially by promoting strand-displacement synthesis (2). This process is controlled by homologs of *Escherichia coli* MutS and MutL, which will be called "MutS" and "MutL" in this commentary instead of "MutS homolog" and "MutL homolog" for brevity. The fact that MutS recognizes mispairs and subsequently recruits MutL to mediate downstream events has been understood for decades. What is unclear, however, is how these steps work; both MutS and MutL form rings around the DNA and can act up to 2 kbp from the mispair in either direction. These complex action-at-a-distance properties have prompted studies by advanced biophysical techniques and led to a proliferation of models, including the "molecular switch/sliding clamp" and "MutL polymerization" models and the now disfavored "hydrolysis-dependent translocation" and "static transactivation" models (described in refs. 2 and 3). In PNAS, Hao et al. (4) use single-molecule fluorescence resonance energy transfer (smFRET) with MutS and MutL from the thermophilic bacterium *Thermus aquaticus* to propose a "MutL arrest" model.

There is general consensus about how MutS recognizes mispairs. Unbound MutS rapidly hydrolyzes adenosine 5′-triphosphate (ATP) and primarily exists in an adenosine 5′-diphosphate (ADP)-bound mispair-searching conformation. In vitro, mispairs are found by three-dimensional collisions with DNA combined with one-dimensional searches involving rotation-coupled diffusion along the DNA backbone (5, 6). In vivo MutS associates with DNA replication forks and likely scans newly synthesized DNA (7). Mispair-searching complexes have short half-lives, but mispair-recognition complexes are long-lived (5, 6). Mispair recognition by homodimeric bacterial MutS and human MSH2–MSH6 (MutSα) and MSH2–MSH3 (MutSβ) is asymmetric; only one subunit recognizes mispairs by base flipping (MutS and MSH6) or insertion of residues between the DNA strands (MSH3) (8). These interactions bend the DNA by 45 to 60°, which helps distinguish mispairs from normal DNA.

There is also general consensus that mispair-bound MutS rapidly exchanges ADP for ATP and undergoes a conformational change to a "sliding clamp." Sliding clamps have increased solvent accessibility for DNA-proximal domains and do not bend DNA (9, 10). ATP hydrolysis is not required, based on results using nucleotide analogs and mutant proteins. Sliding clamps have reduced ATP hydrolysis and are proposed to be either ATP–ATP- or ADP–ATP-bound dimers. Sliding clamps rapidly diffuse bidirectionally along DNA with discontinuous contact with the DNA backbone (5, 6) and rapidly dissociate from DNAs with a free end but are trapped on circular or end-blocked DNAs. The structure of the ATP–ATP *E. coli* MutS dimer cross-linked to the N-terminal domain of MutL (8) has many biophysically predicted sliding clamp features. These features include tilting of the ATPase domains as well

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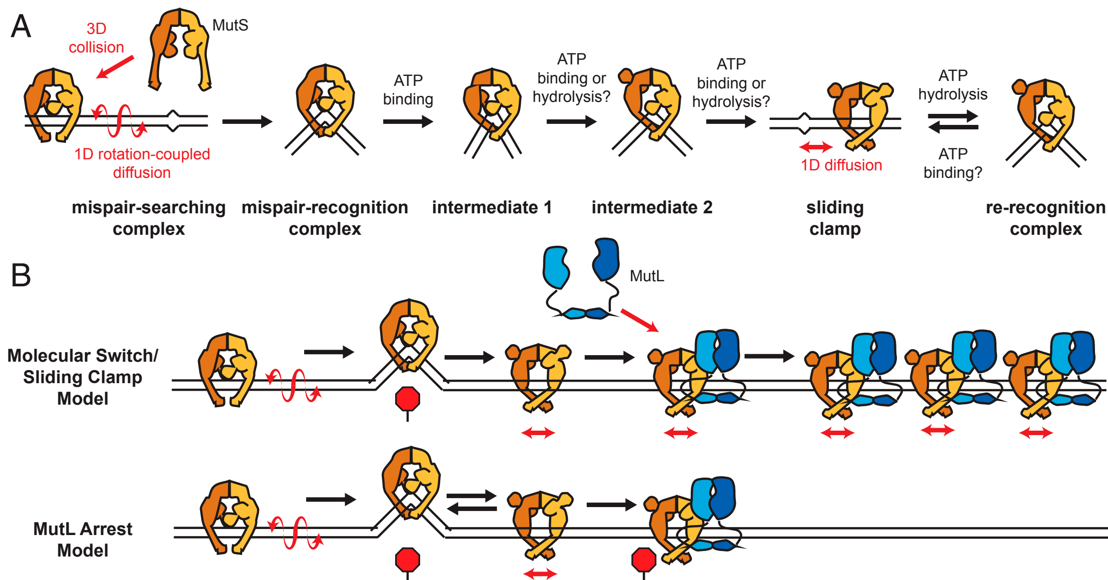

Fig. 1. (*A*) **MutS adopts multiple states during mispair recognition and sliding clamp formation. Intermediate states and mispair rerecognition have only been established for *T. aquaticus* MutS. (*B*) Mispair-proximal repair is proposed to be due to high concentrations of MutS–MutL complexes in the "molecular switch/sliding clamp" model and due to trapping of MutS by mispair rerecognition and MutS–MutL complex formation in the "MutL arrest" model.**

as exclusion of the mispair-binding and "connector" domains from the plane of the MutS ring. These conformational changes are likely driven by relaxation of the protein around ATP, consistent with other proteins with motions driven by cycles of ATP binding and hydrolysis. Analysis of smFRET of *T. aquaticus* MutS indicates the existence of two intermediates between the mispair-recognition complex ("FRET 0.7" in ref. 4) and the sliding clamp ("FRET 0"): Intermediate 1 ("FRET 0.7*") has a larger bend at the mispair, whereas intermediate 2 ("FRET 0.5") has a reduced DNA bend, migration of DNA toward the ATPase domains, and movement of at least one mispair-binding domain (11). ATP binding likely induces intermediate 1, and a burst of ATP hydrolysis in presteady-state kinetics (4) suggests ATP hydrolysis in the intermediate-1-to-2 or the intermediate-2-to-sliding-clamp transition and an ADP–ATP sliding clamp.

What is the role of the MutS sliding clamp? The sliding clamp, and potentially intermediate 2, recruit MutL. If a single MutL recruitment event was the only role for MutS, it seems unlikely that sliding clamps would be conserved during billions of years of evolution. Remarkably, some *Saccharomyces cerevisiae* MutS mutants, which recruit MutL but cannot transition to sliding clamps, support MMR reactions in vitro but are completely defective for MMR in vivo (12). Thus, sliding clamps play a crucial in vivo function, and this role is not recapitulated by current in vitro reconstituted MMR assays.

Sliding clamp movement exposes the mispair, allows recruitment of additional MutS complexes, interferes with DNA binding by other proteins, and would be necessary if diffusing MutS–MutL complexes are required for MMR (3, 13). These observations form the core of the "molecular switch/sliding clamp" model (3). In this model, multiple rounds of loading MutS sliding clamps at mispairs combined with diffusion along the DNA generate a local concentration gradient of MutS–MutL complexes that is highest around the mispair (Fig. 1). Consistently, comigrating *E. coli* and *S. cerevisiae* MutS–MutL complexes have been observed using single-molecule total internal reflection fluorescence microscopy (smTIRFM) (6, 14), in which MutS sliding clamps originate at mispairs but the MutS–MutL interactions occur randomly on the target DNA after sliding clamp formation (*E. coli*) or only at the mispair (*S. cerevisiae*).

Three observations by Hao et al. (4) for *T. aquaticus* MutS provide insights into the MutS sliding clamp intermediate. First, MutS sliding clamps can rebind the mispair. Based on the diffusion rate and lifetime of the sliding clamp, MutS likely passes over the mispair many times before rebinding occurs. Second, rerecognition requires ATP hydrolysis. Intriguingly, the FRET efficiency and lifetime of the rerecognition complex match intermediate 2, but not the original ADP-bound mispair-recognition complex. Intermediate 2 is better characterized than the rerecognition complex (11), so it is unclear if these states have the same conformation. Third, MutL appears to trap MutS sliding clamps on DNA, which contrasts with reported comigrating MutS–MutL complexes (6, 14). Based on these observations and atomic force microscopy of human MutS–MutL complexes (15), Hao et al. (4) propose a "MutL arrest" model in which MutS sliding clamps are constrained near the mispair by protein barriers, mispair rerecognition, and MutL binding. Together, these factors are predicted to ensure that MMR occurs near the mispair.

These different MMR models could reflect biological differences; MutH cleavage sites in *E. coli* methyl-directed MMR may be distant from the mispair, whereas activation of the MutL endonuclease by mobile PCNA or beta clamps could, in principle, involve less-mobile MutS–MutL complexes. However, these models may not be mutually exclusive. Reduced, but not eliminated, ATPase activity is common for MutS sliding clamps. Differences in mispair rerecognition observed by smFRET and smTIRFM (4, 6, 14) may result from the relative sensitivity of the techniques to rebinding events and relative differences in the reduction of ATP hydrolysis in MutS sliding clamps. Similarly, formation of the MutS–MutL complex, which traps *T. aquaticus* MutS on DNA (4), decreases the diffusion rate of *E. coli* MutS by 10-fold (14). Thus, both mechanisms could act in concert; loading of multiple MutS complexes, rerecognition of the mispair by sliding clamps,

and reduced migration of MutL-bound MutS could all increase the concentration of complexes and direct MMR in the vicinity of the mispair.

By focusing on the MutS sliding clamps and MutS–MutL complexes, could both models be incomplete? At sites of repair in vivo, MutL is in excess over MutS (7, 16), suggesting that MutS–MutL complexes might not be obligatory downstream signaling components. MutL has a long unstructured linker that connects the N- and C-terminal domains, which allows MutL to bypass DNA-bound proteins and mediate MMR even when the mispair and MMR-initiating sites are separated by roadblocks that would be problematic for MutS–MutL complexes (14, 17, 18). Could MutS–MutL complexes simply be MutL-loading intermediates? Interactions required for DNA-bound MutL clamps may cause the reduced diffusion of MutS–MutL complexes. Additionally, an ATP-binding defective MutL mutant, which is defective in vivo and cannot form independent MutL clamps, can still form comigrating MutS–MutL complexes (14). The requirement of multiple MutL clamps in MMR, as predicted from in vivo observations, would improve specificity. Each cycle of mispair (re-)recognition interrogates the energetic difference of mispair vs. base-pair binding, similar to multiple recognition events used by transfer RNA synthetases and DNA polymerases (19, 20). Reducing biological "noise" may avoid MMR-induced DNA damage, as mispair-free DNAs can recruit MutS–MutL in vitro, albeit at low levels. Ultimately, additional insights into MMR in vivo will require more studies that apply the same techniques to multiple experimental systems, resolve the relative roles of MutS–MutL complexes and MutL clamps in downstream events, develop MMR assays that better reflect in vivo phenomena, and test the resulting predictions in vivo.

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