

TIRF(ing) reveals Msh2-Msh6 surfing on DNA

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Faced with the thermodynamic and kinetic challenge of finding a few specific sites on DNA among millions of nonspecific sites, within a limited amount of time, what's a protein to do? Single-molecule studies show that some proteins have selected sliding on DNA as a solution.

The idea that mismatch repair (MMR) could increase the fidelity of DNA replication was proposed three decades ago by Meselson and colleagues¹ and validated by findings that MMR is essential for maintaining genomic integrity in organisms from bacteria to mammals. MMR proteins detect postsynthetic errors in DNA, such as base-pair mismatches and insertion/deletion loops (IDLs); distinguish the newly synthesized strand from the template strand; and direct excision of the misincorporated bases as well as DNA resynthesis (reviewed in refs. 2,3). MMR proteins also perform other important tasks in the cell, such as detecting and mediating apoptotic response to DNA damage⁴. Central to MMR is MutS (MutS homolog, Msh, in eukaryotes), a highly conserved protein that is responsible for locating the mismatches, IDLs and damaged sites and for signaling initiation of repair or other cellular responses. The structure and dynamics of MutS/Msh interaction with mismatches and IDLs have been studied extensively^{5–12}, but the mechanism by which such sites are found in the genome remains unresolved. The search for postreplicative errors is challenging, because replicative DNA polymerases have a low error rate of about 1 in 10⁷ incorporated bases¹³, meaning that in the 6-billion-base-pair human diploid genome, only ~600 errors occur in each replication cycle. Moreover, these errors have to be discovered and dealt with before transient markers identifying the newly synthesized strand are lost. In a recent issue of *Molecular Cell*, Gorman *et al.* present research on the *Saccharomyces cerevisiae* MutS homolog, Msh2-Msh6, aimed at understanding how this protein can quickly locate a few aberrant base pairs against the background of millions of Watson-Crick base pairs in DNA¹⁴.

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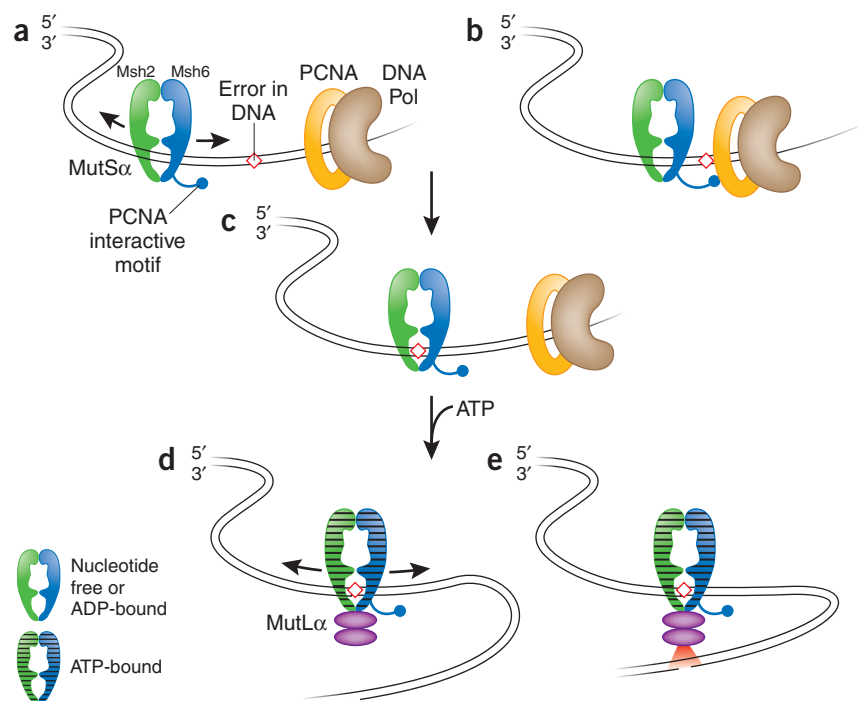


Figure 1 A model depicting Msh2-Msh6 movement on DNA. Shown are pre-mismatch recognition (a,b) and, after ATP binding (c), post-mismatch recognition events (d); Msh2-Msh6 may also interact with distal sites on DNA through space (e).

The authors labeled the Msh2-Msh6 dimer with antibody-conjugated quantum dots and used total internal reflection fluorescence (TIRF) microscopy to visualize single molecules interrogating the length of bacteriophage λ DNA. Their experiments revealed Msh2-Msh6 proteins sliding rapidly on DNA, apparently by one-dimensional diffusion, including rotation along the helical path of the phosphate backbone. All MutS proteins possess ATPase activity, but Msh2-Msh6 sliding was detected both in the absence of nucleotides and in the presence of ADP, which supports the conclusion that the movement is diffusion based. The average cumulative distance traveled by Msh2-Msh6 proteins was about 100 kilobases in 2 min, which yields an apparent velocity of close to 800 base pairs per second and affirms sliding as an important, if not dominant, component of the Msh2-Msh6 search mechanism¹⁴.

In vivo, DNA-bound proteins, such as histones, are expected to block MutS/Msh proteins from diffusing over extended lengths of the double helix, but these proteins can interact with the replisome through the sliding clamp (proliferating cell nuclear antigen, PCNA)¹⁵, suggesting that the DNA synthesis and search machineries may be coupled, by constant or intermittent contact, to facilitate rapid scanning of newly synthesized DNA (Fig. 1).

Another intriguing feature of Msh2-Msh6 behavior revealed by the single-molecule analysis is that these proteins pause for prolonged periods in the midst of sliding on DNA¹⁴. It is important to note that the bacteriophage λ DNA used in the study did not contain mismatches or IDLs; nevertheless, given that the DNA in MutS/Msh-DNA complexes is often found in a bent state^{5–8}, it is likely that flexible regions and/or regions

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prone to bending in the λ DNA serve as energy traps to halt Msh2-Msh6 diffusion. The authors propose that the long dwell-time of Msh2-Msh6 at such sites would facilitate the recruitment of additional proteins necessary to complete MMR¹⁴. We do not know, however, whether the sites on λ DNA where Msh2-Msh6 is entrapped are equivalent to the mismatch/IDL sites that are *bona fide* substrates for MMR. It would be counterproductive for MutS/Msh proteins to incorrectly initiate repair of every mismatch-like site at which they happened to pause while scanning the duplex. The potential for promiscuous repair was considered to be less of a problem after Wang *et al.* used unzipping force analysis to measure *S. cerevisiae* Msh2-Msh6 binding to single DNA molecules and reported barely detectable binding to sites other than mismatches and IDLs⁹. We now know, however, that Msh2-Msh6 proteins bound to nonspecific sites might have diffused away ahead of the unzipping fork and therefore been undetectable in that study. Thus, given the findings of the study by Gorman *et al.*¹⁴, the question of how MutS/Msh proteins select DNA substrates for repair has resurfaced.

In this context, it might be useful to remember an earlier proposal by Junop *et al.* that MutS/Msh proteins use their ATP-binding activity for mismatch verification¹⁶. According to their research, ATP binding results in lower affinity of the protein for matched as compared to mismatched DNA and thereby serves as a proofreading mechanism to favor repair of mismatch/IDL sites over other sites on DNA. Indeed, Gorman *et al.* observed that exchanging ADP in the reaction for ATP (or non-hydrolyzable ATP γ S) rendered many of the immobile Msh2-Msh6 proteins on λ DNA mobile again¹⁴, which suggests that ATP binding to Msh2-Msh6 nonspecifically trapped on DNA can trigger its return to scanning mode. Our kinetic analyses of *Thermus aquaticus* MutS^{17,18} and *S. cerevisiae* Msh2-Msh6 (ref. 12) activities show that

ADP-to-ATP exchange leads to transient stabilization of these proteins in ATP-bound state, specifically at mismatch/IDL sites in DNA. Thus, it is possible that differences in the ATP-coupled dynamic interactions of MutS/Msh with various DNA sequences and/or structures provide the basis for selective authorization of MMR at sites of mismatches and IDLs.

Long lifetimes of specific MutS/Msh-mismatch/IDL complexes could certainly promote MMR by facilitating recruitment of additional repair proteins to the site of the error. However, it is clear that MutS/Msh movement on DNA is important even in the post-mismatch recognition phase of the MMR reaction. In this phase, mismatch/IDL recognition and ATP binding by MutS/Msh proteins activates MutL proteins (MutL homolog, Mlh, in eukaryotes) to direct excision of the error-containing nascent DNA strand²⁻³. In *S. cerevisiae* and other eukaryotes, this activation step results in Mlh1-Pms1 (human Mlh1-Pms2) nicking one DNA strand in the vicinity of the mismatch/IDL to create a start site for exonuclease-catalyzed excision of a section of the strand¹⁹. This nicking activity is biased strongly toward the strand that contains a pre-existing nick, and the current view is that breaks in the newly synthesized DNA strand *in vivo* could serve as strand-discrimination signals that mark it for excision and resynthesis. The mechanism of communication between proteins at the mismatch/IDL site and distal sites involved in the repair reaction (such as a distal pre-existing break in nascent DNA) is still under investigation, with experimental evidence available to support both a '*cis*' mechanism, involving rapid movement of ATP-bound MutS/Msh proteins between the sites, and a '*trans*' mechanism in which MutS/Msh proteins remain bound at the mismatch/IDL site and interact with distal sites through space via looping DNA (Fig. 1)²⁰⁻²². The study by Gorman *et al.*¹⁴ provides real-time views of ATP γ S-bound Msh2-Msh6 moving on DNA, strengthening the case for the *cis* mechanism, although it is likely that both mechanisms

are used in a synergistic manner *in vivo* (for example, a *trans* mechanism would be useful when the movement of MutS/Msh on DNA is occluded by other DNA-bound proteins).

Using TIRF-based single-molecule techniques, Gorman *et al.* have caught Msh2-Msh6 in the act of diffusing on DNA¹⁴ and have significantly enhanced our understanding of how this protein can find rare errors in DNA and direct their repair. A recent study of human oxoguanine DNA glycosylase 1 (hOgg1) also revealed this enzyme sliding via one-dimensional diffusion on DNA²³, suggesting that multiple proteins responsible for DNA repair may have the capacity to conduct rapid, redundant scans of DNA in order to locate their sites of action in good time.

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