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Single-molecule views of MutS on mismatched DNA

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ABSTRACT

Base-pair mismatches that occur during DNA replication or recombination can reduce genetic stability or conversely increase genetic diversity. The genetics and biophysical mechanism of mismatch repair (MMR) has been extensively studied since its discovery nearly 50 years ago. MMR is a strand-specific excision-resynthesis reaction that is initiated by MutS homolog (MSH) binding to the mismatched nucleotides. The MSH mismatch-binding signal is then transmitted to the immediate downstream MutL homolog (MLH/PMS) MMR components and ultimately to a distant strand scission site where excision begins. The mechanism of signal transmission has been controversial for decades. We have utilized single molecule Forster Resonance Energy Transfer (smFRET), Fluorescence Tracking (smFT) and Polarization Total Internal Reflection Fluorescence (smP-TIRF) to examine the interactions and dynamic behaviors of single *Thermus aquaticus* MutS (TaqMutS) particles on mismatched DNA. We determined that TaqMutS forms an incipient clamp to search for a mismatch in ~1 s intervals by 1-dimensional (1D) thermal fluctuation-driven rotational diffusion while in continuous contact with the helical duplex DNA. When MutS encounters a mismatch it lingers for ~3 s to exchange bound ADP for ATP (ADP → ATP exchange). ATP binding by TaqMutS induces an extremely stable clamp conformation (~10 min) that slides off the mismatch and moves along the adjacent duplex DNA driven simply by 1D thermal diffusion. The ATP-bound sliding clamps rotate freely while in discontinuous contact with the DNA. The visualization of a train of MSH proteins suggests that dissociation of ATP-bound sliding clamps from the mismatch permits multiple mismatch-dependent loading events. These direct observations have provided critical clues into understanding the molecular mechanism of MSH proteins during MMR.

1. Introduction

DNA polymerases catalyze the pairing and covalent incorporation of complementary nucleotides into a transient single-stranded DNA (ssDNA) template within the replication fork [1]. The fidelity of this process is remarkable, resulting in less than one error per million nucleotides copied [2]. However, even that low error rate in human cells would lead to perhaps 1000 errors per cell division, which would ultimately have catastrophic consequence for genome integrity. Evelyn Witkin and Robin Holliday independently proposed the existence of mismatch repair (MMR) in 1964 to explain bromo-deoxyuracil incorporation in bacteria and gene conversion in fungi, respectively [3,4]. MMR primarily corrects polymerase misincorporation errors enhancing the overall fidelity of DNA replication up to 1000-fold. Loss of the MMR genes increases cellular mutation rates (Mutator or Mut) [5,6]. Defects in the human MMR machinery have been linked to the most common hereditary cancer predisposition syndrome hereditary non-polyposis colorectal cancer or Lynch syndrome (LS/HNPCC) as well as sporadic colorectal, endometrial, ovarian and upper urinary tract tumors [7]. The 20th anniversary of this connection was marked in 2013; a discovery that also established the concept that Mutators and genomic instability are a central cause of tumorigenesis [8].

The core components of MMR have been conserved throughout evolution [9]. MMR is an excision-resynthesis reaction that begins at a distant strand scission that may be located several

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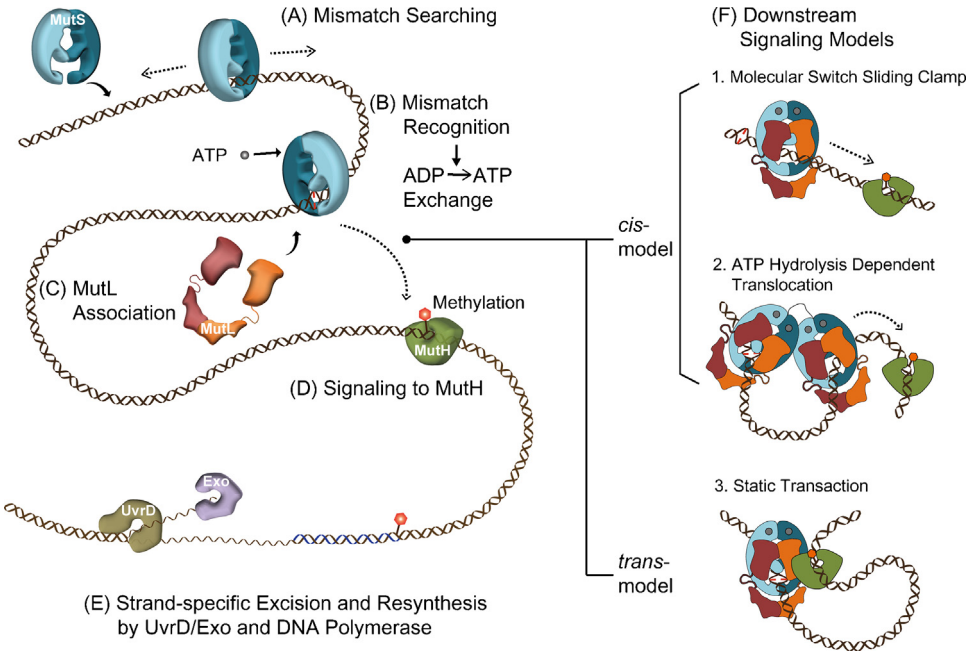

Fig. 1. The mechanism of mismatch repair. (A) Mismatch searching performed by MutS homologs (MSH) occurs on duplex DNA. (B) Mismatch binding by an MSH provokes the exchange of bound ADP for ATP (ADP → ATP exchange). (C) The MutL homolog (MLH/PMS) associates with the MSH to transmit the mismatch binding signal. (D) In bacteria signal, the first downstream signaling component appears to be MutH that is responsible for introducing a strand scission on the newly synthesized unmethylated DNA strand. Methylation is controlled by the DNA adenine methylase (Dam) at GATC sequences. (E) Excision of the newly replicated strand is controlled by the UvrD helicase along with one of four exonucleases (see text) in concert with the MSH-MLH/PMS complex. Resynthesis of the single-stranded gap is performed by the replicative DNA polymerase. (F) Models for mismatch signal transmission: (1) Molecular Switch Sliding Clamp where ATP-bound MSH along with MLH/PMS moves by 1-dimensional thermal diffusion to downstream mismatch repair (MMR) machinery, (2) ATP-Hydrolysis Dependent Translocation where the MSH and MLH/PMS form a multimeric complex that translocates to the downstream machinery using the energy of ATP hydrolysis forming a bidirectional loop structure, and (3) Static Transactivation where an MSH-MLH/PMS complex is formed at the mismatch that then associates with downstream MMR machinery by 3D collision forming a static loop structure.

thousand nucleotides either 3′ or 5′ from the mismatch. The excision tract extends uniquely from the strand scission to just past the mismatch suggesting a highly coordinated degradation process. MutS homologs (MSH) initiate MMR by recognizing mismatched nucleotides and then transmitting this discovery to downstream excision machinery (Fig. 1A and B). Bacterial MutS protein detects mismatches on DNA as a homodimer [10]. Eukaryotes appear to have duplicated and refined the MutS gene to form MSH heterodimers [11–13]. The MSH2-MSH6 heterodimer primarily recognizes base-base mispairs and single insertion/deletion unpaired nucleotides. In contrast, the MSH2-MSH3 heterodimer binds a few bases-base mispairs as well as unpaired nucleotides containing up to 8–12 insertion/deletion loop-type (IDL) mismatches [14–17].

Structural analysis has revealed that MSH proteins are members of the AAA ATPase family [18,19]. MSH homodimer/heterodimer proteins appear to retain at least one ADP [20–22] that appears to control unregulated ATP hydrolysis [20]. Mismatch binding triggers the release of the bound ADP, allowing ATP binding by both subunits (ADP → ATP exchange) [17,20,22–24] that results in dissociation from the mismatch. MutL homologs (MLH/PMS) are the first downstream MMR component that associates with ATP-bound MSH (Fig. 1C) [23,25]. While a GHKL (DNA Gyrase, *H*sp90, Histidine *K*inase and Mut*L*) ATP binding and hydrolysis motif is a defining feature of MLH/PMS proteins [26,27], its role in MLH/PMS functions during MMR remains obscure. Bacterial MutL functions as a homodimer while eukaryotic MLH/PMS function as heterodimers. The major MLH/PMS in *Saccharomyces cerevisiae* (Sc) is ScMlh1-ScPms1 and in human hMLH1-hPMS2 (ScPms1 and hPMS2 are conserved homologs) [28–31].

The MMR excision tract occurs uniquely on the newly synthesized DNA strand. This *strand specificity* is an essential property of MMR that decreases the potential for mutations. In *Escherichia coli* (Ec), the MutS-MutL complex activates the EcMutH endonuclease that then introduces a single-strand scission on the unmethylated strand in a nearby hemimethylated GATC site (Fig. 1D). The *D*NA *a*denine *m*ethylase (Dam) symmetrically methylates GATC sequences throughout the genome [32], which then become transiently unmethylated on the newly replicated strand following DNA replication. The EcMutH strand scission serves as an entry site for the EcUvrD helicase and one of four exonucleases (Fig. 1E; 3′ → 5′ exonucleases, EcRecJ and EcExoVII; 5′ → 3′ exonucleases, EcExoI and EcExoX) [33] that remove the newly replicated strand to just past the mismatch [34–36]. The ability to perform *Bidirectional excision* from either the 3′ or 5′ direction toward the mismatch is a second defining property of MMR. The resulting DNA gap is resynthesized by the replication machinery and sealed by DNA ligase [36].

Outside of Gram-negative enteric bacteria such as *E. coli*, the mechanism of introducing strand specificity and bidirectionality is a mystery. An interesting hypothesis suggests that RNaseH introduces a strand specific scission in eukaryotes during removal of randomly incorporated ribonucleotides that have occurred during replication [37,38]. However, the mutator activity of the EcMutH strand-specific scission component in *E. coli* is nearly equivalent to EcMutS and EcMutL [5]. In contrast, the mutator activity of *rnh* (RNaseH) deletions is at least 100-fold less than ScMsh2 or ScPms1 deletions [15,38]. These observations suggest that components associated with eukaryotic strand-specific scission during MMR are at the very least redundant. A role of MSH proteins in directing strand specificity is unknown.

The mechanism of *bidirectional excision* in eukaryotes is also a puzzle since the 5′ → 3′ exonuclease, ExoI, has been the only exonuclease linked to eukaryotic MMR [39–41]. Interestingly, the 5′ → 3′ excision reaction does not require an MLH/PMS [42], while the 3′ → 5′ excision reaction requires MLH/PMS [43]. Several years ago the human hPMS2 protein was shown to contain a cryptic

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