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Review

# Mismatch repair in Gram-positive bacteria

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**Abstract**

DNA mismatch repair (MMR) is responsible for correcting errors formed during DNA replication. DNA polymerase errors include base mismatches and extra helical nucleotides referred to as insertion and deletion loops. In bacteria, MMR increases the fidelity of the chromosomal DNA replication pathway approximately 100-fold. MMR defects in bacteria reduce replication fidelity and have the potential to affect fitness. In mammals, MMR defects are characterized by an increase in mutation rate and by microsatellite instability. In this review, we discuss current advances in understanding how MMR functions in bacteria lacking the MutH and Dam methylase-dependent MMR pathway.


*Keywords:* Mismatch repair; MutS; MutL; *Bacillus subtilis*

## 1. Mismatch repair in *Escherichia coli*

The Gram-negative bacterium *E. coli* has served as the traditional model for the study of bacterial MMR. Experiments in this model system include the extensive biochemical characterization of all MMR components, including a successful reconstitution of the pathway *in vitro* [1] (see for review Refs. [2,3]).

Upon the identification of a replication error, *E. coli* MMR is initiated by the mismatch sensing protein MutS (Fig. 1A) [4]. There are a number of models that have been proposed to describe the initial phase of mismatch recognition and the transmission of this signal to downstream proteins in the pathway [for review [5]]. Briefly, the "translocation model", suggests that energy released by ATP hydrolysis drives translocation from the mismatch stimulating formation of a loop [6]. The "sliding clamp or molecular switch" model, proposes that upon mismatch binding, ADP is exchanged for ATP, eliciting a conformational change in MutS converting it to a sliding clamp allowing for diffusion along the DNA helix [7,8]. The third model "static transactivation" suggests that mismatch bound MutS remains bound to the mismatch and facilitates communication with downstream events through DNA looping [9]. Although each model has experimental support, based on considerable *in vitro* and the *in vivo* observations of MMR complexes, we favor, the molecular "sliding clamp model" where MutS converts to a sliding clamp and diffuses along the DNA in search of MutL.

Based on the molecular switch model, after mismatch detection, MutS converts to a sliding clamp followed by recruitment of MutL through an interaction with residues Q211 and Q212 located in the connector domain of MutS [7,10,11] (Fig. 1B). A third principal component of MMR in

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*E. coli* is the restriction endonuclease-like protein MutH. MutH follows behind the ongoing replication fork through association with the hemi-methylated d(GATC) sites [12]. The MutS·MutL binary complex forms allowing for MutL to active MutH [13]. Upon activation, MutH specifically nicks the unmethylated strand of the hemimethylated d(GATC) site [7]. The *E. coli* combination of the Dam methylase and MutH activities provides a signal that directs the MMR pathway to the nascent strand in a process termed strand discrimination [1,14]. MutL loads the UvrD helicase at the newly incised nick on either the continuous strand or the incised strand, depending on whether the MutH-directed nick exists 5′ or 3′ to the mismatch, allowing for bidirectional excision (Fig. 1) [15]. Helicase loading ensures that helicase movement occurs toward the detected mismatch by unwinding the strand with the replication error, followed by degradation by one of several single-stranded exonucleases (ExoI, RecJ, ExoVII, ExoX) [16]. In the final correction step, DNA polymerase III holoenzyme replicates the ssDNA gap and DNA ligase seals the nick in the sugar-phosphate backbone (Fig. 1D) (see for review Ref. [17]). In total, the repair of replication errors *in vitro* and *in vivo* relies on a minimum set of protein activities: MutS, MutL, MutH, Dam, UvrD, exonucleases (RecJ, Exo I, VII, X), the Pol III holoenzyme, DNA ligase, and SSB [1] (Table 1).

## 2. Mismatch repair in MutH-independent bacteria

In considering bacterial MMR, it is important to mention that *E. coli* and a few closely related gamma proteobacteria use a MMR pathway where the strand-discrimination signal is known: methylation of adenine in d(GATC) sequences by the Dam methylase marks the template strand [18]. At the start of the cell cycle, d(GATC) sites are fully methylated; however upon replication, most d(GATC) sites exist in a transient hemimethylated state for >2 min [19]. As described above, the brief hemimethylated state is exploited by MutH to incise the DNA and target mismatch correction to the base located in the unmethylated strand, representing the newly synthesized strand.

*E. coli* has traditionally served as the bacterial model for MMR studies; however, most prokaryotes and all eukaryotes rely on a MutH- and Dam methylation-independent pathway (see for review Ref. [20]). In place of the MutH endonuclease activity, most prokaryotic and eukaryotic MutL homologs (MLHs) contain a highly conserved endonuclease active site, where extensive conservation is apparent even among distantly related organisms including human, *Arabidopsis* and the Gram-positive bacterium *Bacillus subtilis* [21,22]. Even *Pseudomonas* species, which have Dam-methylase, but lack MutH contain a MutL-endonuclease active homolog [23]. Despite this extensive evolutionary conservation of MLHs, *E. coli* MutL lacks the intrinsic endonuclease activity that defines eukaryotic, and even most prokaryotic, MMR pathways [21]. Therefore, though *E. coli* has certainly served as an important organism for studying the mechanistic steps of MMR since the discovery of mutator alleles [24,25], *E. coli* uses a methylation-directed MMR pathway, which is rare in biology.

Therefore, to understand how MMR functions in bacteria that lack MutH and Dam, other experimentally tractable bacterial systems, including *Streptococcus pneumoniae* and

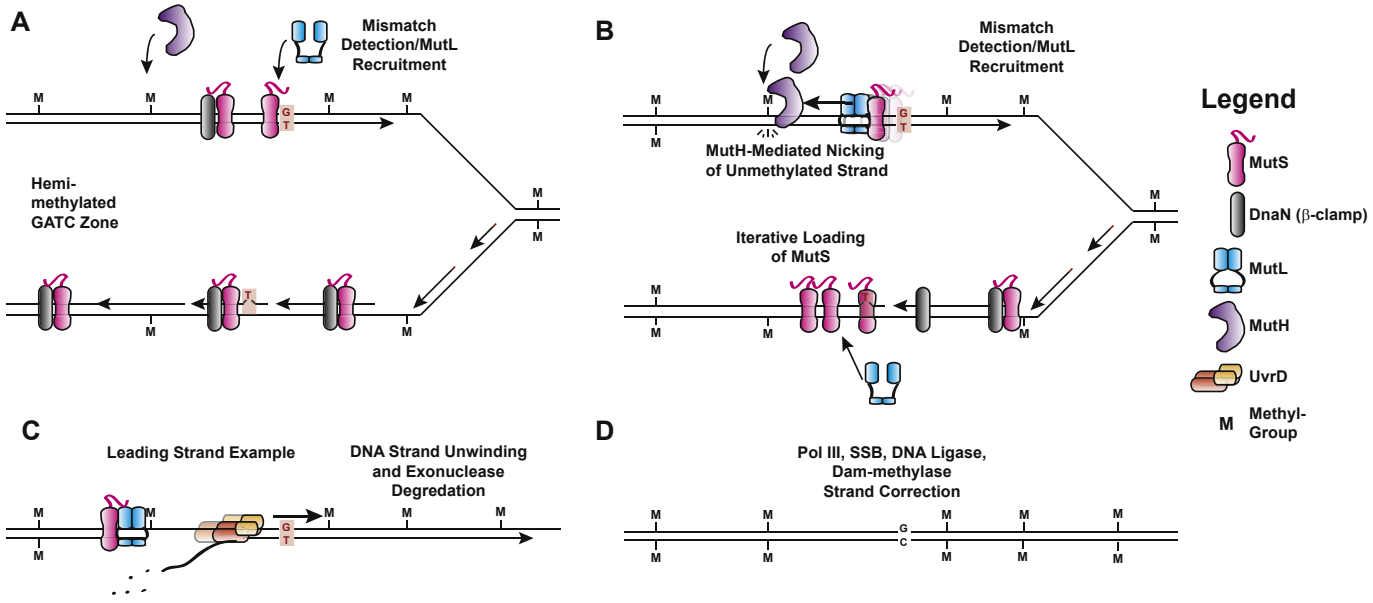


Fig. 1. Model of *Escherichia coli* methyl-directed repair. (A) A replication error is detected by MutS within a narrow window (≤2 min) after the progression of the replication fork where d(GATC) sites are hemimethylated. To assist with the detection of the mismatch, the DnaN clamp positions MutS on nascent DNA. MutL is recruited by mismatch-bound MutS. (B) The MutS·MutL binary complex diffuses away from the mismatch, colliding with and activating MutH. MutH is positioned at hemimethylated d(GATC) sites, and upon activation, MutH nicks the unmethylated strand. (C) Mismatch repair occurs on both the leading and lagging strands. In (C) we show correction on the leading strand where MutL recruits the UvrD helicase to the nick, where it unwinds the unmethylated strand through the mismatch. Simultaneously, exonucleases degrade the strand. (D) SSB bound ssDNA surrounding the recently removed mismatch is replicated by the Pol III holoenzyme. Ligase seals the remaining nick, completing repair.