Mismatch Repair System in Prokaryotes

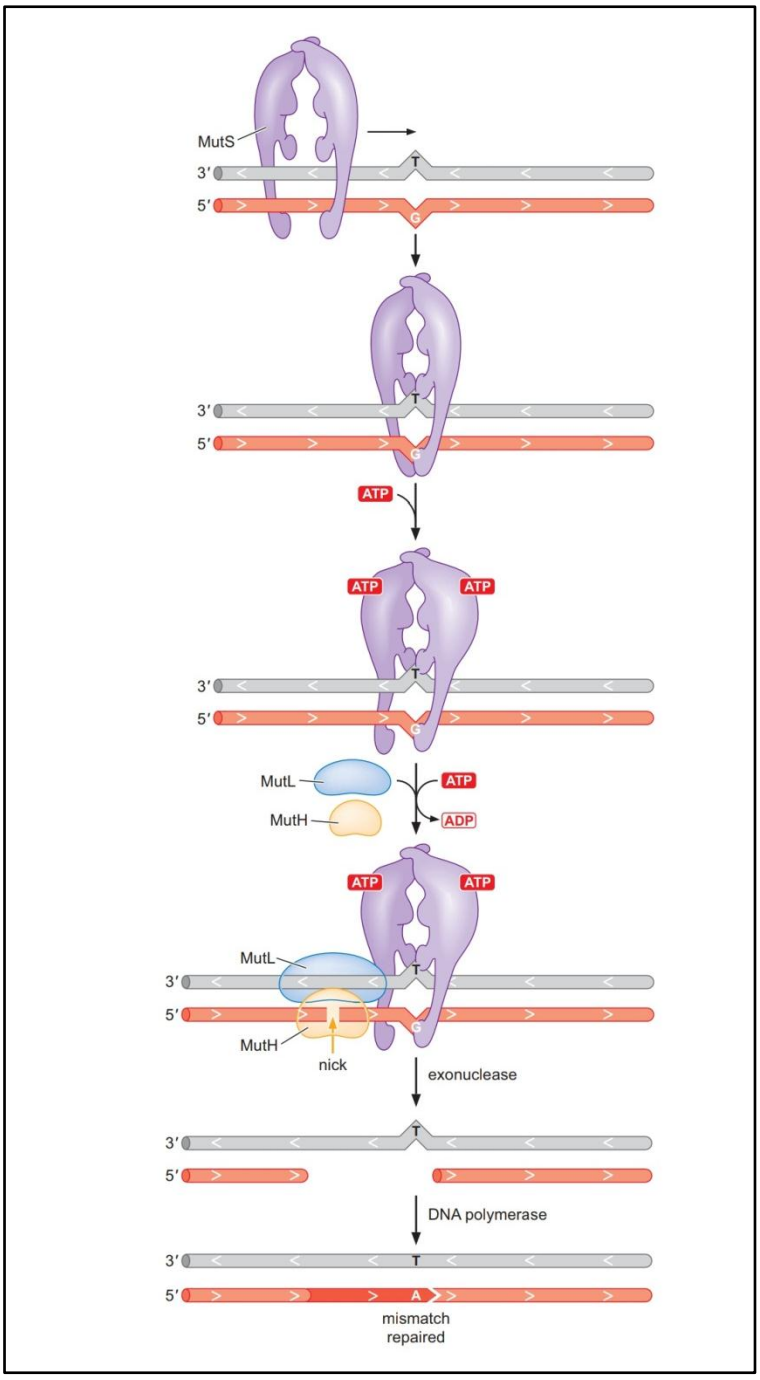
Replication machinery achieves remarkably a high degree of accuracy using a proofreading mechanism, the 3'→5' exonuclease component of the replisome, which removes wrongly incorporated nucleotides. Proofreading improves the fidelity of DNA replication by a factor of 100. The proofreading exonuclease is not, however, foolproof. Some misincorporated nucleotides escape detection and become a mismatch between the newly synthesized strand and the template strand. If the misincorporated nucleotide is not detected and replaced, it will have resulted in a permanent change (a mutation) in the DNA sequence.

Fortunately, a mechanism known as mismatch repair system exists for detecting mismatches and repairing them. Final responsibility for the fidelity of DNA replication rests with this mismatch repair system, which increases the accuracy of DNA synthesis by an additional two to three orders of magnitude.

The mismatch repair system faces two challenges. First, it must scan the genome for finding mismatches. Mismatches are transient (they are eliminated following a second round of replication when they result in mutations), so, must rapidly find and repair. Second, the system must correct the mismatch accurately; that is, it must replace the misincorporated nucleotide in the newly synthesized strand and not the correct nucleotide in the parental strand.

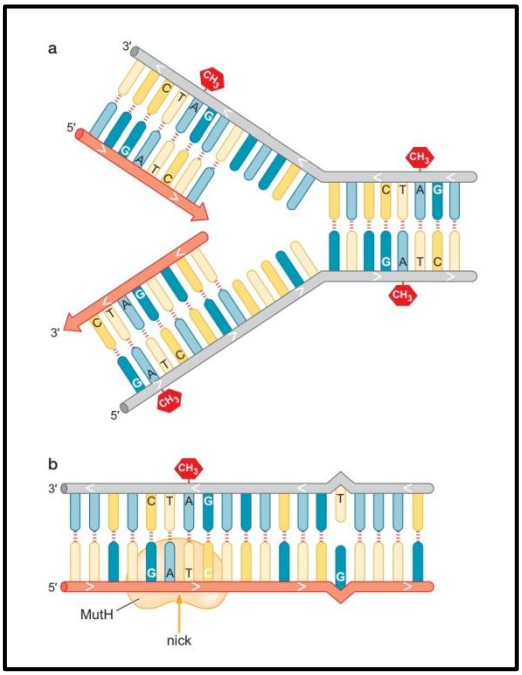
In Escherichia coli, mismatches are detected by a dimer of the mismatch repair protein MutS. MutS scans the DNA, recognizing mismatches from the distortion they cause in the DNA backbone. MutS embraces the mismatch-containing DNA, inducing a pronounced kink in the DNA and a conformational change in MutS itself. A key to the specificity of MutS is that DNA containing a mismatch is much more readily distorted than properly base-paired DNA.

MutS has an ATPase activity that is required for mismatch repair, but its precise role in repair is not understood. The complex of MutS and the mismatch-containing DNA recruits MutL, a second protein component of the repair system. MutL, in turn, activates MutH, an enzyme that causes an incision or nick on one strand near the site of the mismatch. Nicking is followed by the action of a specific helicase (UvrD) and one of three exonucleases. The helicase unwinds the DNA, starting from the incision and moving in the direction of the site of the mismatch, and the exonuclease progressively digests the displaced single strand, extending to and beyond the site of the mismatched nucleotide. This action produces a single-strand gap, which is then filled in by DNA polymerase III and sealed with DNA ligase. The overall effect is to remove the mismatch and replace it with the correctly base-paired nucleotide.



The parental strands are tagged by transient hemimethylation. The E. coli enzyme Dam methylase methylates Adenine residues on both strands of the sequence 5'-GATC-3'. The GATC sequence is widely distributed along the entire genome (occurring at about once every 256 bp), and all of these sites are methylated by the Dam methylase. When a replication fork passes through DNA that is methylated at GATC sites on both strands (fully methylated DNA), the resulting daughter DNA duplexes will be hemimethylated (i.e., methylated on only the parental strand). Thus, for a few minutes, until the Dam methylase catches up and methylates the newly synthesized strand, daughter DNA duplexes will be methylated only on

the strand that served as a template. Thus, the newly synthesized strand is marked (it lacks a methyl group) and hence can be recognized as the strand for repair.



The MutH protein binds at such hemimethylated sites, but its endonuclease activity is normally latent. Only when MutH is contacted by MutL and MutS located at a nearby mismatch (which is likely to be within a distance of a few hundred base pairs) does MutH become activated as described above. Just how this interaction takes place over distances of up to several hundred base pairs is uncertain, but recent evidence indicates that the MutS–MutL complex leaves the mismatch and moves along the DNA contour to reach MutH at the site of hemimethylation. Once activated, MutH selectively nicks the unmethylated strand, thus only newly synthesized DNA in the vicinity of the mismatch is removed and replaced. Methylation is therefore a “memory” device that enables the E. coli repair system to retrieve the correct sequence from the parental strand if an error has been made during replication.

Different exonucleases are used to remove single-stranded DNA between the nick created by MutH and the mismatch, depending on whether MutH cuts the DNA on the 5' or the 3' side of the misincorporated nucleotide. If the DNA is cleaved on the 5' side of the mismatch, then exonuclease VII or RecJ, which degrades DNA in a 5'→3' direction, removes the stretch of DNA from the MutH-induced cut through the misincorporated nucleotide. Conversely, if the nick is on the 3' side of the mismatch, then the DNA is removed by exonuclease I, which degrades DNA in a 3'→5' direction. After removal of the mismatched base, DNA Pol III fills in the missing sequence.

Selection of error prone DNA polymerase in eukaryotes

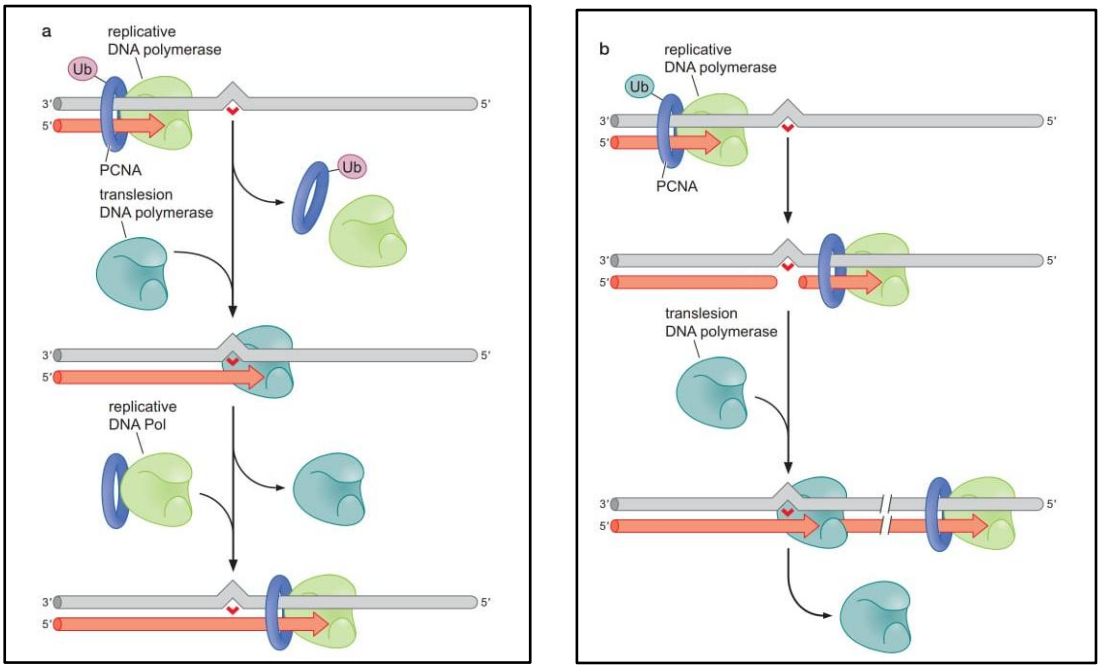
Sometimes a replicating DNA polymerase encounters a lesion, such as a pyrimidine dimer or an apurinic site, that has not been repaired. Because such lesions are obstacles to progression of the DNA polymerase, the replication machinery must attempt to copy across the lesion or be forced to cease replication. Even if cells cannot repair these lesions, there is a fail-safe mechanism that allows the replication machinery to bypass these sites of damage or tolerate the DNA damage. One mechanism of DNA damage tolerance is translesion synthesis. Although this mechanism is highly error-prone and thus likely to introduce mutations, translesion synthesis spares the cell the worse fate of an incompletely replicated chromosome.

Translesion synthesis is catalyzed by a specialized class of DNA polymerases that synthesize DNA directly across the site of the damage. In E. coli, DNA Pol IV (DinB) or DNA Pol V (a complex of the proteins UmuC and UmuD') performs translesion synthesis. DinB and UmuC are members of a distinct family of DNA polymerases found in many organisms known as the Y family of DNA polymerases. There are five translesion polymerases known in humans, four of which belong to the Y family.

An important feature of these polymerases is that although they are template-dependent, they incorporate nucleotides in a manner that is independent of base pairing. This explains how the enzymes can synthesize DNA over a lesion on the template strand. As the enzyme is not "reading" sequence information from the template, translesion synthesis is often highly error-prone. Nonetheless, the nucleotide incorporated may not be random—some translesion polymerases incorporate specific nucleotides. For example, in human, a member of the Y family of translesion polymerases (DNA Pol h) correctly inserts two A residues opposite a thymine dimer. Structural studies show that the active site of DNA Pol h is better at accommodating a thymine dimer than is the active site of another translesion DNA polymerase (DNA Pol k).

Because of its high error rate, translesion synthesis can be considered a system of last resort. It enables the cell to survive what might otherwise be a catastrophic block to replication, but the price that is paid is a higher level of mutagenesis. For this reason, translesion DNA polymerases must be tightly regulated. In E. coli, the translesion polymerases are not present under normal circumstances. Rather, their synthesis is induced only in response to DNA damage. Thus, the genes encoding the translesion polymerases are expressed as part of a pathway known as the SOS response.

Translesion polymerase gains access to the stalled replication machinery at the site of DNA damage. In mammalian cells, entry into the translesion synthesis pathway is triggered by chemical modification of the sliding clamp. The sliding clamp, which is known as PCNA in eukaryotes, anchors the replicative polymerase to the DNA template. The chemical modification is the covalent attachment to the sliding clamp of a peptide known as ubiquitin in a process known as ubiquitination.



Once ubiquitinated, the sliding clamp recruits a translesion polymerase, which contains domains that recognize and bind to ubiquitin. The translesion polymerase, in turn, somehow displaces the replicative polymerase from the 3' end of the growing strand and extends it across the site of the damage. Ubiquitination of the sliding clamp is therefore a distress signal that recruits a translesion polymerase to rescue a replication machine that is stalled at a site of DNA damage. In addition to a polymerase switching mechanism, data support that translesion synthesis also uses a mechanism of gap filling. Following replication, a gap results from the replicative DNA polymerase skipping over the DNA lesion and continuing replication through repriming events or by starting a new Okazaki fragment. Translesion polymerases have low processivity, thus perhaps they simply dissociate from the template shortly after copying across a lesion. Yet several unanswered questions remained to understand the exact translesion mechanism.