Mismatch repair

This is backup to replicative proofreading by correcting mismatched nucleotides remaining in DNA after replication. Mismatches often involve the normal four bases in DNA. For example, a T may be mis-paired with a G. Because both T and G are normal components of DNA, mismatch repair systems need some way to determine whether the T or the G is the correct base at a given site. The repair system makes this distinction by identifying the template strand, which contains the original nucleotide sequence, and the newly synthesized strand, which contains the misincorporated base (the error). In bacteria, this distinction can be made based on the pattern of methylation in newly replicated DNA.

In *E. coli*, the A in GATC sequences is methylated subsequent to its synthesis. Thus, a time interval occurs during which the template strand is methylated, and the newly synthesized strand is unmethylated. The mismatch repair system uses this difference in methylation state to excise the mismatched nucleotide in the nascent strand and replace it with the correct nucleotide by using the methylated parental strand of DNA as template.

- In *E. coli*, mismatch repair requires the products of four genes, mutH, mutL, mutS, and mutU (uvrD).
- The MutS protein recognizes mismatches and binds to them to initiate the repair process.
- MutH and MutL proteins then join the complex.
- MutH contains a GATC-specific endonuclease activity that cleaves the unmethylated strand at hemimethylated (that is, half methylated) GATC sites either 5’ or 3’ to the mismatch. The incision sites may be 1000 nucleotide pairs or more from the mismatch.
- The subsequent excision process requires MutS, MutL, DNA helicase II (MutU), and an appropriate exonuclease. If the incision occurs at a GATC sequence 5’ to the mismatch, a 5’ → 3’ exonuclease like *E. coli* exonuclease VII is required. If the incision occurs 3’ to the mismatch, a 3’ → 5’ exonuclease activity like that of *E. coli* exonuclease I is needed.
- After the excision process has removed the mismatched nucleotide from the unmethylated strand, DNA polymerase III fills in the large—up to 1000 bp—gap, and DNA ligase seals the nick.
- Homologues of the *E. coli* MutS and MutL proteins have been identified in fungi, plants, and mammals—an indication that similar mismatch repair pathways occur in eukaryotes. In fact, mismatch excision has been demonstrated *in vitro* with nuclear extracts prepared from human cells. Thus, mismatch repair is probably a universal or nearly universal mechanism for safeguarding the integrity of genetic information stored in double-stranded DNA.

Post replication repair

In *E. coli*, light-dependent repair, excision repair, and mismatch repair can be eliminated by mutations in the phr (photoreactivation), uvr, and mut genes, respectively. In mutants deficient in more than one of these repair mechanisms, still another DNA repair system, called post replication repair, is operative.

- When DNA polymerase III encounters a thymine dimer in a template strand, its progress is blocked.
- DNA polymerase restarts DNA synthesis at some position past the dimer, leaving a gap in the nascent strand opposite the dimer in the template strand.
- At this point, the original nucleotide sequence has been lost from both strands of the progeny double helix.
- The damaged DNA molecule is repaired by a recombination-dependent repair process mediated by the *E. coli* recA gene product.
- The RecA protein, which is required for homologous recombination, stimulates the exchange of single stunds between homologous double helices.
- During post-replication repair, the RecA protein binds to the single strand of DNA at the gap and mediates pairing with the homologous segment of the sister double helix. The gap opposite the dimer is filled with the homologous DNA strand from the sister DNA molecule.
- The resulting gap in the sister double helix is filled in by DNA polymerase, and the nick is sealed by DNA ligase.
- The thymine dimer remains in the template strand of the original progeny DNA molecule, but the complementary strand is now intact.
- If the thymine dimer is not removed by the nucleotide excision repair system, this postreplication repair must be repeated after each round of DNA replication. The DNA repair systems described so far are quite accurate.

SOS response

When the DNA of *E. coli* cells is heavily damaged by mutagenic agents such as UV light, the cells take some drastic steps in their attempt to survive. They go through a so-called SOS response, during which a whole battery of DNA repair, recombination, and replication proteins are synthesized.

Two of these proteins, encoded by the umuC and umuD (UV mutable) genes, are subunits of DNA polymerase V, an enzyme that catalyzes the replication of DNA in damaged regions of the chromosome—regions where replication by DNA polymerase III is blocked. DNA polymerase V allows replication to proceed across damaged segments of template strands, even though the nucleotide sequences in the damaged region cannot be replicated accurately. This error-prone repair system eliminates gaps in the newly synthesized strands opposite

damaged nucleotides in the template strands but, in so doing, increases the frequency of replication errors.

The mechanism by which the SOS system is induced by DNA damage has been worked out in considerable detail.

- Two key regulatory proteins—LexA and RecA control the SOS response.
- Both are synthesized at low background levels in the cell in the absence of damaged DNA. Under this condition, LexA binds to the DNA regions that regulate the transcription of the genes that are induced during the SOS response and keeps their expression levels low.
- When cells are exposed to ultraviolet light or other agents that cause DNA damage, the RecA protein binds to single-stranded regions of DNA caused by the inability of DNA polymerase III to replicate the damaged regions.
- The interaction of RecA with DNA activates RecA, which then stimulates LexA to inactivate itself by self-cleavage.
- With LexA inactive, the level of expression of the SOS genes—including recA, lexA, umuC, umuD, and others— increases and the error-prone repair system is activated. The SOS response appears to be a risky attempt to escape the lethal effects of heavily damaged DNA. When the error-prone repair system is operative, mutation rates increase sharply.