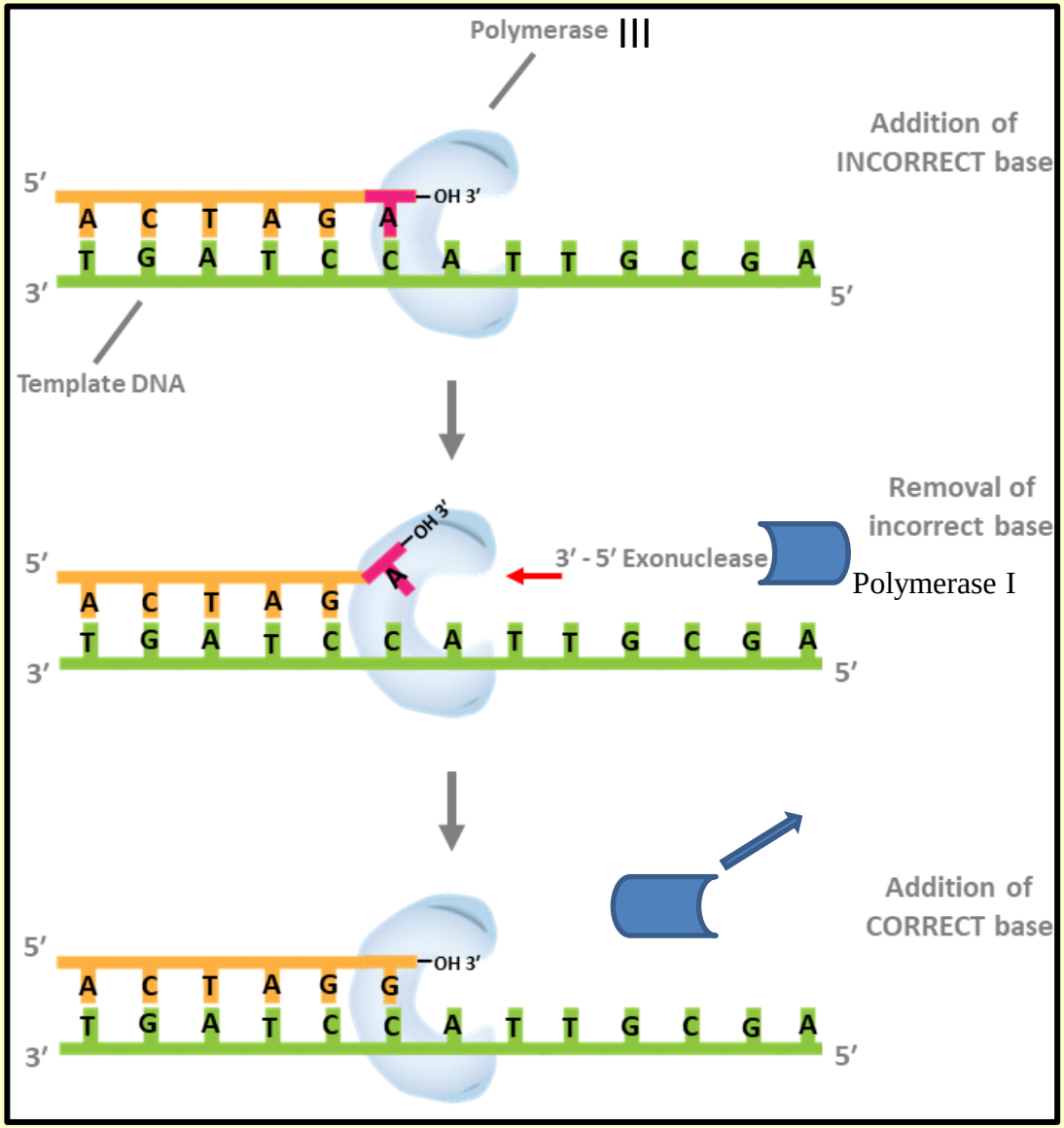
Fidelity of DNA replication

1. Nucleotide selection: **Correct & precise base pairing-** A=T G≡C

2. Proof reading :

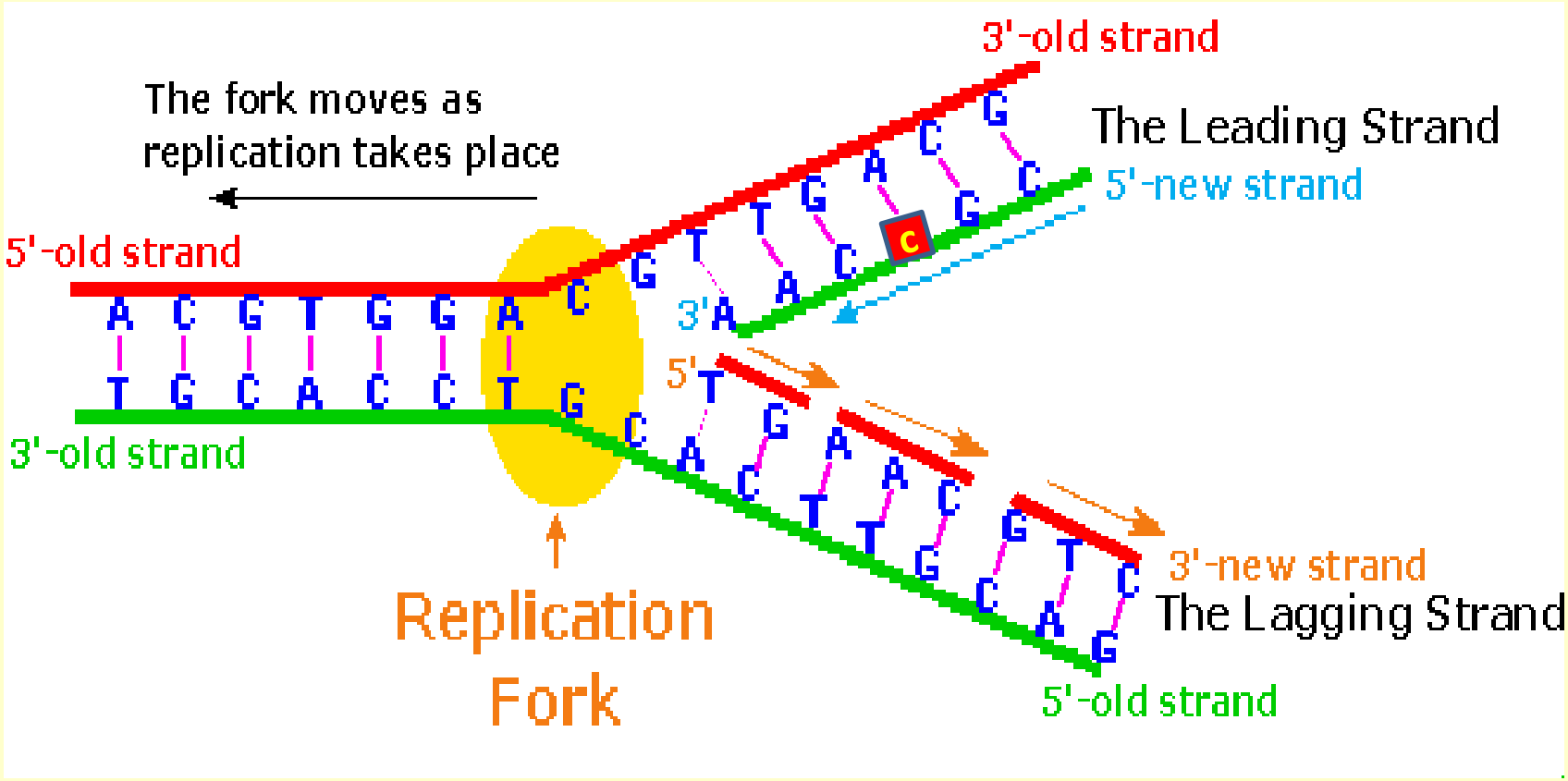


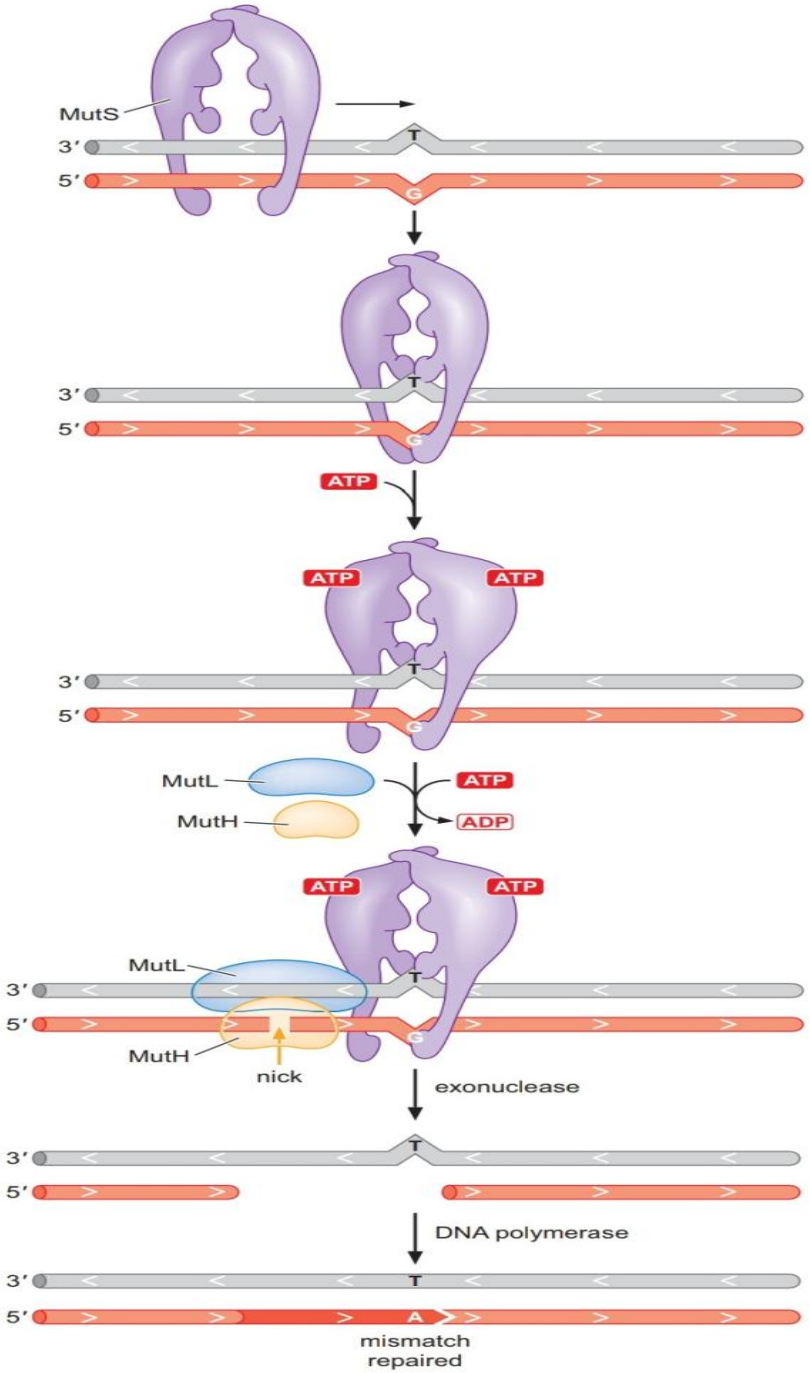
3. Mismatch Repair System in Prokaryotes

- High degree of accuracy using a proofreading mechanism, improves the fidelity of DNA replication by a factor of 100
- Not, however, foolproof
- Some undetected one may cause mutation
- Mismatch repair system exists for detecting mismatches and repairing them
- Final responsibility for 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.

Two challenges:

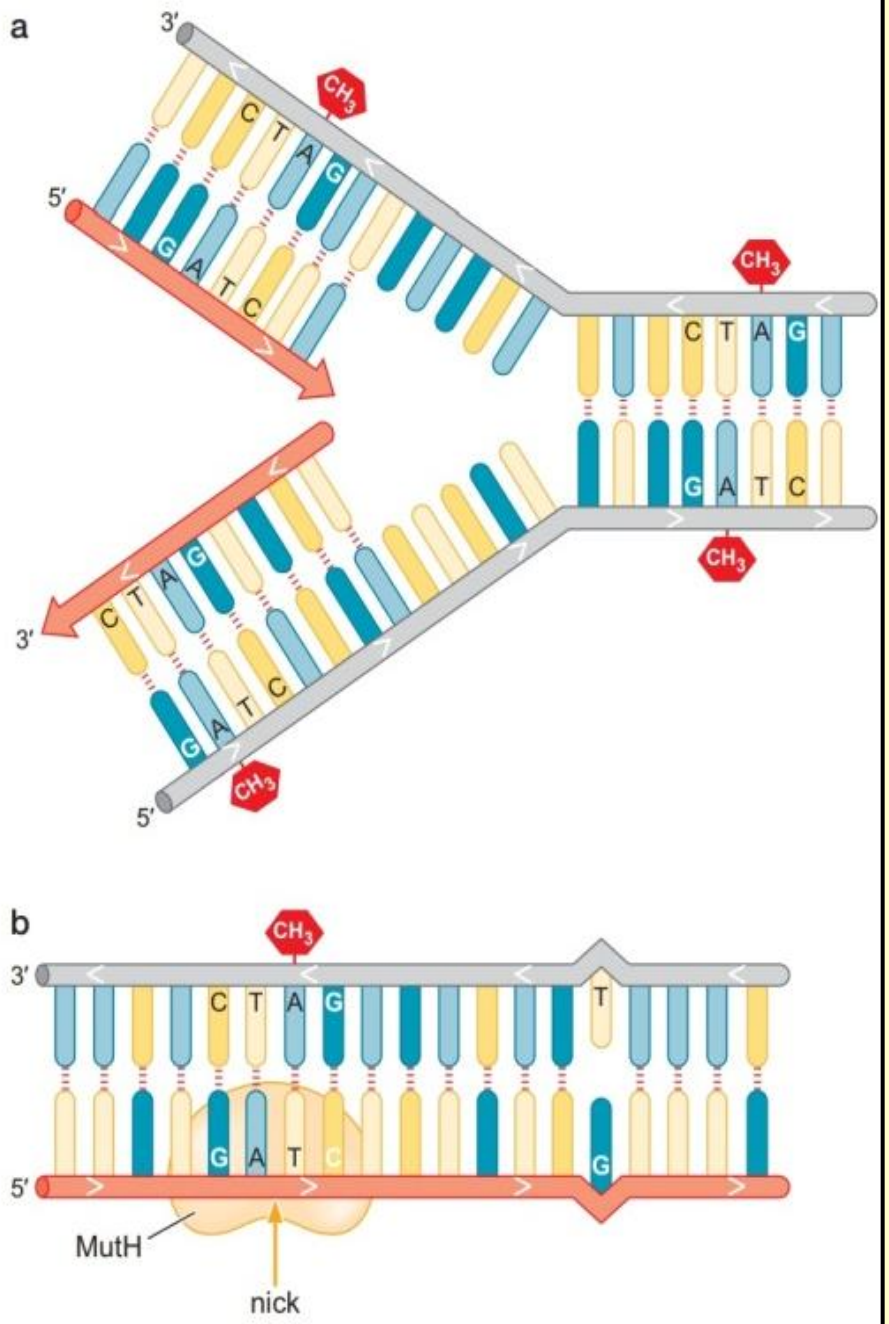
1. **Must scan the genome for finding mismatches. Mismatches are transient. So, must rapidly find and repair.**
2. **Must replace the misincorporated nucleotide in the newly synthesized strand and not the correct nucleotide in the parental strand.**





In *E. coli*

- Mismatches detected by a dimer of protein MutS.
- MutS scans the DNA, recognizing mismatches.
- Conformational change in MutS itself.
- MutS has an ATPase activity.
- Complex of MutS and the mismatch-containing DNA recruits MutL.
- MutL, activates MutH, an enzyme that causes incision near the site of the mismatch.
- Nicking is followed by action of a specific helicase (UvrD) and one exonucleases.
- Helicase unwinds the DNA, from incision to mismatch
- Exonuclease progressively digests the displaced mismatched nucleotide.
- Produces a gap, which then filled in by DNA polymerase III and sealed with DNA ligase.



Parental strands tagged by hemimethylation.

E. coli enzyme Dam methylase methylates Adenine residues on both strands of the sequence 5'-GATC-3'.

After passing of replication fork, the resulting daughter DNA duplexes will be hemimethylated.

MutH protein binds at hemimethylated sites, contacted by MutL and MutS located at a nearby mismatch, become activated & selectively nicks the unmethylated strand.

5' side nick - exonuclease VII or RecJ degrades DNA in a 5'→3' direction

3' side nick - exonuclease I degrades DNA in a 3'→5' direction.

After removal of the mismatched base, DNA Pol III fills in the missing sequence.

4. Selection of error prone DNA polymerase in eukaryotes

Sometimes lesion, such as a pyrimidine dimer or an apurinic site, not repaired. If cells cannot repair these lesions, there is a fail-safe mechanism to bypass these sites of damage.

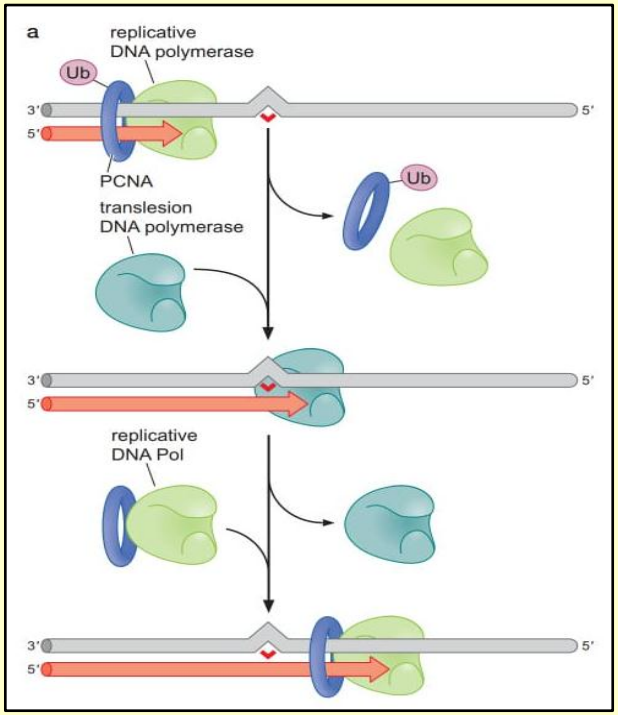
One mechanism of DNA damage tolerance is translesion synthesis. This mechanism is highly error-prone

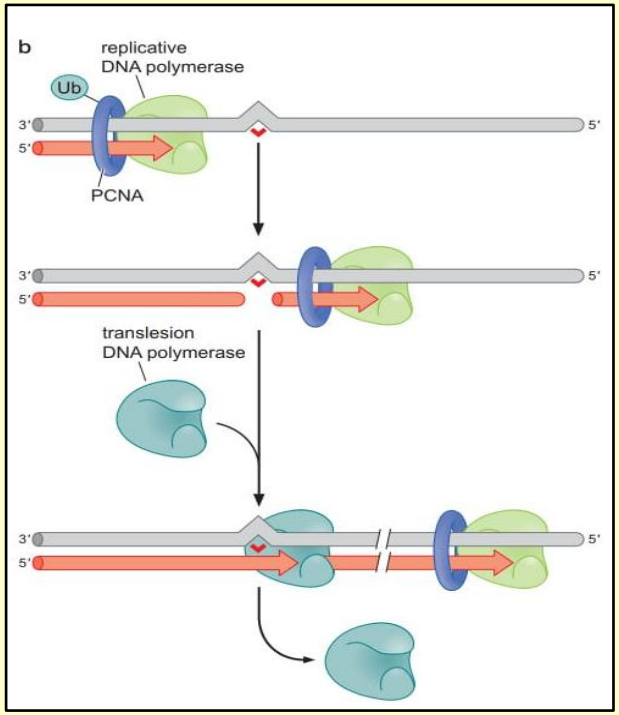
Translesion synthesis is catalyzed by a specialized class of DNA polymerases that synthesize DNA directly across the site of the damage. Many of them belongs to Y family of DNA polymerases

They are template-dependent, but the enzyme is not read sequence information from template, thus synthesis is highly error-prone.

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 than another translesion DNA polymerase (DNA Pol k).

It enables the cell to survive from catastrophic block to replication, but the price paid is a higher level of mutagenesis. For this reason, translesion DNA polymerases must be tightly regulated and use as the **SOS response**.





Translesion polymerase gains access to the stalled replication machinery triggered by chemical modification by ubiquitination of the sliding clamp PCNA.

Then translesion polymerase, somehow displaces the replicative polymerase from the 3' end of the growing strand and extends it across the site of the damage.

Translesion polymerase may rescue a replication machine that is stalled at a site of DNA damage or 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 simply dissociate from the template shortly after copying across a lesion.

Several unanswered questions remained to the exact mechanism.