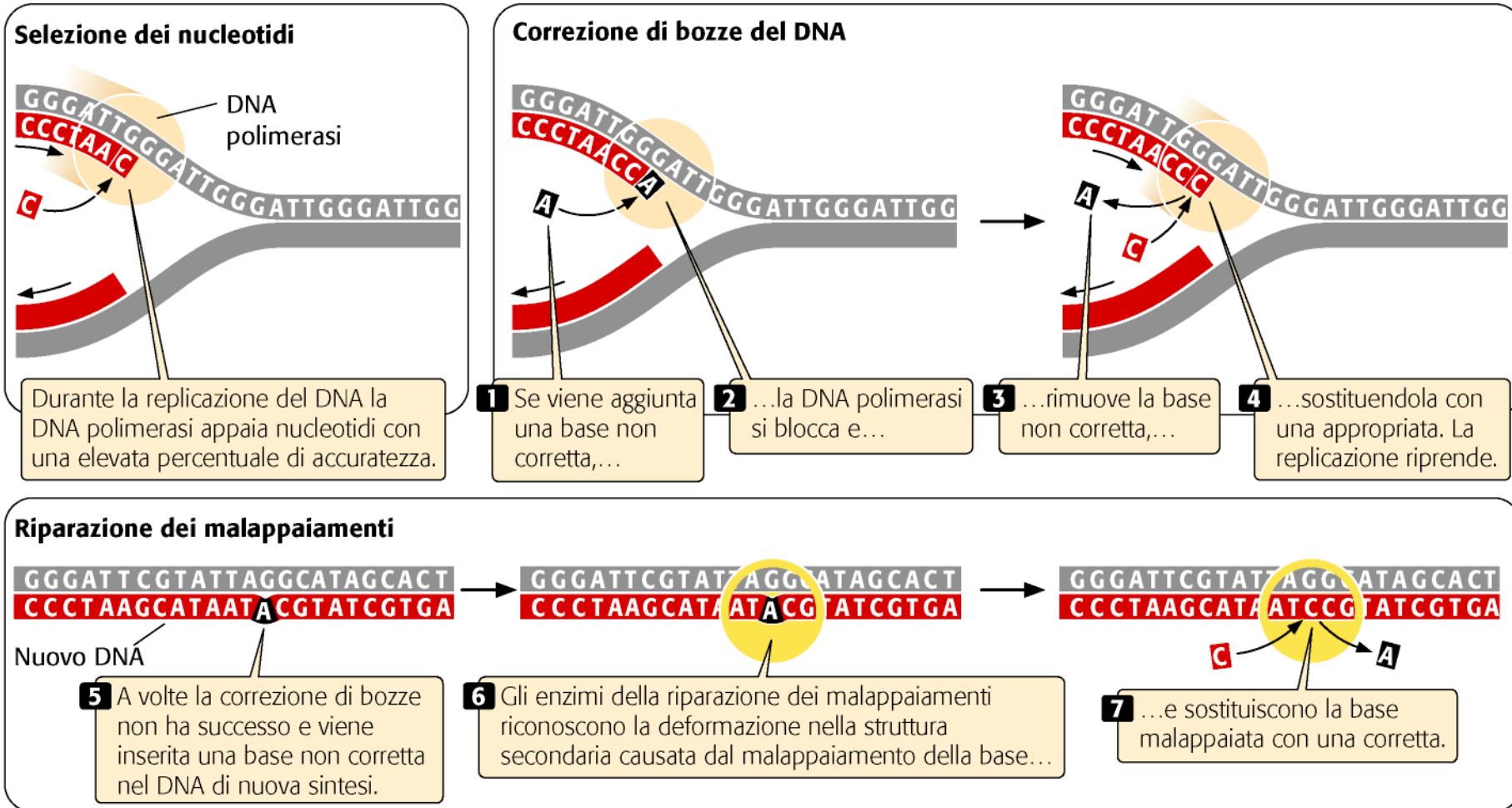
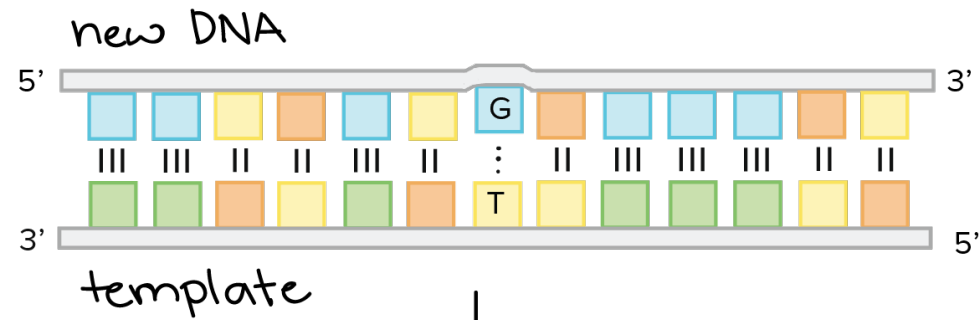


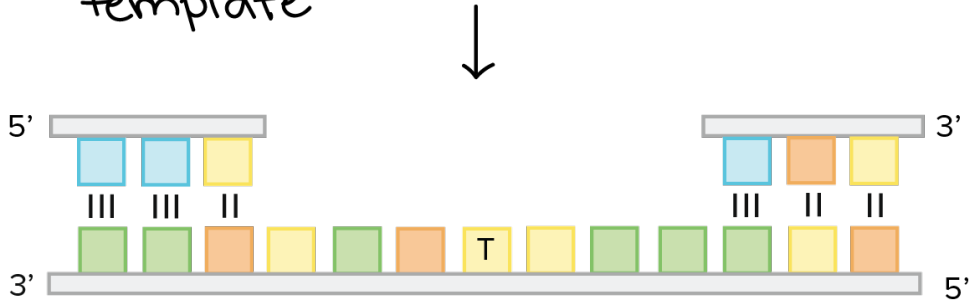
LA FEDELTA' DELLA REPLICAZIONE DEL DNA

1. -selezione dei nucleotidi
2. -correzione di bozze
3. -riparazione dei malappaiamenti

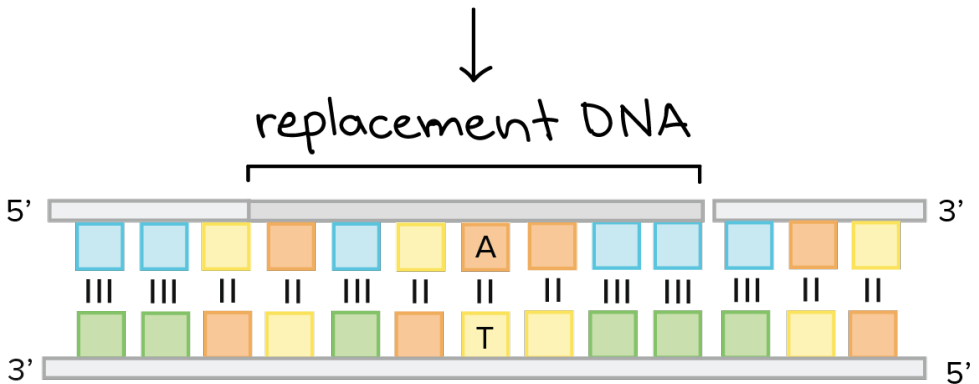




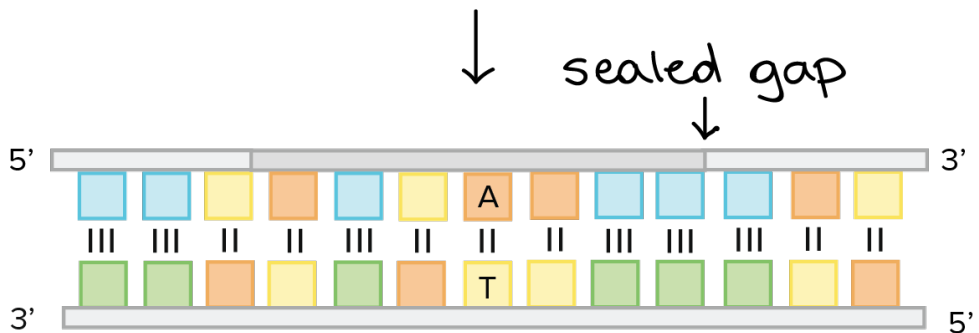
A mismatch is detected in newly synthesized DNA.



The new DNA strand is cut, and the mispaired nucleotide and its neighbors are removed.



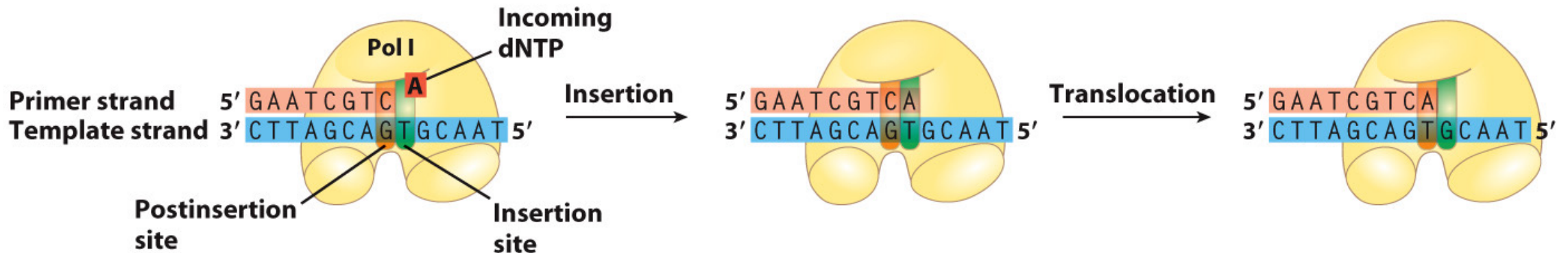
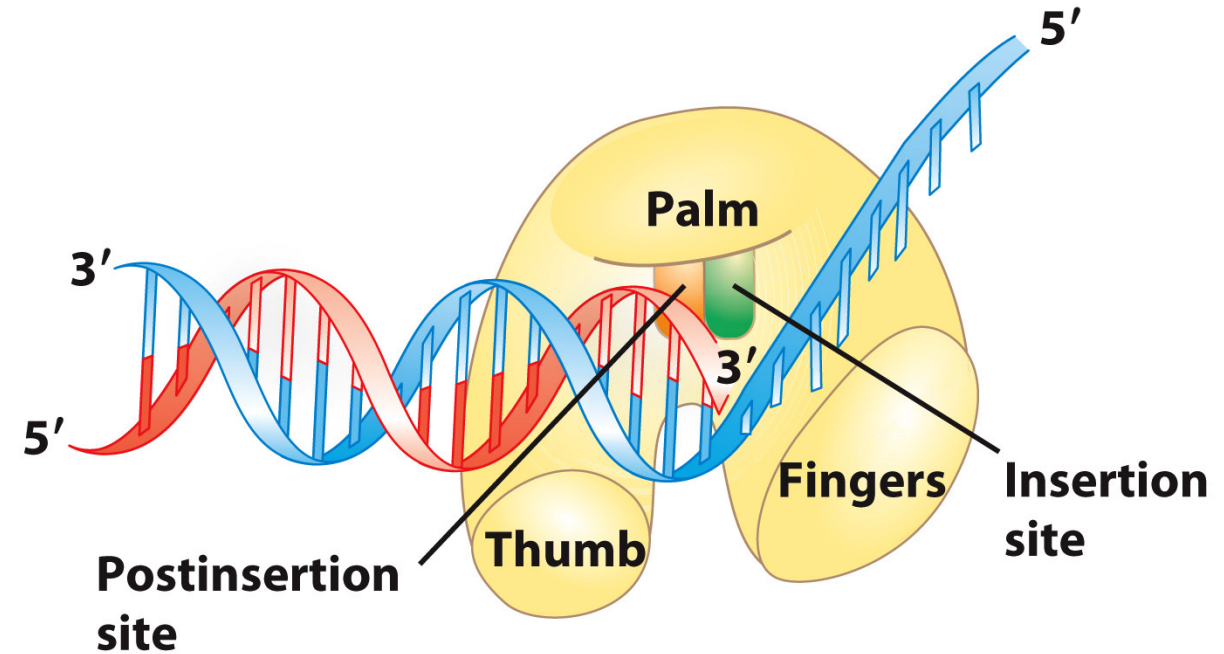
The missing patch is replaced with correct nucleotides by a DNA polymerase.



A DNA ligase seals the gap in the DNA backbone.

1-Selezione di nucleotidi

- DNA Pol has a pocket with two regions:
 - **insertion site**: where the incoming nucleotide binds
 - **Post-insertion site**: where the newly made base pair resides when the polymerase moves forward



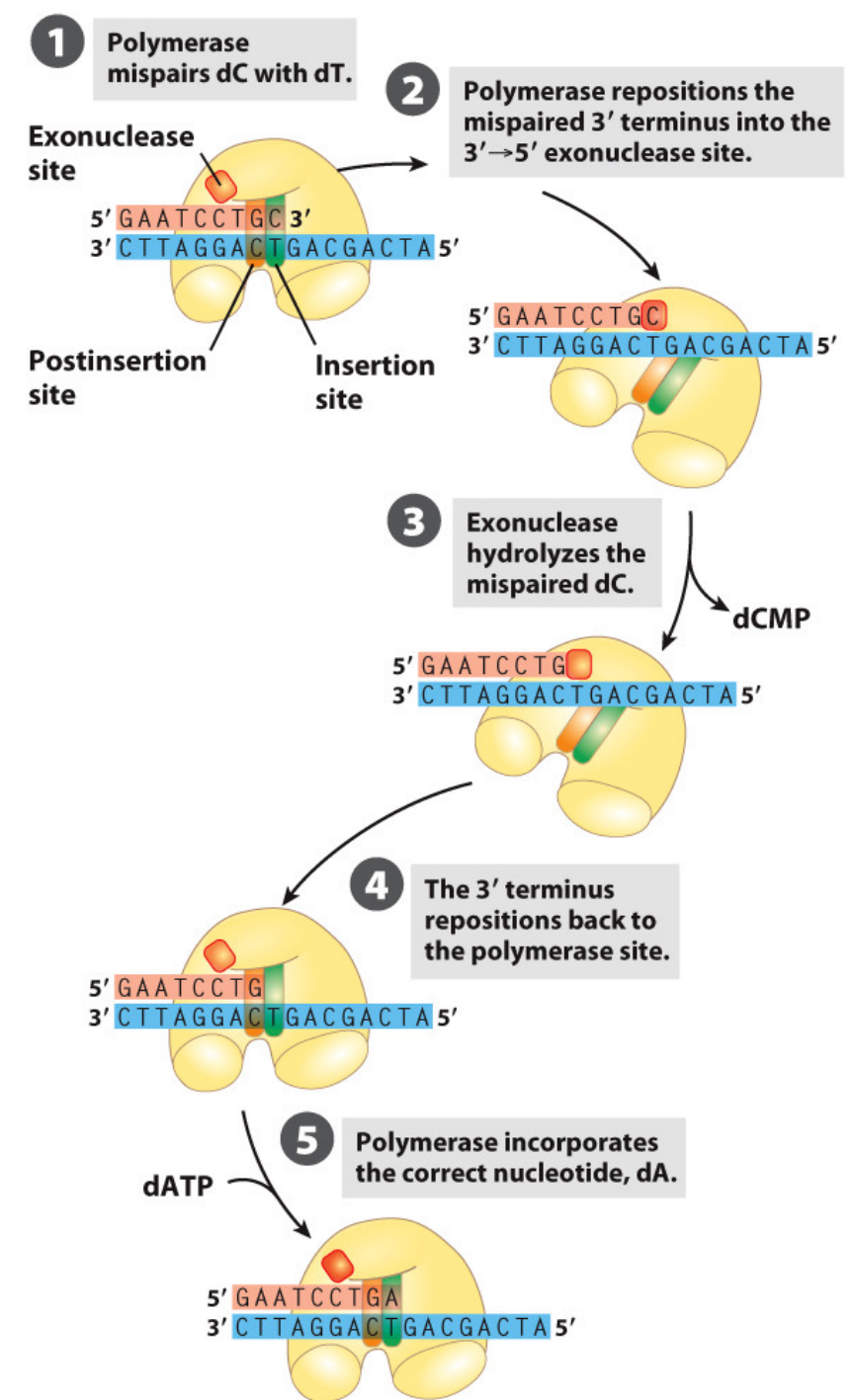
2- L'attività *proof-reading*

La DNA polimerasi possiede due attività enzimatiche distinte, espletate da due domini diversi:

- attività polimerasica in direzione 5' -3'
- attività esonucleasica in direzione 3' -5'

Quando la polimerasi rileva un errore di appaiamento tra le basi, **dopo che il legame PDE e' stato creato**, il complesso stampo-innesco si avvicina al dominio con attività esonucleasica, dove viene eliminato il nucleotide errato, permettendo alla polimerasi di riprendere velocemente la sintesi, senza provocare la dissociazione dell'intero complesso.

Questa attività *proof reading* riduce la frequenza di errore a uno ogni 10^7 nucleotidi



3- Sistema di riparazione degli appaiamenti sbagliati

Sistema di enzimi che rimuove i mal-appaiamenti dopo che il DNA è stato duplicato, aumentando la fedeltà di replicazione da 2 a 3 ordini di magnitudine:

1. **Riconosce la distorsione dell'elica** dovuta al mismatch (distinguendo il filamento nuovo dal vecchio);
2. Taglia il segmento di DNA contenente il mismatch;
3. Risintetizza il segmento tagliato usando il filamento vecchio come stampo.

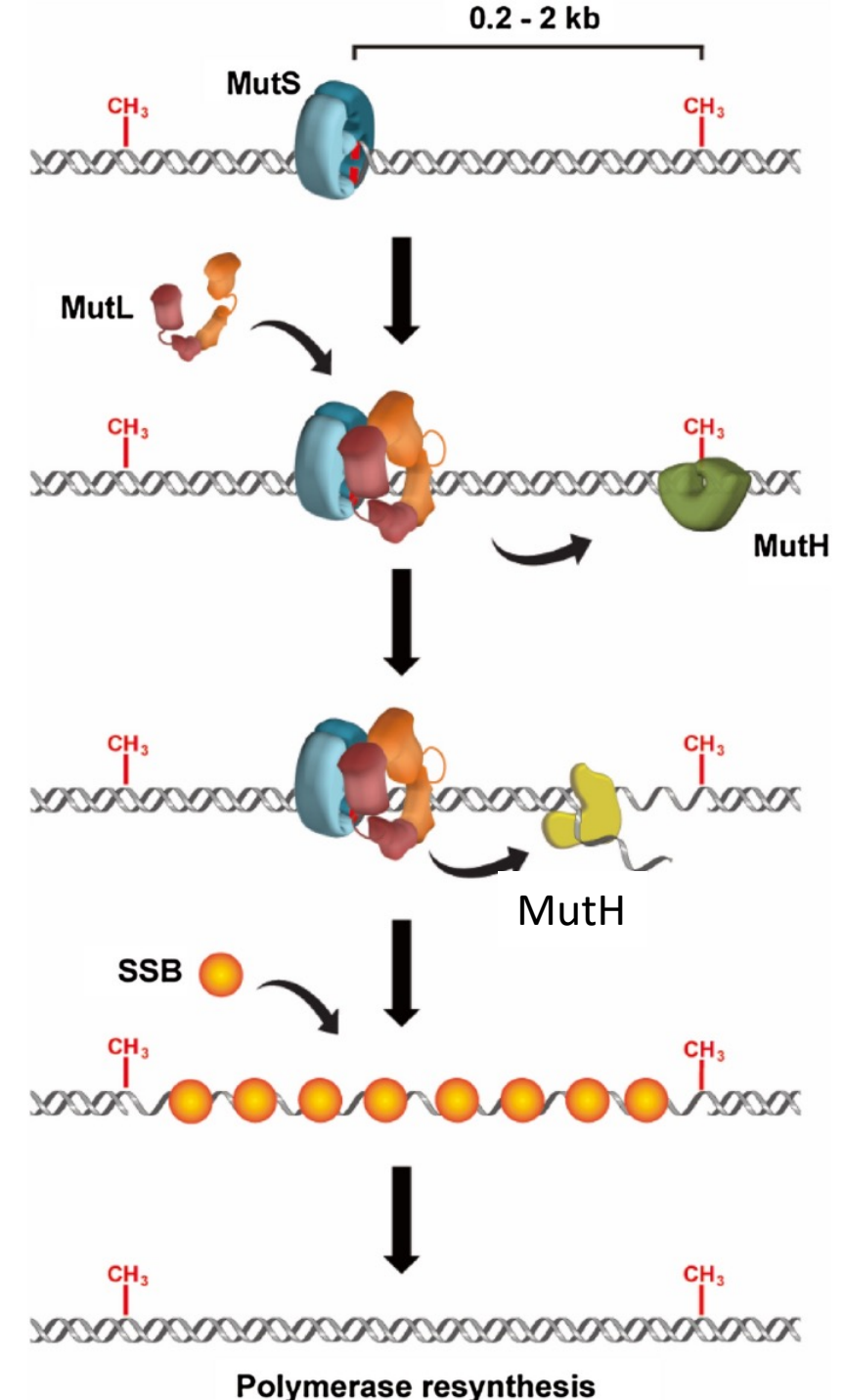
Nei procarioti **MutS** scorre lungo il DNA e riconosce il mismatch

MutH e **MutL** vengono reclutate

MutH taglia un segmento di filamento neosintetizzato, il gap viene riempito dalla DNA polimerasi e le estremità saldate dalla DNA ligasi.

MutL is a Multidomain Protein

MutL is composed of two structured domains connected by a flexible linker. The N-terminal region of the protein (~ 330 residues) encompasses an ATPase domain that is highly conserved from *E. coli* to humans.

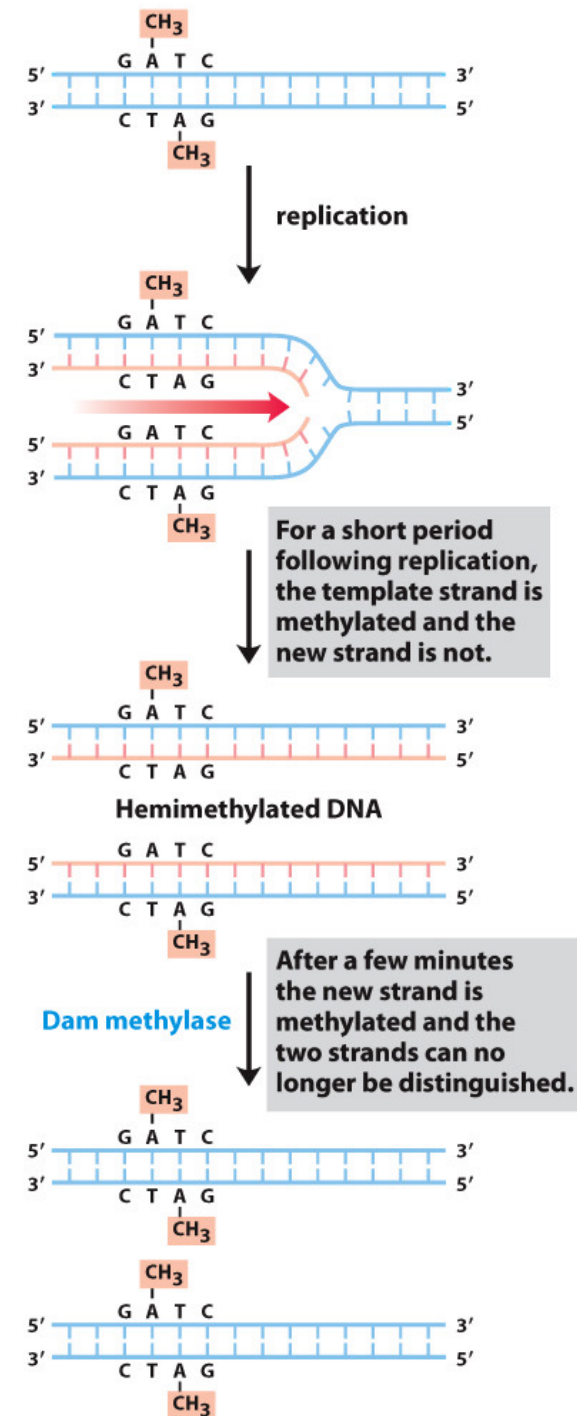


Mismatch Repair

How do repair enzymes “know” which strand is the correct one?

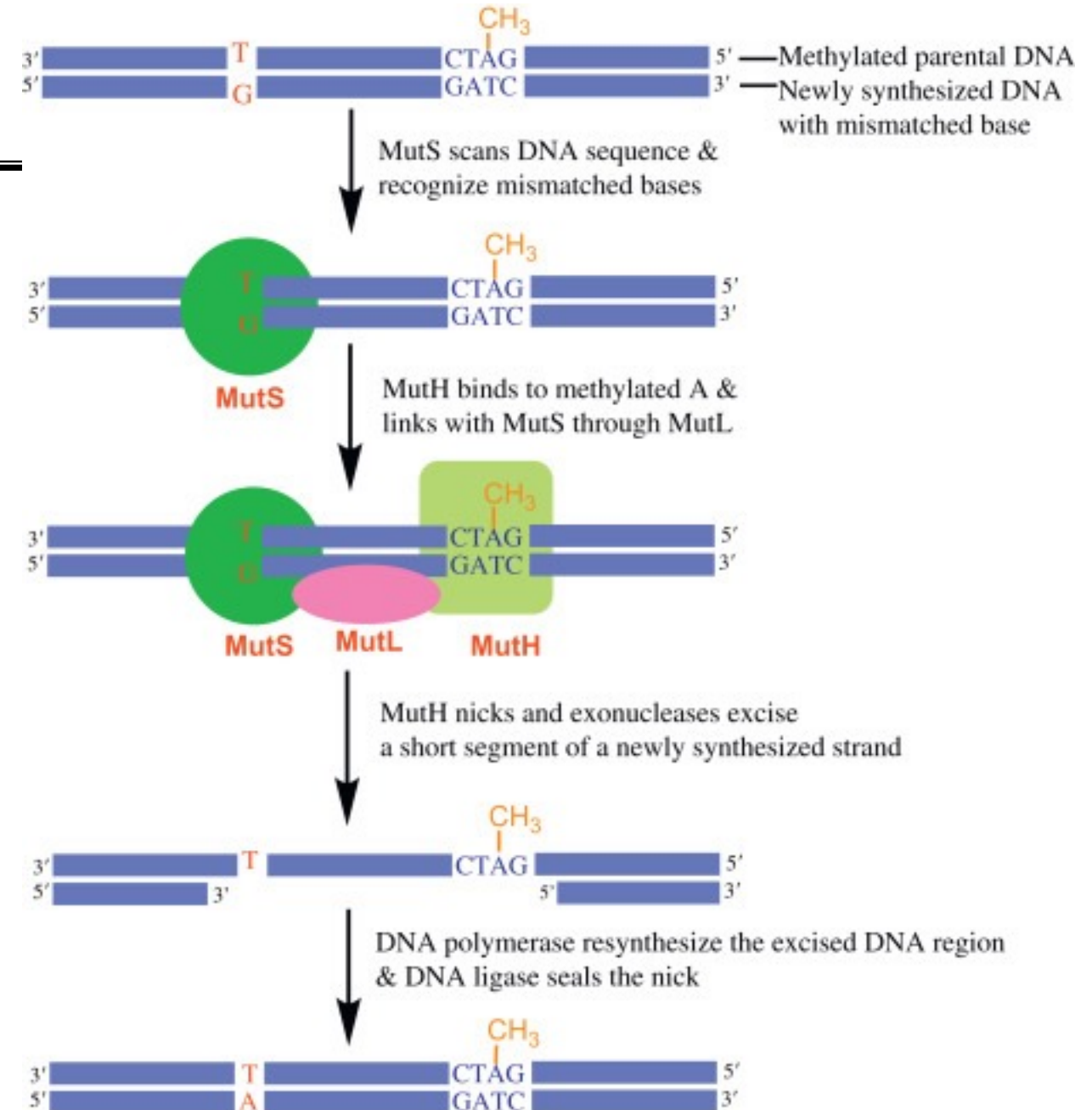
Mismatch Repair and Methylation

- In *E. coli*, the parent strand is methylated.
- **Dam methylase** inserts CH_3 at adenines in the **GATC** sequence.
- Following a short period of time, the daughter strand is then methylated.
- The **newly synthesized strand is unmethylated** for a short period after synthesis.
- Any replication errors must reside in the unmethylated strand.
- The methyl-directed mismatch repair system will **cleave the unmethylated strand** in the initial part of the repair process.

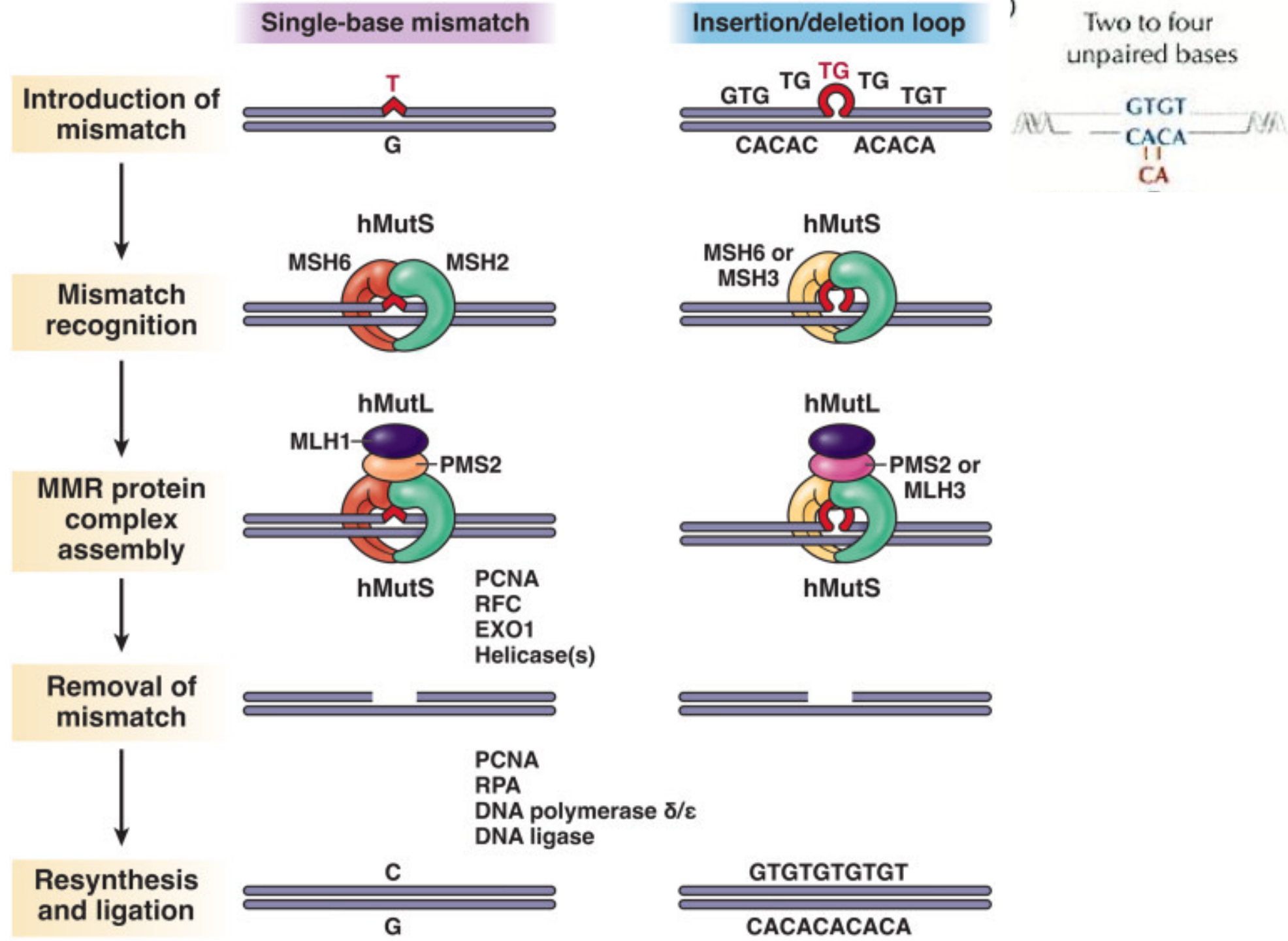


Mechanism of Mismatch Recognition and Repair in *E. coli*

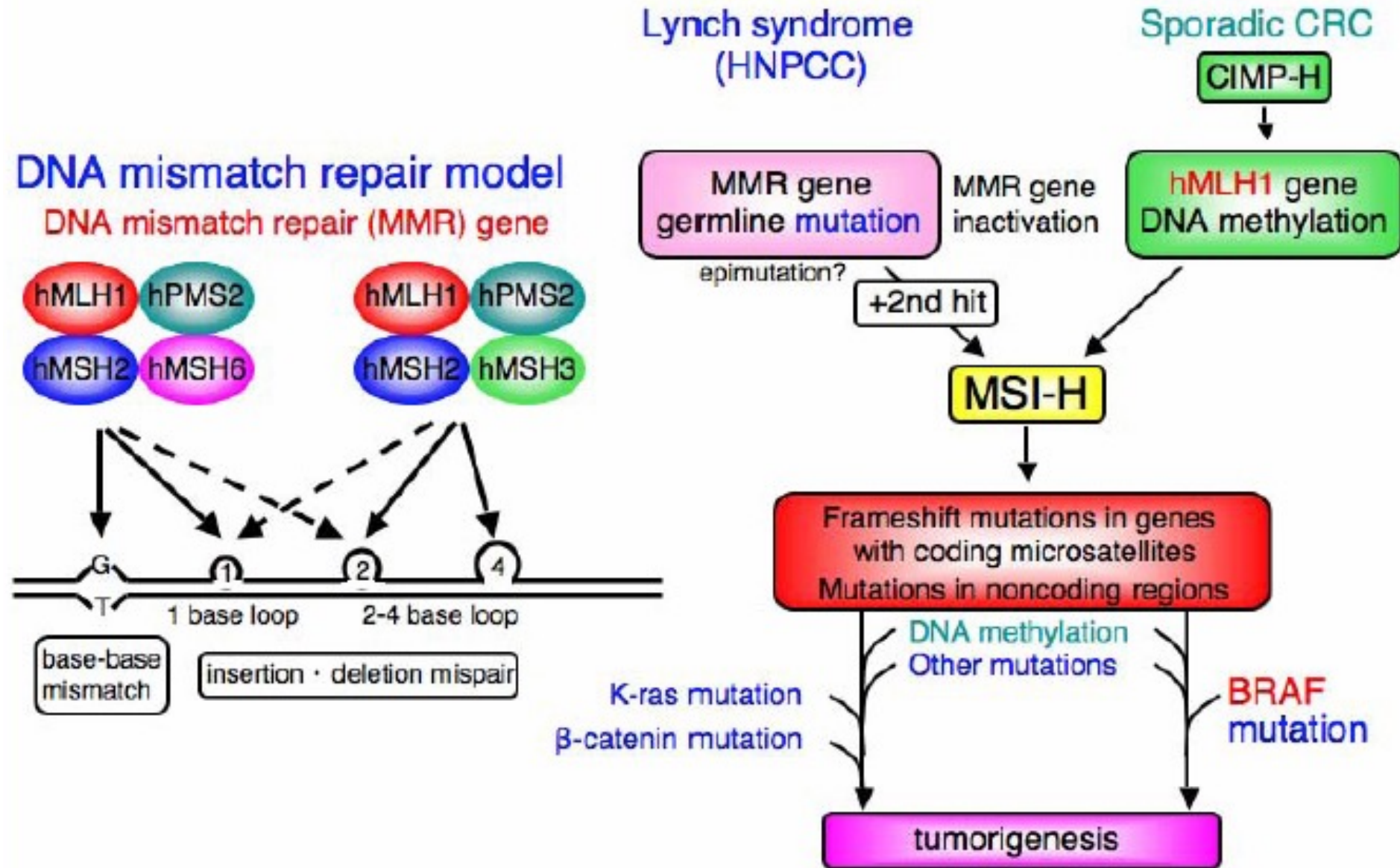
- MutL and MutS proteins recognize methylated GATC.
- MutH binds to MutL-MutS-DNA complex, making a DNA loop.
- MutH cleaves the nonmethylated DNA strand on the 5'-side of the G.
- DNA unwinds and is degraded 3'→5'.
 - Helicase II (UvrD helicase), SSB, and exonucleases work to degrade the nonmethylated DNA toward the mismatch.
- The removed sequence is replaced using DNA Pol III and DNA ligase.

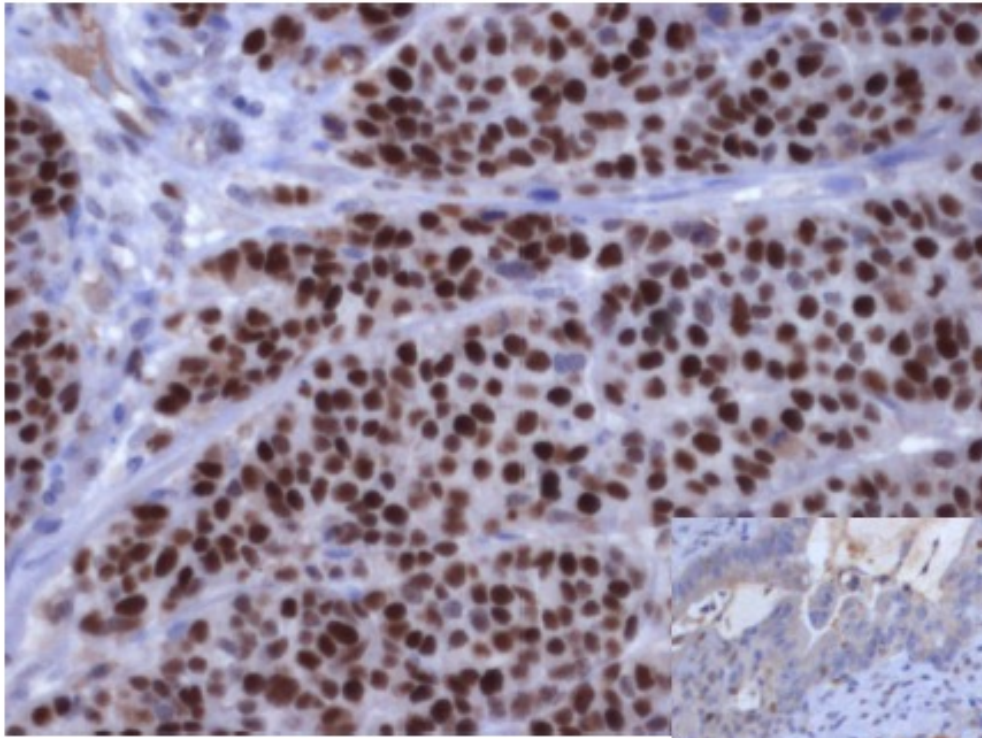


Nelle cellule umane hMSH2 e hMLH1, sono simili a MutS e MutL rispettivamente



Mutazioni nei geni del mismatch repair system causano la predisposizione a certi tipi di cancro (es. HNPCC, carcinoma colonrettale ereditario non poliposico, noto come sindrome di Lynch; test predittivo: mutazioni nei geni MSH2 e MLH1).



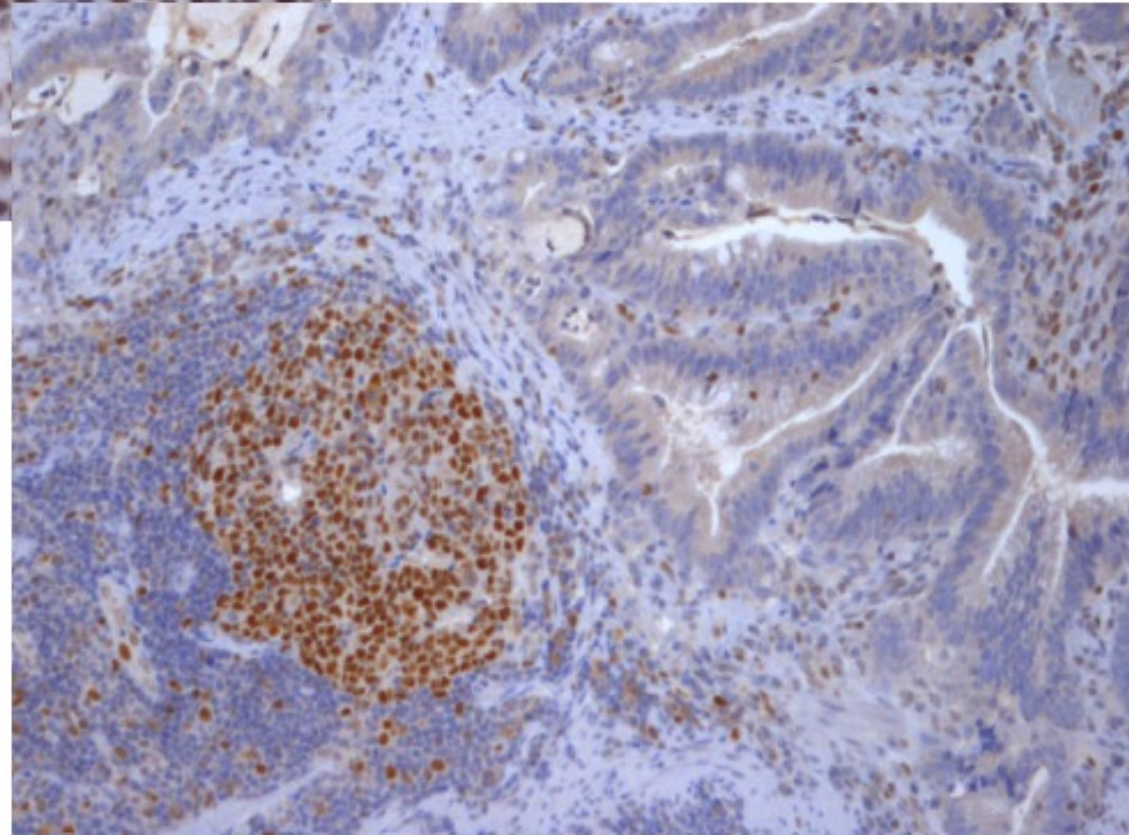


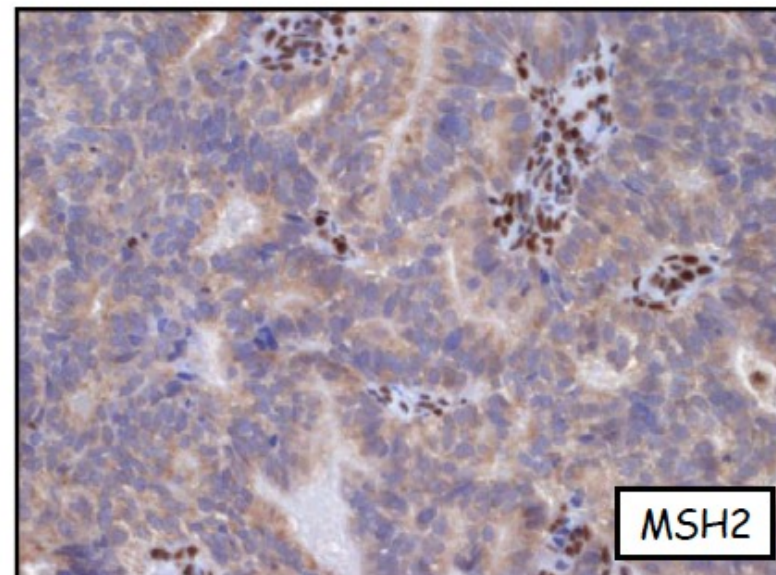
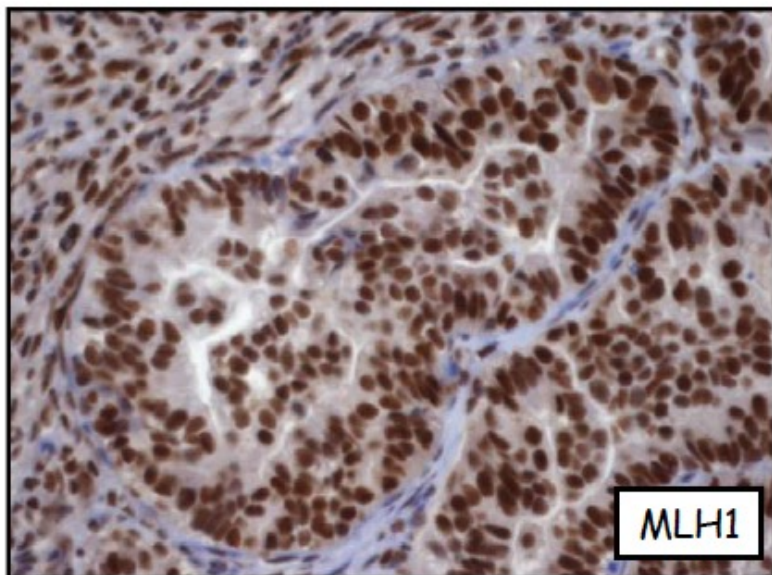
*Normale espressione
di MSH6*

*Perdita completa
di espressione
di MSH6*



Carcinoma colo-rettale





Utero
Adenocarcinoma endometriode
Perdita completa di espressione
di MSH2 e MSH6

