Riparazione degli errori di appaiamento (MMR)

Elimina le singole basi misappaiate ed i loop di inserzione-delezione che si formano durante la replicazione in presenza di brevi sequenze ripetute

Ripara il DNA con un'efficienza pari al 99,9%

Riconosce e ripara solo l'elica neosintetizzata che contiene i nucleotidi errati

E' compiuta da complessi multiproteici

- The Nobel Prize in Chemistry 2015 was awarded jointly to
- Tomas Lindahl,
- Paul Modrich and
- Aziz Sancar

"for mechanistic studies of DNA repair"

- Modrich transformed the field of mismatch repair from genetic observations to a detailed biochemical understanding, first in bacteria, and later in eukaryotic cells.
- Sancar has transformed the field of nucleotide excision repair, from genetics and phenomena in cell extracts, to a detailed molecular description of the mechanisms involved, first in bacteria, and later also in eukaryotic cells. Sancar also explained the molecular mechanisms underlying photoreactivation, the first form of DNA repair described

- Lindahl demonstrated that DNA is an inherently unstable molecule, subject to decay even under physiological conditions. Guided by this observation, Lindahl identified a completely new group of DNA glycosylases and described their role in base excision repair.

- Modrich transformed the field of mismatch repair from genetic observations to a detailed biochemical understanding, first in bacteria, and later in eukaryotic cells.
- Sancar has transformed the field of nucleotide excision repair, from genetics and phenomena in cell extracts, to a detailed molecular description of the mechanisms involved, first in bacteria, and later also in eukaryotic cells. Sancar also explained the molecular mechanisms underlying photoreactivation, the first form of DNA repair described

Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)

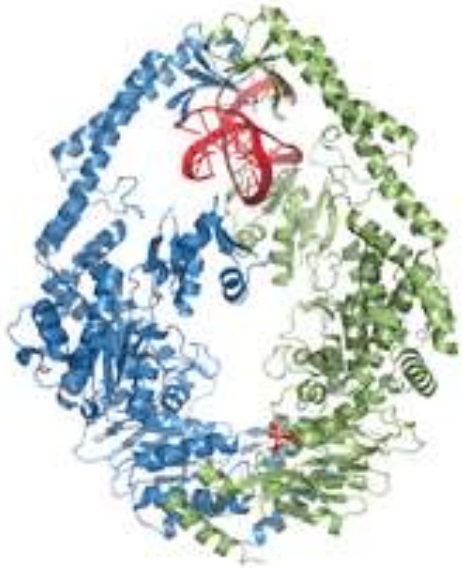
A) MutS binds mismatched base pairs

Apparent affinities of mutS protein for base pair mismatches

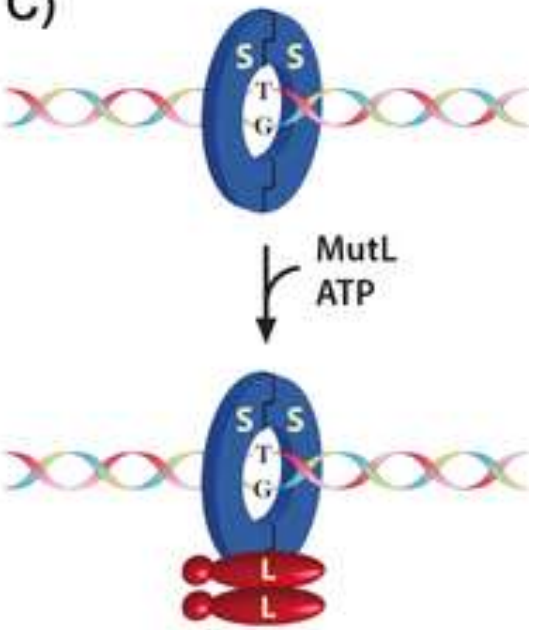
Mismatch	Apparent dissociation constant
	nM
G-T	39 ± 4
A-C	53 ± 4
A-A	110 ± 7
T-T	140 ± 9
G-G	150 ± 10
A-G	270 ± 30
C-T	370 ± 40
C-C	480 ± 50

AFFINITA'

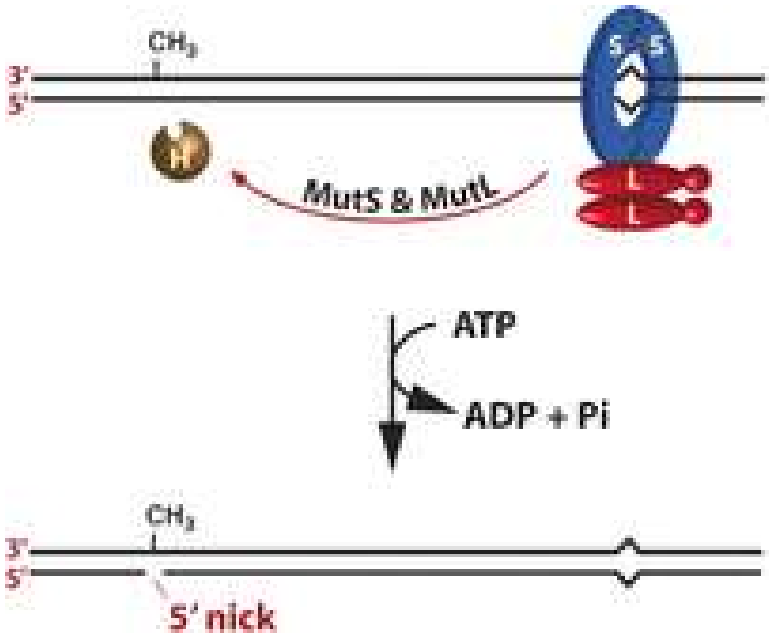
B)

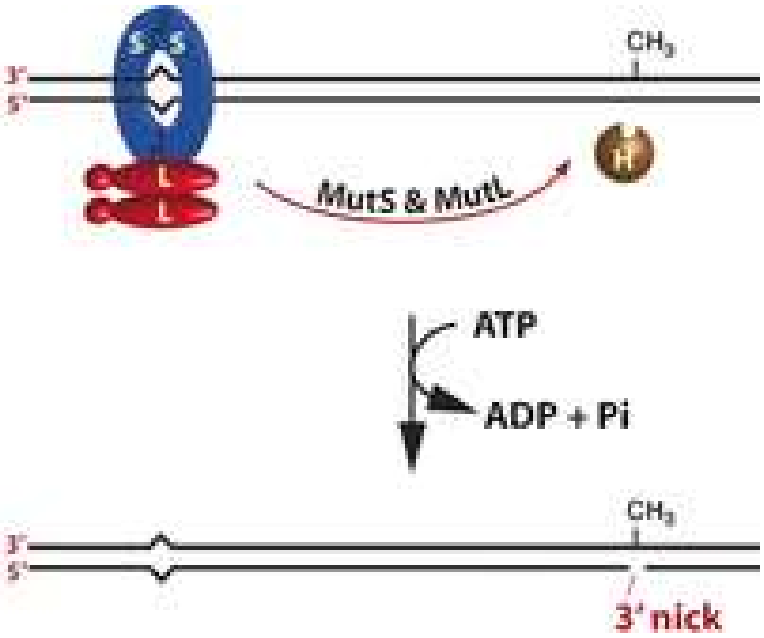


C)



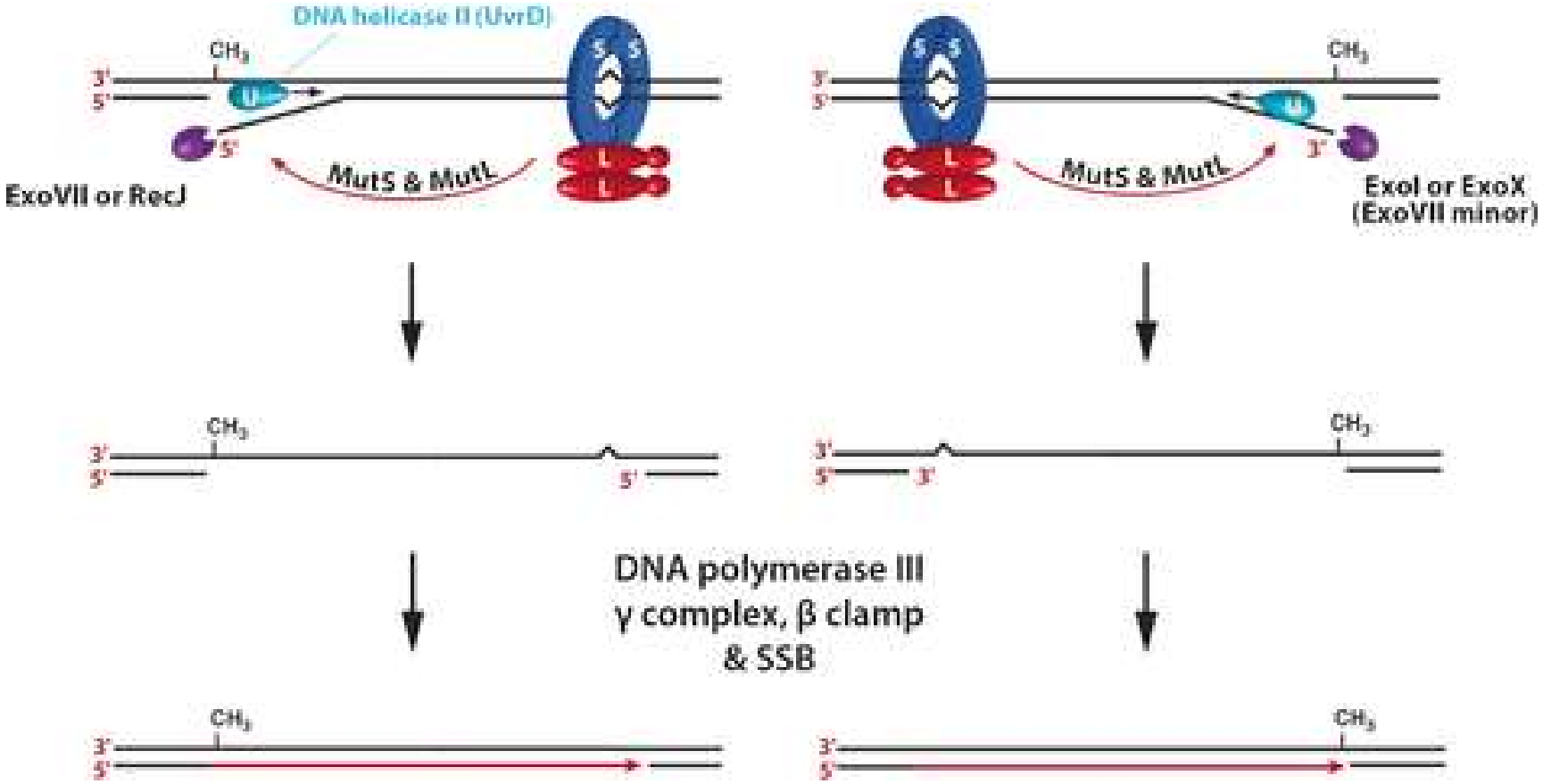
Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)





Angewandte Chemie International Edition
Volume 55, Issue 30, pages 8490-8501, 20 MAY 2016 DOI: 10.1002/anie.201601412
http://onlinelibrary.wiley.com/doi/10.1002/anie.201601412/full#anie201601412-fig-0006

Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)



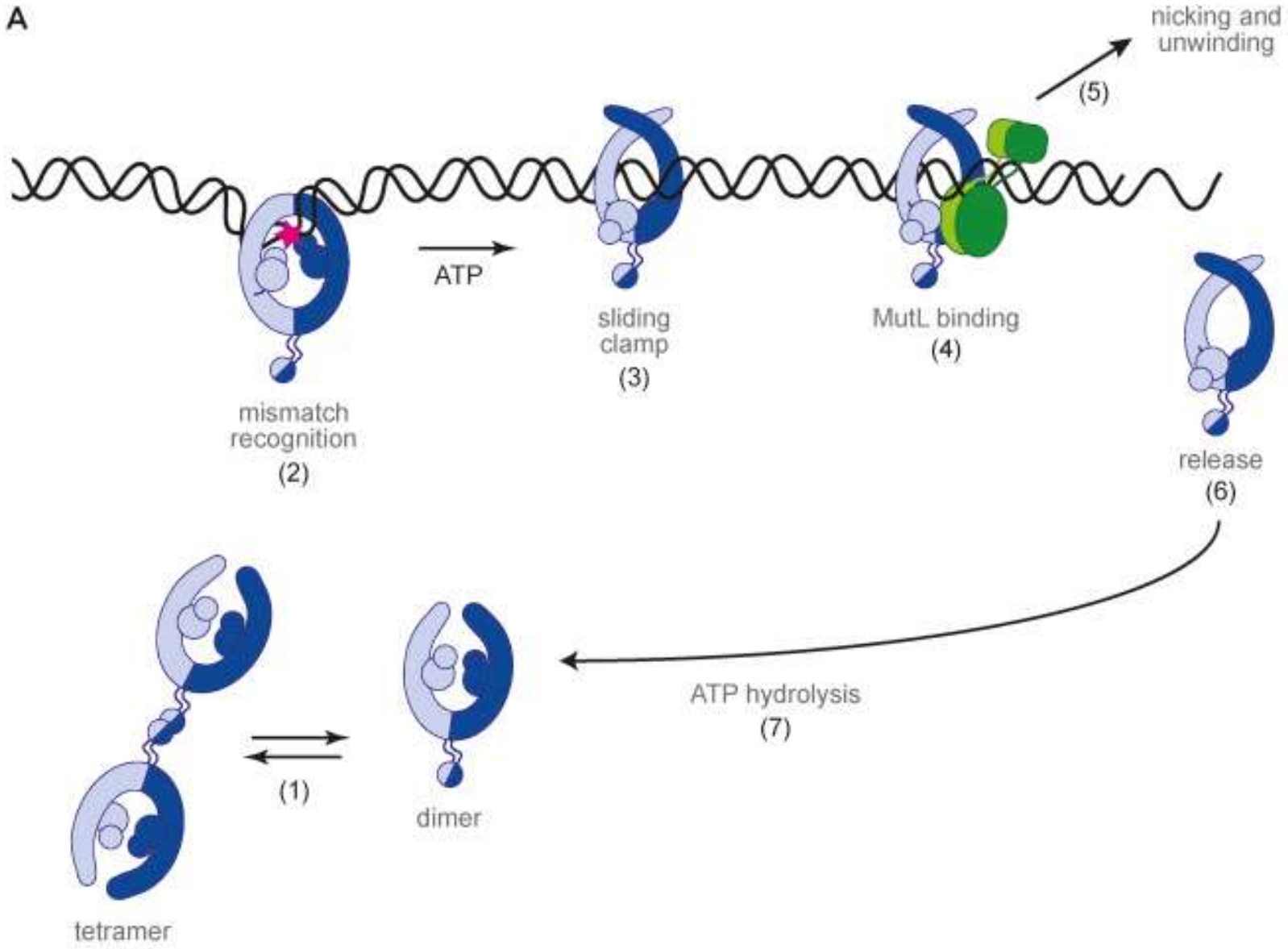
Angewandte Chemie International Edition
Volume 55, Issue 30, pages 8490-8501, 20 MAY 2016 DOI: 10.1002/anie.201601412
http://onlinelibrary.wiley.com/doi/10.1002/anie.201601412/full#anie201601412-fig-0007

Table 1

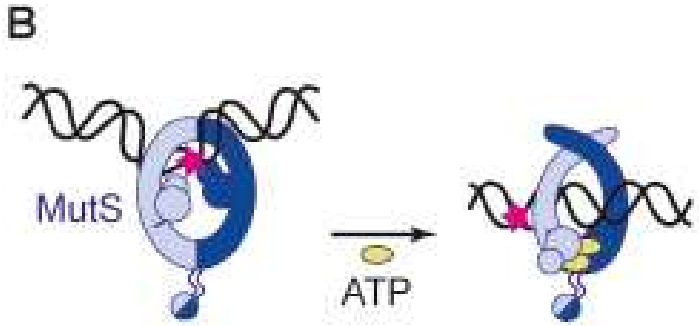
MMR proteins in *E. coli, S. cerevisiae* and *H. sapiens*

E. coli		*H. sapiens*	Comments
MutS-MutS		Msh2-Msh6 (MutSα) Msh2-Msh3 (MutSβ)	Mispair recognition complex—homodimer in *E. coli* and a heterodimer in eukaryotes. MutSα and MutSβ have overlapping mispair recognition specifi
		Mlh1-Pms2 (MutLα)	Homodimer in *E. coli* and heterodimer in eukaryotes. MutL (*E. coli*) and MutLα (eukaryotes) play a central role during MMR. In *E. coli*, MutL promo whereas in eukaryotes MutLα possess an intrinsic endonuclease activity
MutL-MutL		Mlh1-Pms1 (MutLβ)	MutLβ is an accessory factor for MMR
		Mlh1-Mlh3 (MutLγ)	MutLγ substitutes for MutLα in the repair of a minor fraction of mispairs, but primarily acts in the resolution of meiotic recombination intermediates
Dam methylase		Absent	Promotes N^6-adenine methylation at d(GATC) sites, serves as strand discrimination signal in *E. coli*
MutH		Absent[a]	Endonuclease, nicks daughter strand using d(GATC) hemi-methylated sites as strand discrimination signal
none		Exo1	5′-3′ dsDNA exonuclease, acts in the excision reaction
RecJ, ExoVII		None	5′-3′ ssDNA exonuclease, acts in the excision reaction
ExoI, ExoVII, ExoX		None	3′-5′ ssDNA exonuclease, acts in the excision reaction
UvrD	)wn	None or unknown	DNA helicase II, promotes excision reaction, activated by MutS
β-clamp		PCNA	DNA polymerase processivity factor. In eukaryotes stimulates Mutα endonuclease activity. The gene encoding PCNA in *S. cerevisiae* is *POL30*
γ-Complex		RFC	Loading of β-clamp/PCNA
SSB		RPA1-3	ssDNA binding protein, acts in the excision and DNA resynthesis reactions. The genes encoding RPA subunits in *S. cerevisiae* are *RFA1, 2* and, *3*
DNA Pol III		Pol delta	DNA polymerase that acts in the gap-filling step
DNA ligase		Ligase I	Seals nicks after DNA resynthesis

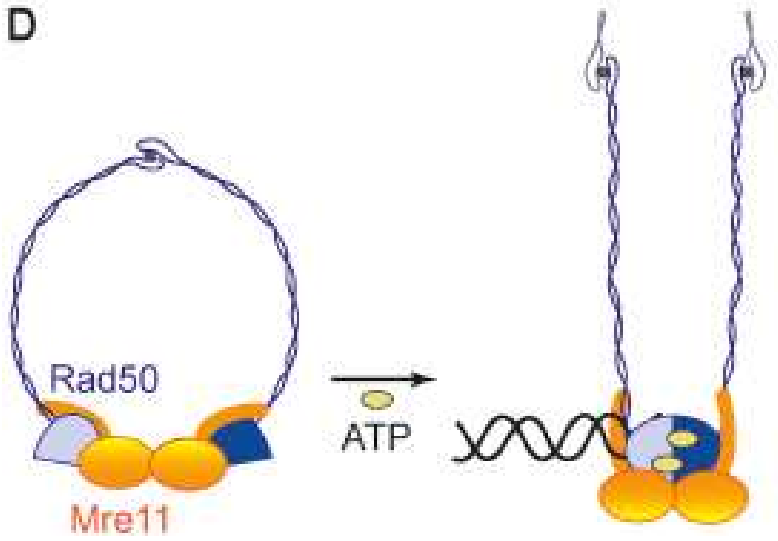
predominant states of the MutS cycle



predominant states of the MutS cycle



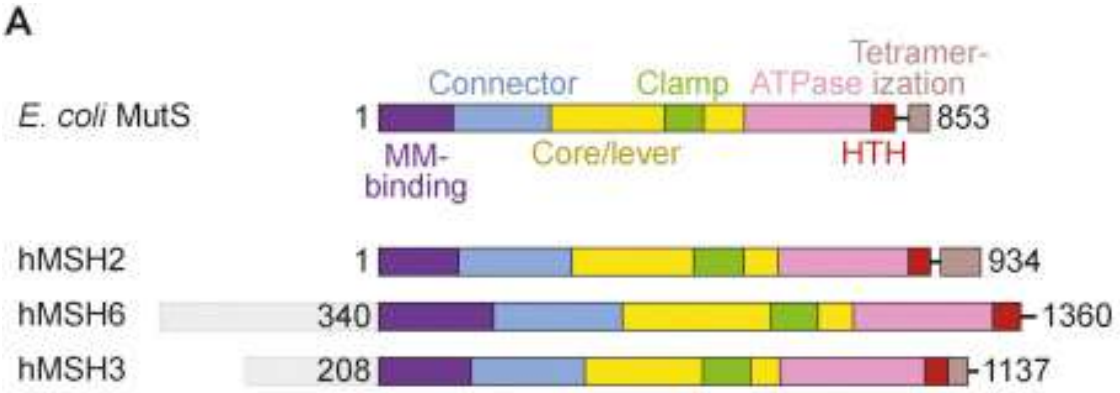
ATP binding induces a hinge motion that translocates mismatched DNA to a new channel in MutS proteins

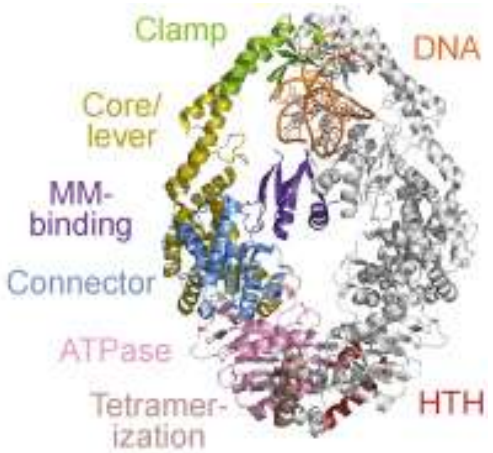


ATP binding by Rad50/Mre11 modulates the protein structure to increase binding to DNA ends

ATP-driven motions in different DNA repair proteins

MutS proteins

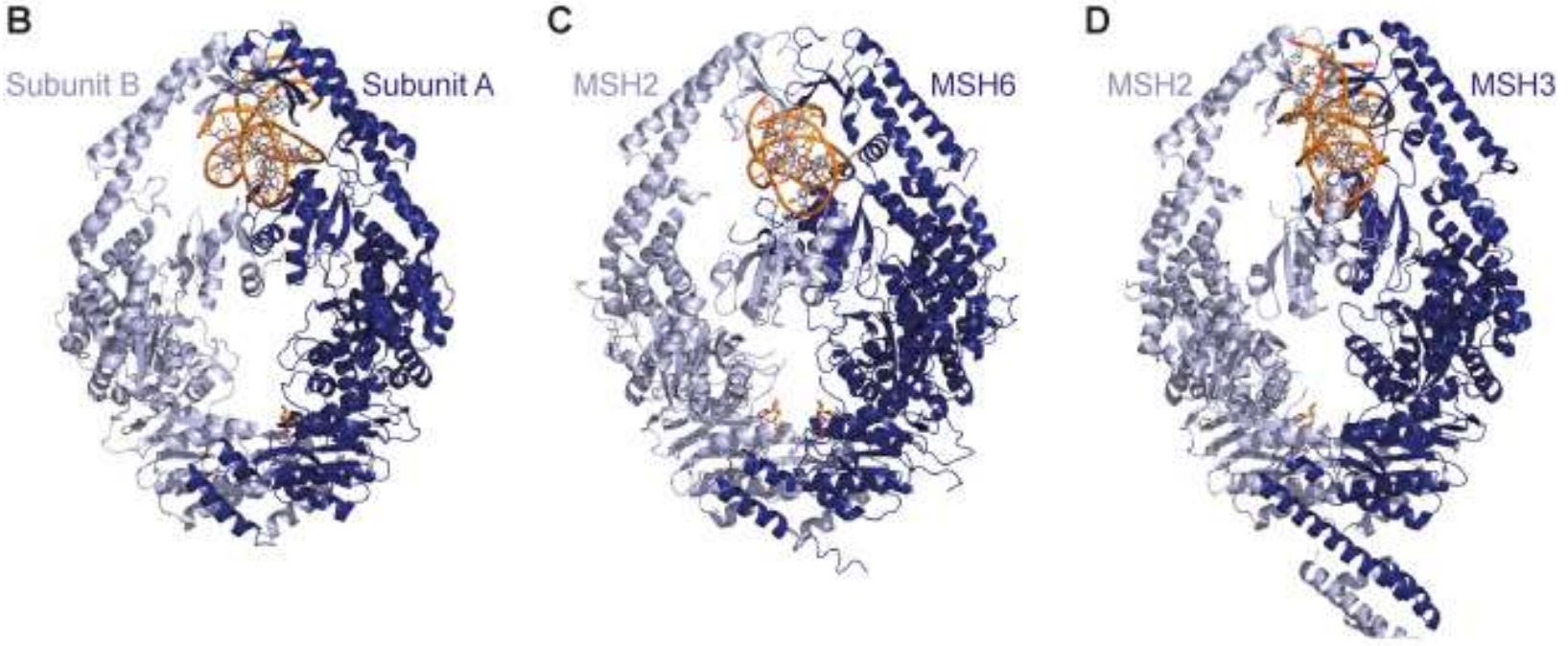




E.Coli MutS bound to a GT mismatch

human MutSα

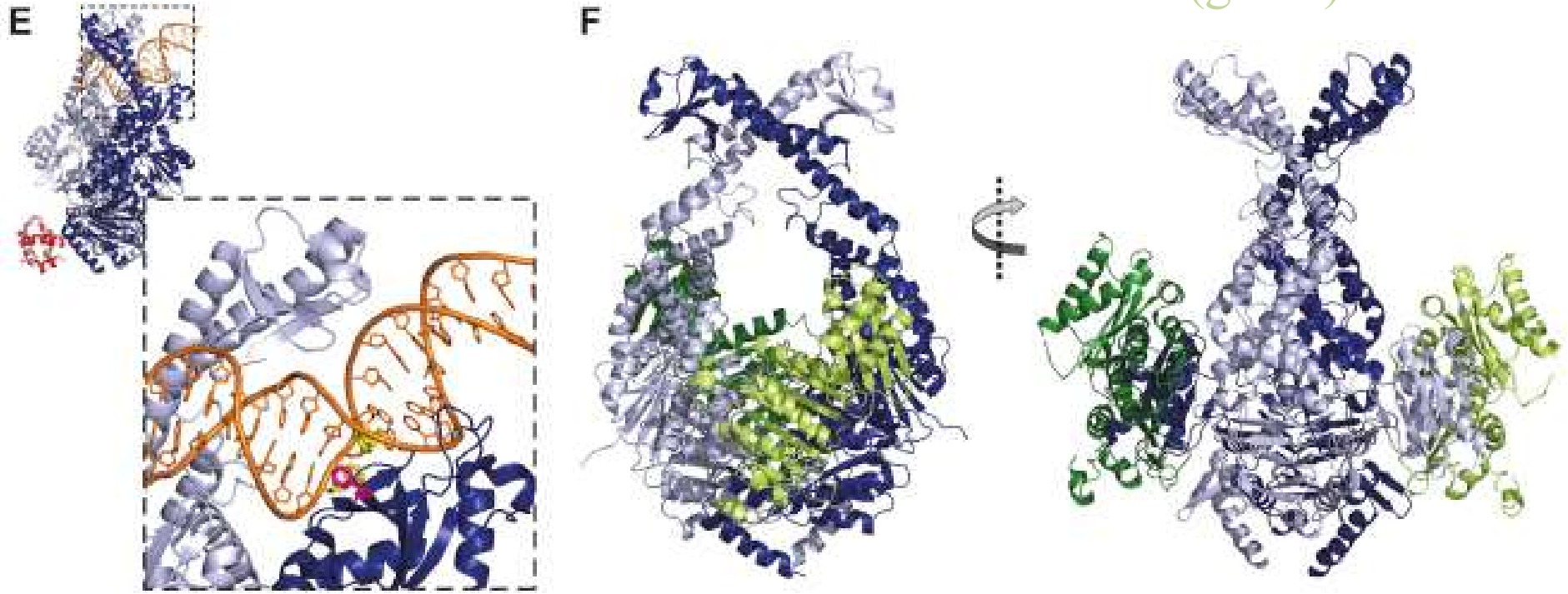
MutSβ bound to a 3-base del



Flora S. Groothuizen, Titia K. Sixma DNA Repair, Volume 38, 2016, 14–23

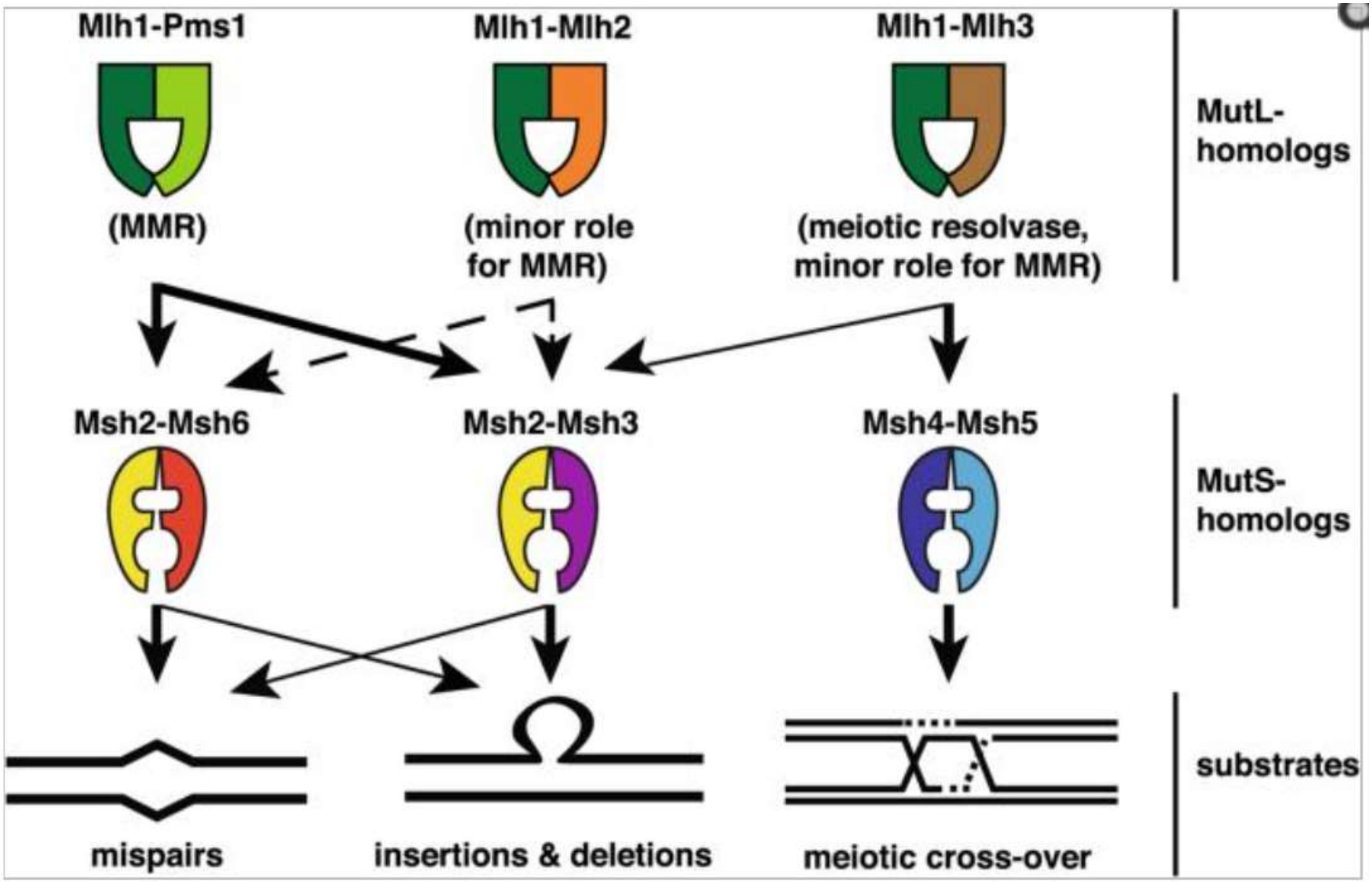
The conserved molecular machinery in DNA mismatch repair enzyme structures

The MutS sliding clamp bound to
the N-terminal domain of MutL (green)



Mismatch yellow;
phe36 pink

Omologia di MutS/MutL negli eucarioti

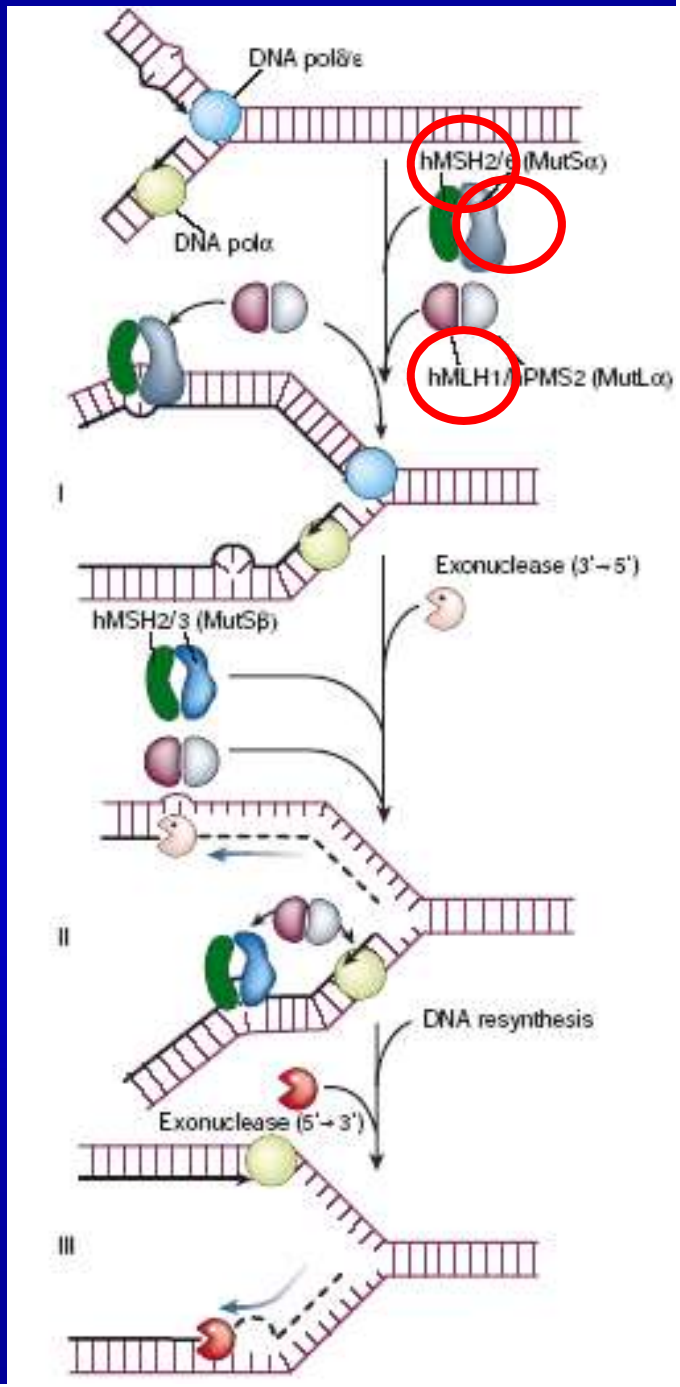


MSH2 forma un eterodimero con **MSH6** (misappaiamento) o **MSH3** (loop di inserzione-delezione) e si lega al DNA segnalando l'elica templato

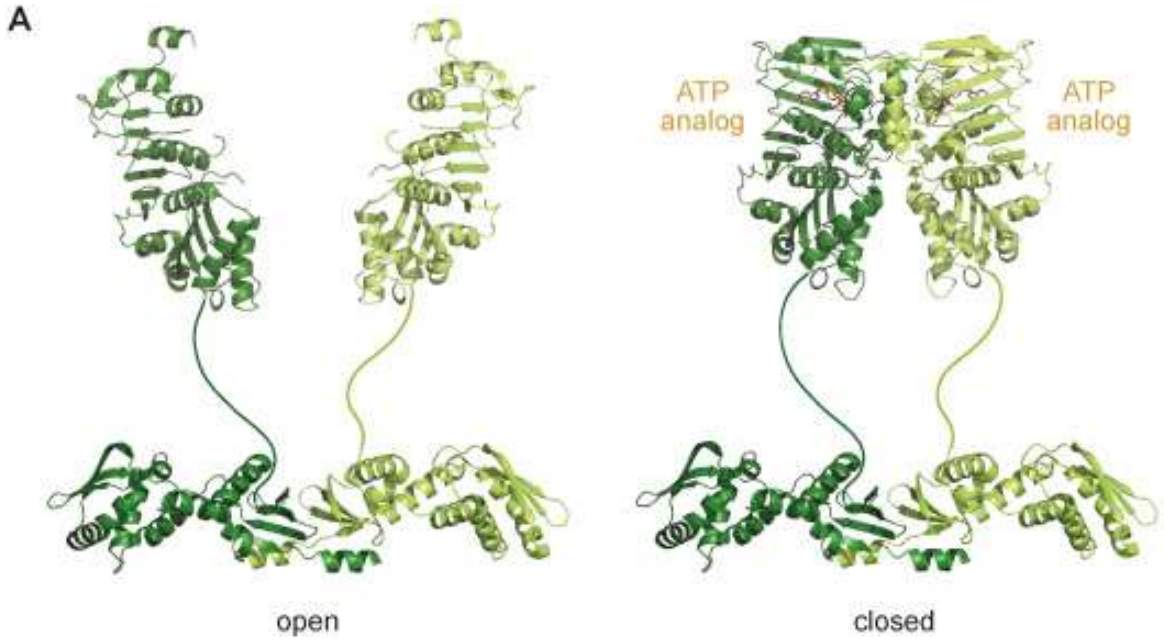
L'eterodimero **MLH1-PMS2**, talvolta legato anche a **PMS1**, incide e coordina il legame con l'esonucleasi **EXO1** 3'- 5' ed una o più elicasi

EXO1 rimuove le basi errate e il gap è riempito da DNA polimerasi e ligasi

MSH2, MSH3 e MSH6 sono omologhi a **mutS** di E.coli; **MLH1**, **PMS1** e **PMS2** sono omologhi a **mutL** di E.coli

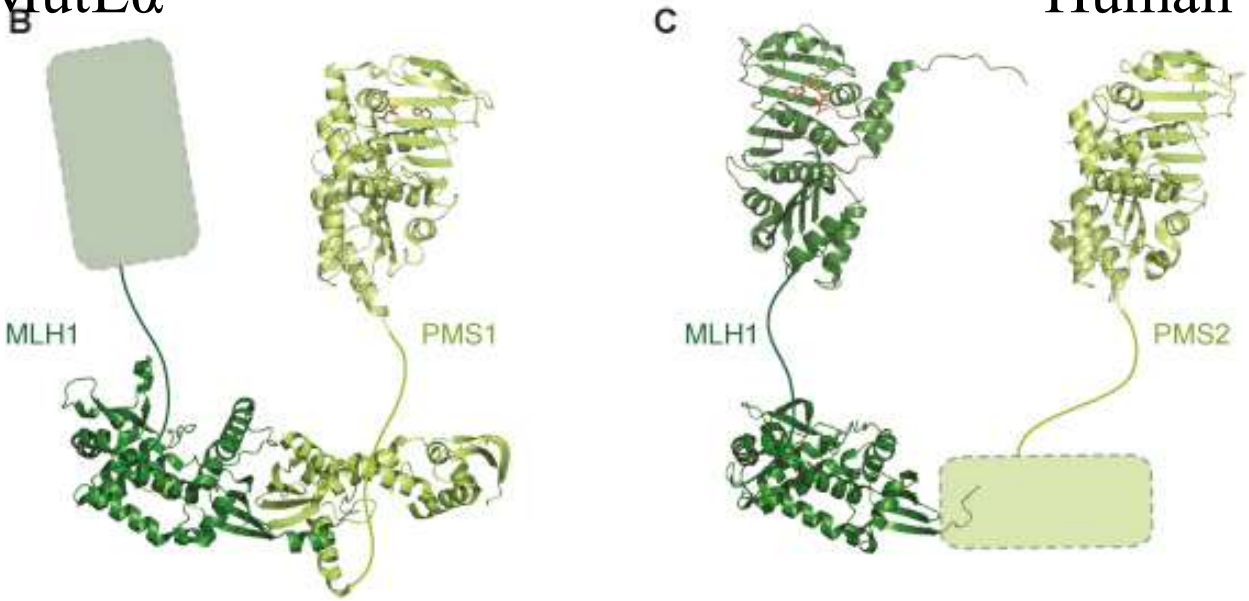


MutL proteins

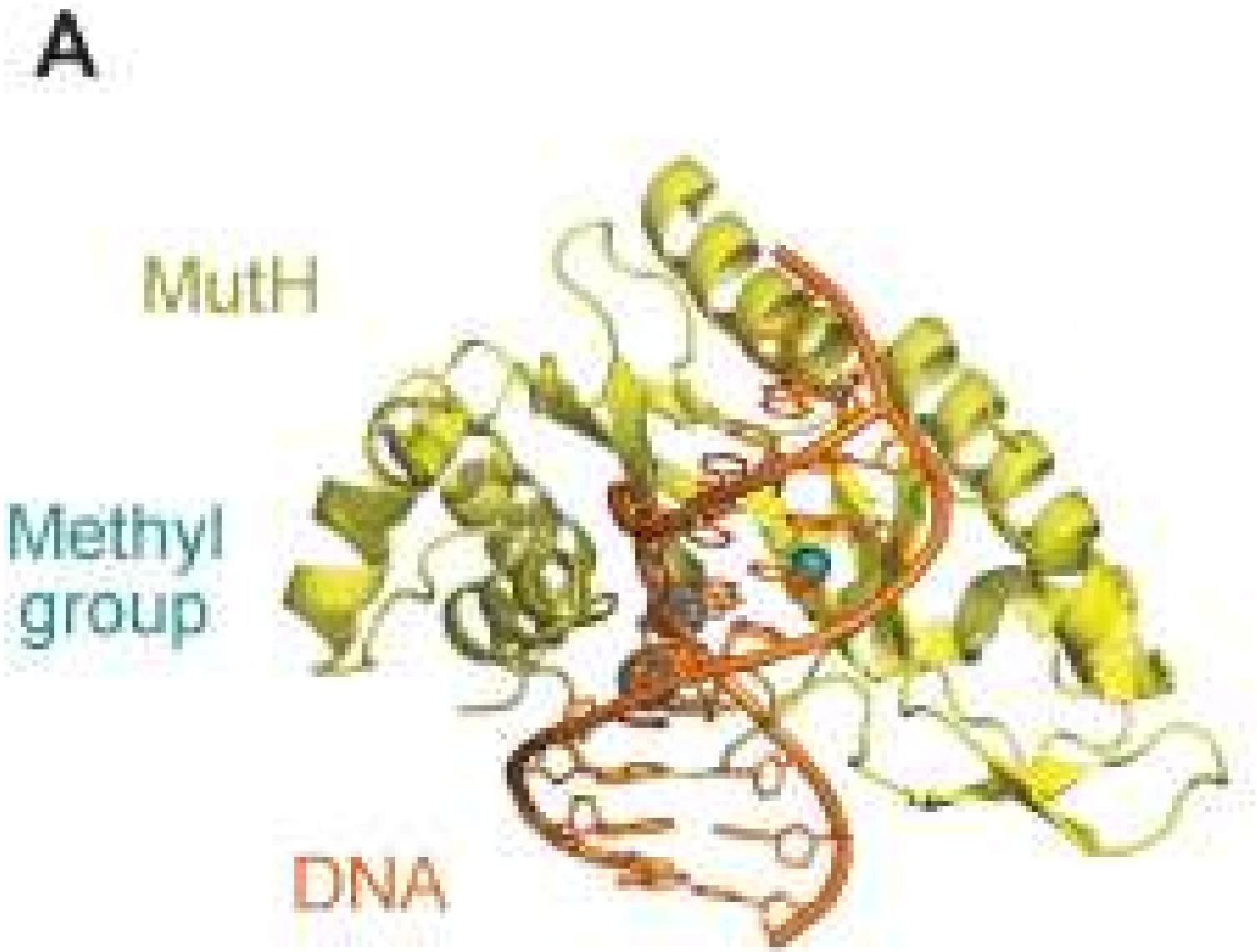


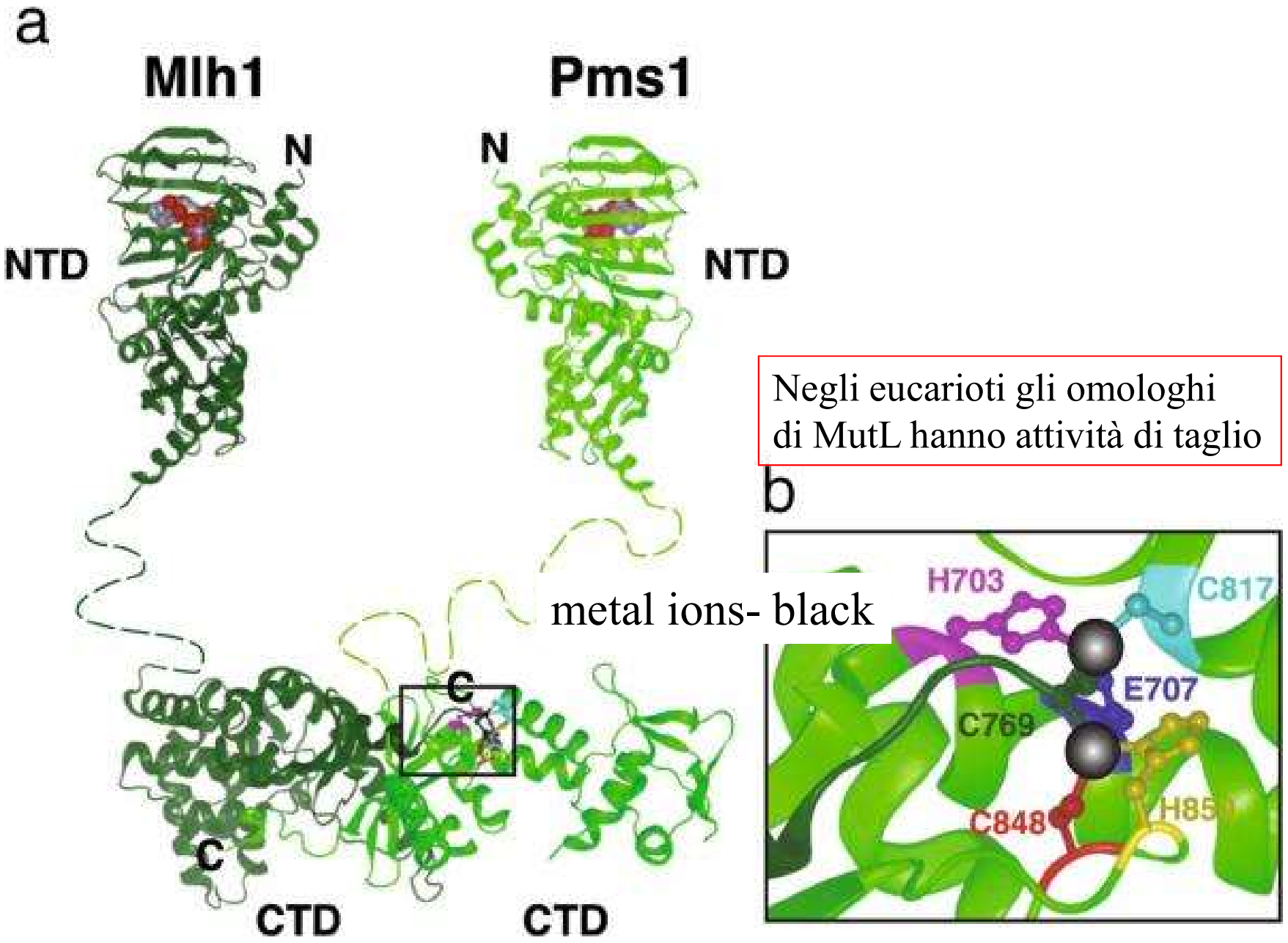
Yeast MutLα

Human MutLα



endonucleases in MMR



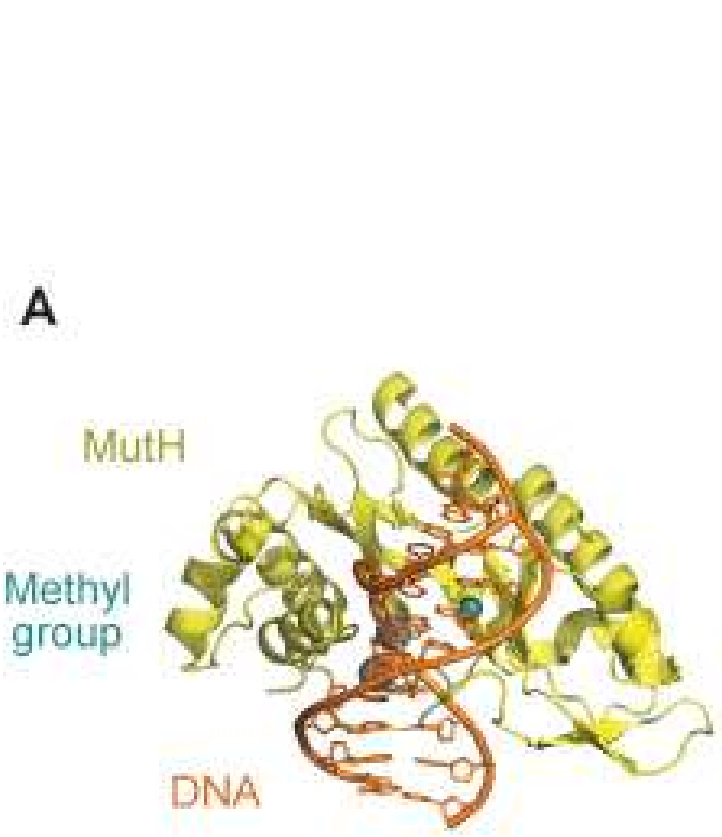


Negli eucarioti gli omologhi di MutL hanno attività di taglio

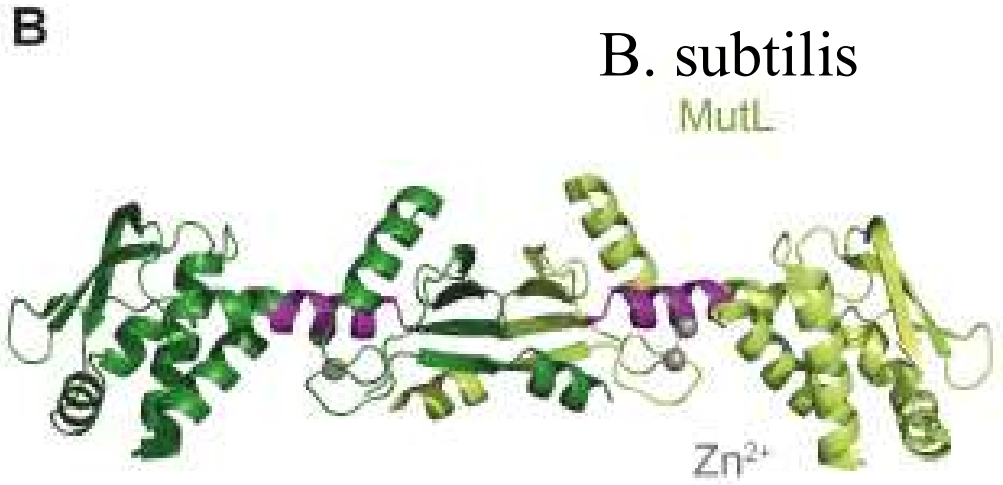
metal ions- black

endonuclease site

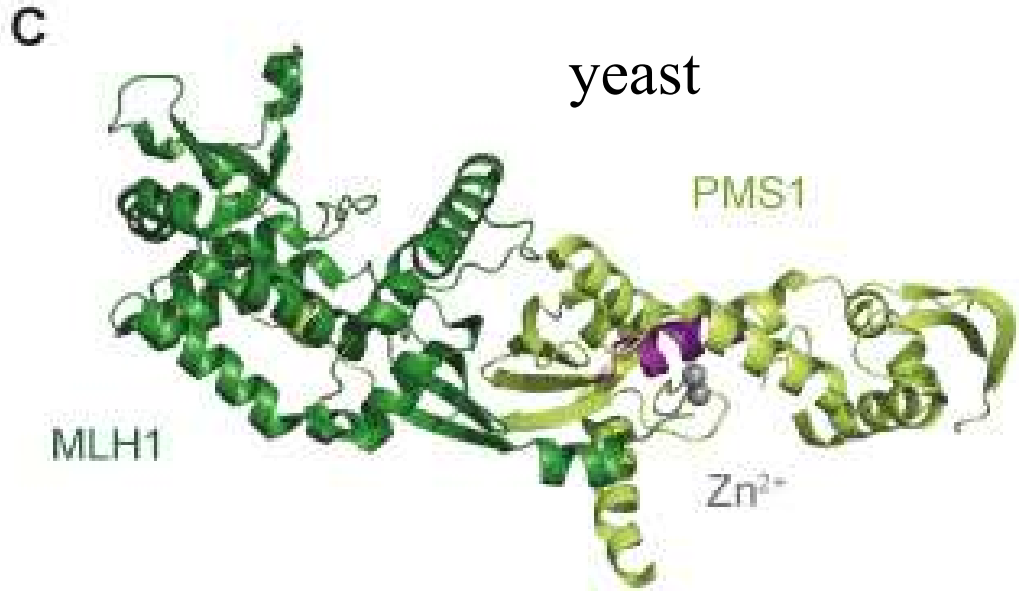
endonucleases in MMR



E. coli

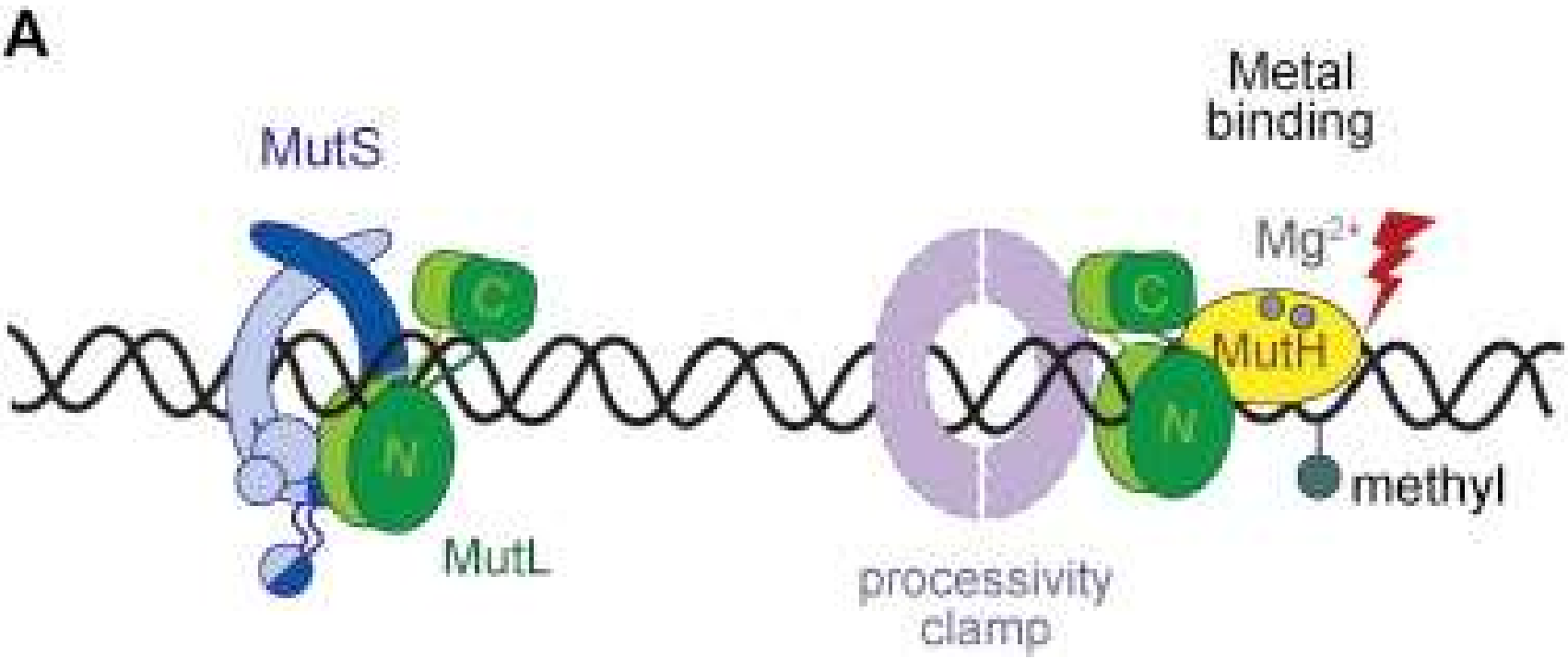


B. subtilis



yeast

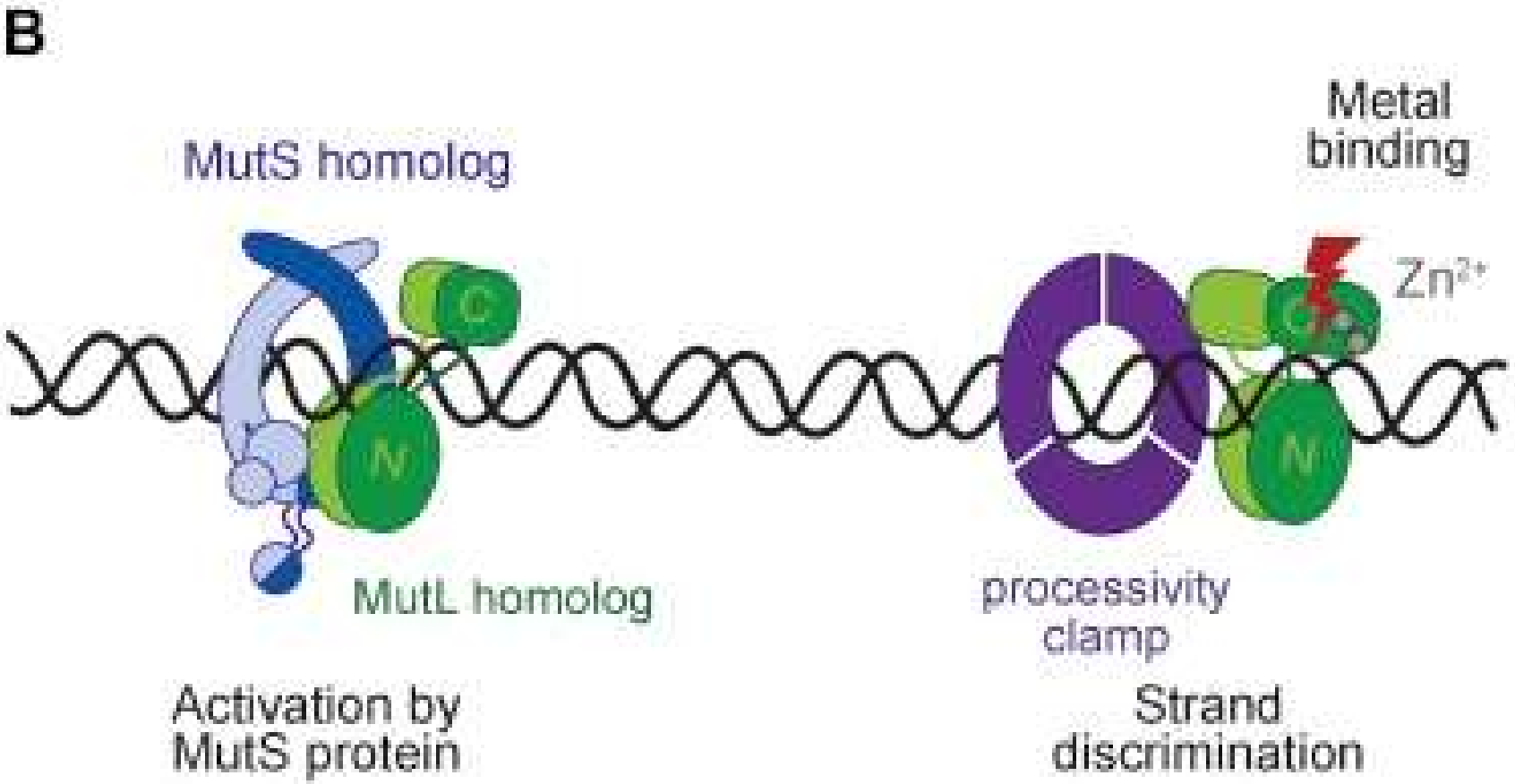
MutH protein dependent endonuclease



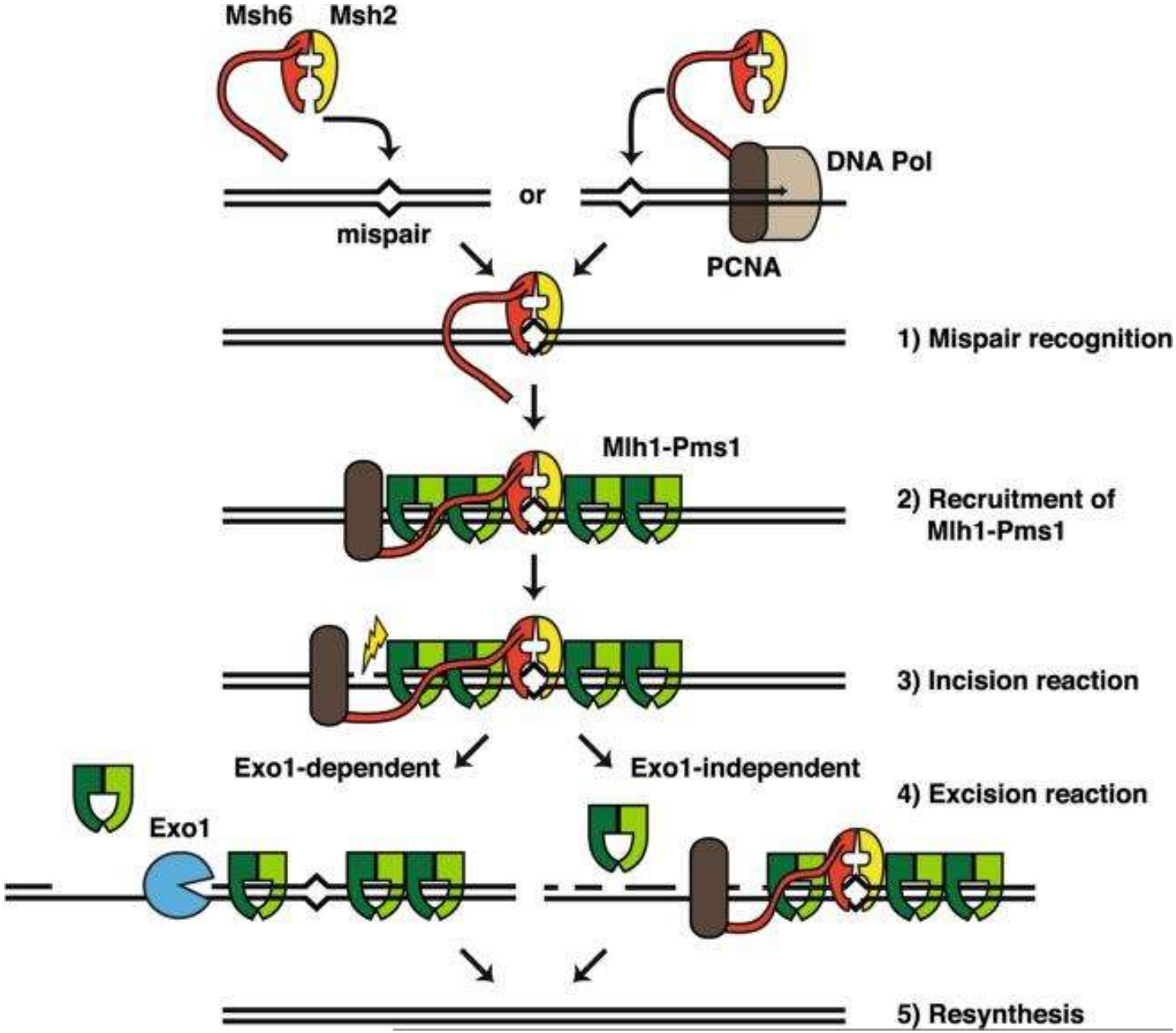
activation of endonuclease
activities in MMR

activation of endonuclease
activities in MMR

MutL endonuclease activity



alternative excision pathways during MMR

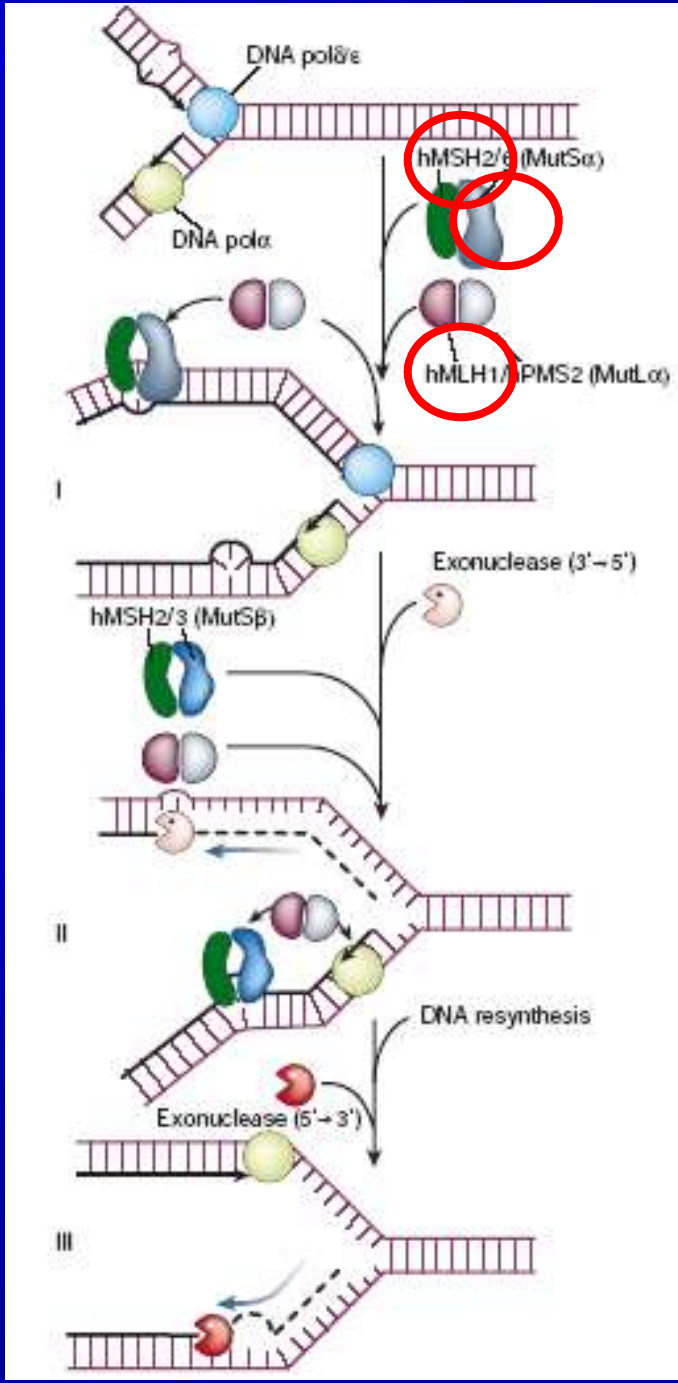


HNPCC
cancro colon-rettale ereditario non poliposico

hMLH1: 50% delle mutazioni

hMSH2: 35%

hMSH6: 10%



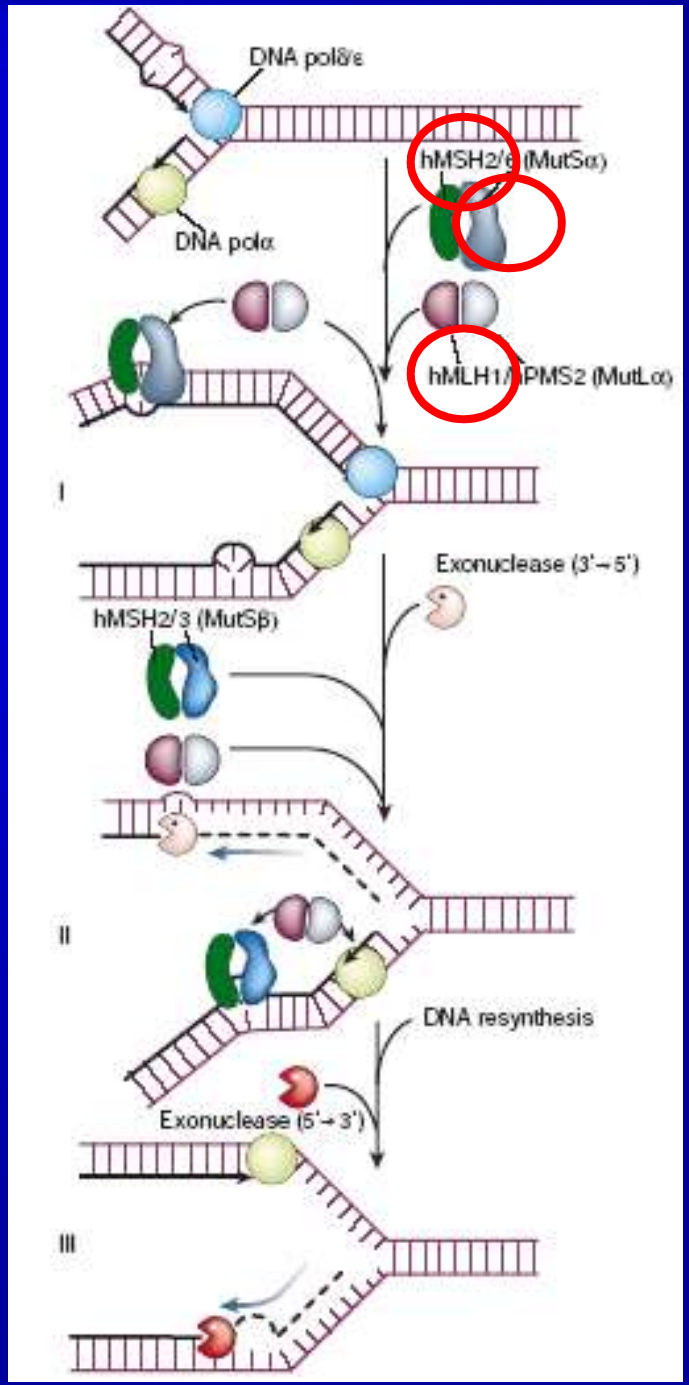
HNPCC
cancro colon-rettale ereditario non poliposico

hMLH1: 50% delle mutazioni

hMSH2: 35%

hMSH6: 10%

Mutazioni polimerasi **D**elta ed **E**psilon



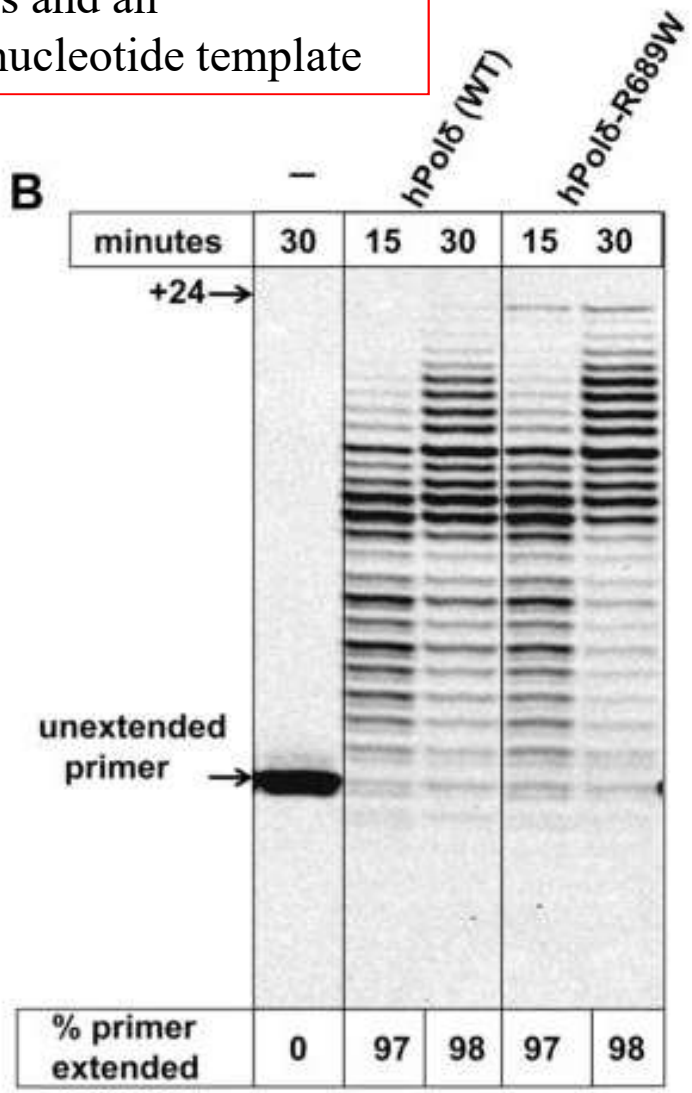
Nucleotide selectivity defect and mutator phenotype conferred by a colon cancer-associated DNA polymerase δ mutation

Germline mutations in the POLD1 and POLE genes encoding the catalytic subunits of replicative DNA polymerases δ (Polδ) and ε (Polε) cause hereditary CRC

POLD1-R689W, encodes an error-prone DNA polymerase and causes a catastrophic increase in spontaneous mutagenesis

incubating the purified enzymes with all four dNTPs and an oligonucleotide template

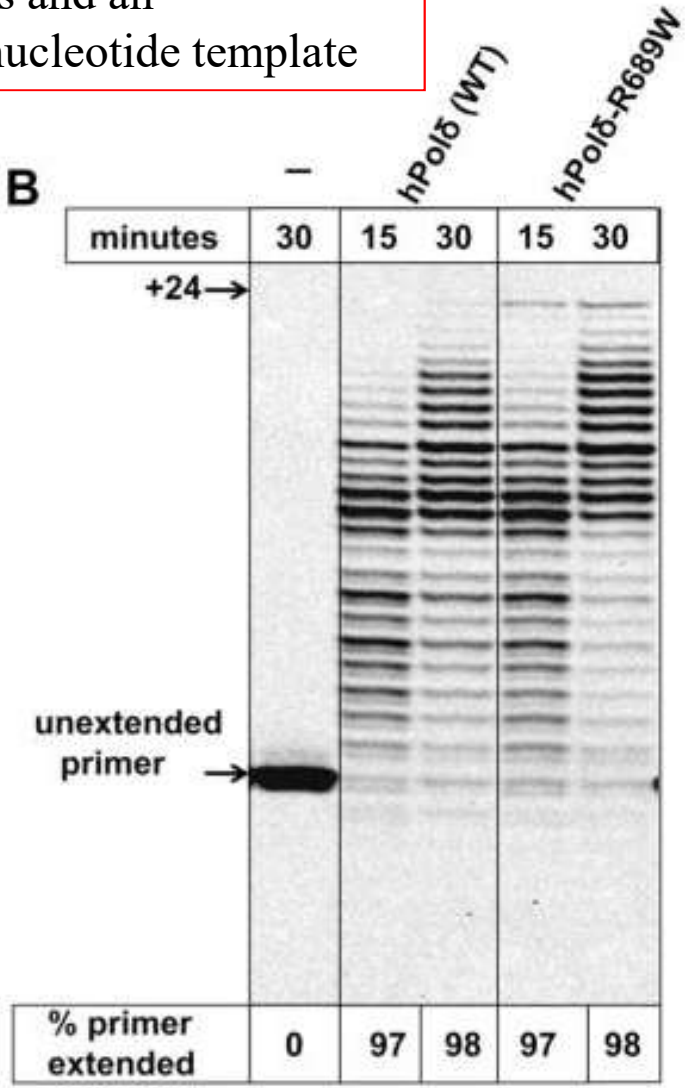
Polδ-R689W is an active and….



DNA synthesis

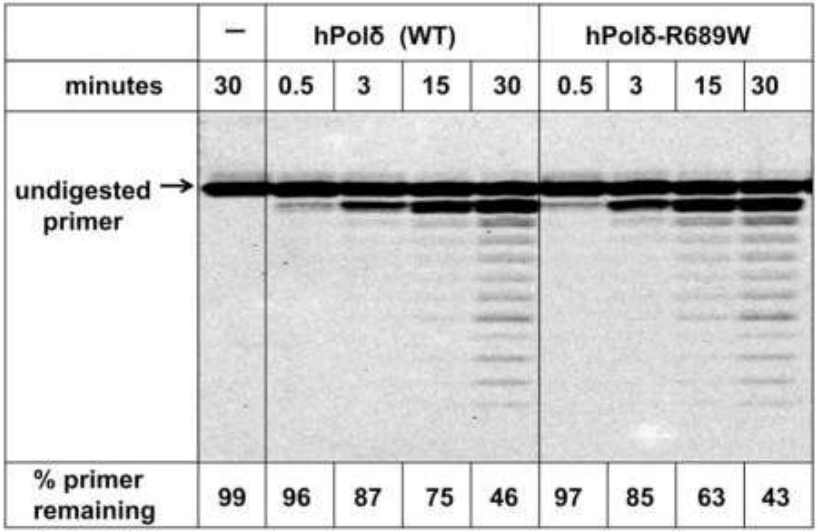
incubating the purified enzymes with all four dNTPs and an oligonucleotide template

Polδ-R689W is an active and….



DNA synthesis

Exonuclease activity

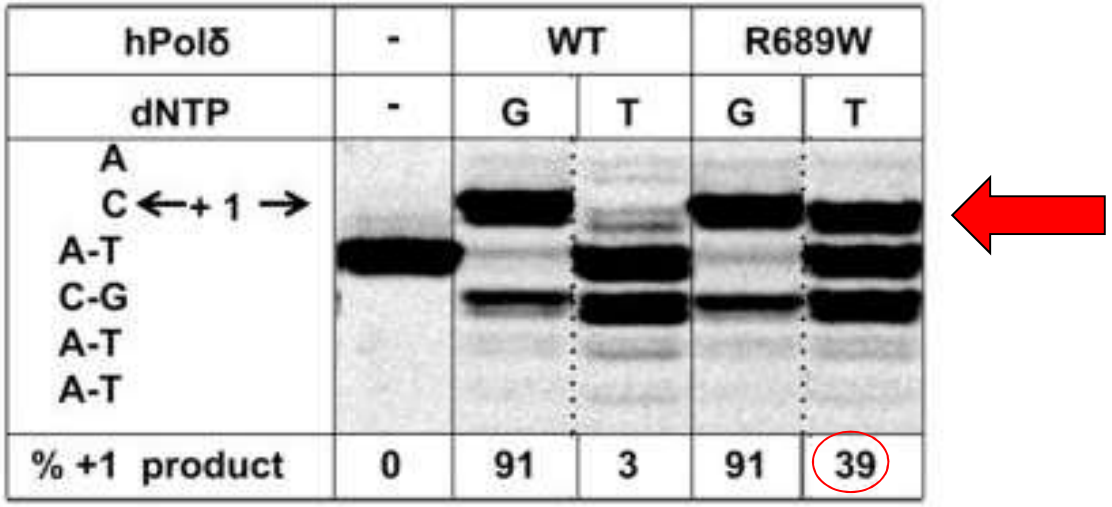


incubating the purified enzymes with the oligonucleotide substrate and no dNTPs

……highly error-prone DNA polymerase

D

template C



efficiency of correct and incorrect nucleotide insertion

incubate the enzymes and the oligonucleotide substrate for 15 min in the presence of dGTP or dTTP

HNPCC
cancro colon-rettale ereditario non poliposico

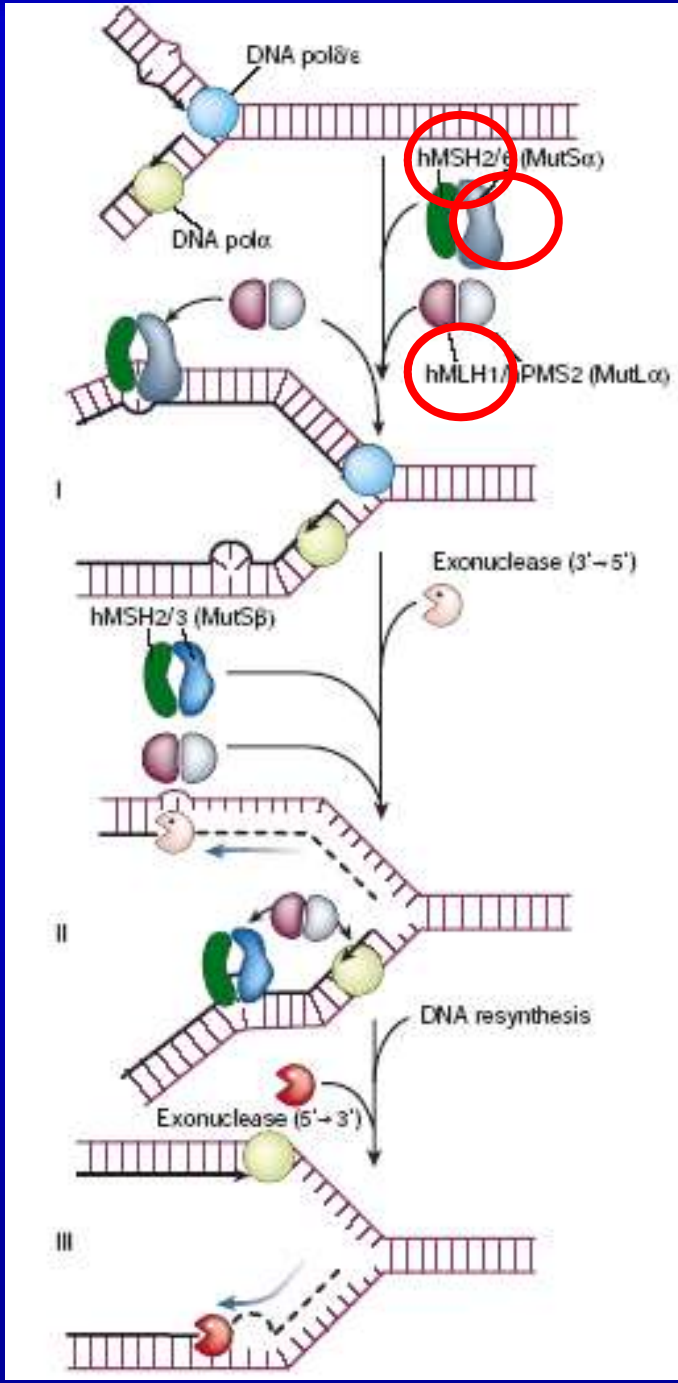
hMLH1: 50% delle mutazioni

hMSH2: 35%

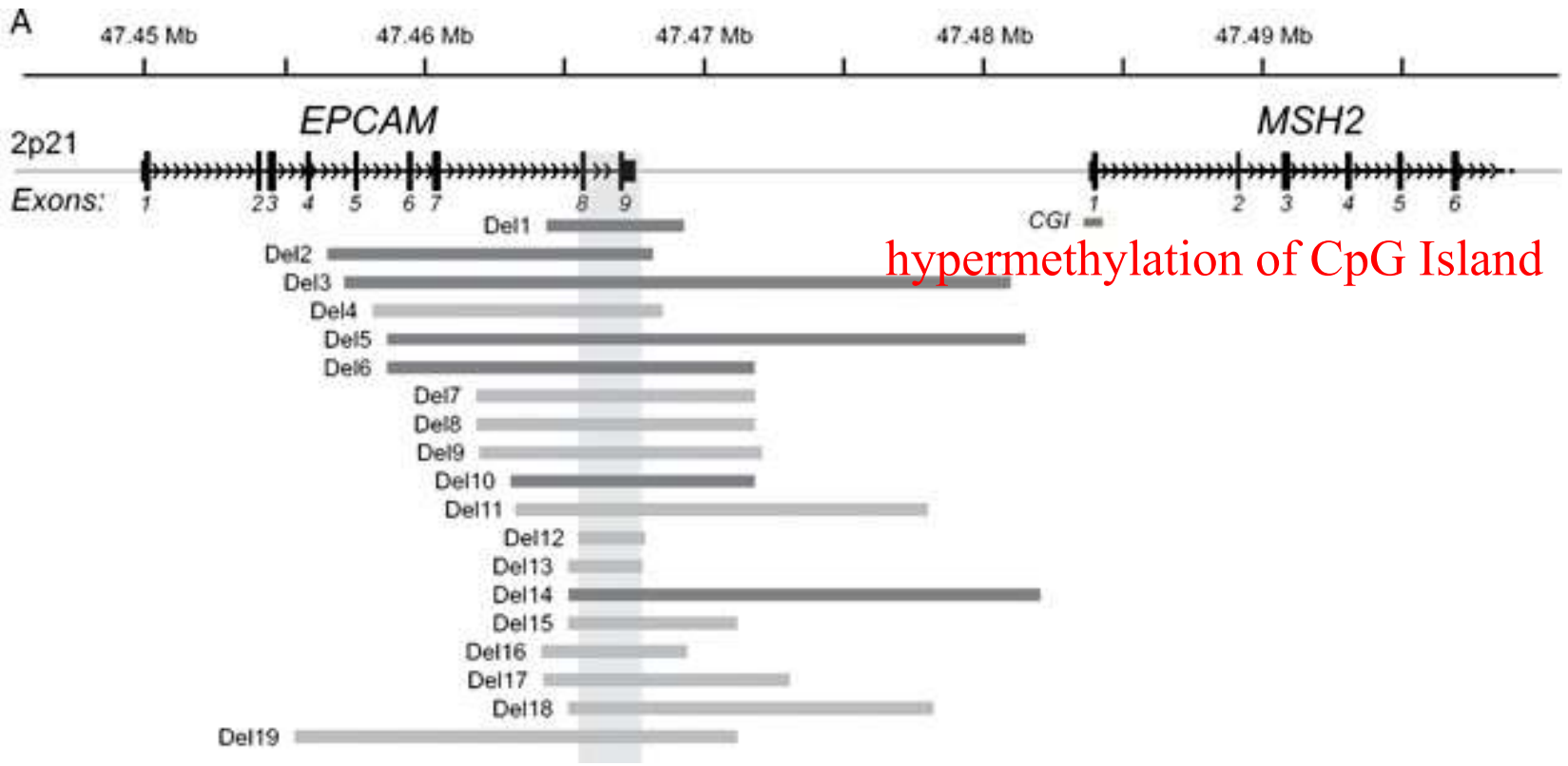
hMSH6: 10%

Mutazioni polimerasi **D**elta ed **E**psilon

epithelial cell adhesion molecule gene **EPCAM Deletion**



Recurrence and variability of germline *EPCAM* deletions in Lynch syndrome



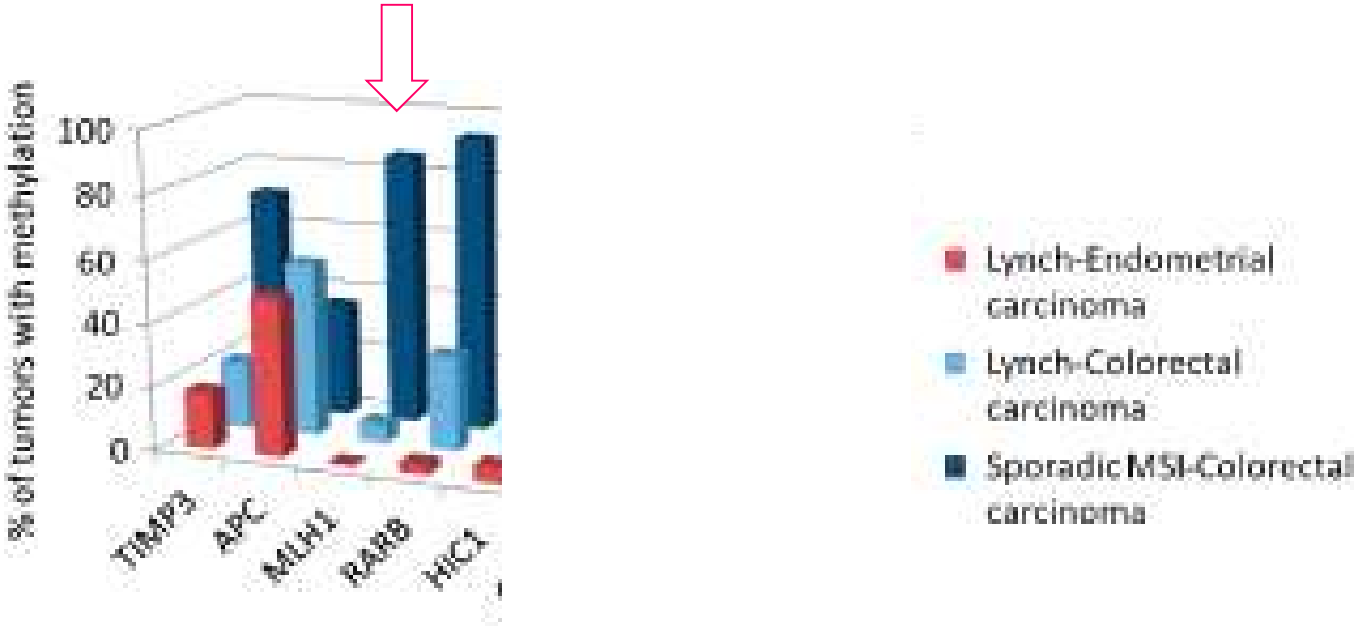
hypermethylation of CpG Island

..result in transcriptional read-through into the MSH2 gene and subsequent hypermethylation of its CpG island promoter in EPCAM-expressing tissues

Human Mutation
Volume 32, Issue 4, pages 407-414, 1 MAR 2011 DOI: 10.1002/humu.21446
http://onlinelibrary.wiley.com/doi/10.1002/humu.21446/full#fig1

METILAZIONE DEL DNA E TUMORI

Epigenetic mechanisms in the pathogenesis of Lynch syndrome



Lynch = HNPCC cancro colon-rettale ereditario non poliposico

Clinical Genetics
Volume 85, Issue 5, pages 403-412, 17 FEB 2014 DOI: 10.1111/cge.12349
http://onlinelibrary.wiley.com/doi/10.1111/cge.12349/full#cge12349-fig-0002

Mutazioni in un gene del MMR → predisposizione a HNPCC (cancro colon-rettale ereditario non poliposico), patologia frequente (1/200), aumento 100-1000X del tasso generale di mutazione, rischio di tumori al colon-retto

hMLH1: 50% delle mutazioni in HNPCC

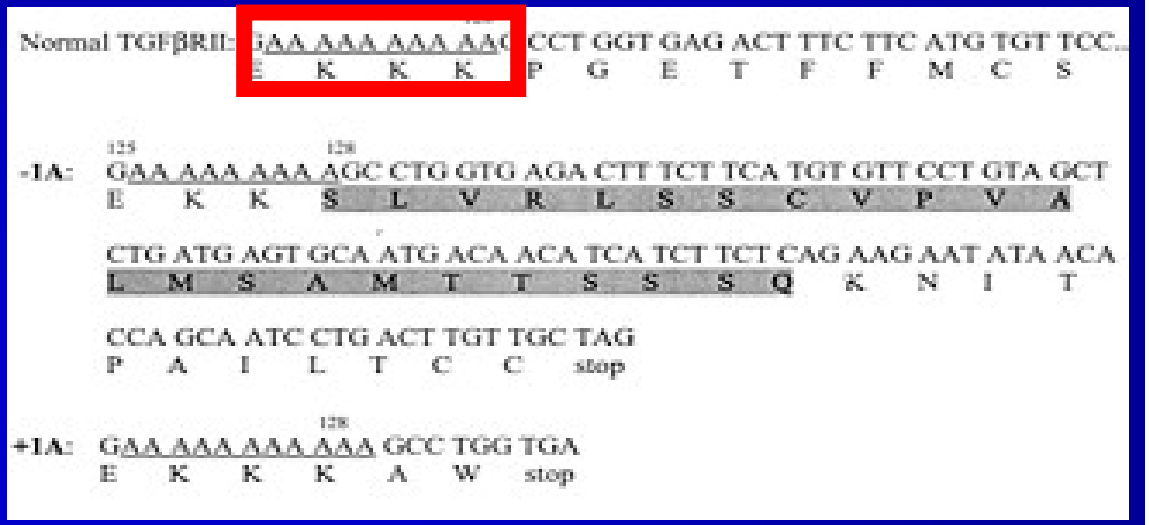
hMSH2: 35%

hMSH6: 10%

Le sostanze chimiche in grado di indurre mutazioni sono contenute soprattutto nel cibo o sono prodotte dal metabolismo alimentare → maggiori probabilità di colpire la mucosa della zona colon-rettale, dove il cibo permane 24-36 ore

L'alterazione del MMR aumenta l'insorgenza di mutazioni nel gene codificante per il recettore di tipo II per il TGFβ (TGFβ è un inibitore della proliferazione cellulare)

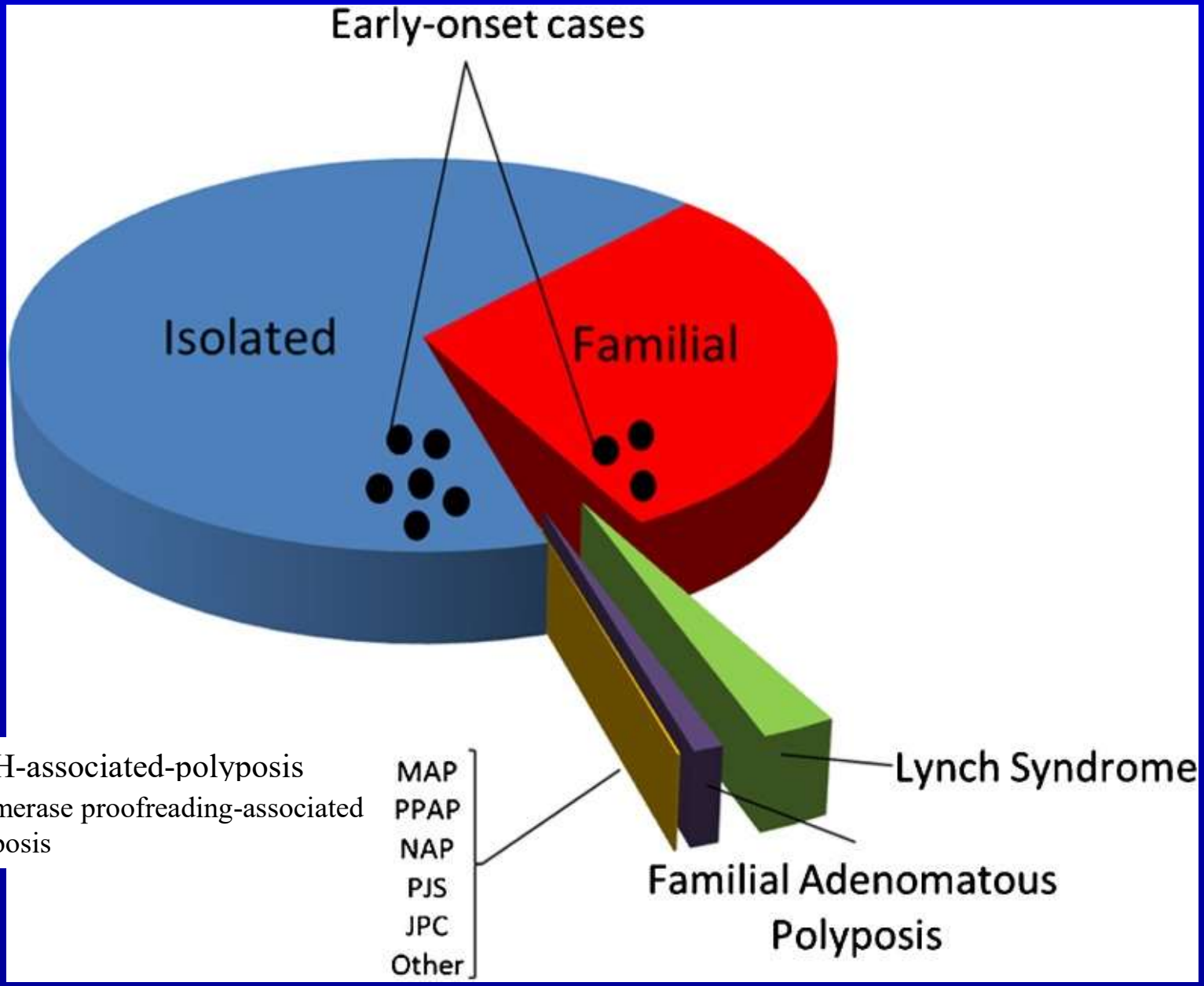
Tale gene contiene una fila di 10 Adenine dove si ha frequente "slittamento" della DNA polimerasi → sequenze con 9 o 11 A, corrette da MMR



Pazienti con alterazioni del MMR: l'errore permane → recettore per TGFβ non funzionale

La mutazione rende le cellule insensibili alla inibizione della crescita indotta da TGFβ → sviluppo incontrollato caratteristico dei tumori

Colorectal cancers



MUTYH-associated-polyposis

polymerase proofreading-associated polyposis