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)

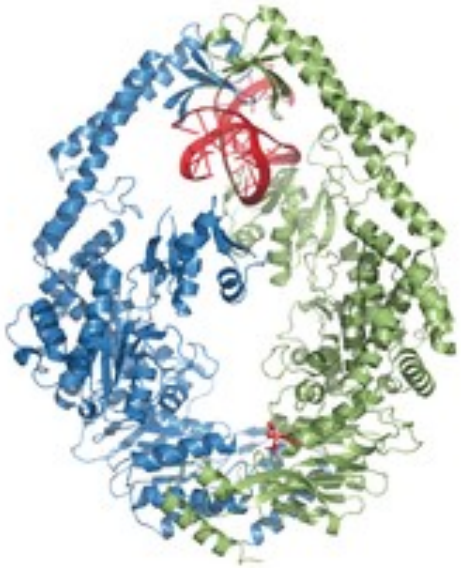
A) MutS binds mismatched base pairs

Apparent affinities of mutS protein for base pair mismatches

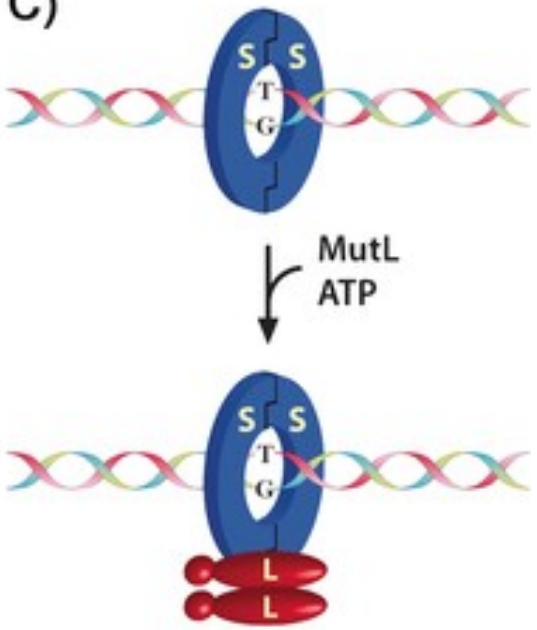
Mismatch	Apparent dissociation constant
	nM
G-T	39 ± 4
A-C	53 ± 4
A-A	110 ± 7
T-T	140 ± 9
G-G	150 ± 10
A-G	270 ± 30
C-T	370 ± 40
C-C	480 ± 50

AFFINITA'

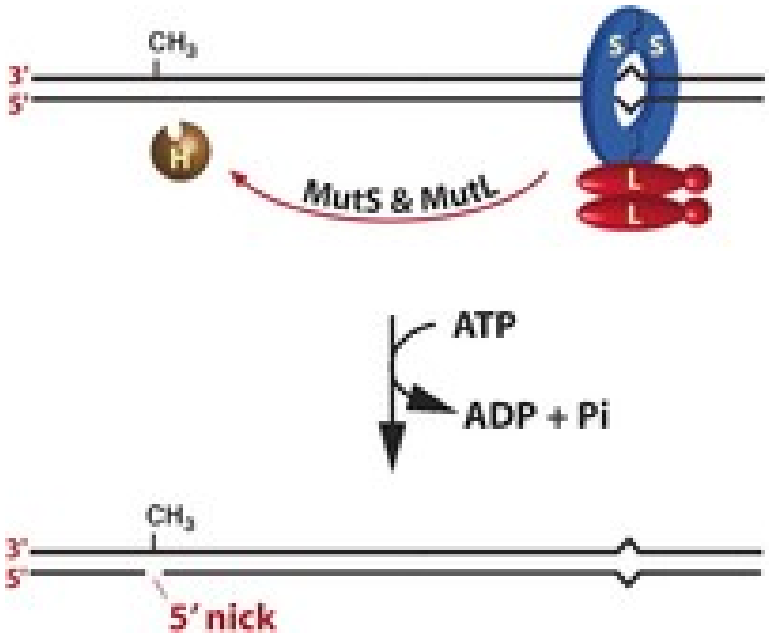
B)

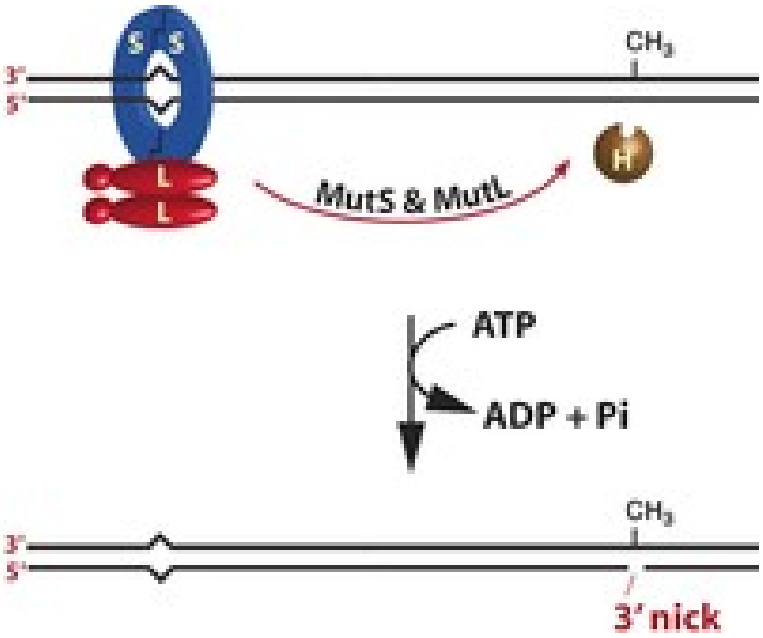


C)



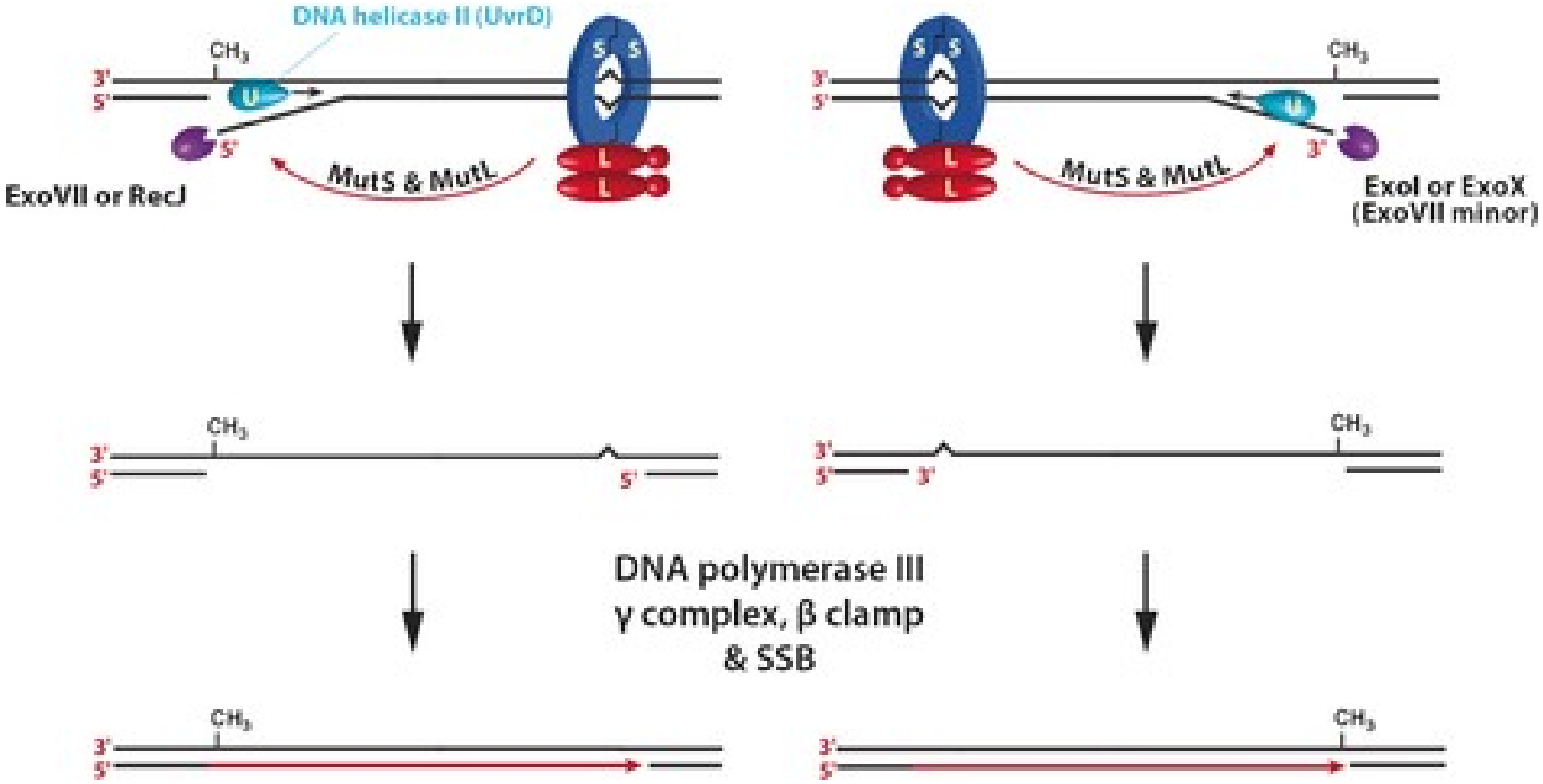
Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)





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)



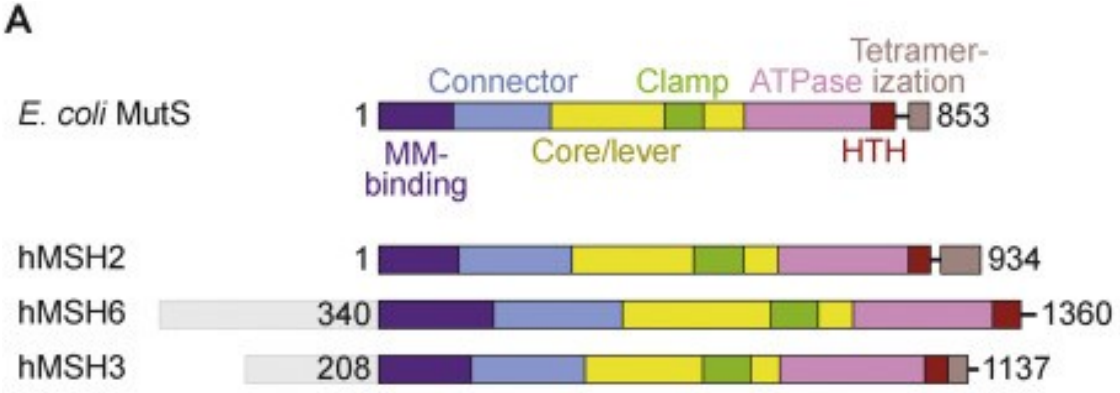
Angewandte Chemie International Edition
Volume 55, Issue 30, pages 8490-8501, 20 MAY 2016 DOI: 10.1002/anie.201601412
http://onlinelibrary.wiley.com/doi/10.1002/anie.201601412/full#anie201601412-fig-0007

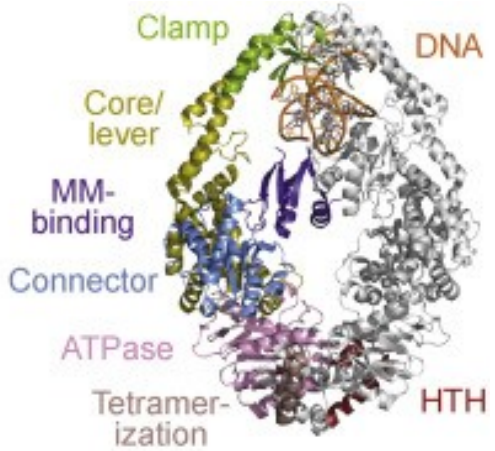
Table 1

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

E. coli	*iae*	*H. sapiens*	Comments
MutS-MutS	ıı6	Msh2-Msh6 (MutSα)	Mispair recognition complex—homodimer in *E. coli* and a heterodimer in eukaryotes. MutSα and MutSβ have overlapping mispair recognition specifi
	ıı3	Msh2-Msh3 (MutSβ)	
	;1	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	ı2	Mlh1-Pms1 (MutLβ)	MutLβ is an accessory factor for MMR
	ı3	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 methylas		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	nknown	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

MutS proteins

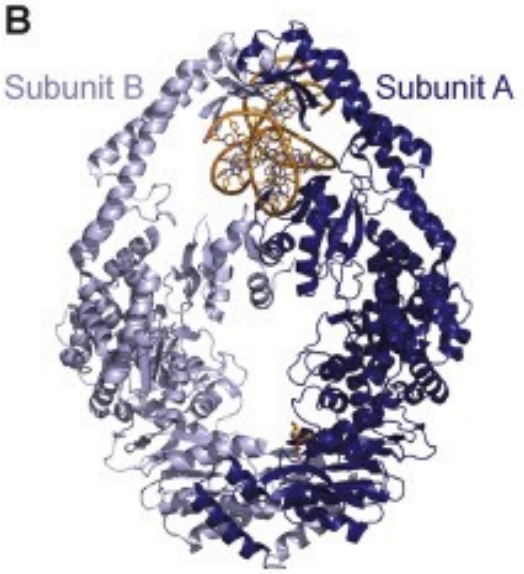


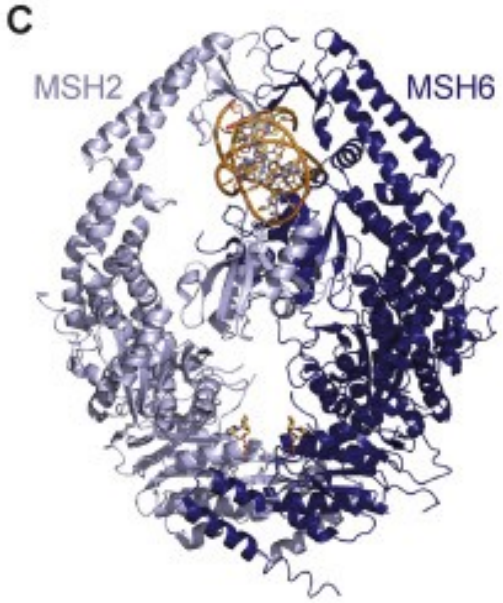


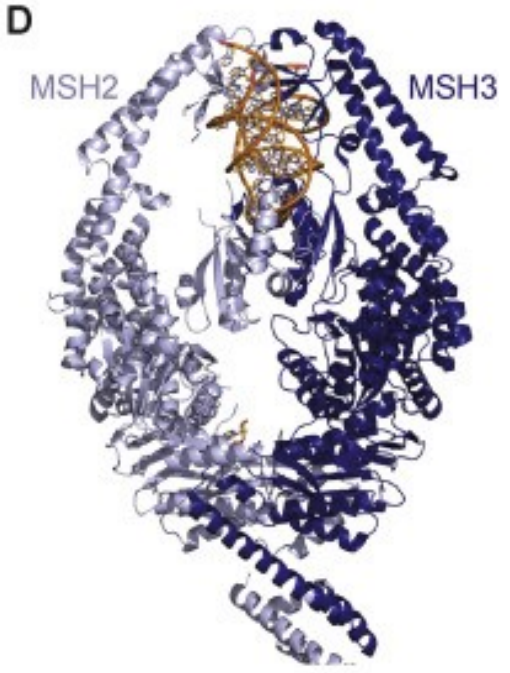
E.Coli MutS bound to a GT mismatch

human MutSα

MutSβ bound to a 3-base del



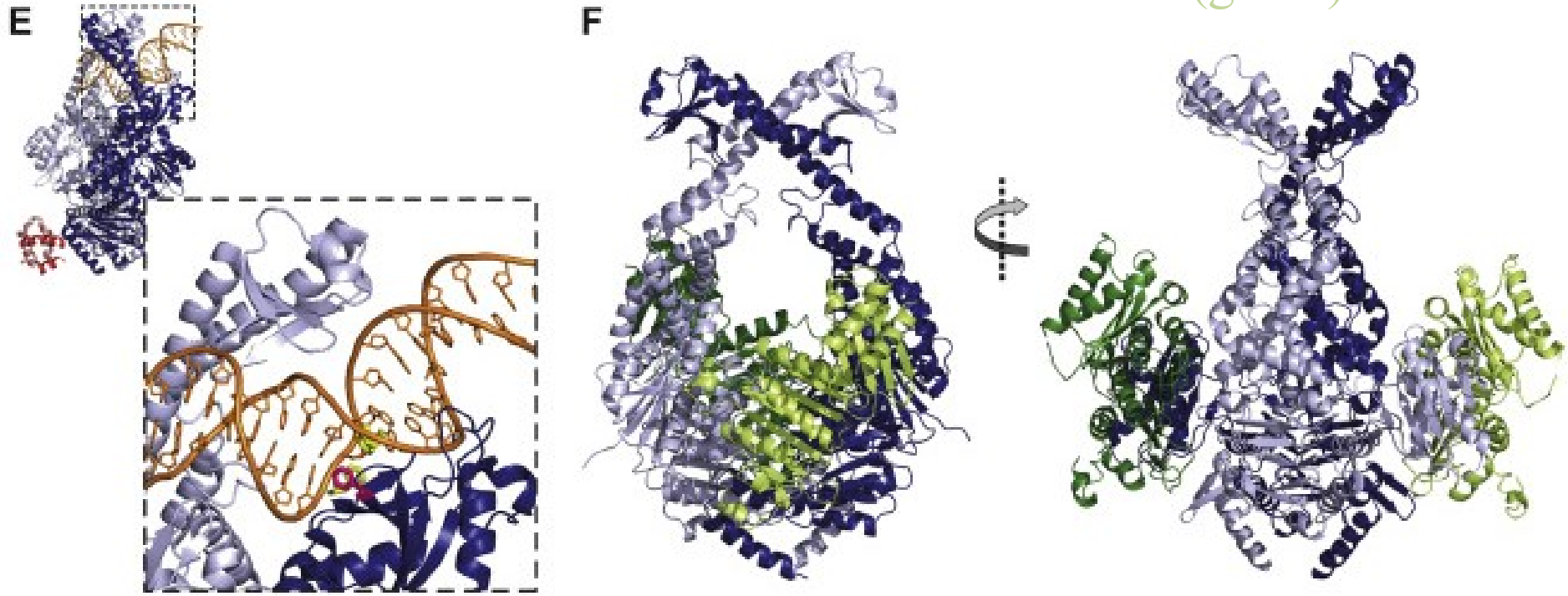




Flora S. Groothuizen, Titia K. Sixma DNA Repair, Volume 38, 2016, 14–23

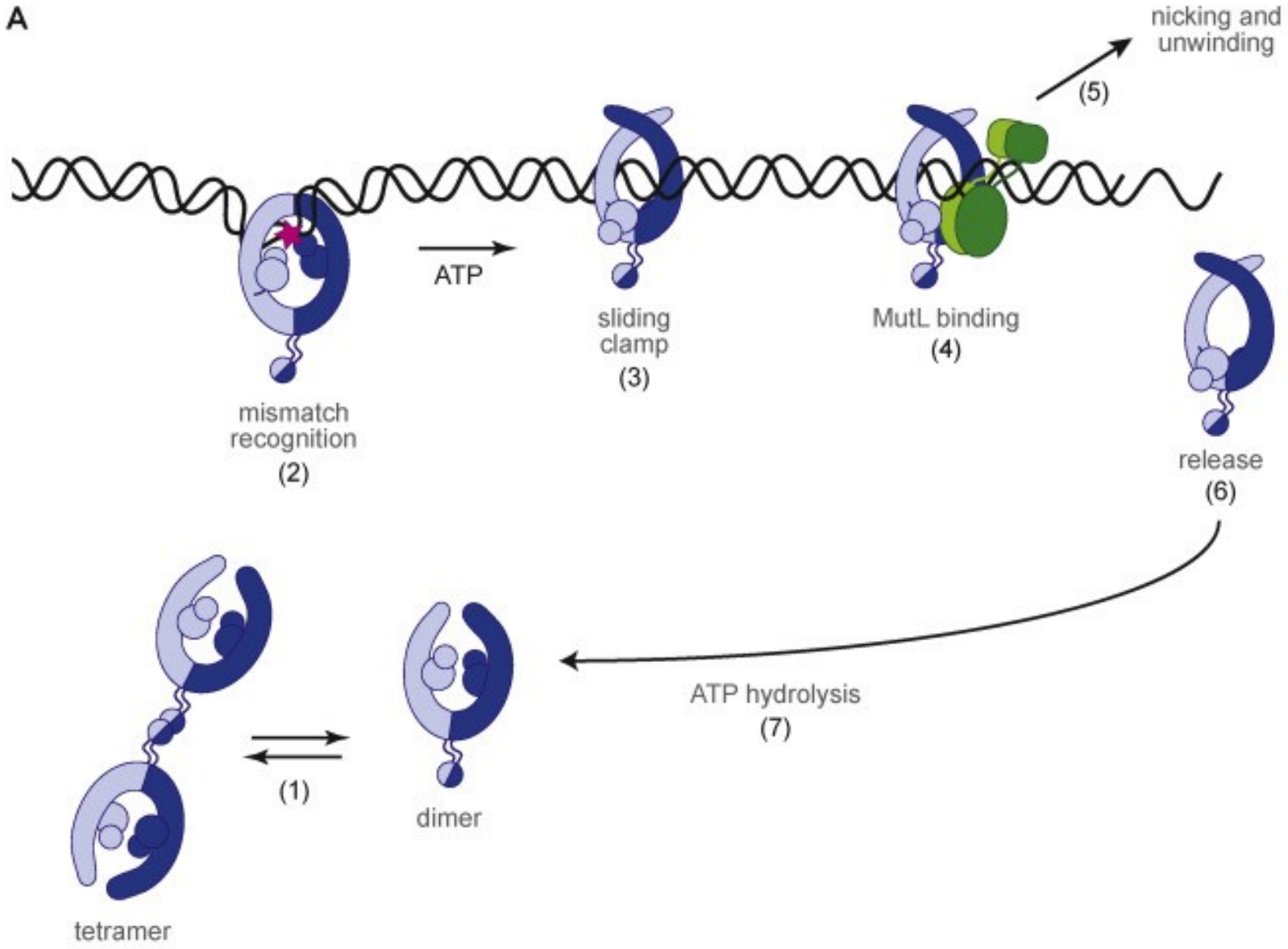
The conserved molecular machinery in DNA mismatch repair enzyme structures

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

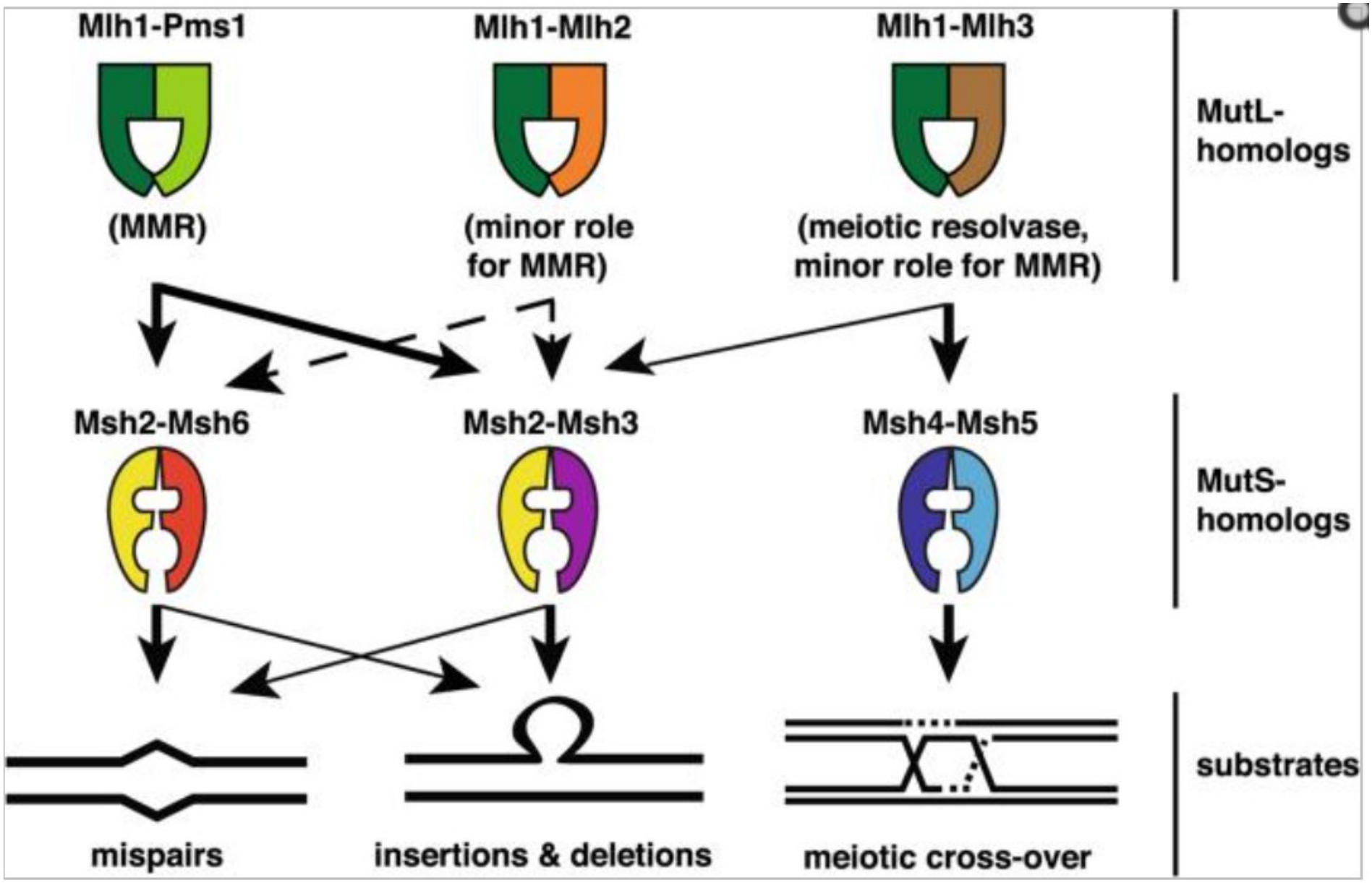


Mismatch yellow;
phe36 pink

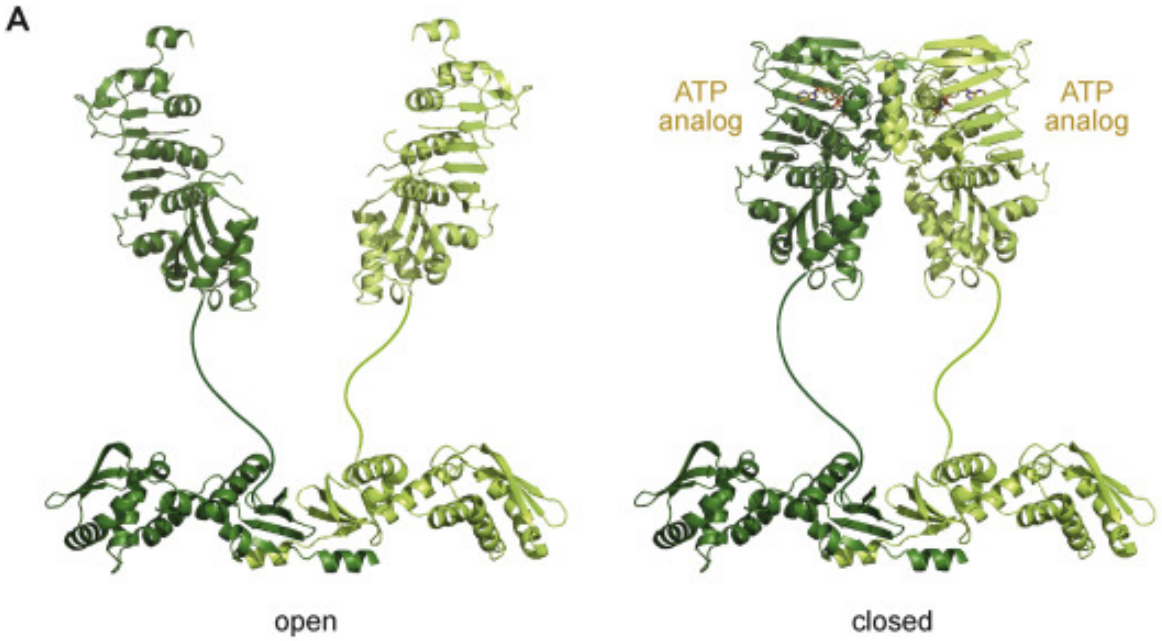
predominant states of the MutS cycle



Omologia di MutS/MutL negli eucarioti

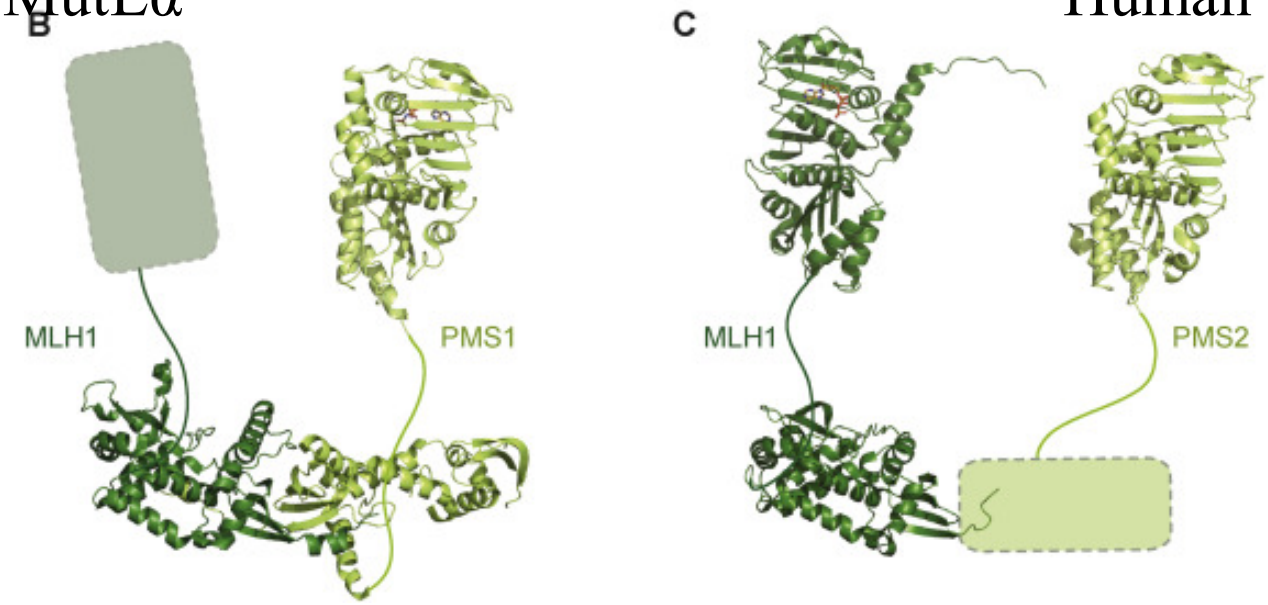


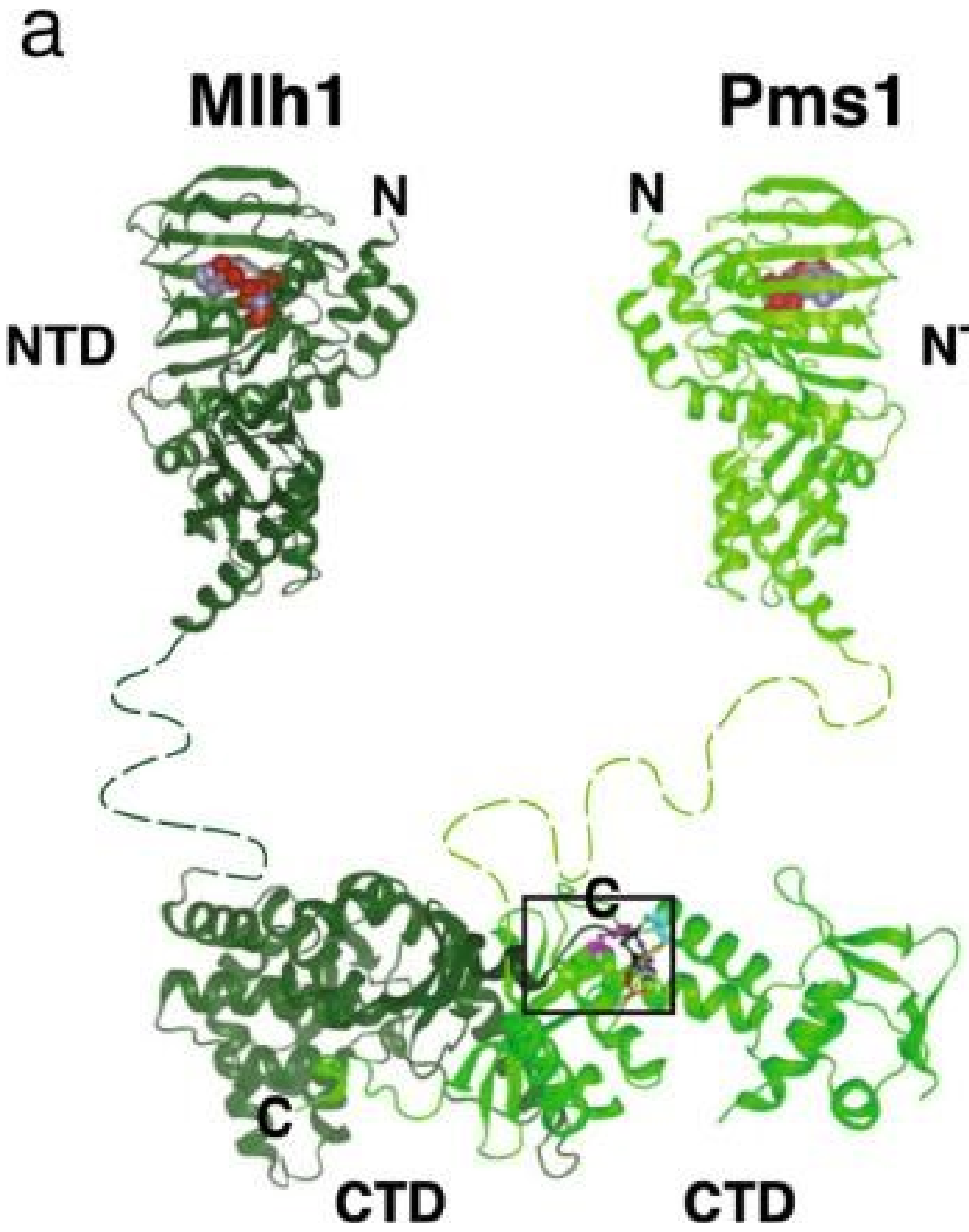
MutL proteins



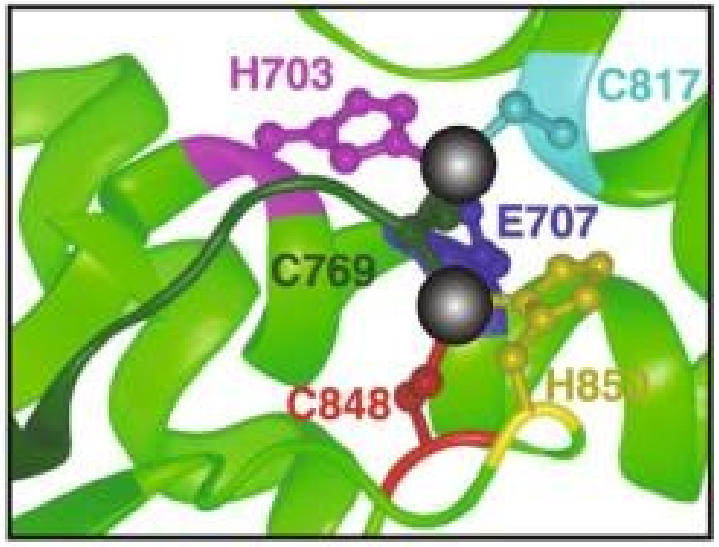
Yeast MutLα

Human MutLα



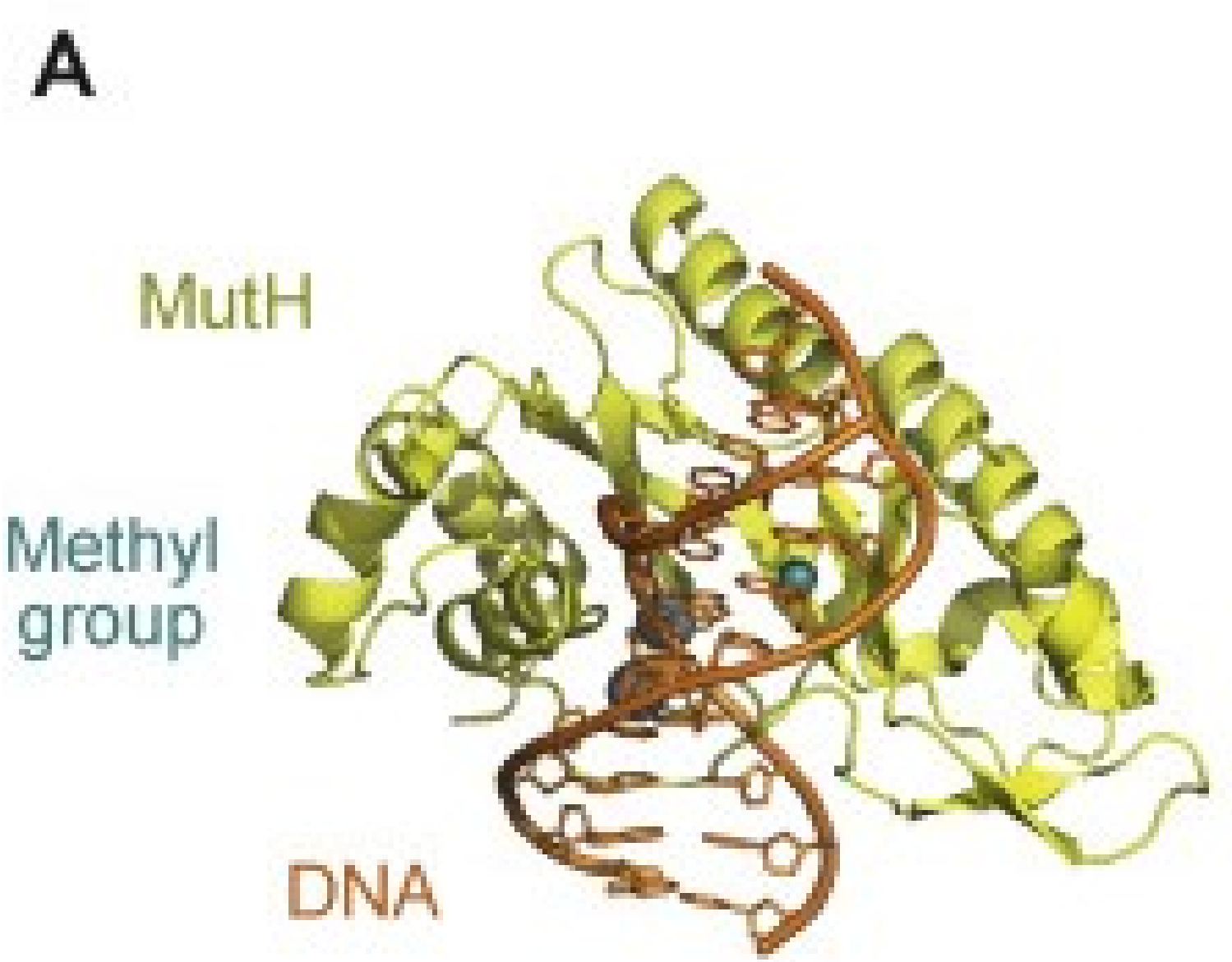


Negli eucarioti gli omologhi
di MutL hanno attività di taglio

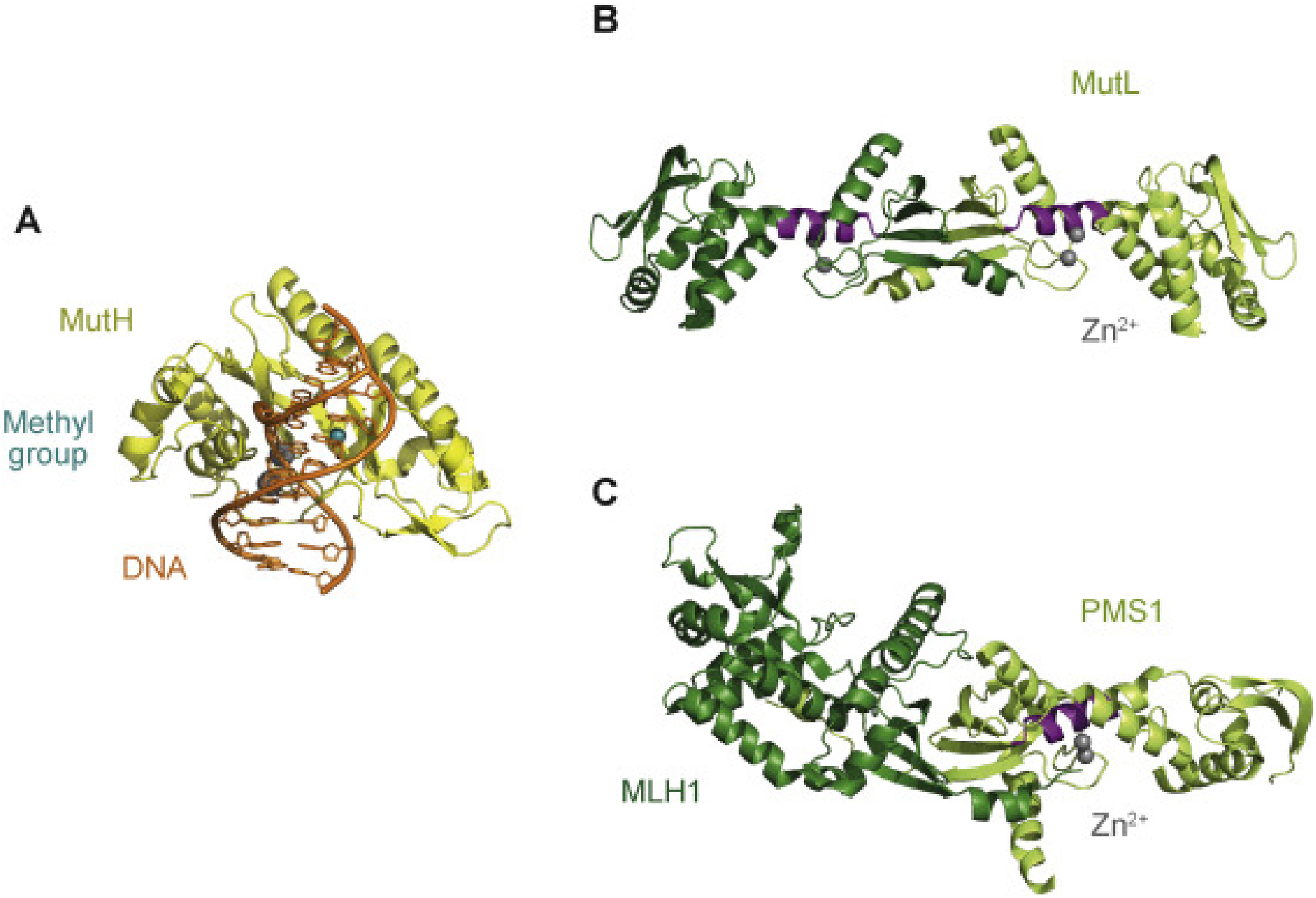


endonuclease site

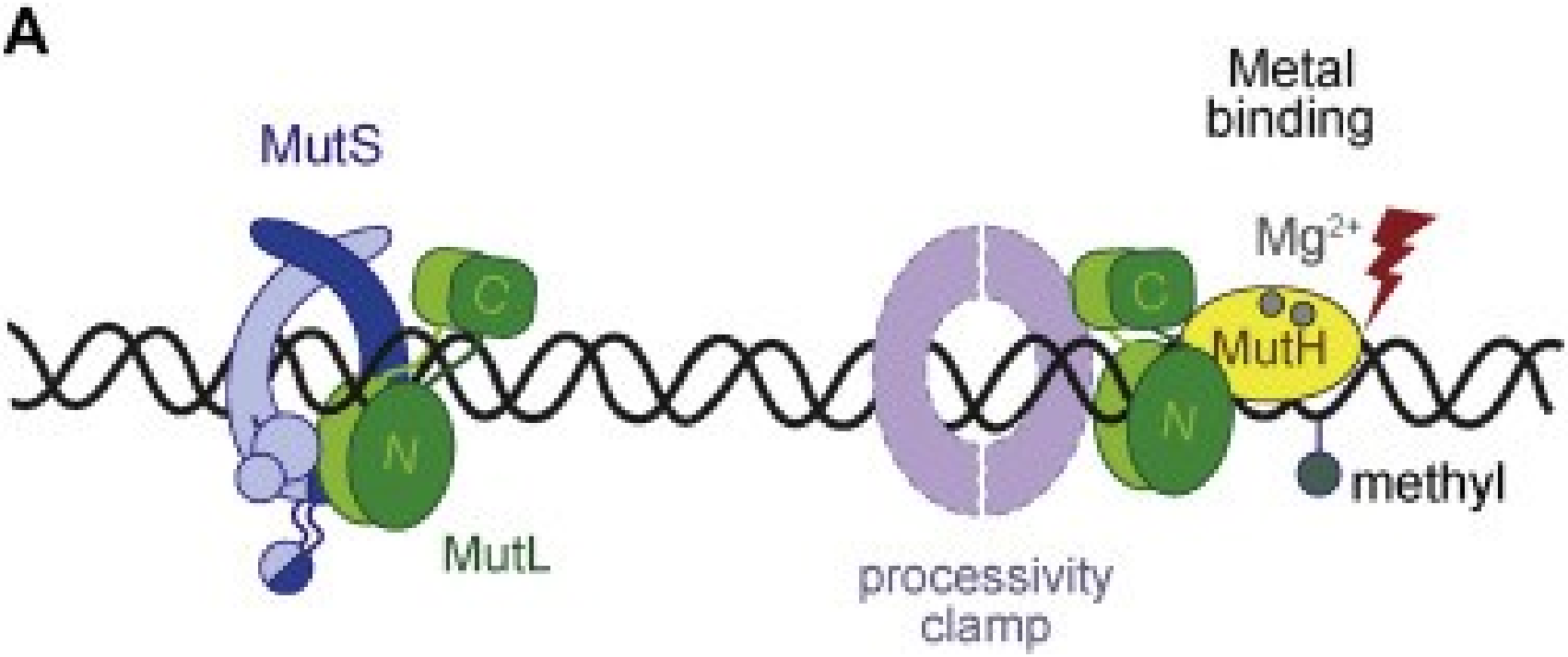
endonucleases in MMR



endonucleases in MMR



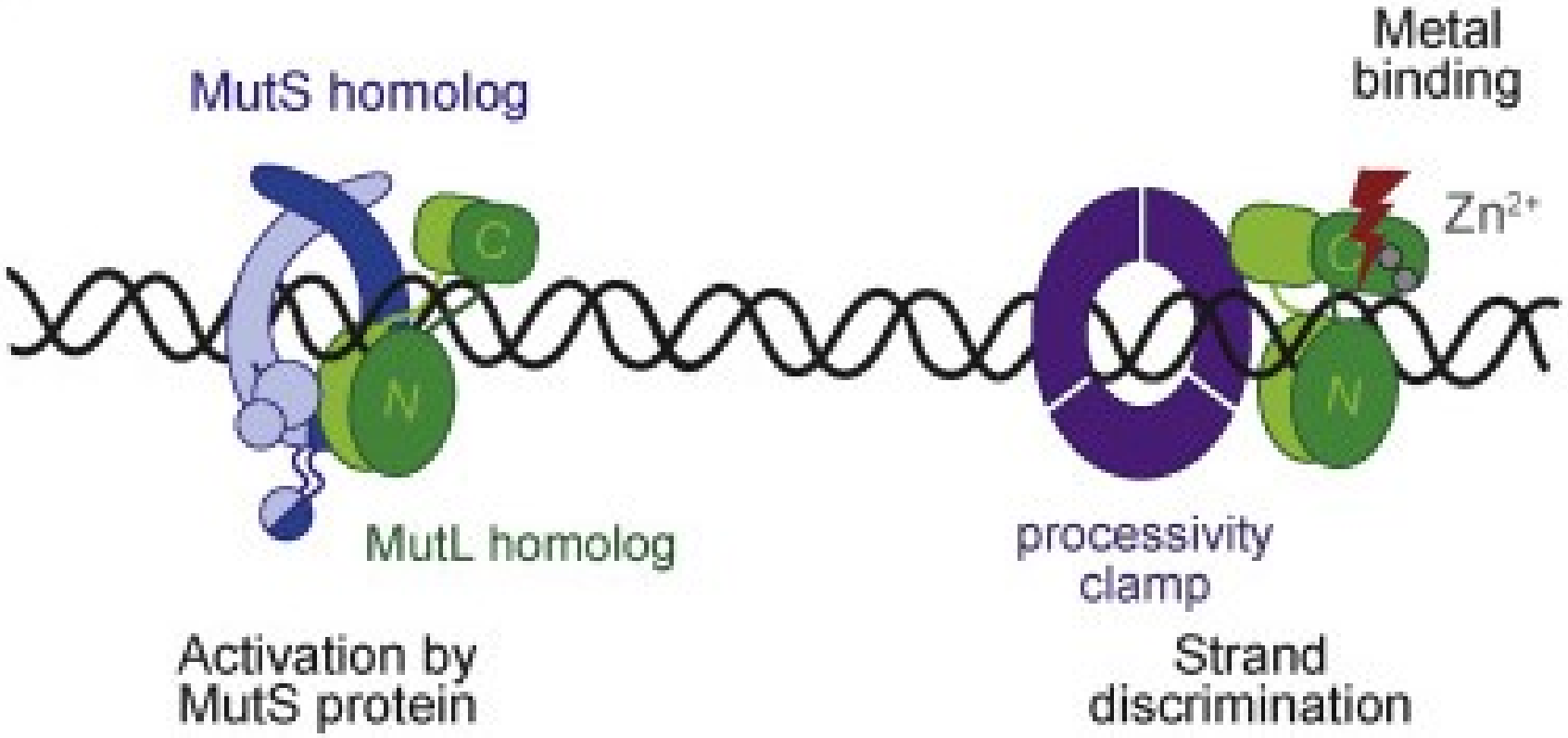
Procarioti



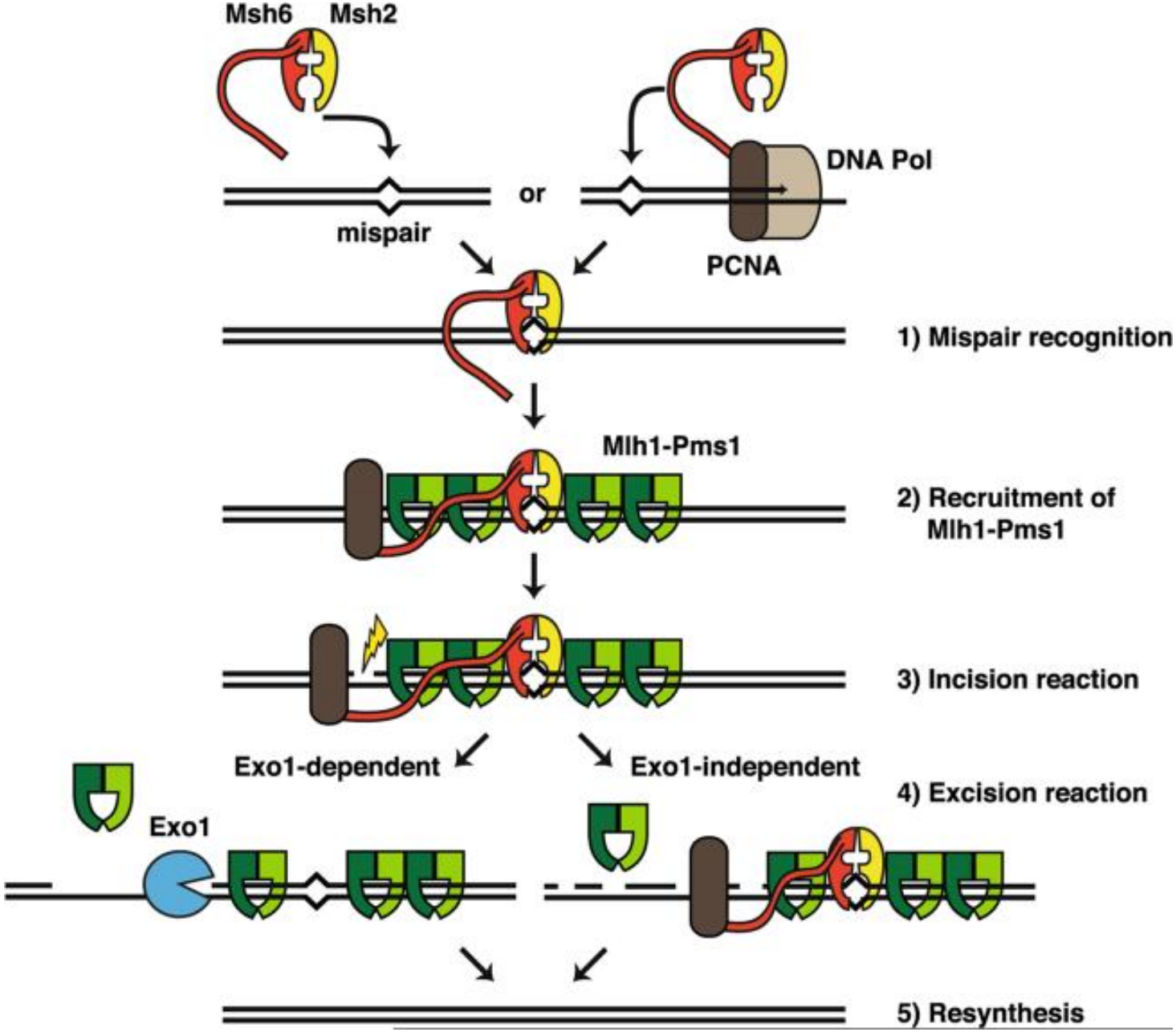
activation of endonuclease
activities in MMR

Eucarioti

activation of endonuclease activities in MMR



alternative excision pathways during MMR

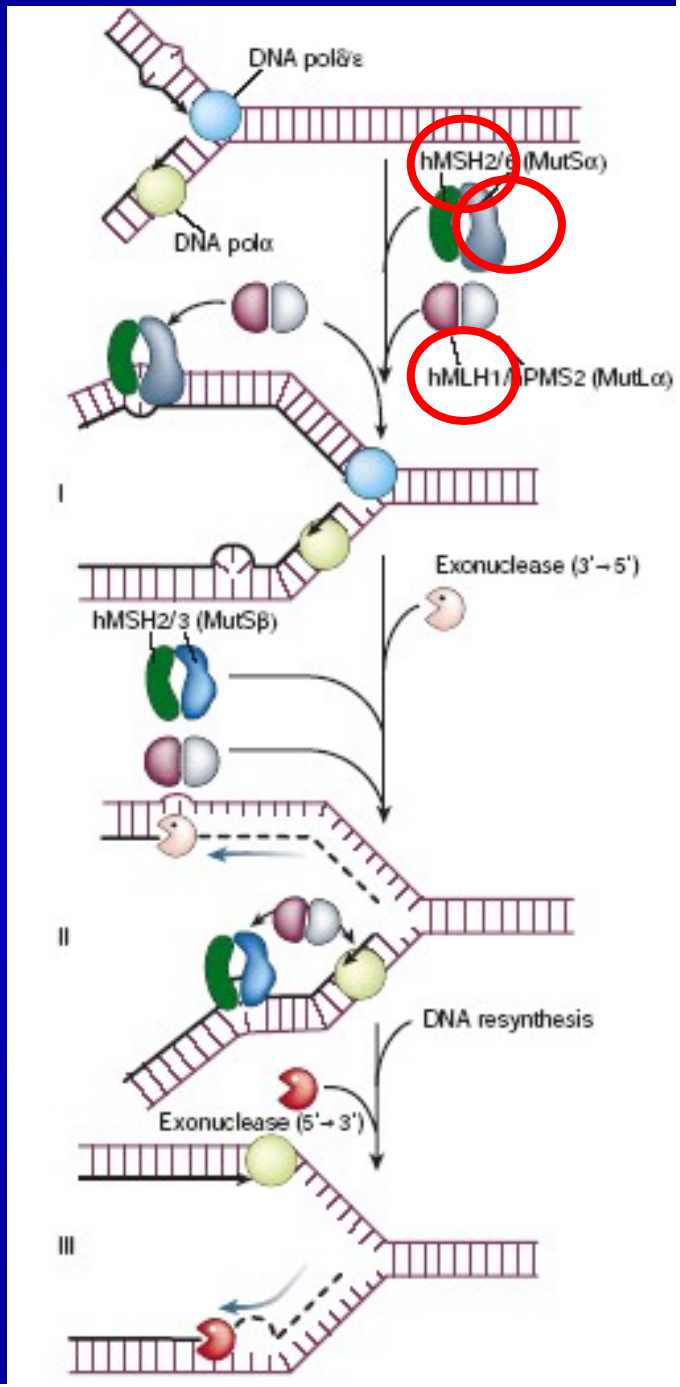


MSH2 forma un eterodimero con **MSH6** (misappaiamento) o **MSH3** (loop di inserzione-delezione) e si lega al DNA segnalando l'elica templato

L'eterodimero **MLH1-PMS2**, talvolta legato anche a **PMS1**, 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

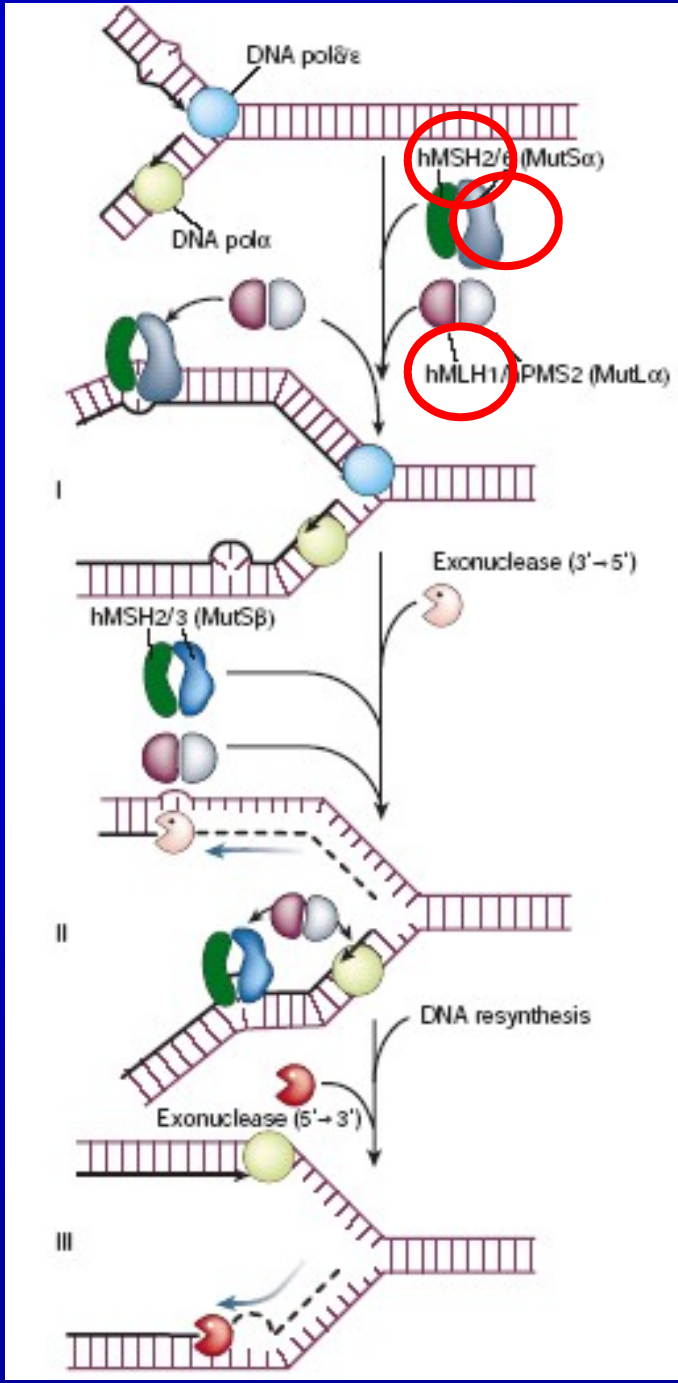


HNPCC
cancro colon-rettale ereditario non poliposico

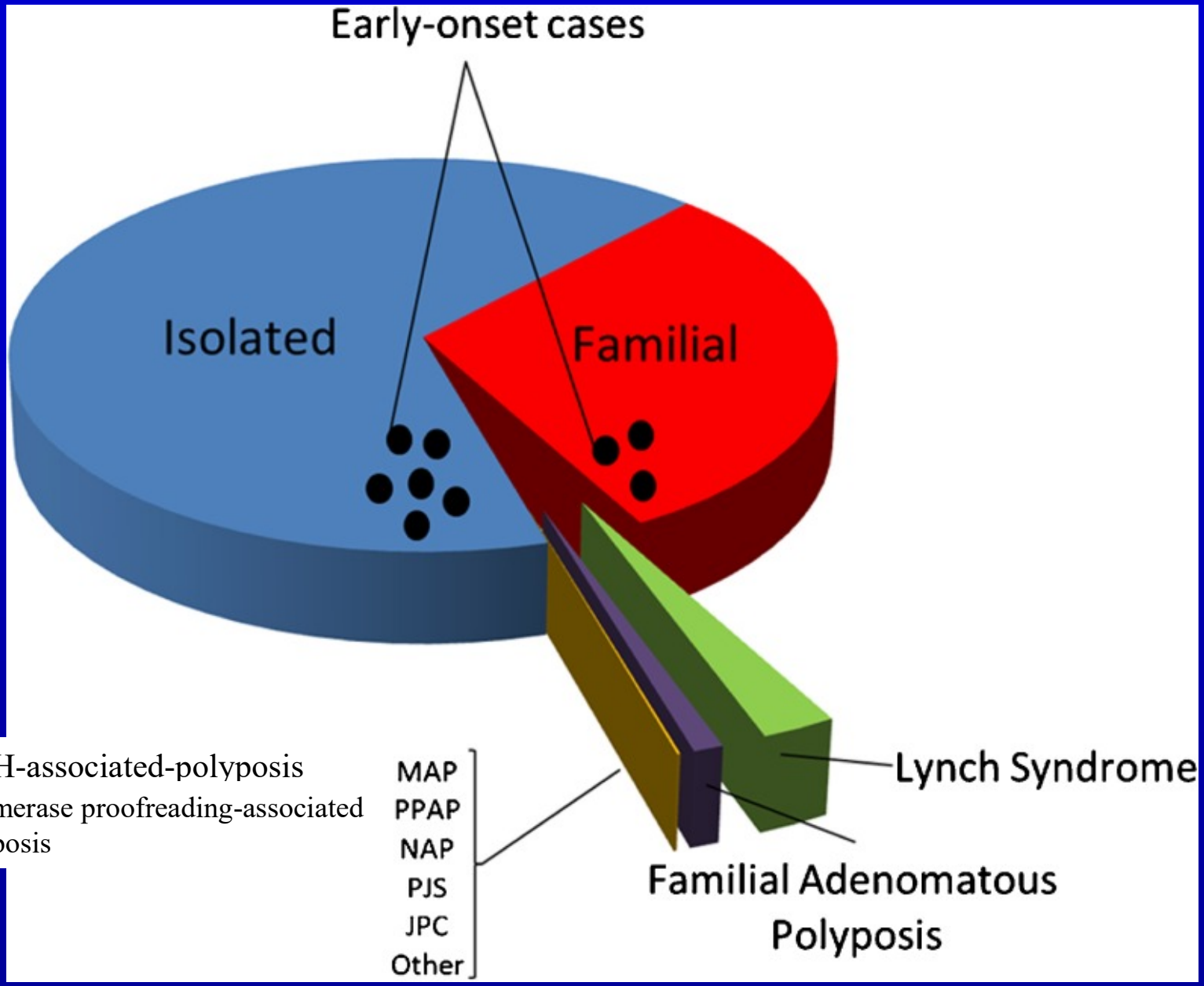
hMLH1: 50% delle mutazioni

hMSH2: 35%

hMSH6: 10%



Colorectal cancers



MUTYH-associated-polyposis

polymerase proofreading-associated polyposis

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

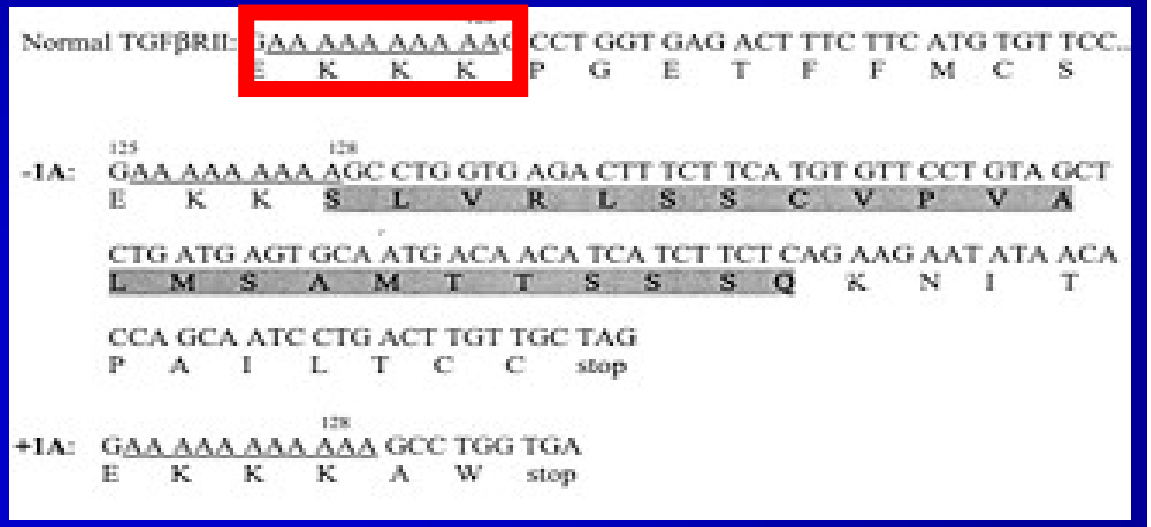
hMSH2: 35%

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



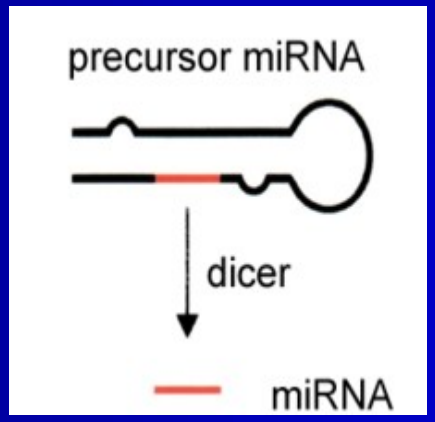
Pazienti con alterazioni del MMR: l'errore permane → recettore per TGFβ non funzionale

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

Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6

Small RNAs

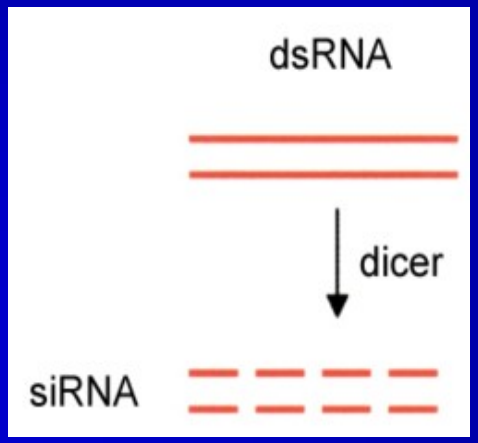
miRNA



Prodotti in modo endogeno

Funzione: regolazione dell'espressione genica sopprimendo la traduzione o la trascrizione di geni target

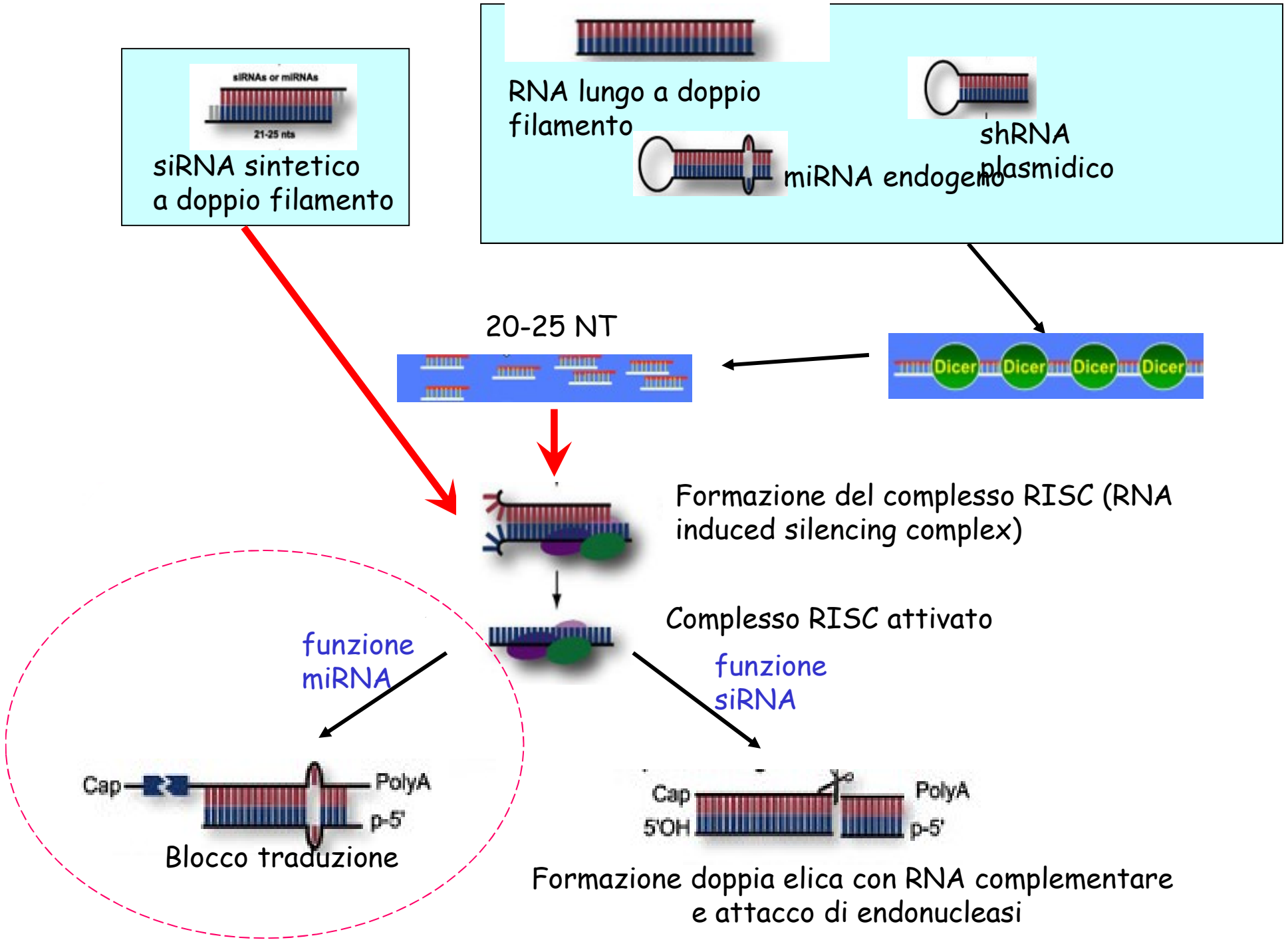
siRNA



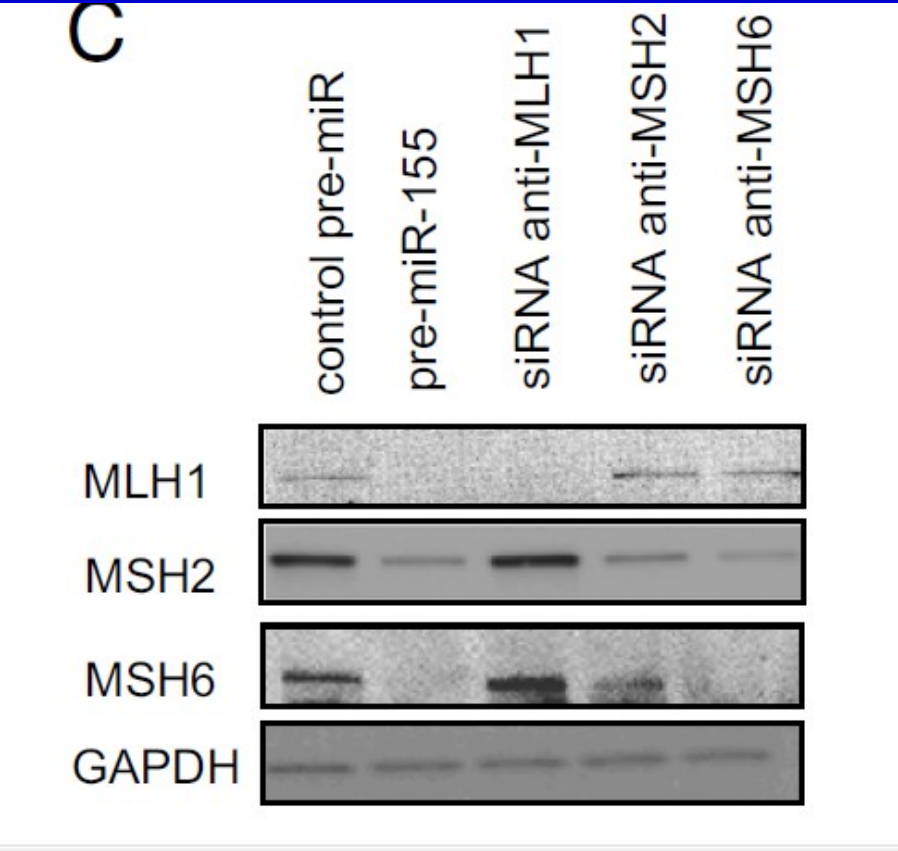
Exo-siRNA:Introdotti in modo esogeno (virus a dsRNA, transposoni, transgeni)

Endo-siRNA: derivati da loci genomici endogeni

Funzione principale: rispondere alle minacce esterne sopprimendo la trascrizione genica dell'"invasore"

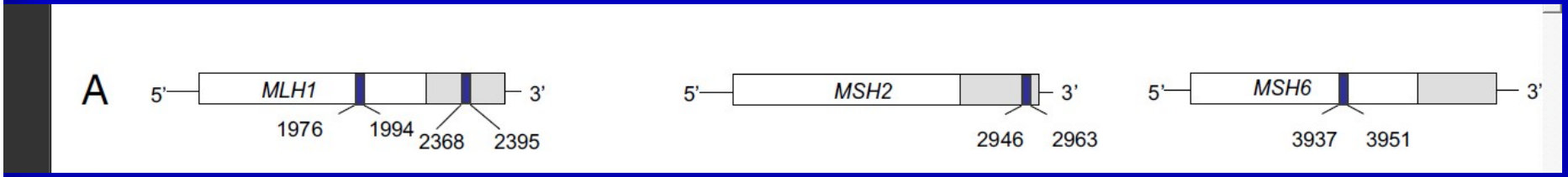

siRNAs or miRNAs
21-25 nts
siRNA sintetico
a doppio filamento
RNA lungo a doppio
filamento
shRNA
plasmidico
miRNA endogeno
20-25 NT
Dicer
Dicer
Dicer
Dicer
Formazione del complesso RISC (RNA
induced silencing complex)
Complesso RISC attivato
funzione
miRNA
funzione
siRNA
Cap
PolyA
p-5'
Blocco traduzione
Cap
5'OH
PolyA
p-5'
Formazione doppia elica con RNA complementare
e attacco di endonucleasi

Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in CRC cells



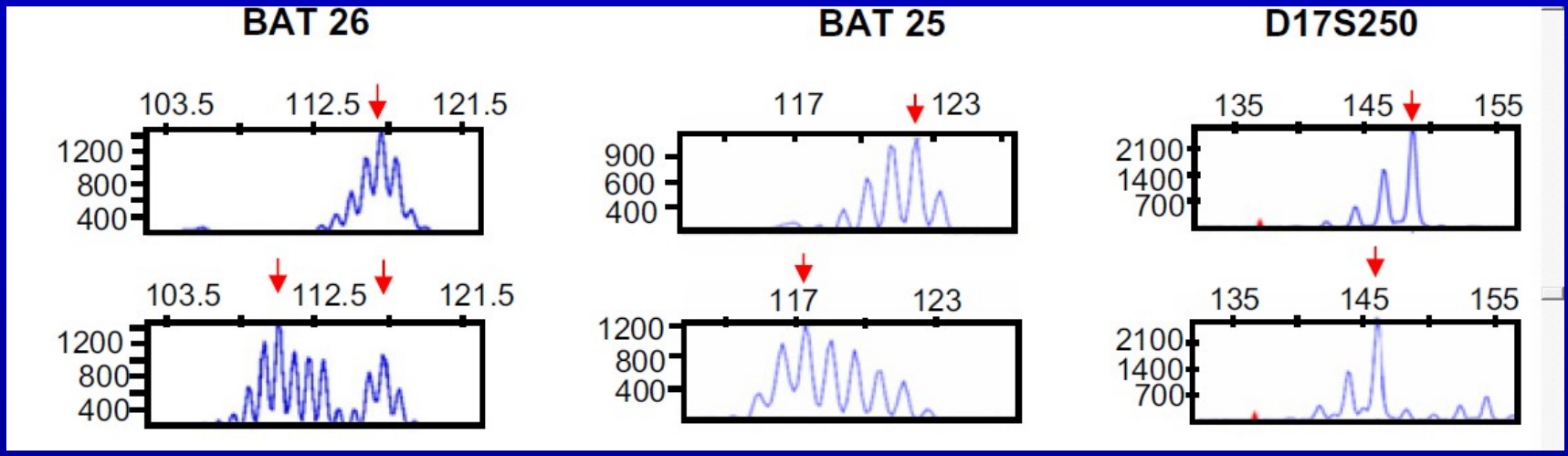
Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in ColoRectal Cancer cells

- hMLH1, hMSH2, and hMSH6 are direct targets of miR-155. (A) Locations of the target sites of miR-155 in the 3′ UTRs and/or the CDS of the indicated genes

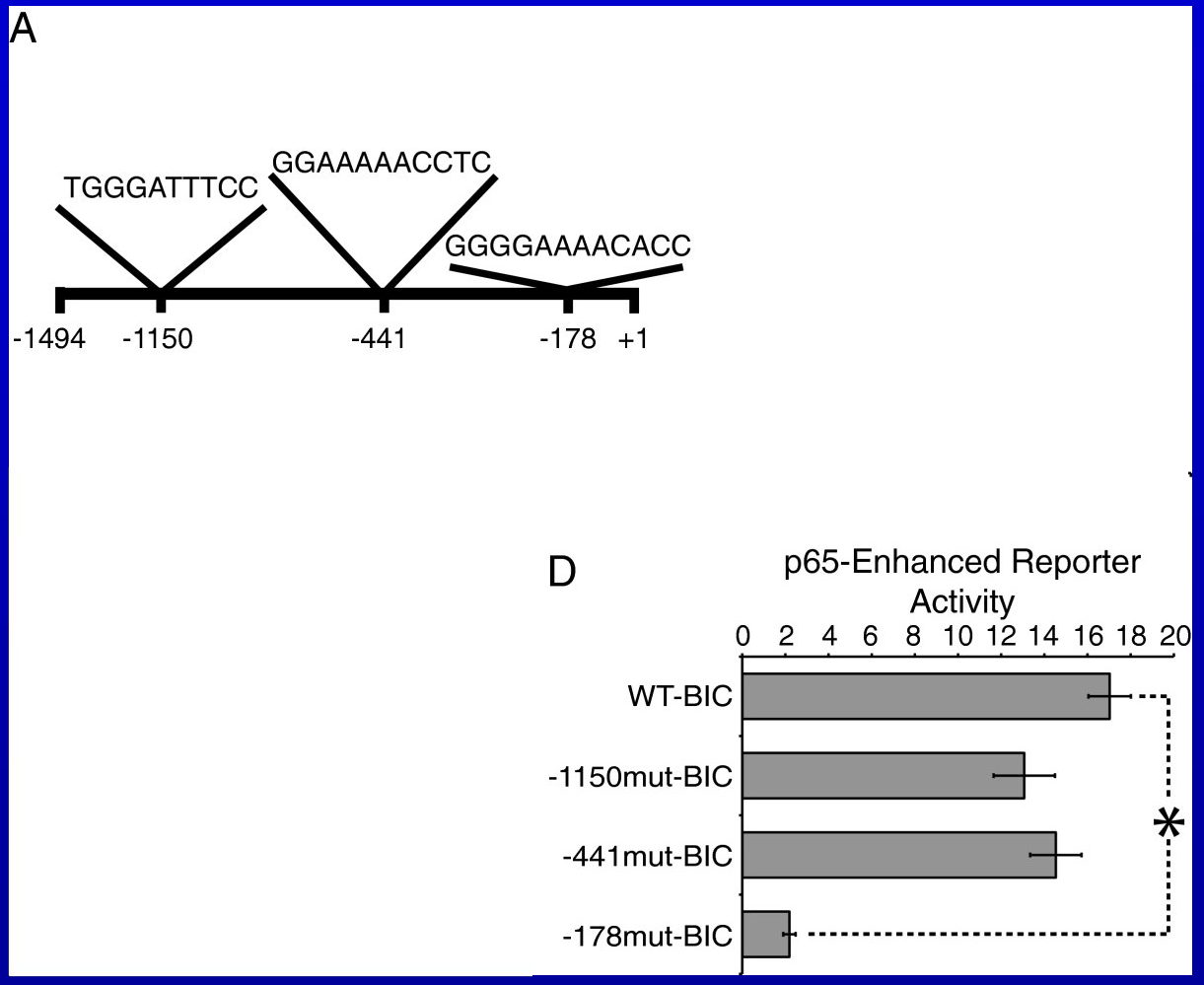


Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in CRC cells

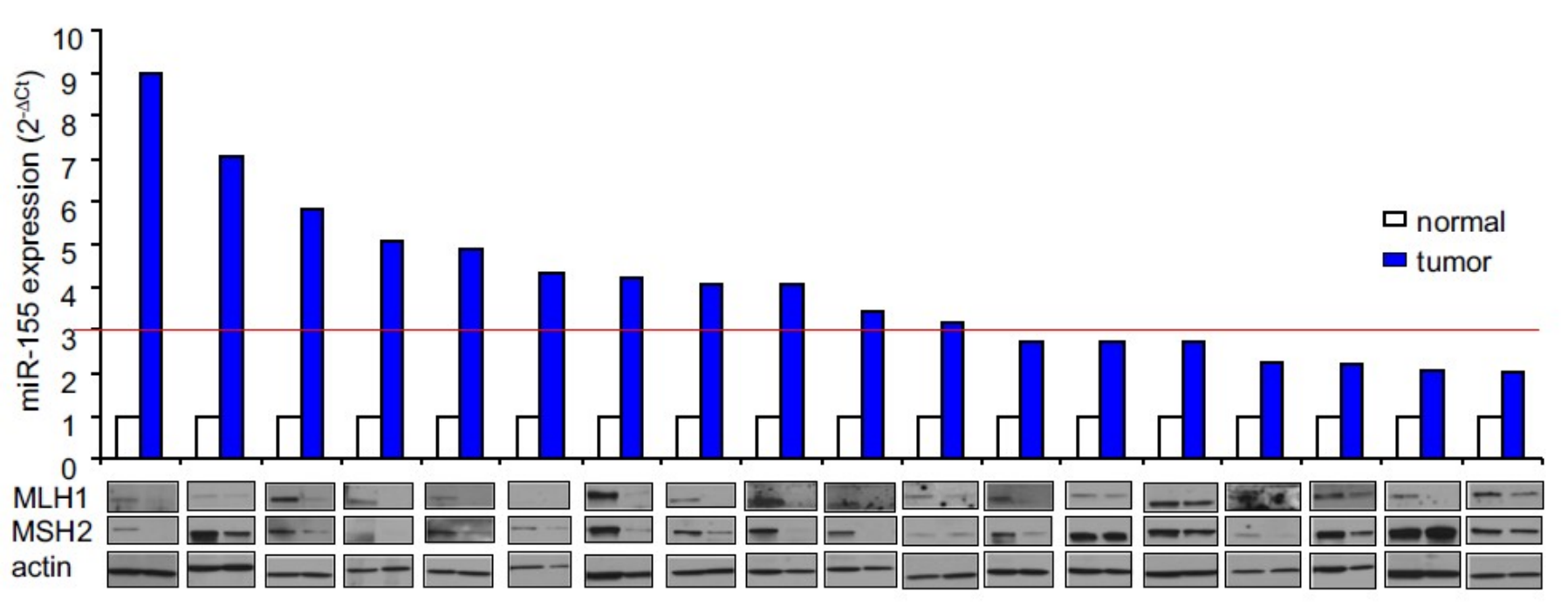
- Microsatellite analysis of Colo 155 (+) overexpression of miR-155) and (-) cells
- BAT-26 and BAT 25 (mononucleotide repeats)
- D17S250 (dinucleotide repeat)



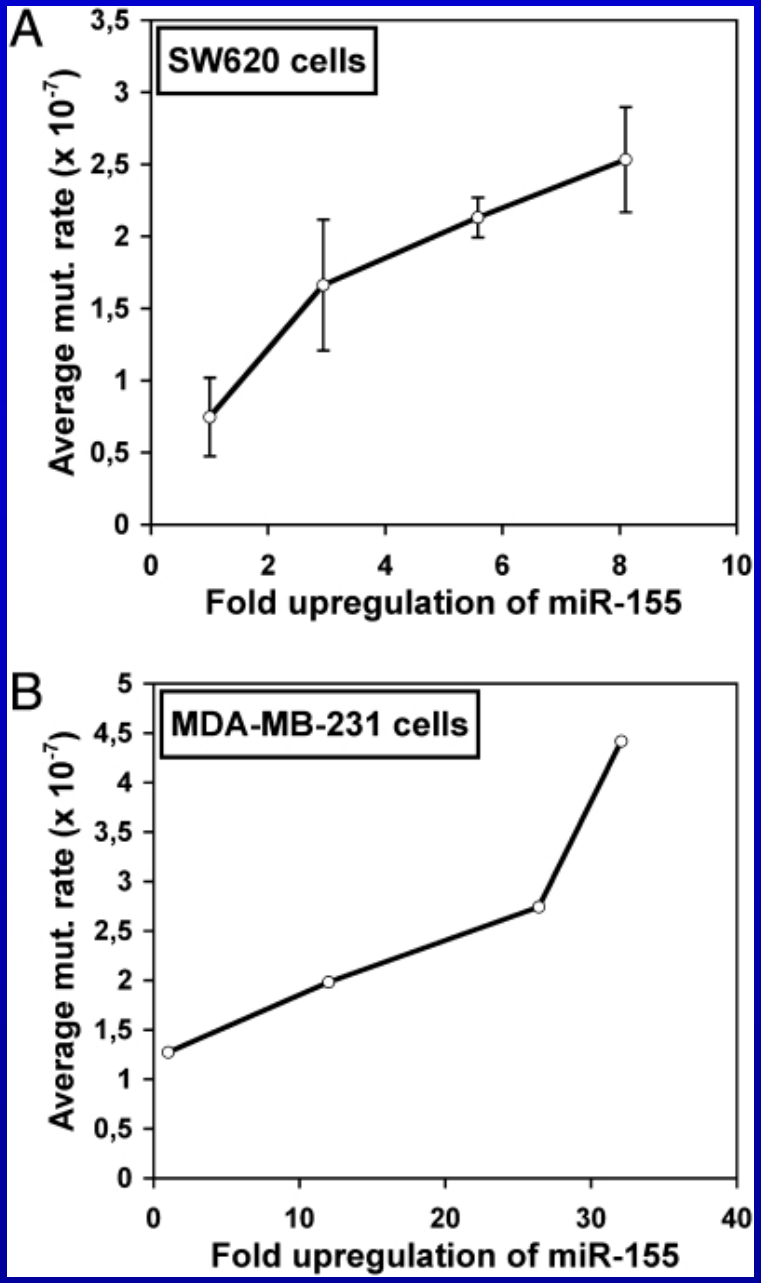
NF-κB p65 up-regulates expression from the MIR155HG promoter through an NF-κB binding site located upstream of the transcription start site BMC Molecular Biology 2013 14:24



miR-155 expression is inversely related to MLH1 and MSH2 in CRC tissues



PNAS 2011

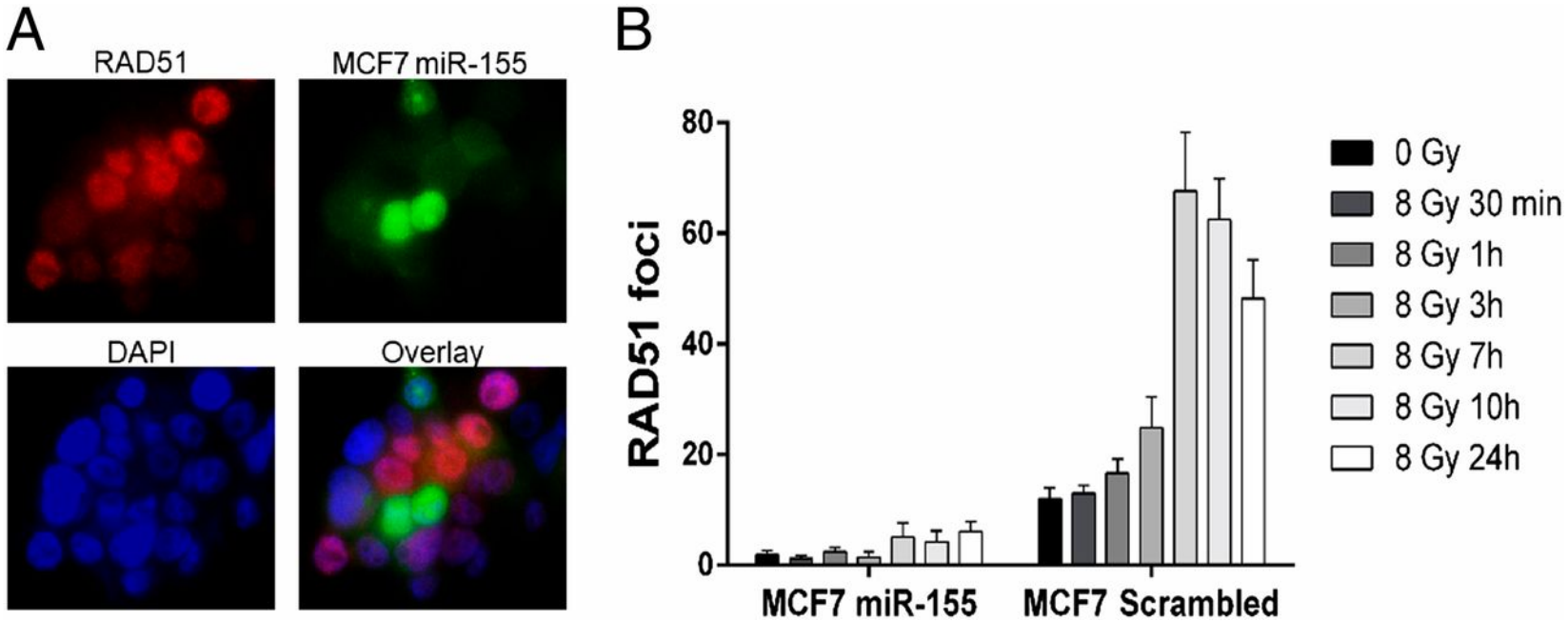


colorectal adenocarcinoma cells

breast adenocarcinoma cells

miR-155 under the control of an inducible system

miR-155 inhibits gamma-rays-induced RAD51 foci formation.



miR-155–overexpressing MCF7 cells

Gasparini P et al. PNAS 2014;111:4536-4541