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

"for mechanistic studies of DNA repair"

- 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.

Fatto!

- 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

- Modrich transformed the field of mismatch repair from genetic observations to a detailed biochemical understanding, first in bacteria, and later in eukaryotic cells.

# 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

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

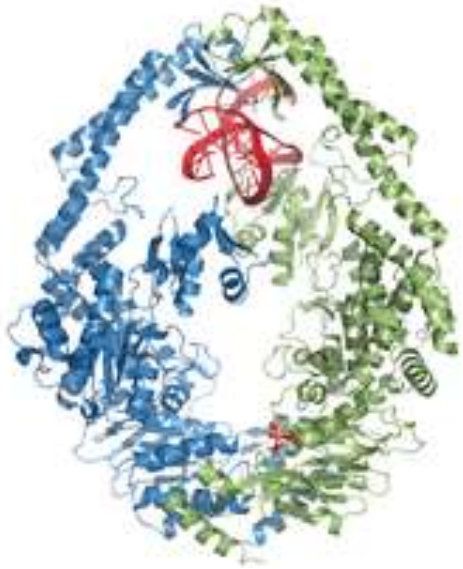
A) MutS binds mismatched base pairs

*Apparent affinities of mutS protein for base pair mismatches*

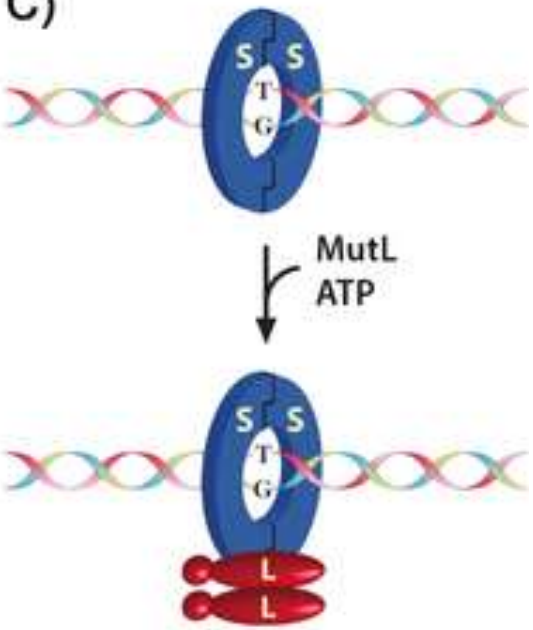
| Mismatch | Apparent dissociation constant |
|---|---|
| | *nM* |
| G-T | 39 ± 4 |
| A-C | 53 ± 4 |
| A-A | 110 ± 7 |
| T-T | 140 ± 9 |
| G-G | 150 ± 10 |
| A-G | 270 ± 30 |
| C-T | 370 ± 40 |
| C-C | 480 ± 50 |

AFFINITA'

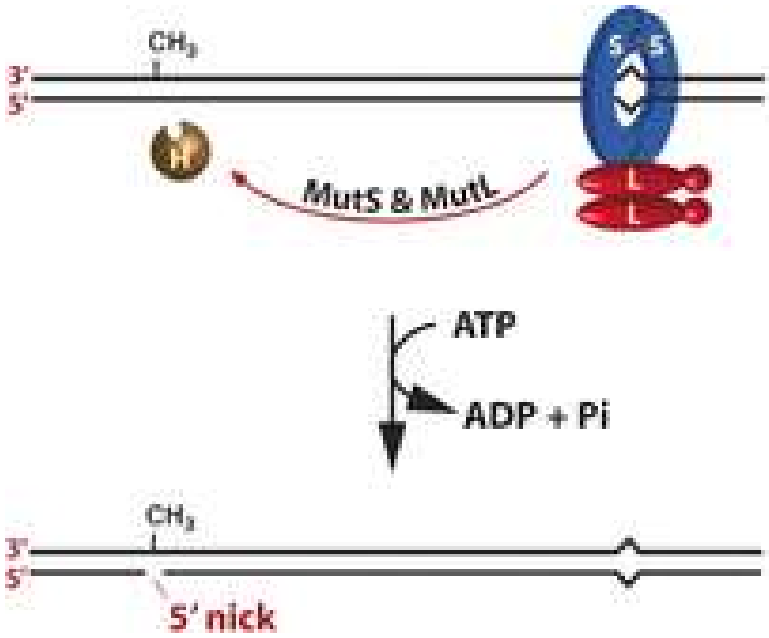
B)

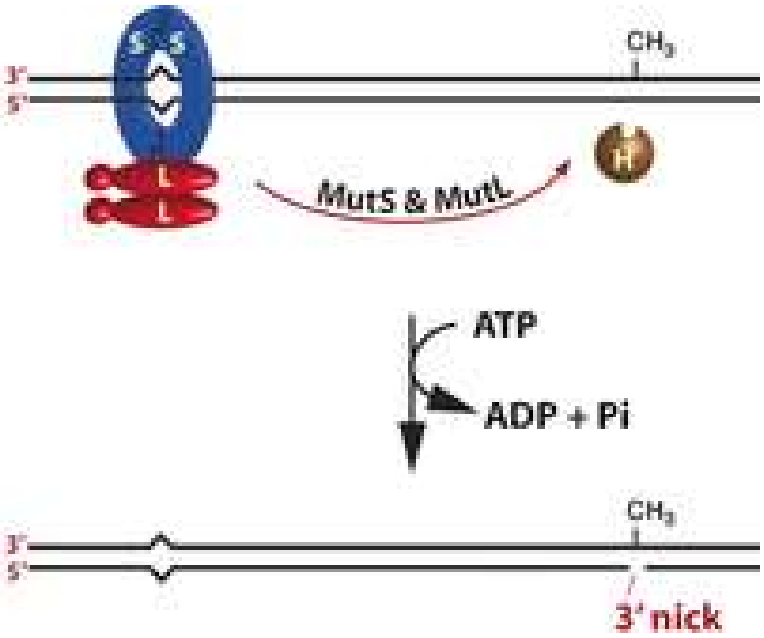


C)



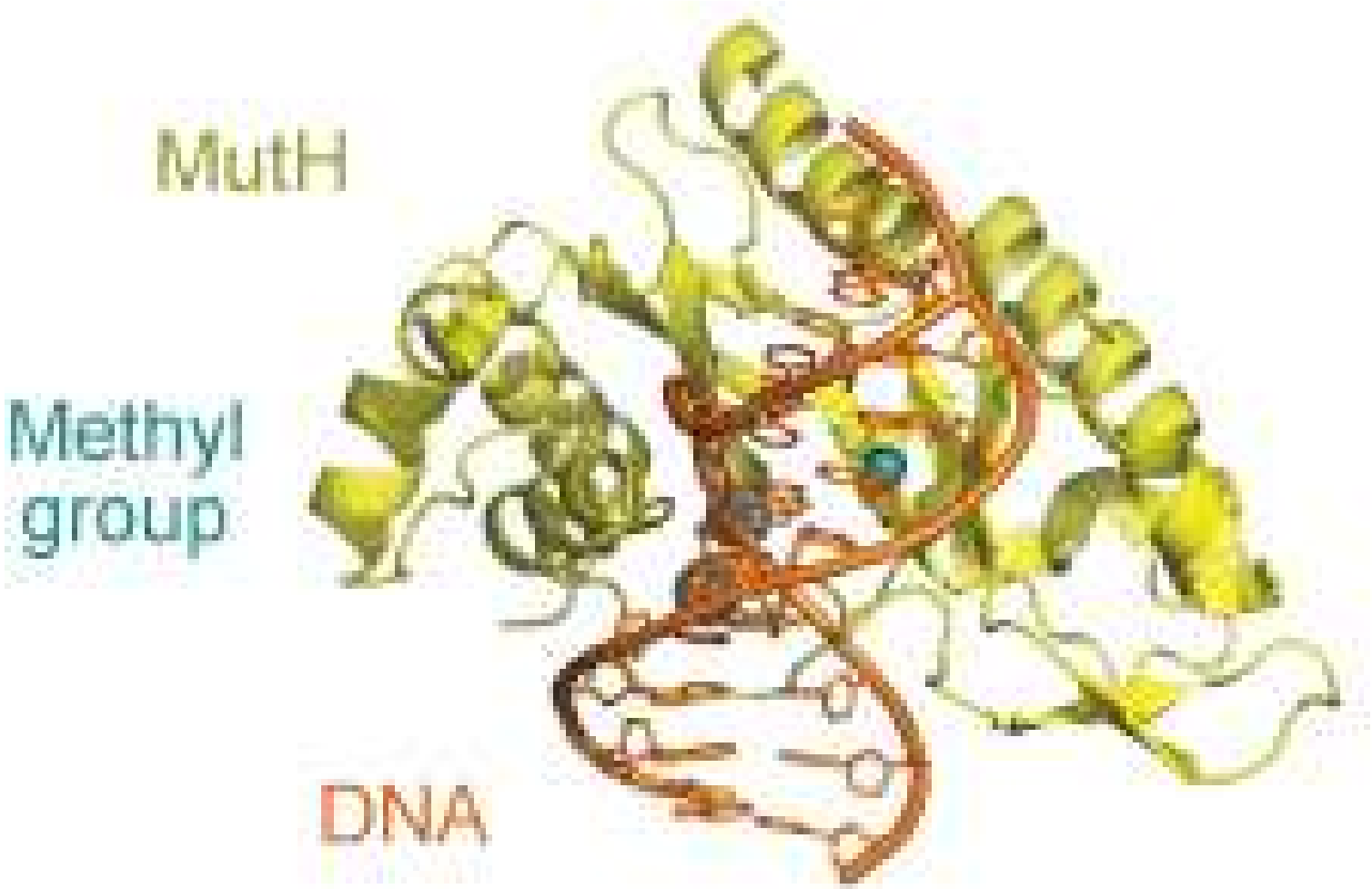
# Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)



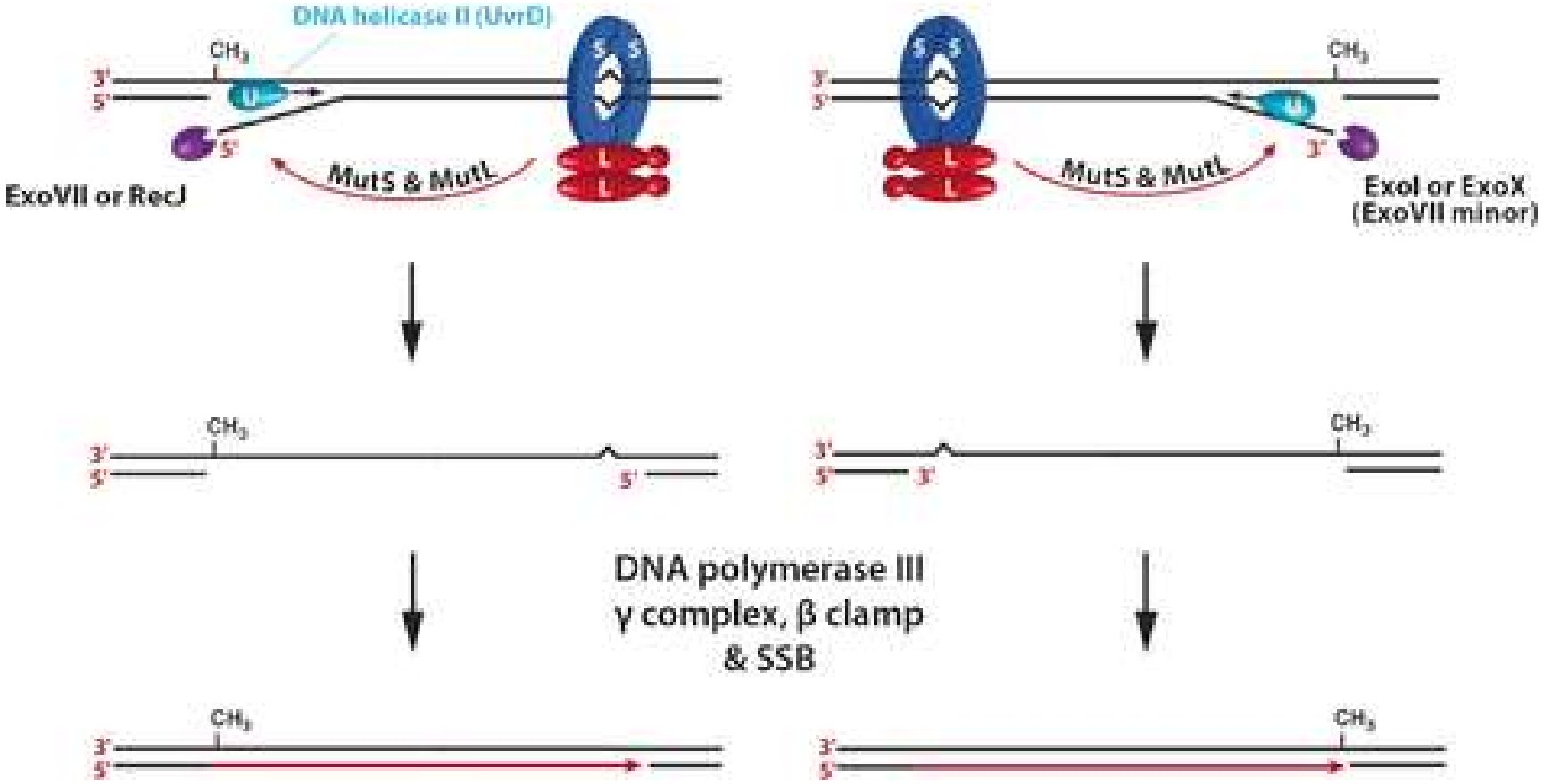




**Angewandte Chemie International Edition**
Volume 55, Issue 30, pages 8490-8501, 20 MAY 2016 DOI: 10.1002/anie.201601412
http://onlinelibrary.wiley.com/doi/10.1002/anie.201601412/full#anie201601412-fig-0006

A MutH protein (yellow) bound to hemimethylated DNA (methyl group shown as sphere)

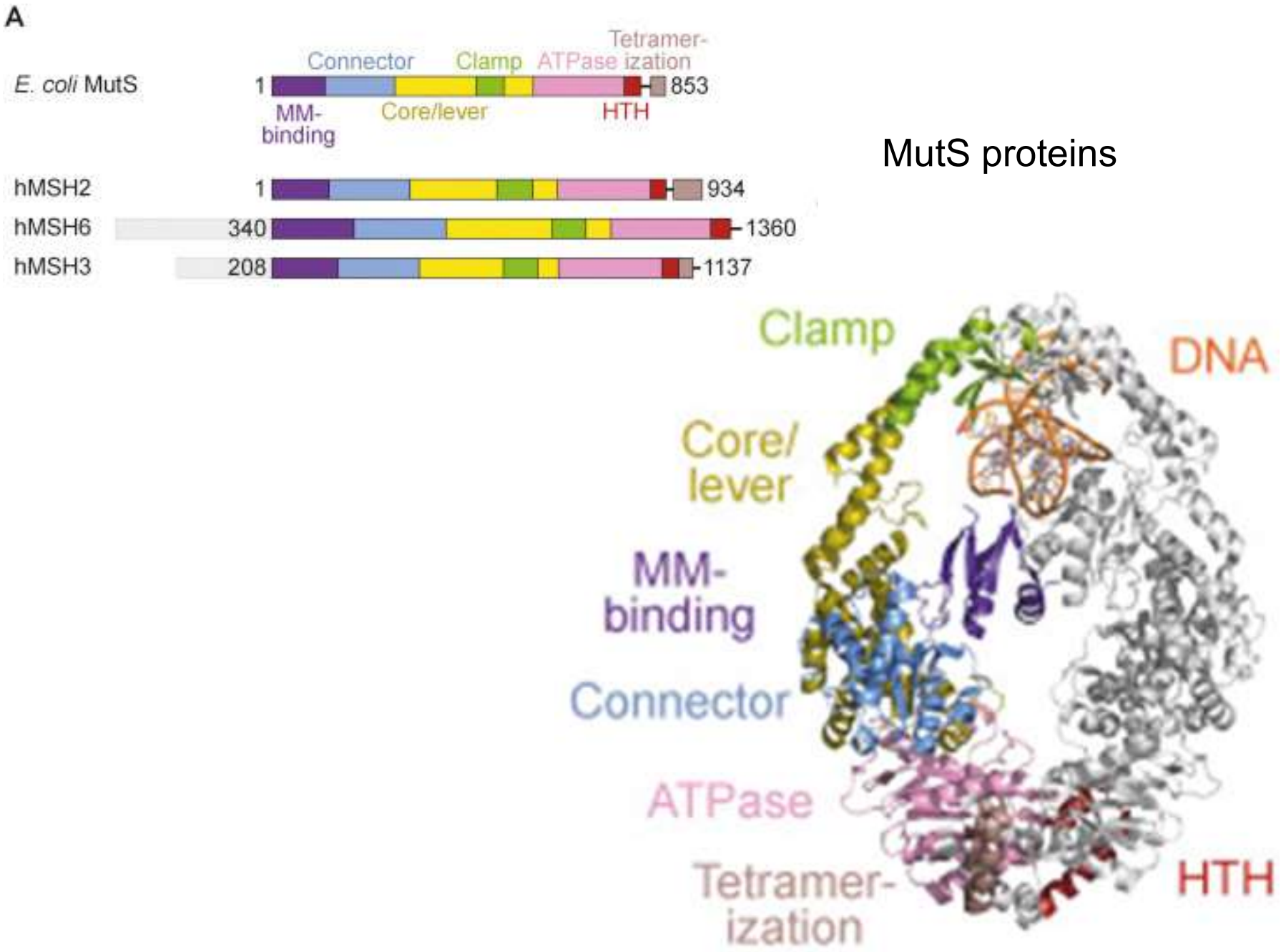


## Mechanisms in *E. coli* and Human Mismatch Repair (Nobel Lecture)




**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

# MutS proteins




Flora S. Groothuizen, Titia K. Sixma DNA Repair, Volum

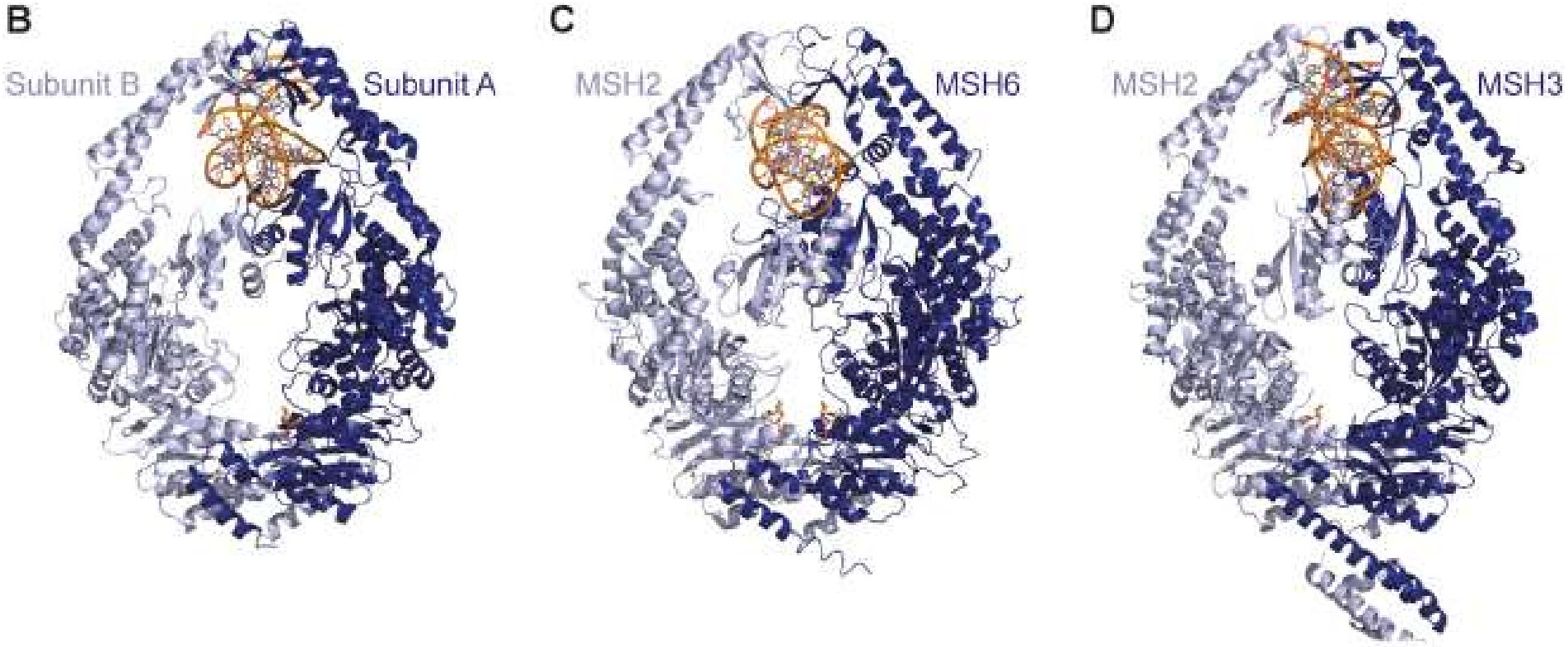
**The conserved molecular machinery in DNA mismatcl**

# MutS proteins

E.Coli MutS bound to a GT mismatch

human MutSα

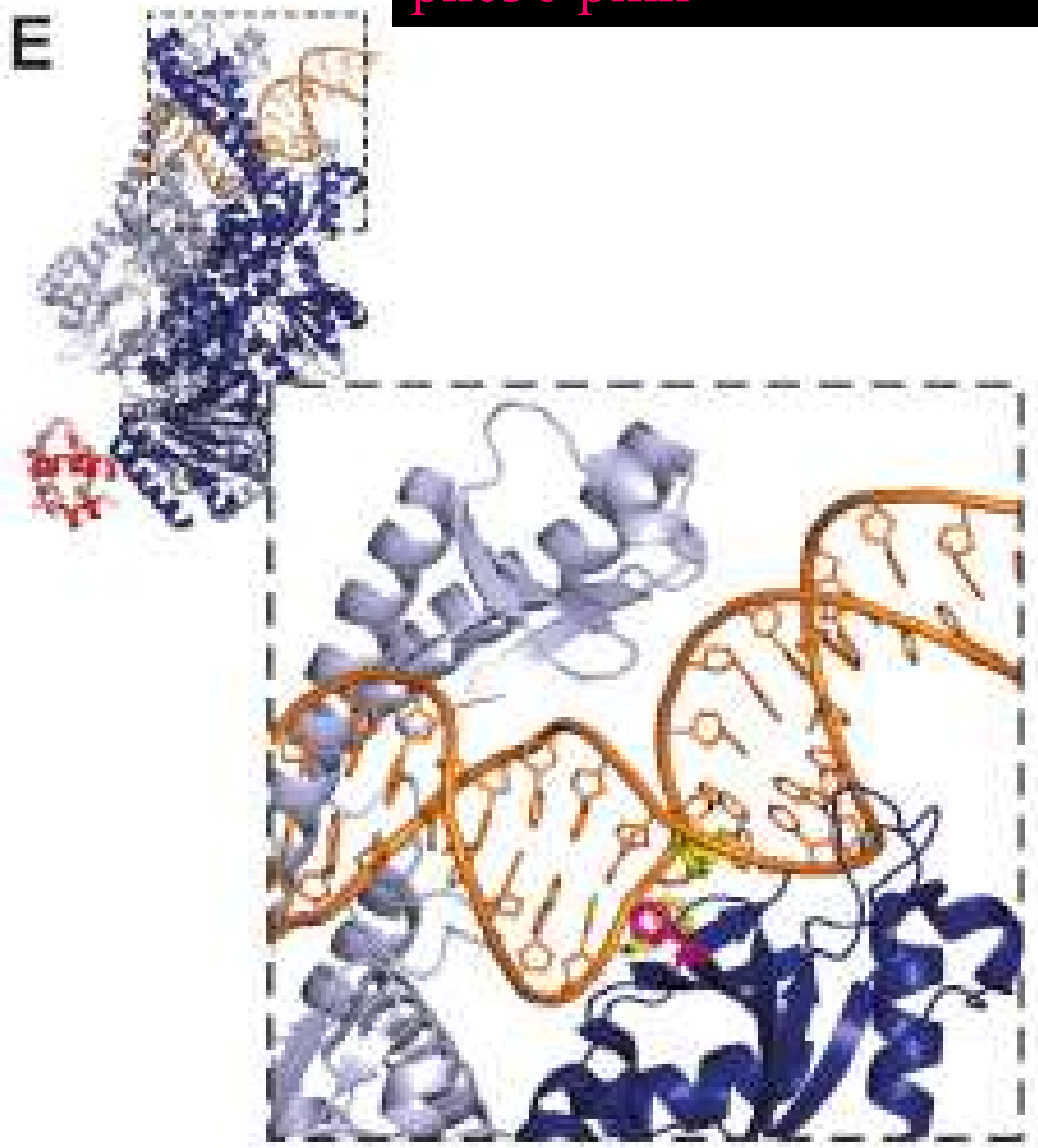
MutSβ bound to a 3-base del




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

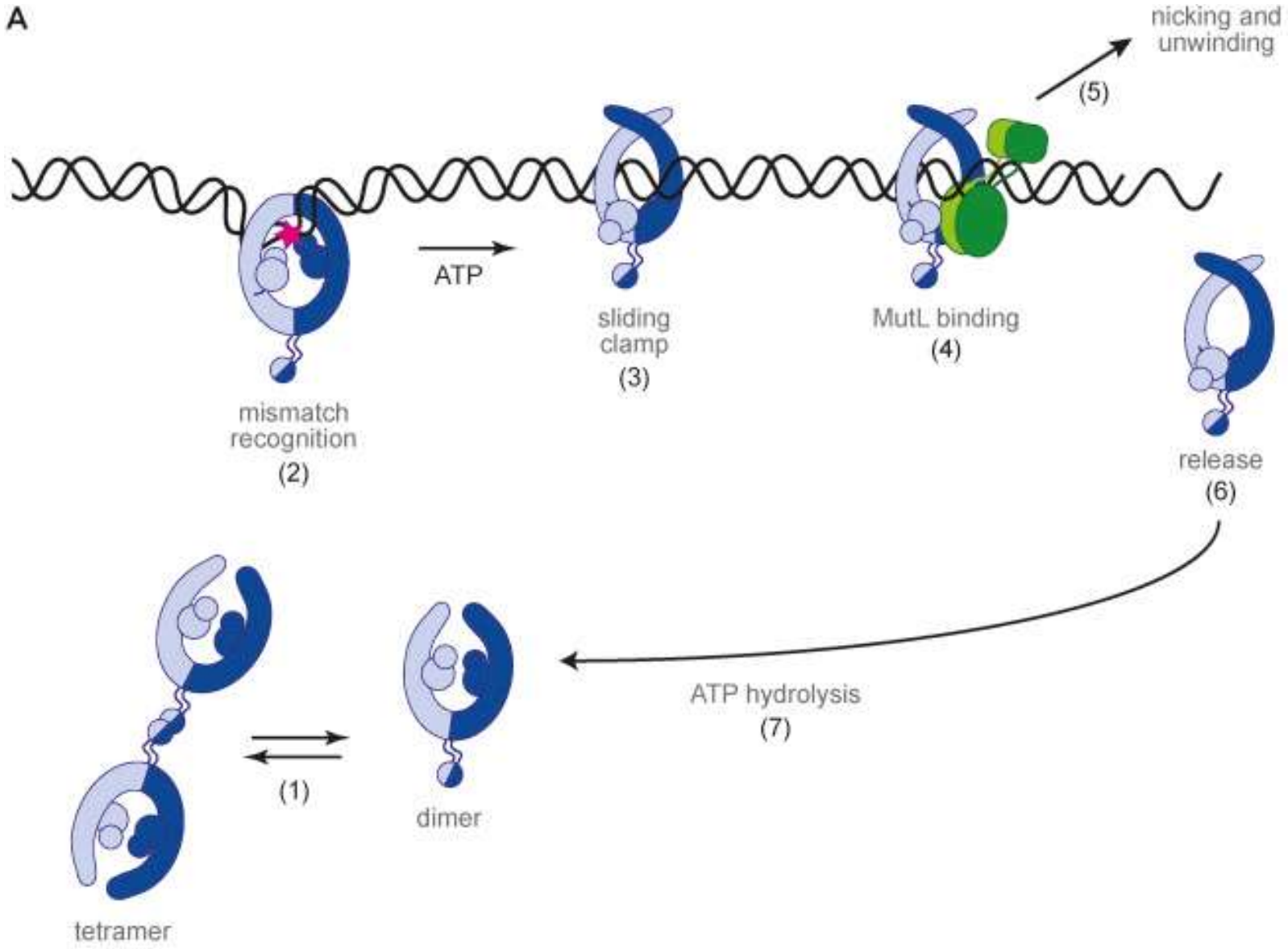
**The conserved molecular machinery in DNA mismatch repair enzyme structures**

Mismatch yellow;
phe36 pink

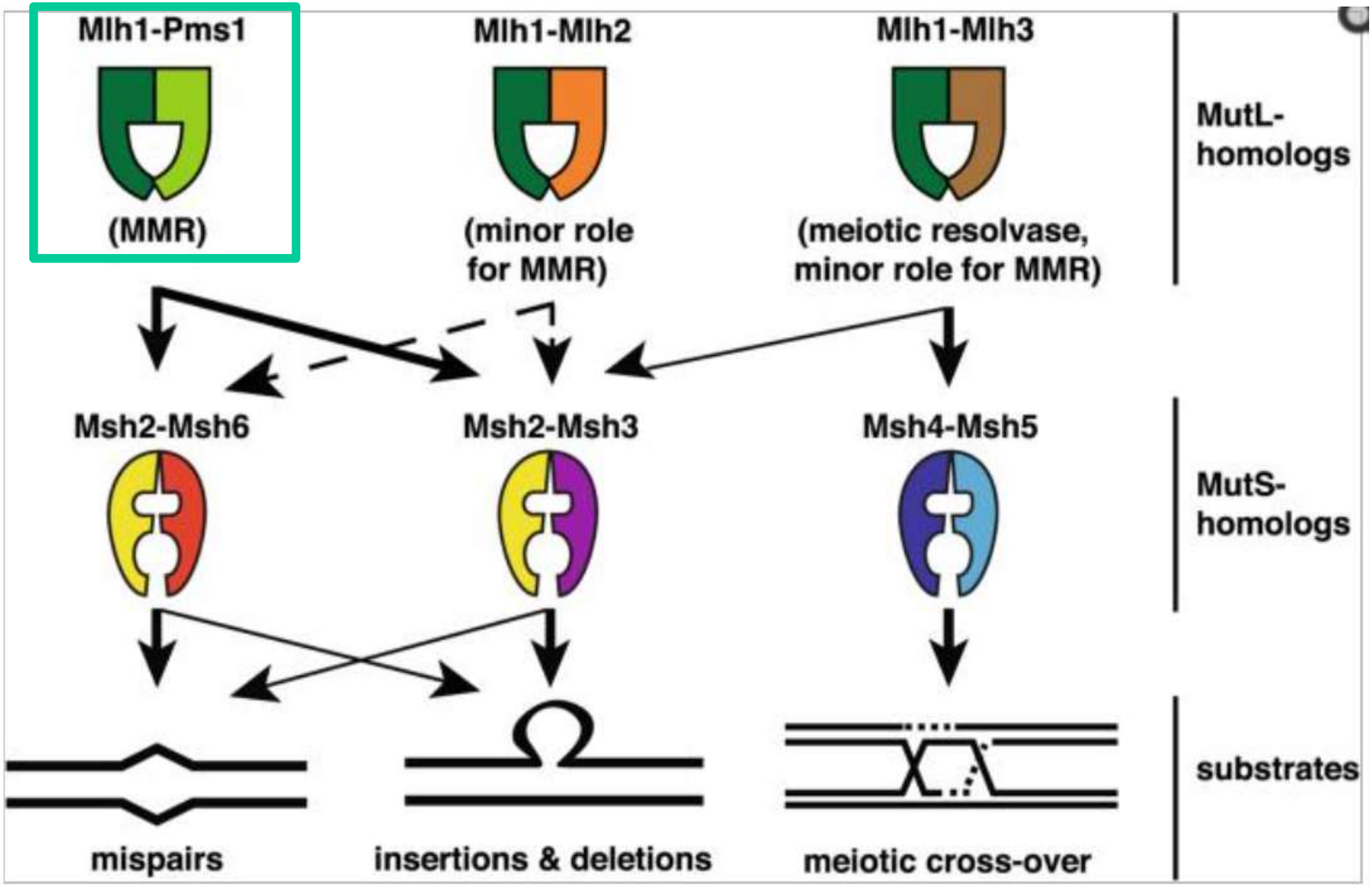


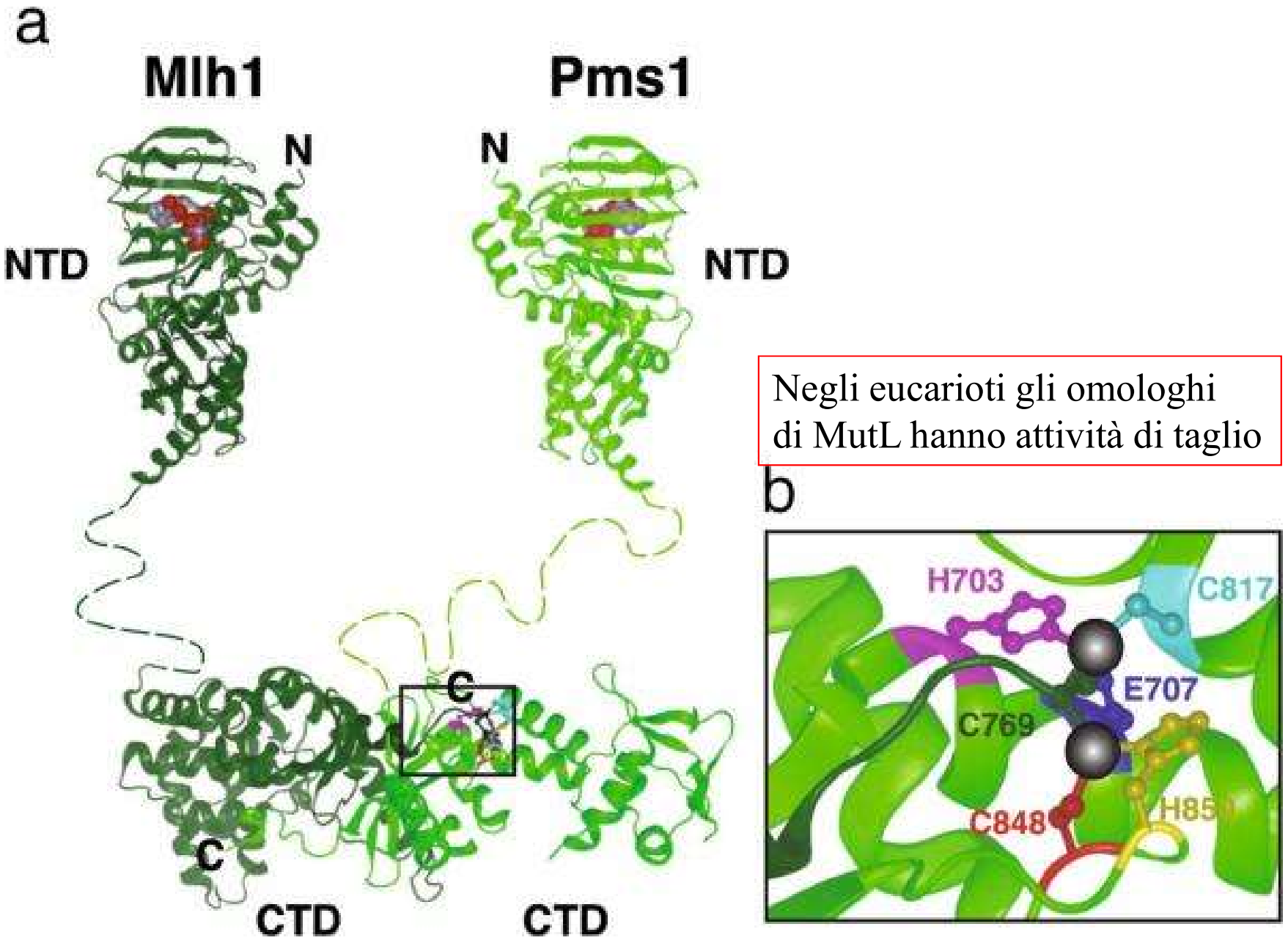

**The conserved molecular machinery in DNA mismatch repair enzyme structures**

predominant states of the MutS cycle



# Omologia di MutS/MutL negli eucarioti



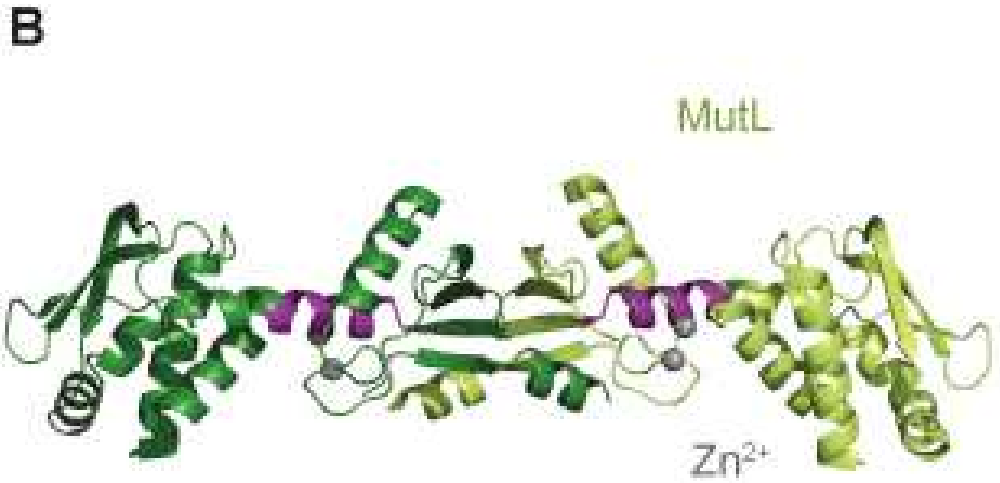



Negli eucarioti gli omologhi di MutL hanno attività di taglio

endonuclease site

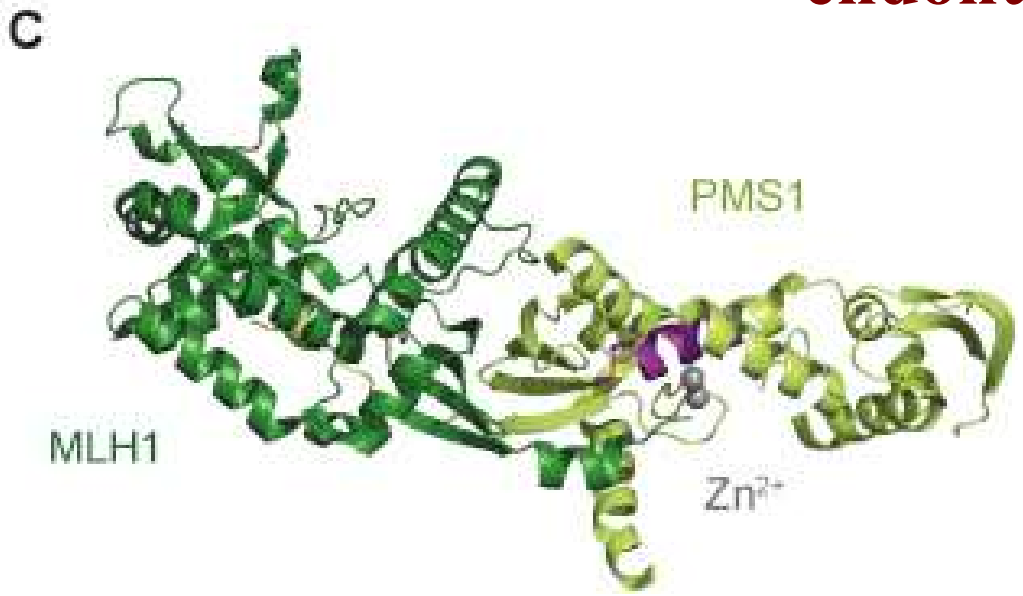
metal binding pocket (metal ions in black)

endonucleases in MMR



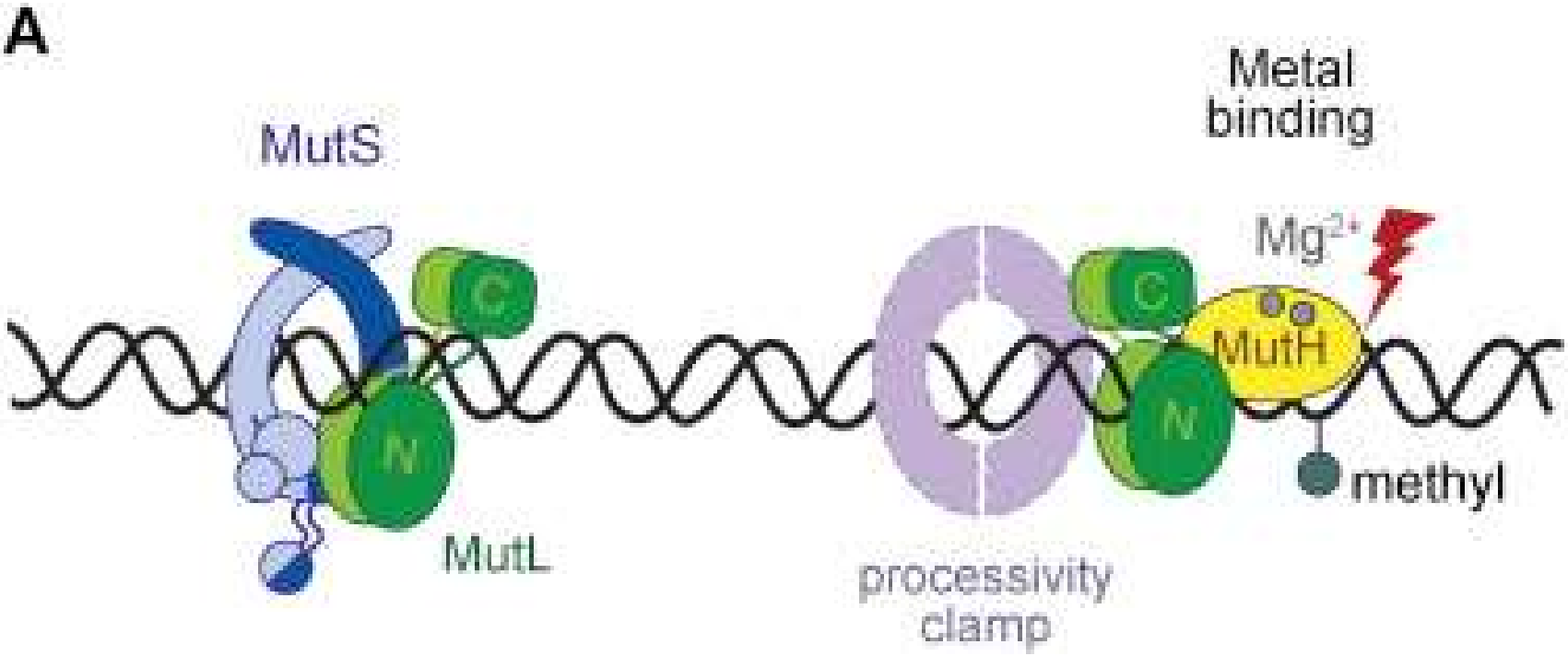

B. subtilis

**endonuclease motif**




yeast

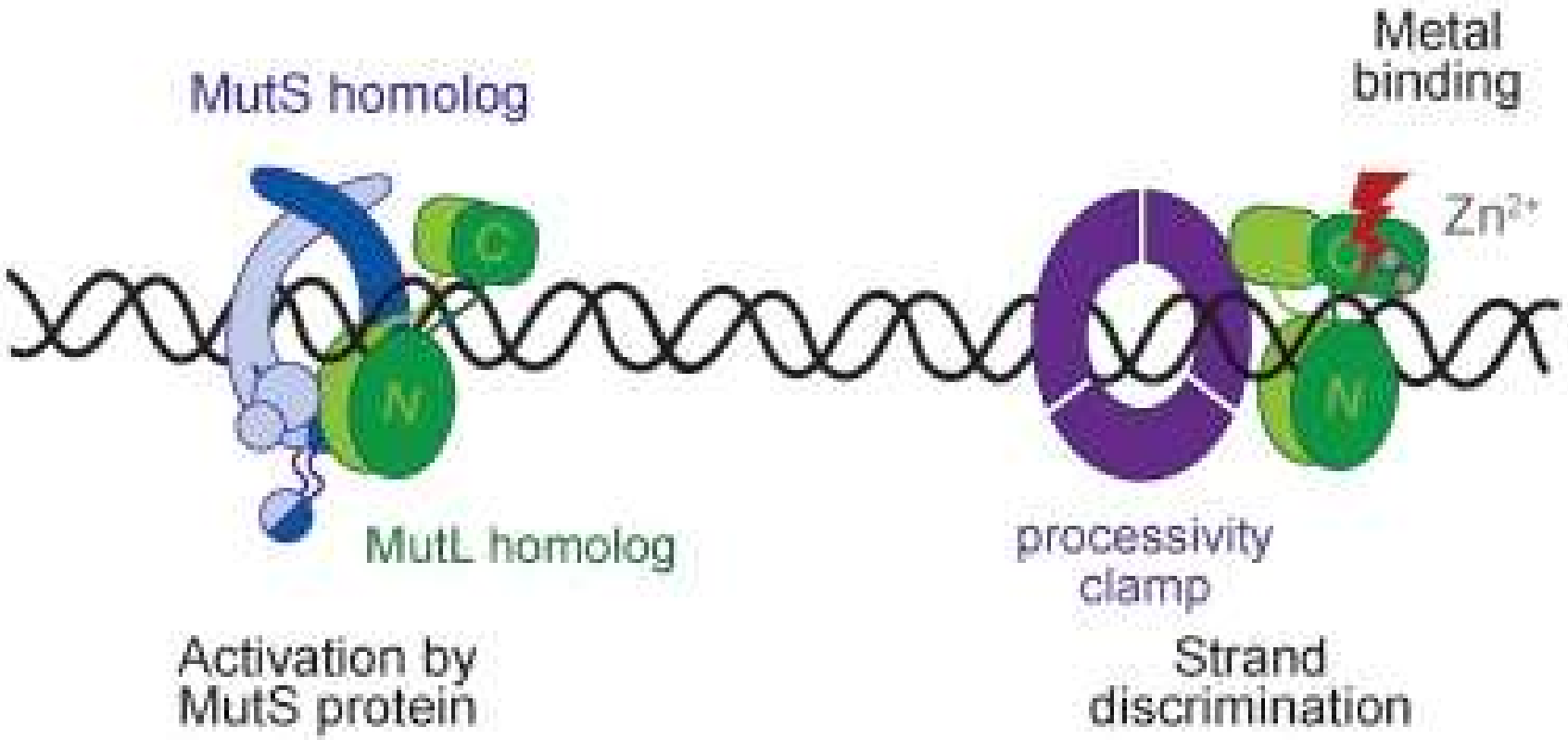
Procarioti



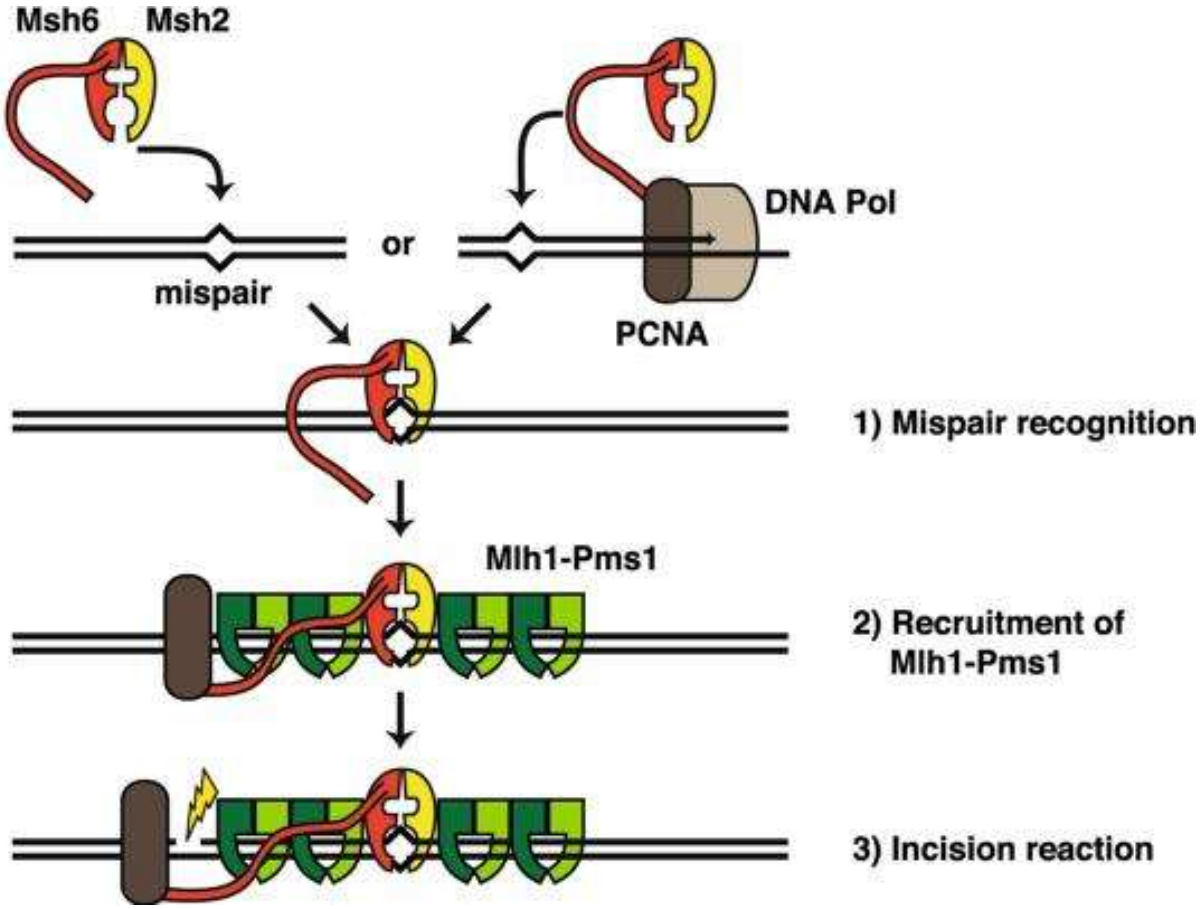

activation of endonuclease
activities in MMR

Eucarioti

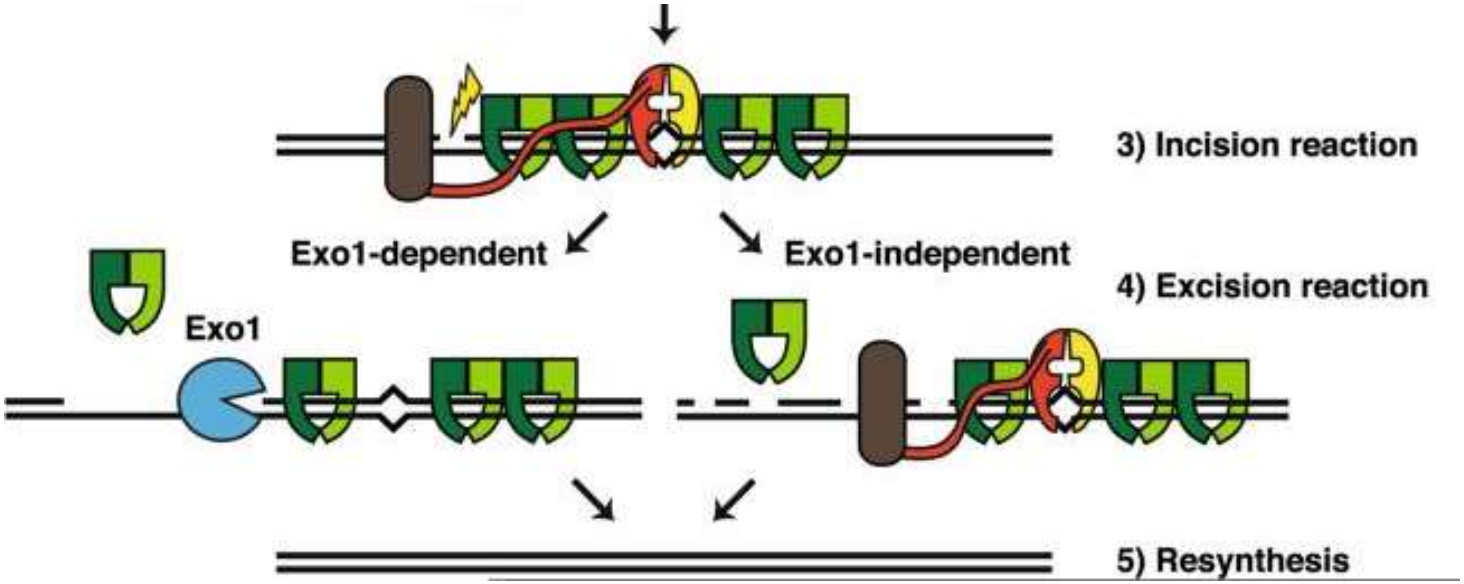
activation of endonuclease activities in MMR



# alternative excision pathways during MMR



# alternative excision pathways during MMR

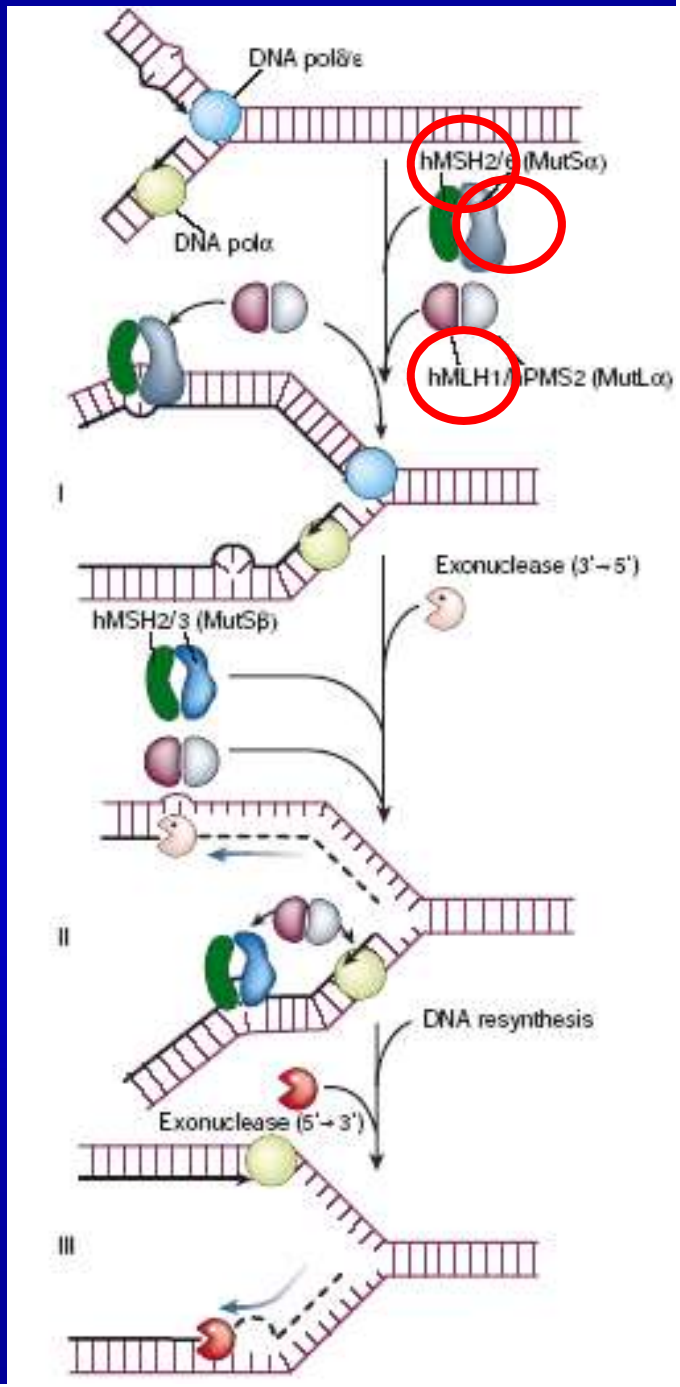


**MSH2** forma un eterodimero con **MSH6** (misappaiamento) o **MSH3** (loop di inserzione-delezione) e si lega al DNA segnalando l'elica templato

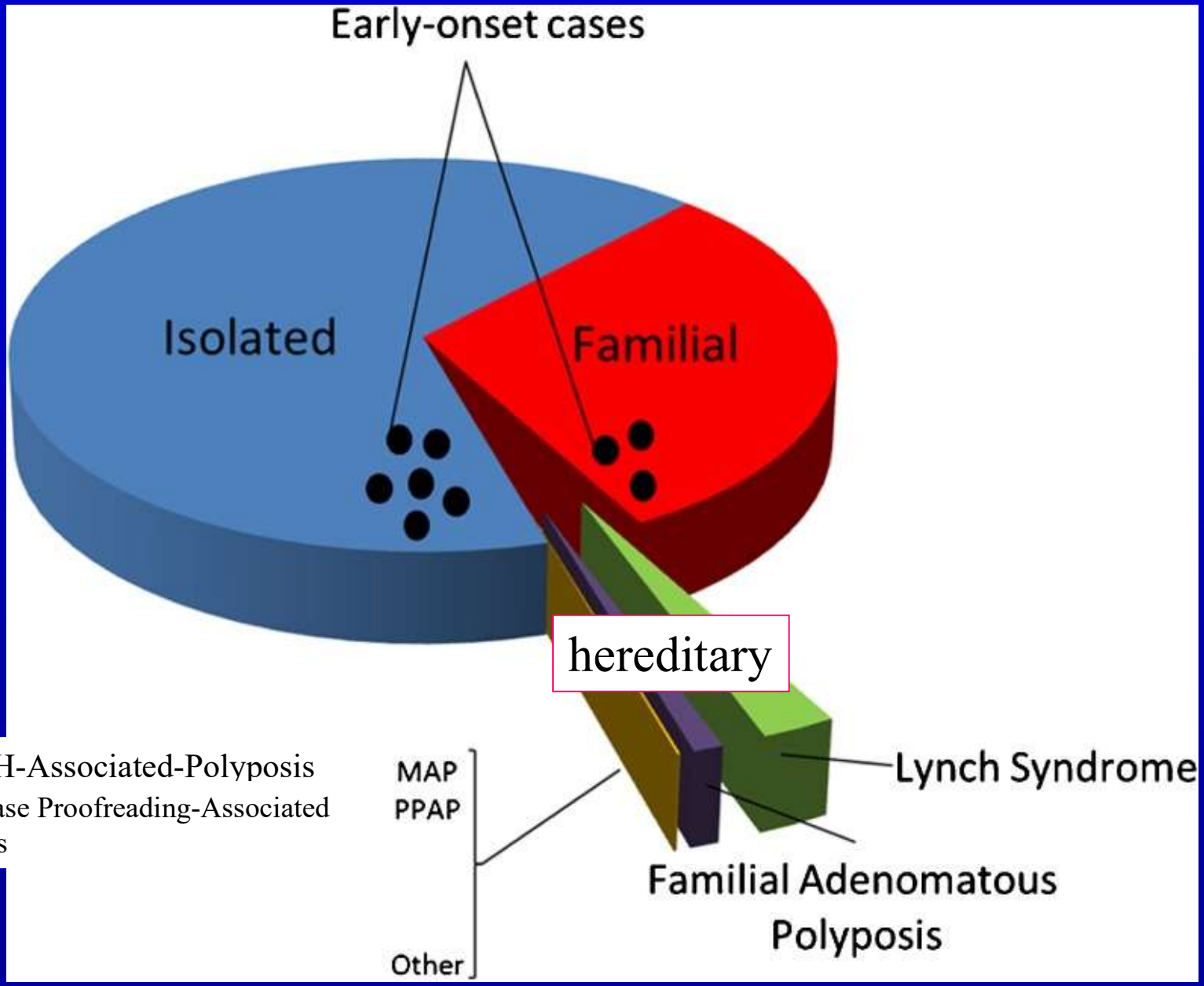
L'eterodimero **MLH1-PMS2**, talvolta legato anche a **PMS1**, coordina il legame con l'esonucleasi **EXO1** 3'- 5' ed una o più elicasi

**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



# Colorectal cancers



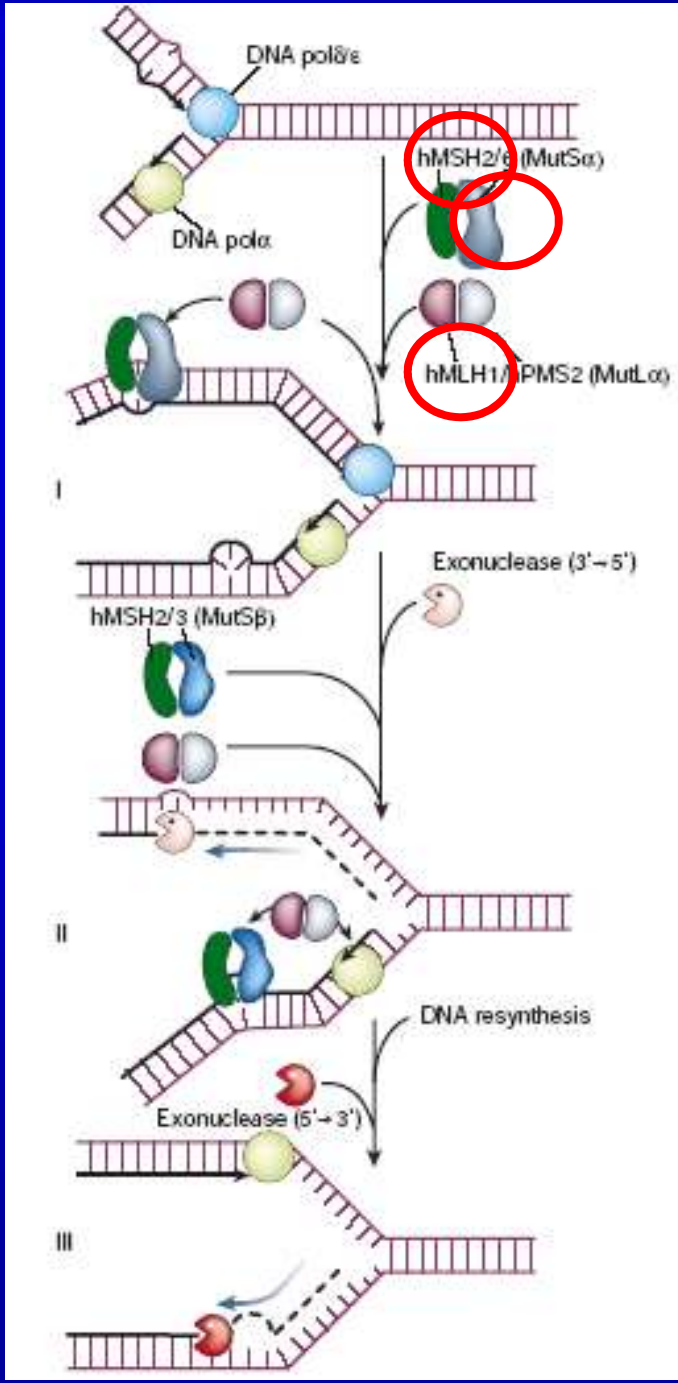

MUTYH-Associated-Polyposis
Polymerase Proofreading-Associated Polyposis

# HNPCC – Lynch syndrome
# cancro colon-rettale
# ereditario non poliposico

**hMLH1:** 50% delle mutazioni

**hMSH2:** 35%

**hMSH6:** 10%



**Mutazioni in un gene del MMR → predisposizione a HNPCC (cancro colon-rettale ereditario non poliposico),**

**aumento 100-1000X del tasso generale di mutazione, rischio di tumori al colon-retto**

**hMLH1: 50% delle mutazioni in HNPCC**

**hMSH2: 35%**

**hMSH6: 10%**

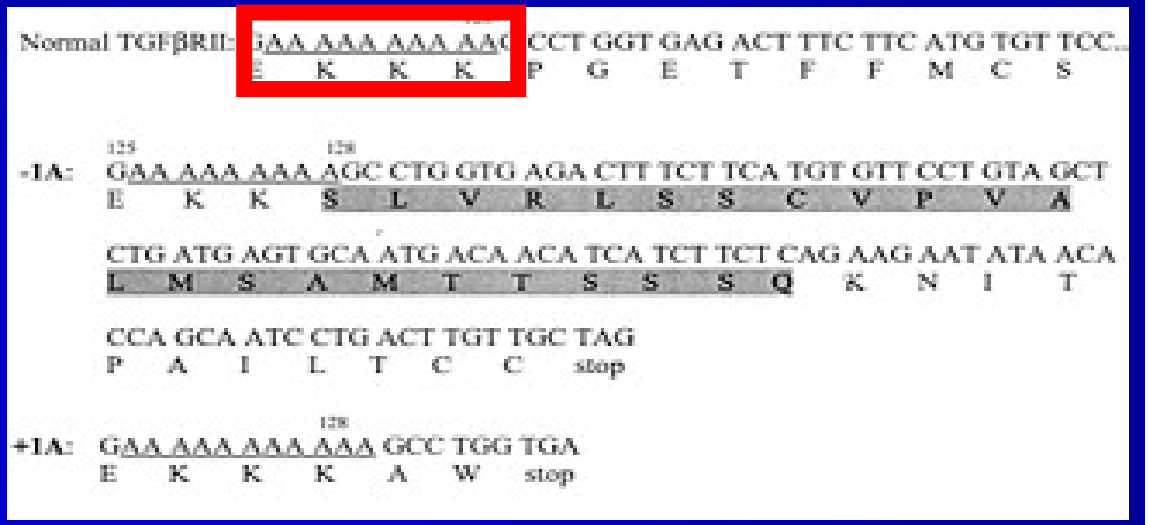
**Le sostanze chimiche in grado di indurre mutazioni sono contenute soprattutto nel cibo o sono prodotte dal metabolismo alimentare → maggiori probabilità di colpire la mucosa della zona colon-rettale, dove il cibo permane 24-36 ore**

| Gene | Total (%) |
|---|---|
| *ACVR2A* | 70/77 (90.9) |
| *TGFBR2* | 69/77 (89.6) |
| *EGFR* | 68/76[a] (88.3) |
| *BMPR2* (A)11 | 57/76[a] (75.0) |
| *E2F4* | 40/75[b] (53.3) |
| *MSH3* | 38/77 (49.4) |
| *BAX* | 34/77 (44.2) |
| *TCF7L2* | 32/77 (41.6) |
| *BMPR2* (A)7 | 27/77 (35.1) |
| *PRDM2* | 22/77 (28.6) |
| *MSH6* | 19/77 (24.7) |
| *IGF2R* | 16/77 (20.8) |
| *B2M* | 7/77 (9.1) |
| *APC* | 6/77 (7.8) |
| *PTEN* | 6/77 (7.8) |
| *AXIN2* | 3/77 (3.9) |

Mutational frequency of the target gene microsatellite sequences

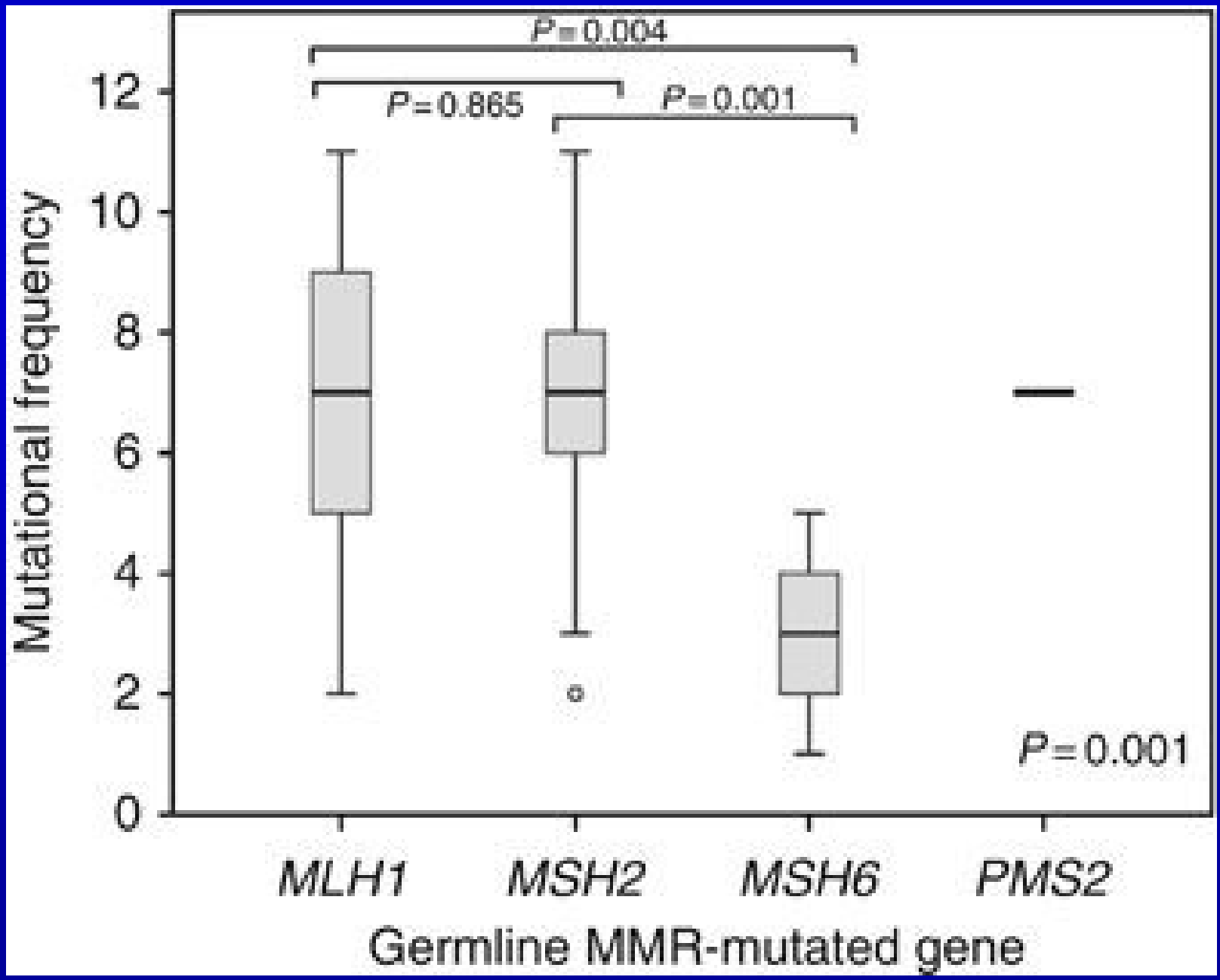
**L'alterazione del MMR aumenta l'insorgenza di mutazioni nel gene codificante per il recettore di tipo II per il TGFβ (TGFβ è un inibitore della proliferazione cellulare)**

**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**

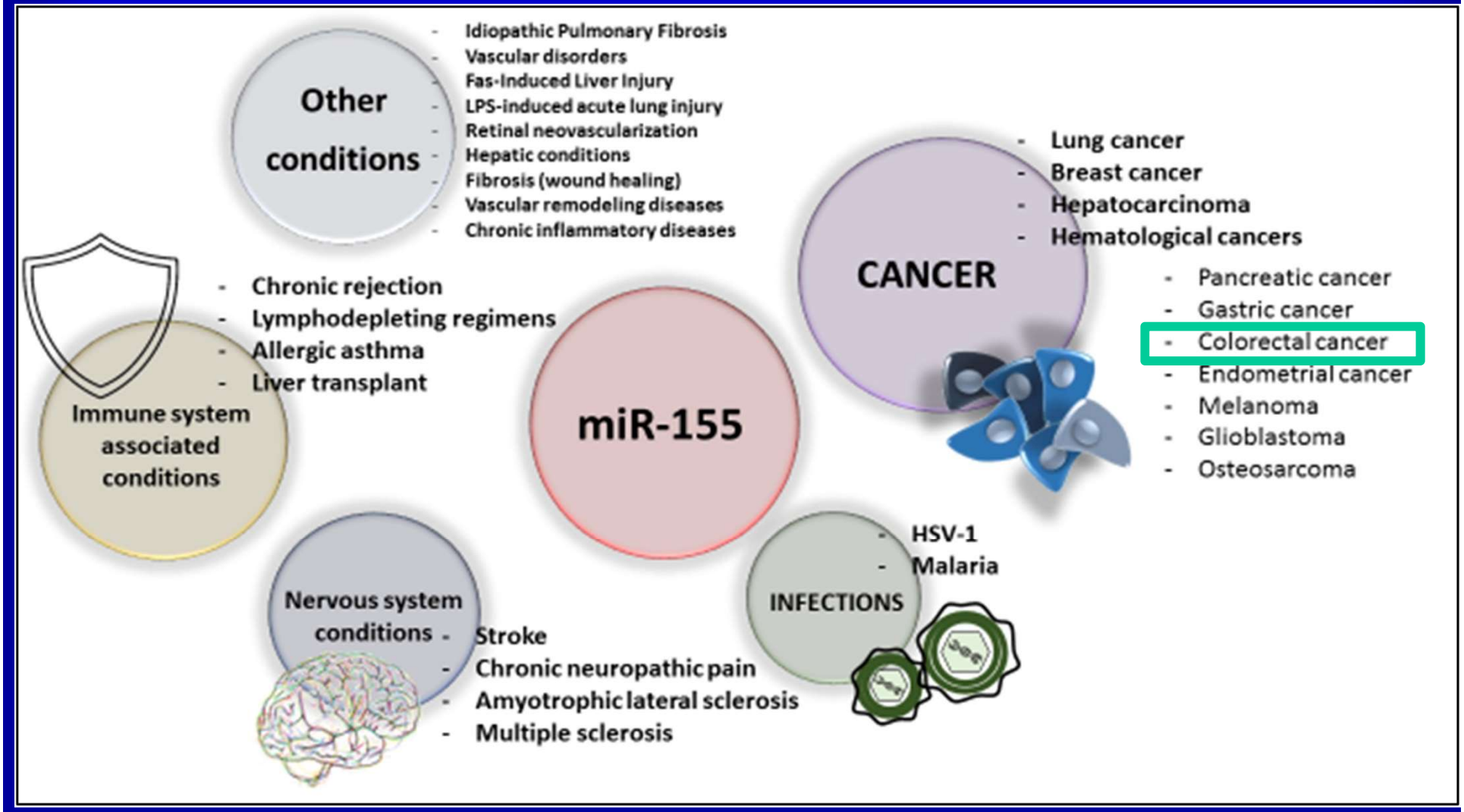


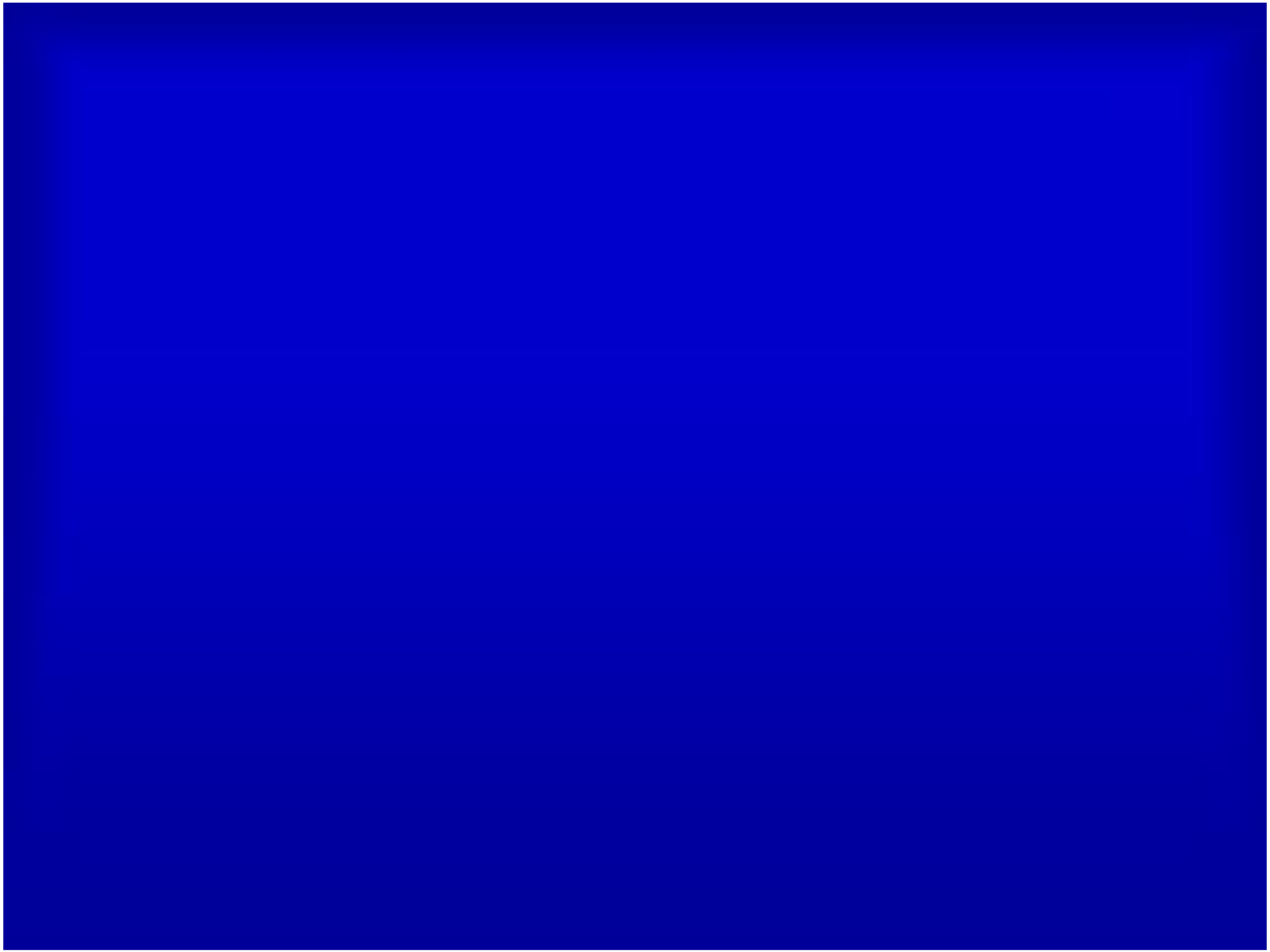

**Pazienti con alterazioni del MMR: l'errore permane → recettore per TGFβ non funzionale**

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


P = 0.004
P = 0.865
P = 0.001
P = 0.001
Mutational frequency
0
2
4
6
8
10
12
MLH1
MSH2
MSH6
PMS2
Germline MMR-mutated gene

# MiR-155 is involved in a broad spectrum of disease states

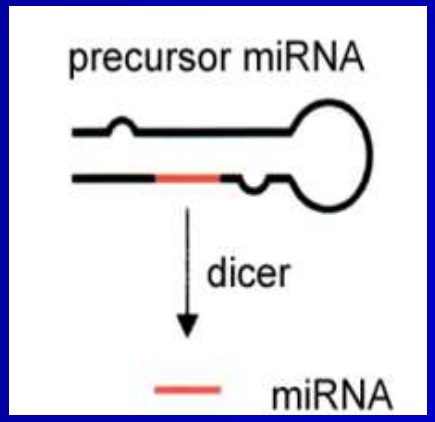




# Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6

# Small RNAs

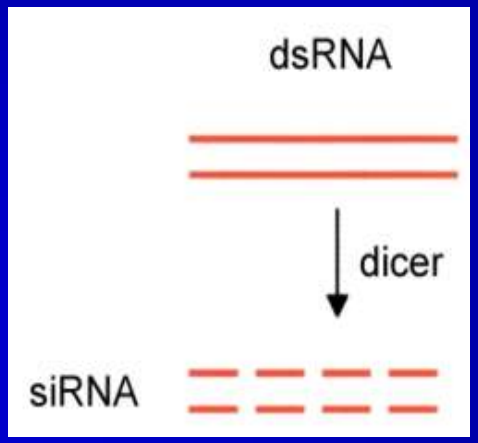
## miRNA




Prodotti in modo endogeno

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

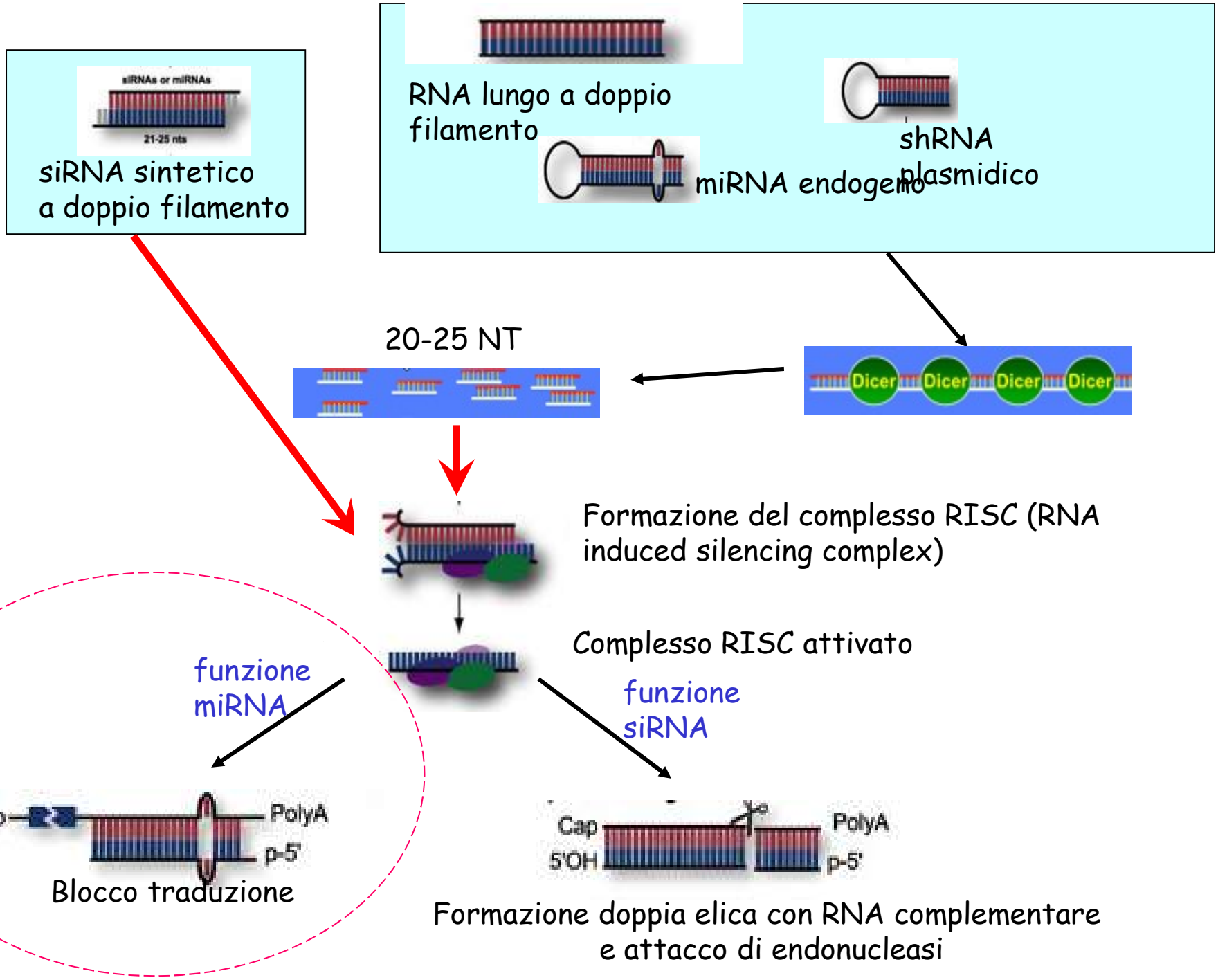
## siRNA



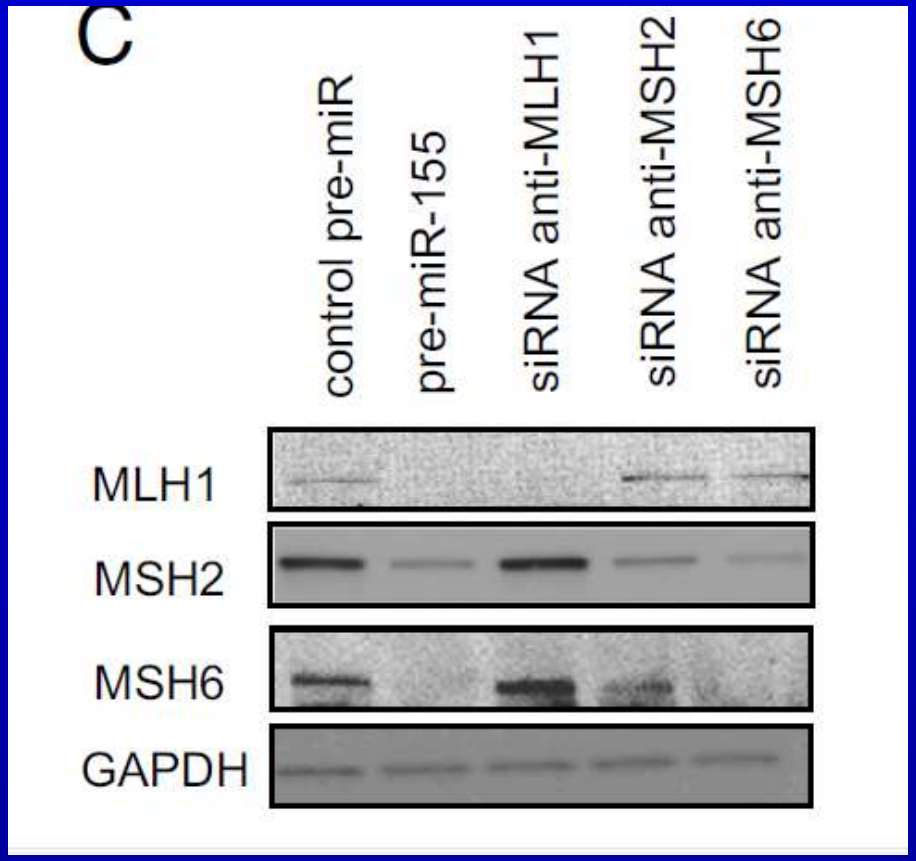

Exo-siRNA:Introdotti in modo esogeno (virus a dsRNA, transposoni, transgeni)

Endo-siRNA: derivati da loci genomici endogeni

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

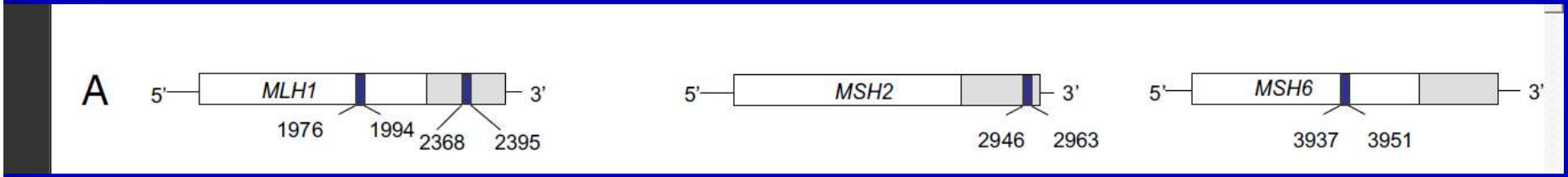

siRNAs or miRNAs
21-25 nts
siRNA sintetico
a doppio filamento
RNA lungo a doppio
filamento
shRNA
plasmidico
miRNA endogeno
20-25 NT
Dicer
Dicer
Dicer
Dicer
Formazione del complesso RISC (RNA
induced silencing complex)
Complesso RISC attivato
funzione
miRNA
funzione
siRNA
Cap
PolyA
p-5'
Blocco traduzione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



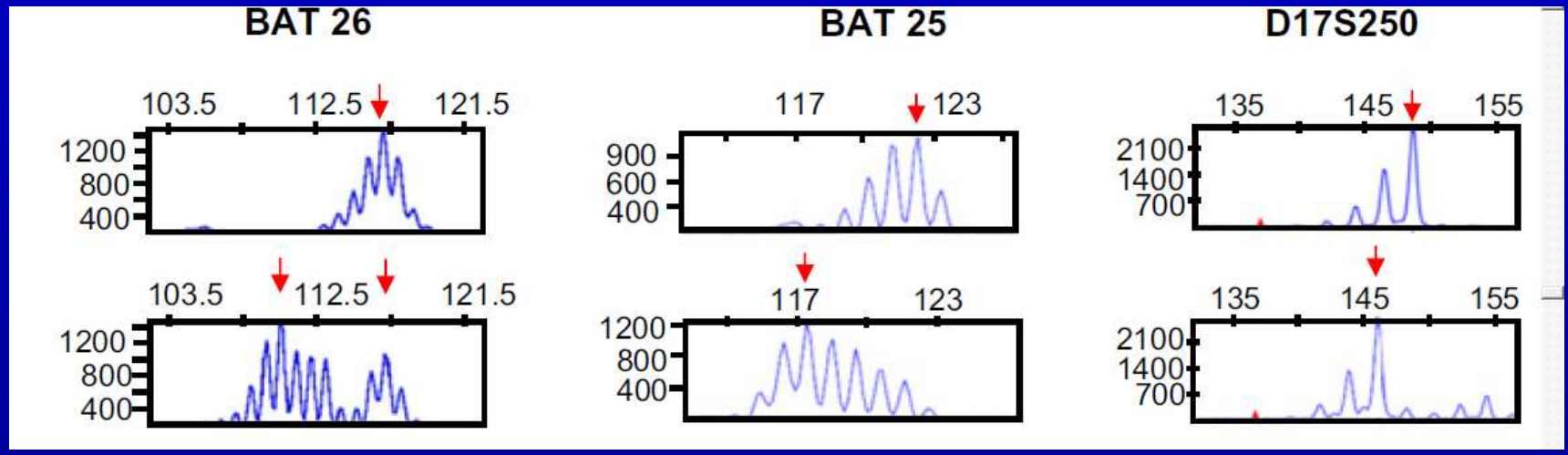
# Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in ColoRectal Cancer cells

- 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

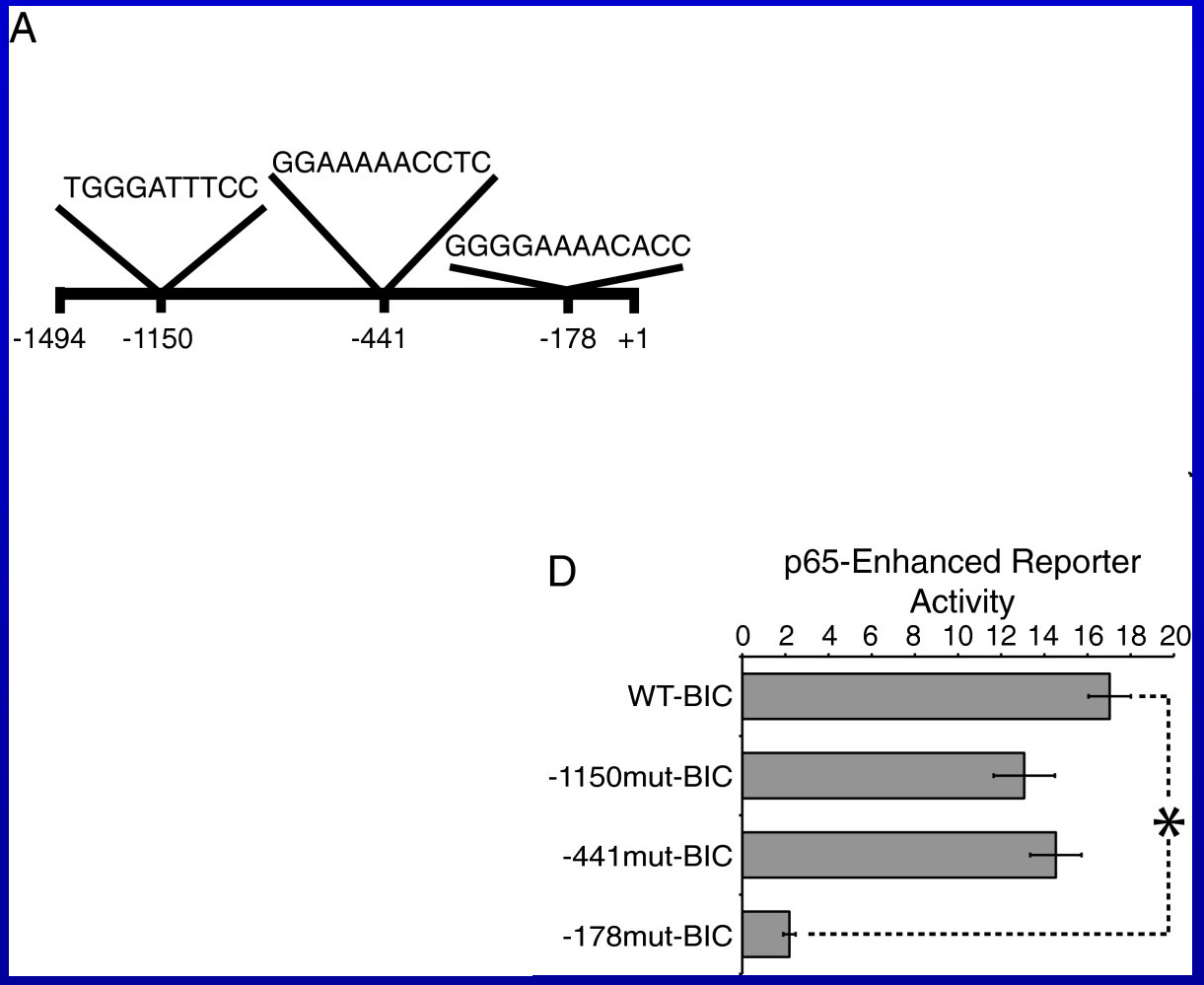


# Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in CRC cells

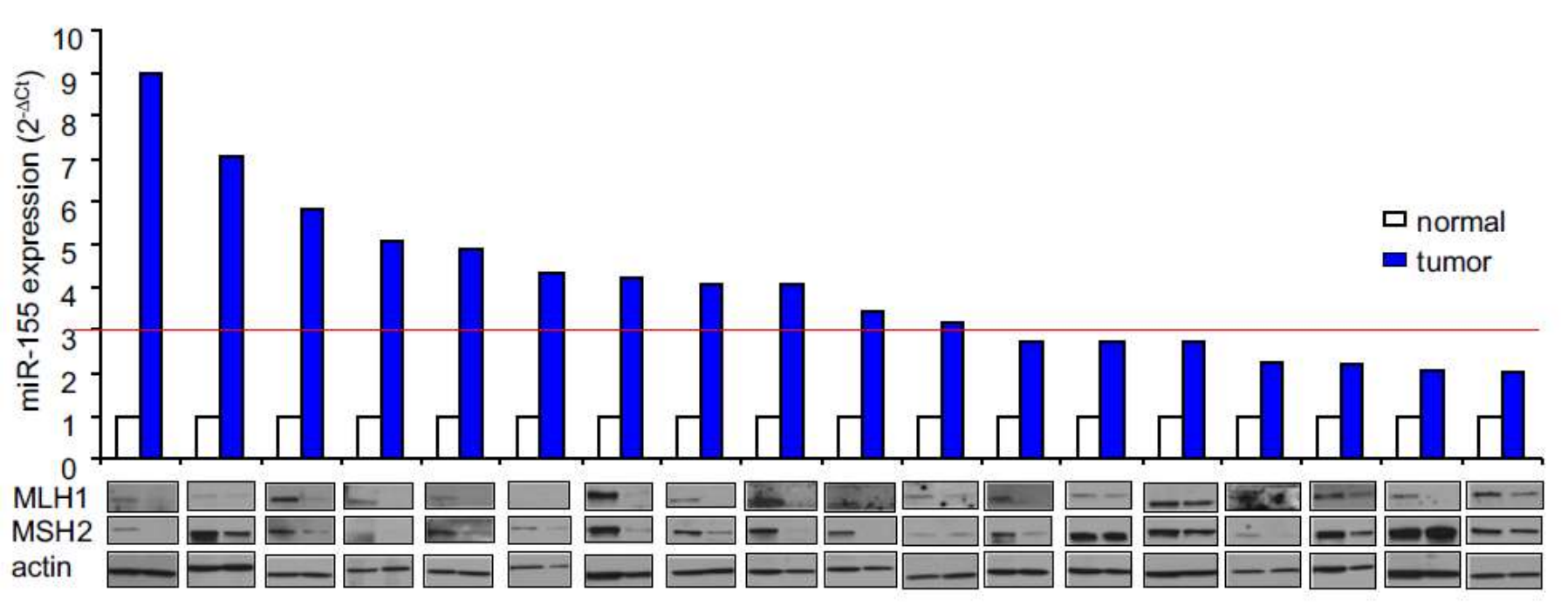
- Microsatellite analysis of Colo 155 (+) overexpression of miR-155) and (- ) cells
- BAT-26 and BAT 25 (mononucleotide repeats)
- D17S250 (dinucleotide repeat)



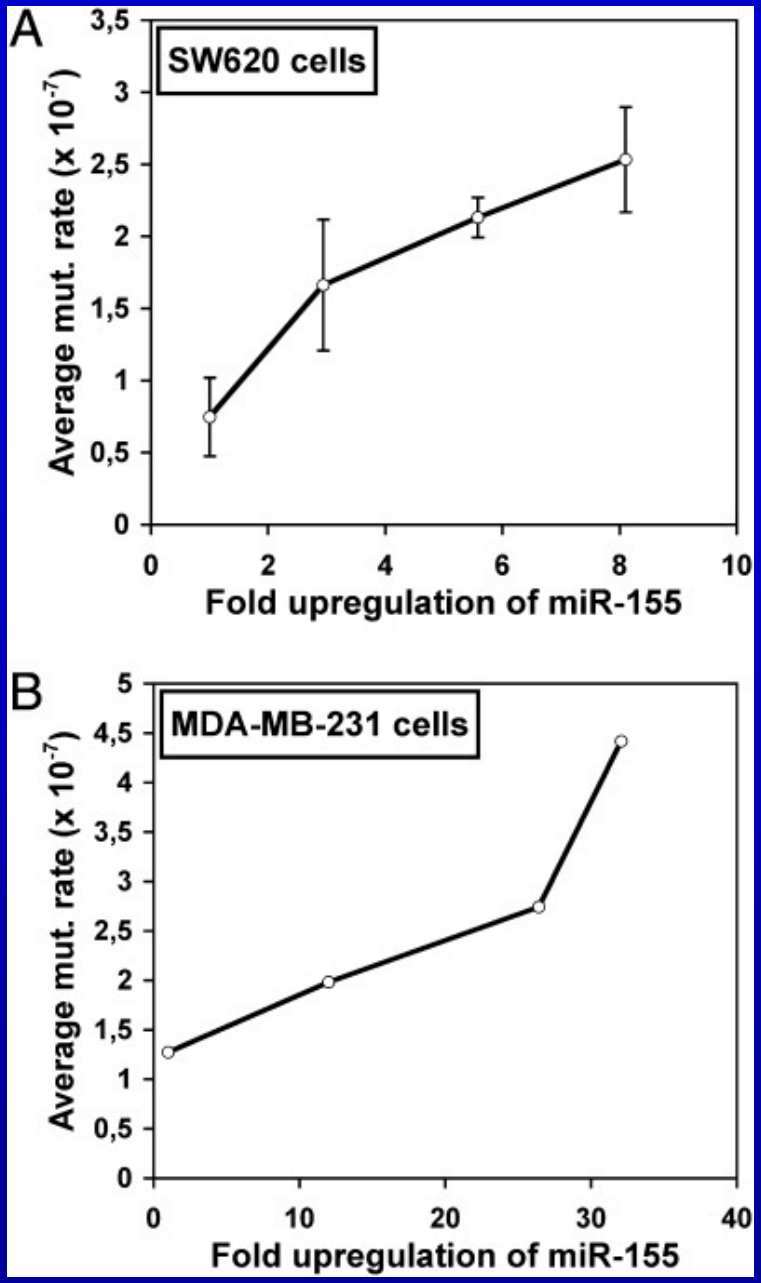
# NF-κB p65 up-regulates expression from the MIR155HG promoter through an NF-κB binding site located upstream of the transcription start site BMC Molecular Biology 2013 14:24



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



PNAS 2011

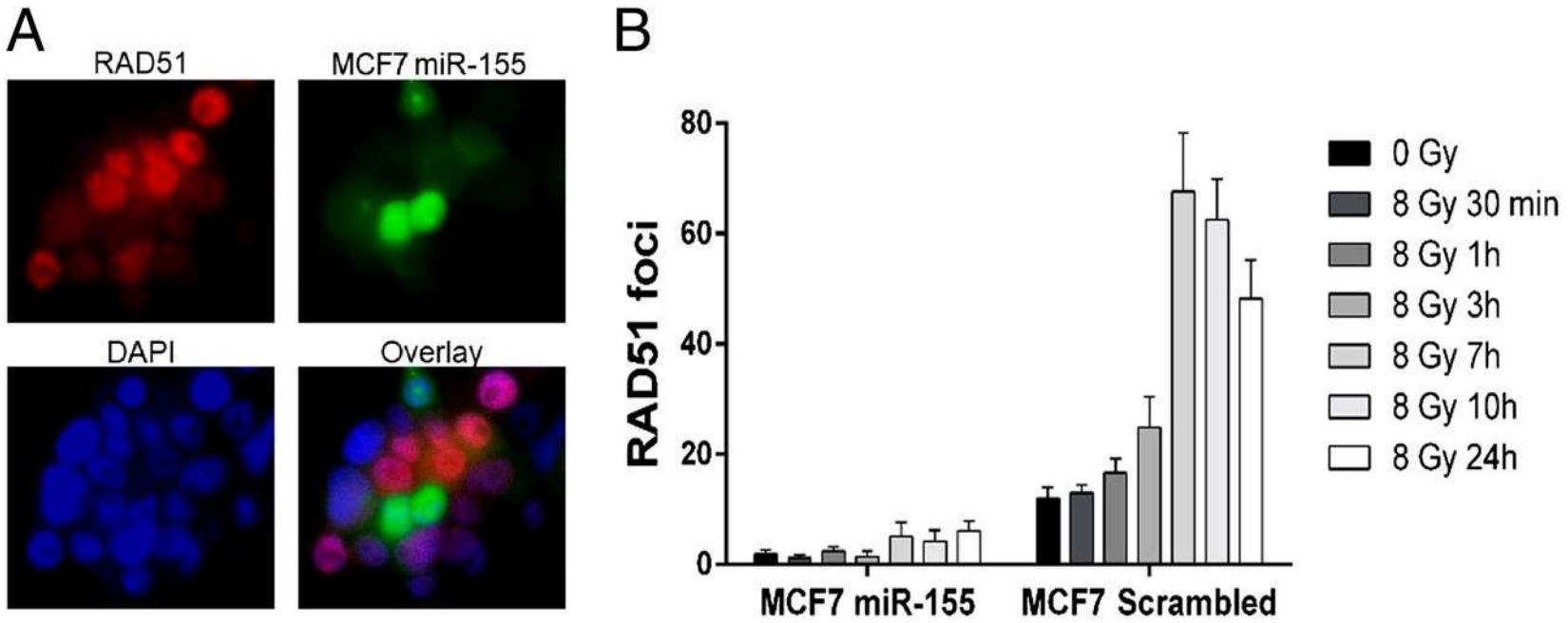



colorectal adenocarcinoma cells

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**