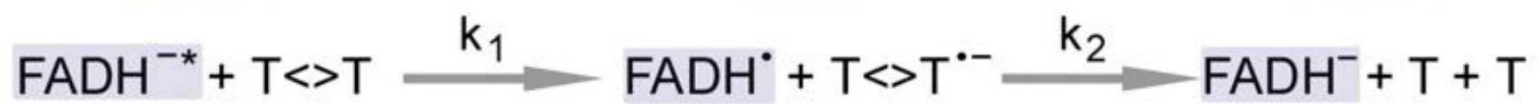
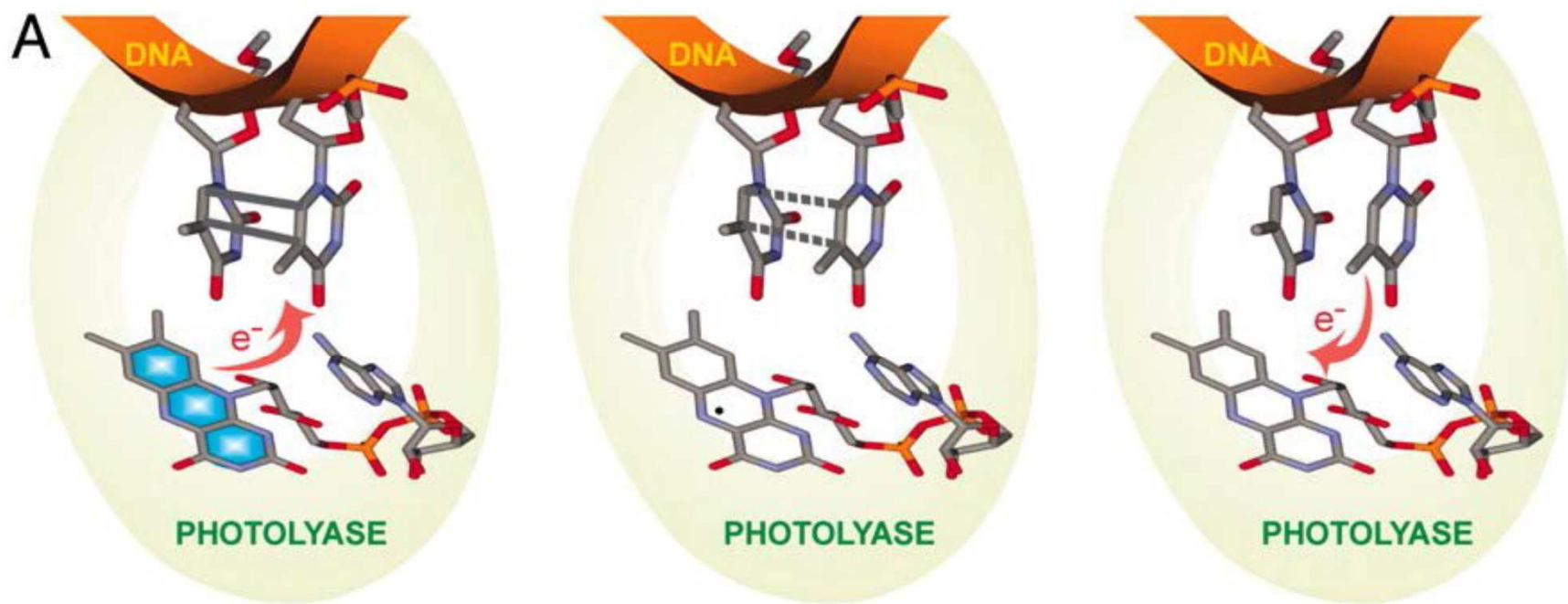


- The Nobel Prize in Chemistry 2015 was awarded jointly to
 - Tomas Lindahl,
 - Paul Modrich and
 - Aziz Sancar
- "for mechanistic studies of DNA repair"

- 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**.
- 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



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 un sistema multiproteico che comprende almeno 6 elementi diversi

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

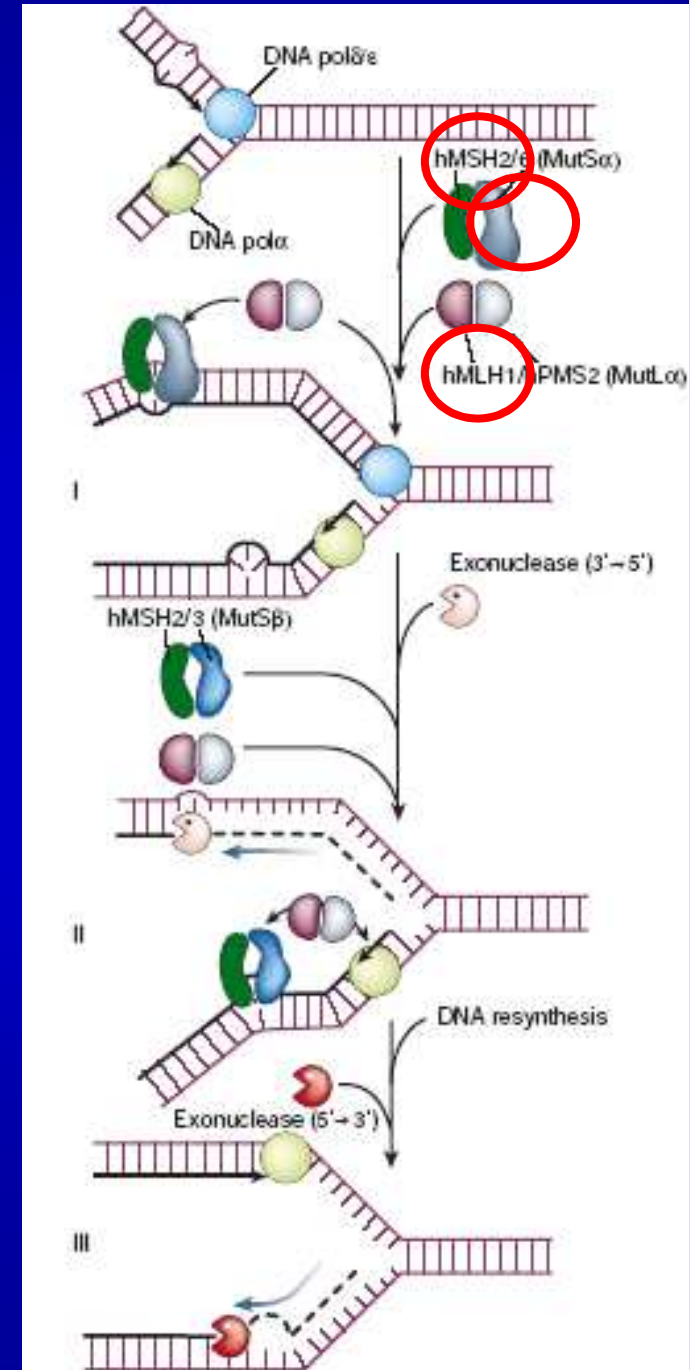


L'eterodimero **MLH1-PMS2**, talvolta legato anche a **PMS1**, coordina il legame con l'esonucleasi **EXO1** 3'-5' ed una o più elicasi



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



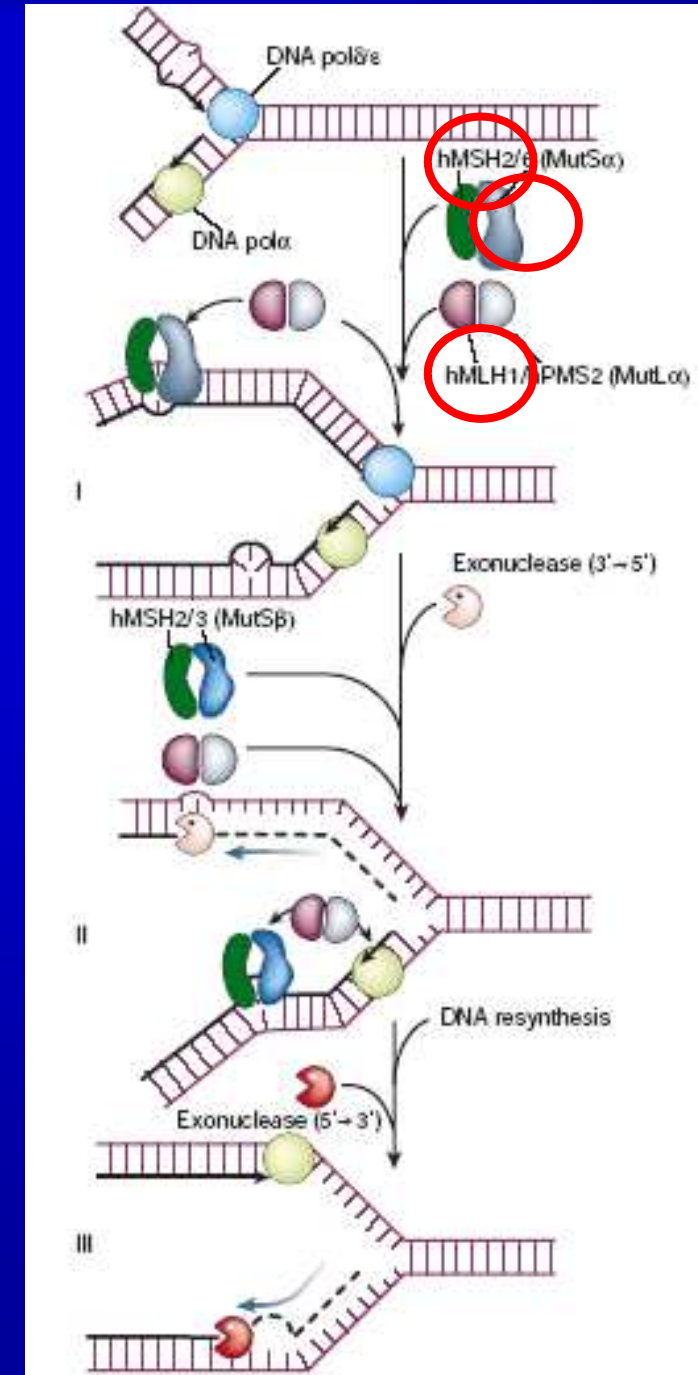
HNPPC

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), patologia frequente (1/200), aumento 100-1000X del tasso generale di mutazione, rischio di tumori al colon-retto

hMLH1: 50% delle mutazioni in HNPCC

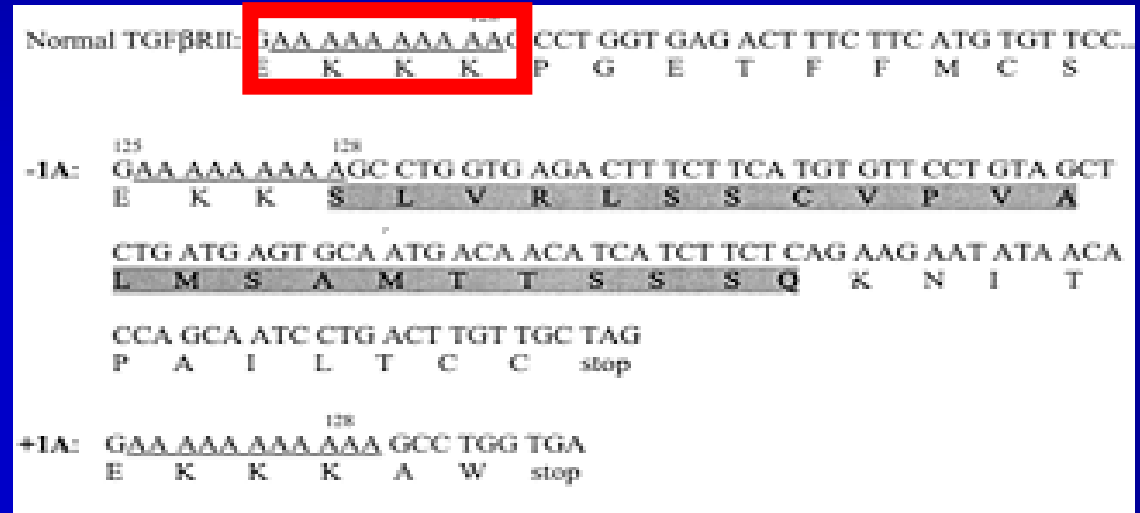
hMSH2: 35%

hMSH6: 10%

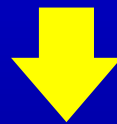
Le sostanze chimiche in grado di indurre mutazioni sono contenute soprattutto nel cibo o sono prodotte dal metabolismo alimentare □ maggiori probabilità di colpire la mucosa della zona colon-rettale, dove il cibo permane 24-36 ore

L'alterazione del MMR aumenta l'insorgenza di mutazioni nel gene codificante per il recettore di tipo II per il TGF β (TGF β 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 TGF β non funzionale

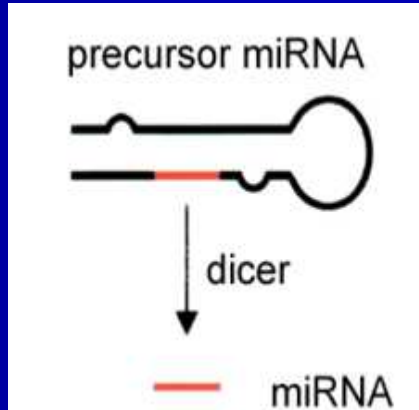


La mutazione rende le cellule insensibili alla inibizione della crescita indotta da TGF β □ sviluppo incontrollato caratteristico dei tumori

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

Small RNAs

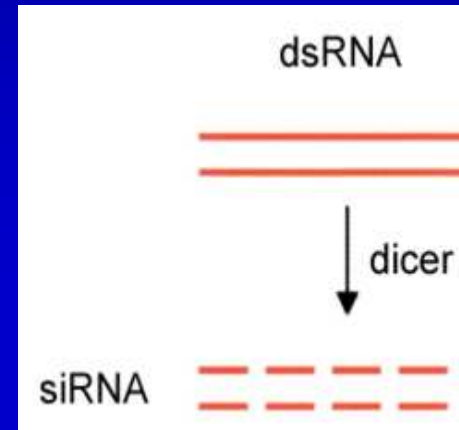
miRNA



Prodotti in modo endogeno

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

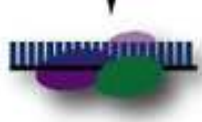
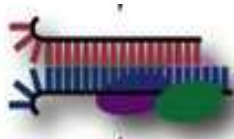
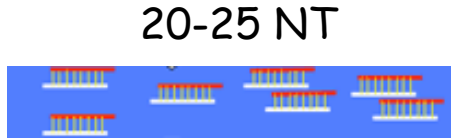
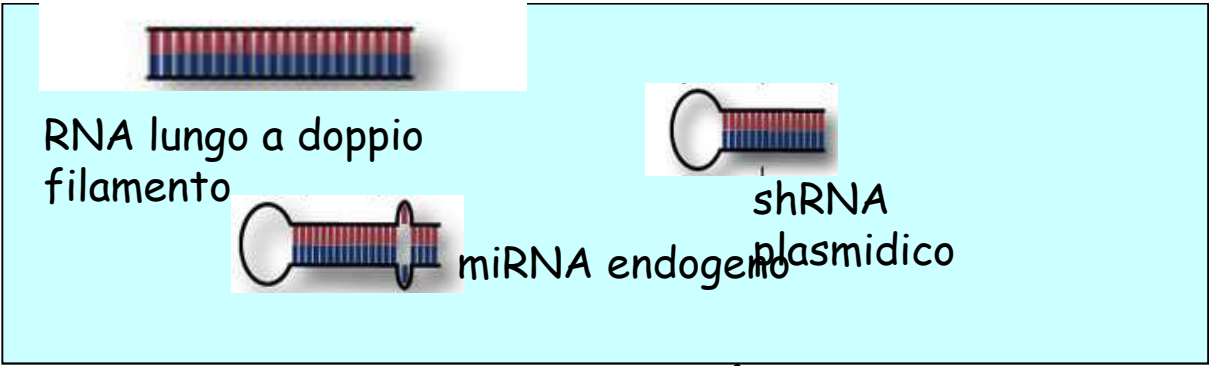
siRNA



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

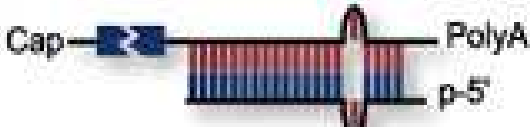
Endo-siRNA: derivati da loci genomici endogeni

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



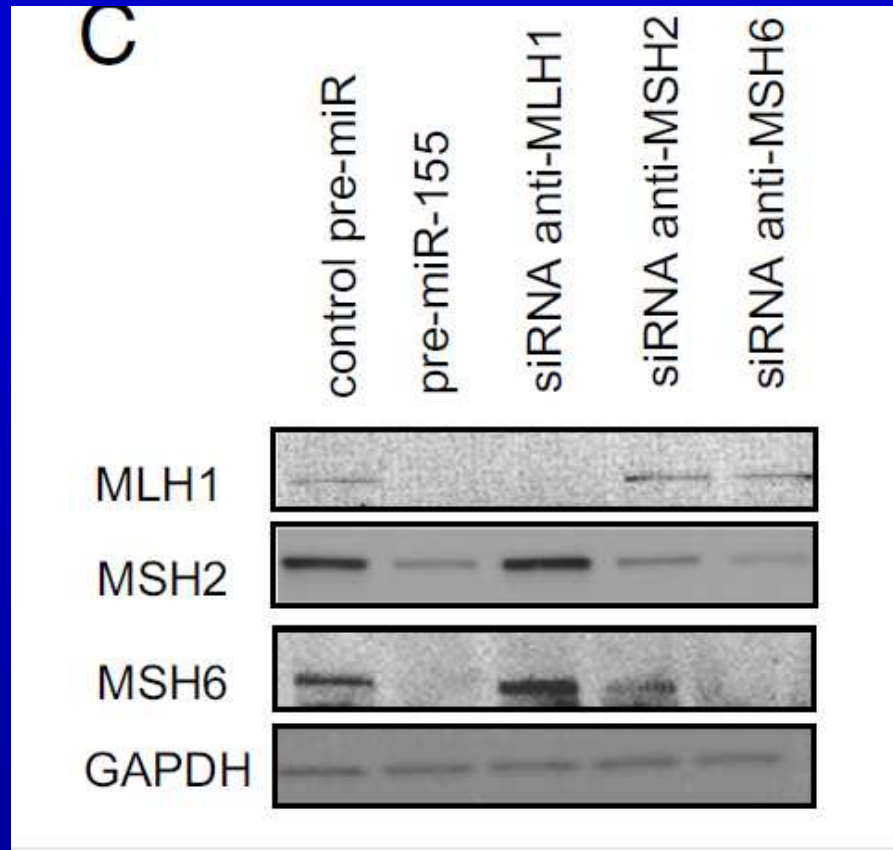
funzione miRNA

funzione siRNA



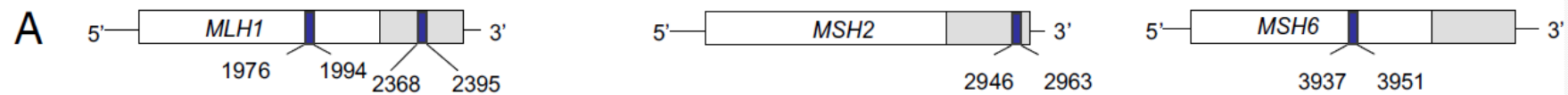
Formazione doppia elica con RNA complementare e attacco di endonucleasi

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



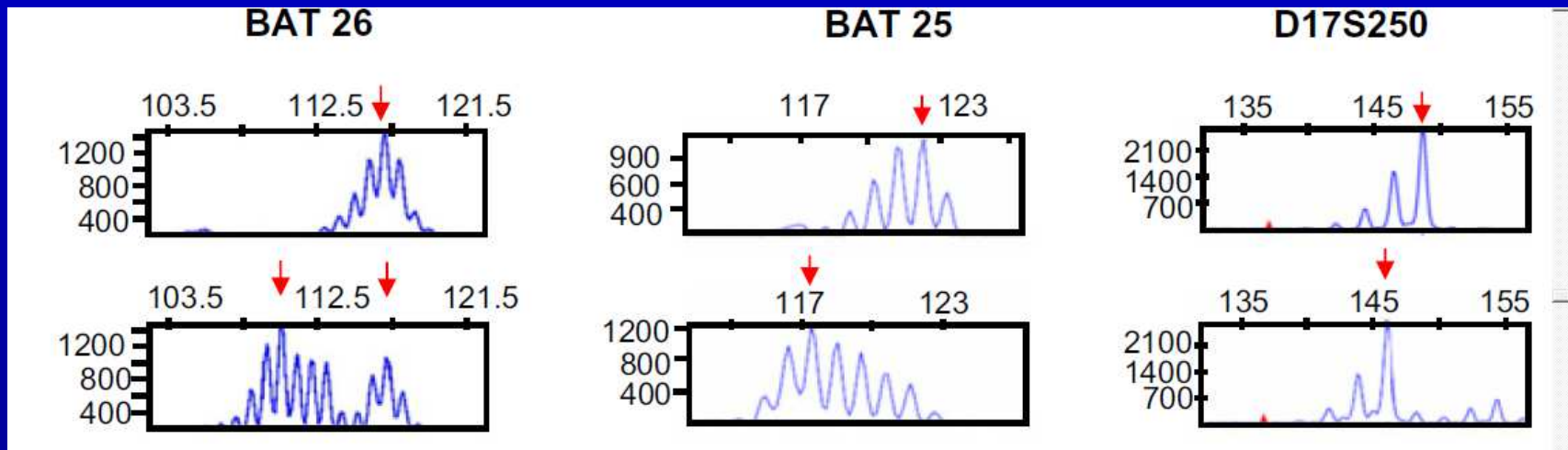
Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in ColoRectal Cancer cells

- 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

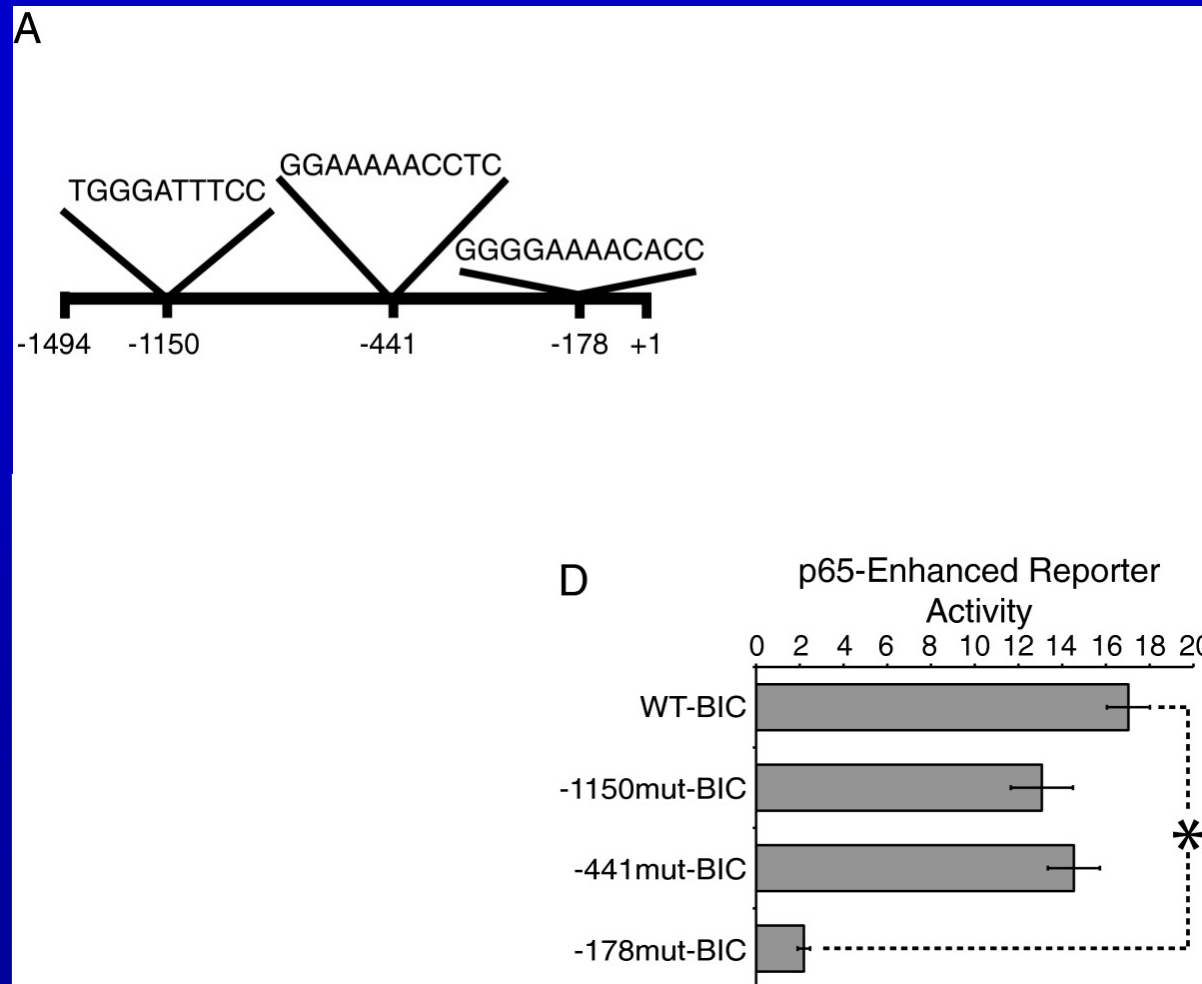


Overexpression of miR-155 decreases the expression of MLH1, MSH2, and MSH6 in CRC cells

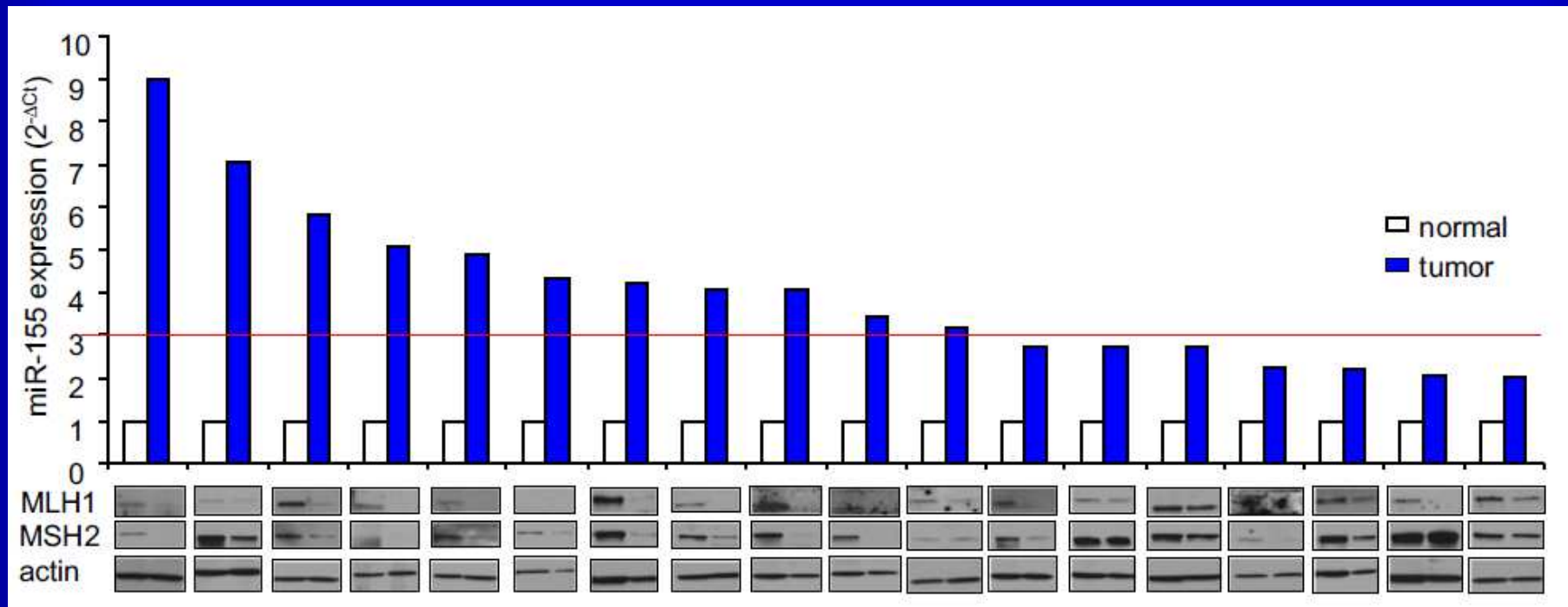
- Microsatellite analysis of Colo 155 (+) overexpression of miR-155) and (-) cells
- BAT-26 and BAT 25 (mononucleotide repeats)
- D17S250 (dinucleotide repeat)



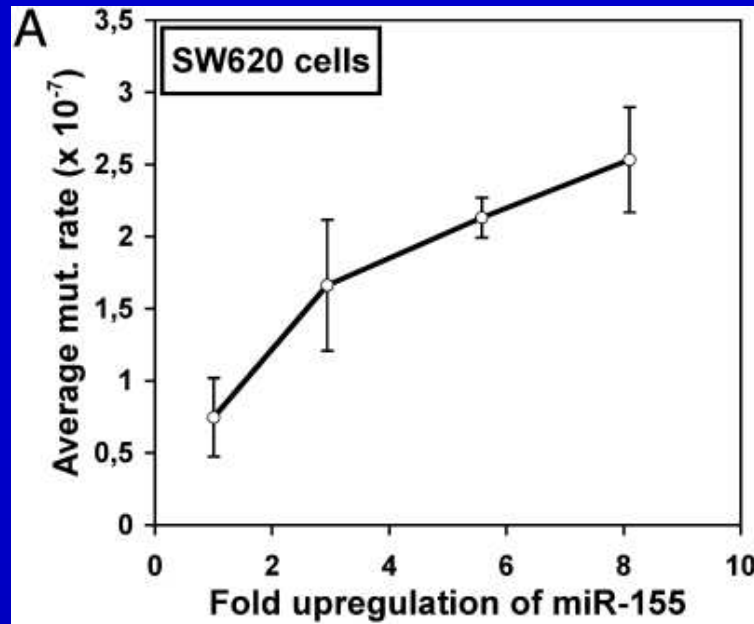
NF- κ B p65 up-regulates expression from the MIR155HG promoter through an NF- κ B binding site located upstream of the transcription start site BMC Molecular Biology 2013 14:24



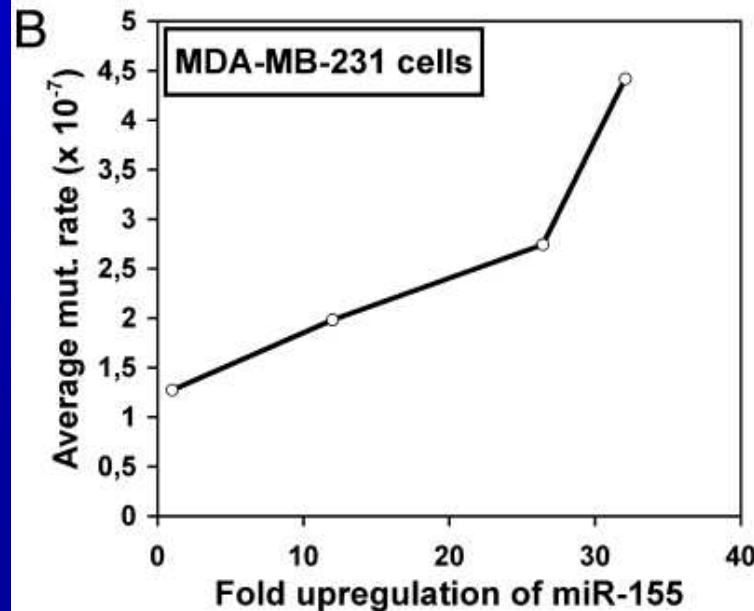
miR-155 expression is inversely related to MLH1 and MSH2 in CRC tissues



colorectal adenocarcinoma cells

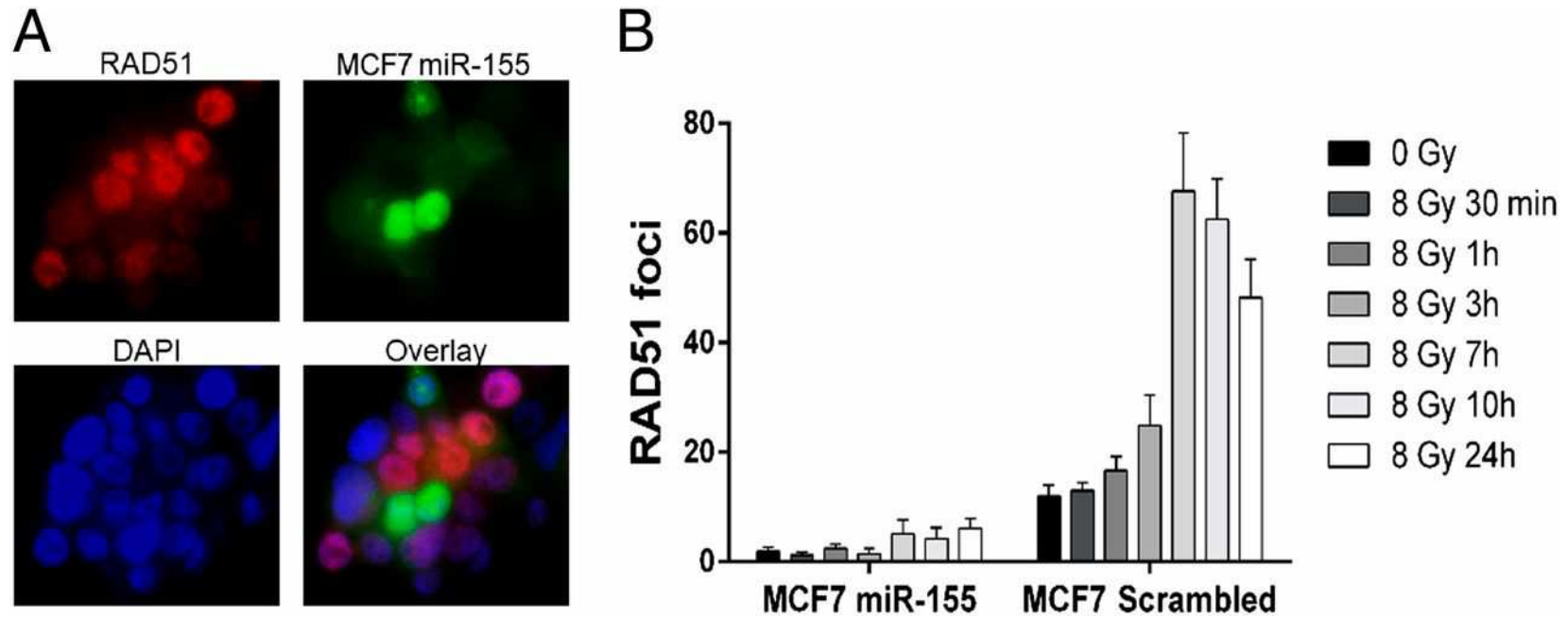


breast adenocarcinoma cells



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

http://www.sabiosciences.com/rt_pcr_product/HTML/PAMM-6002Z.html