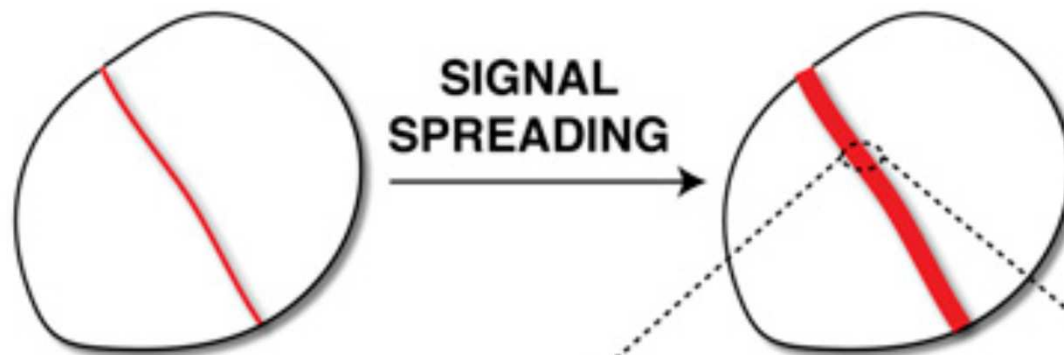
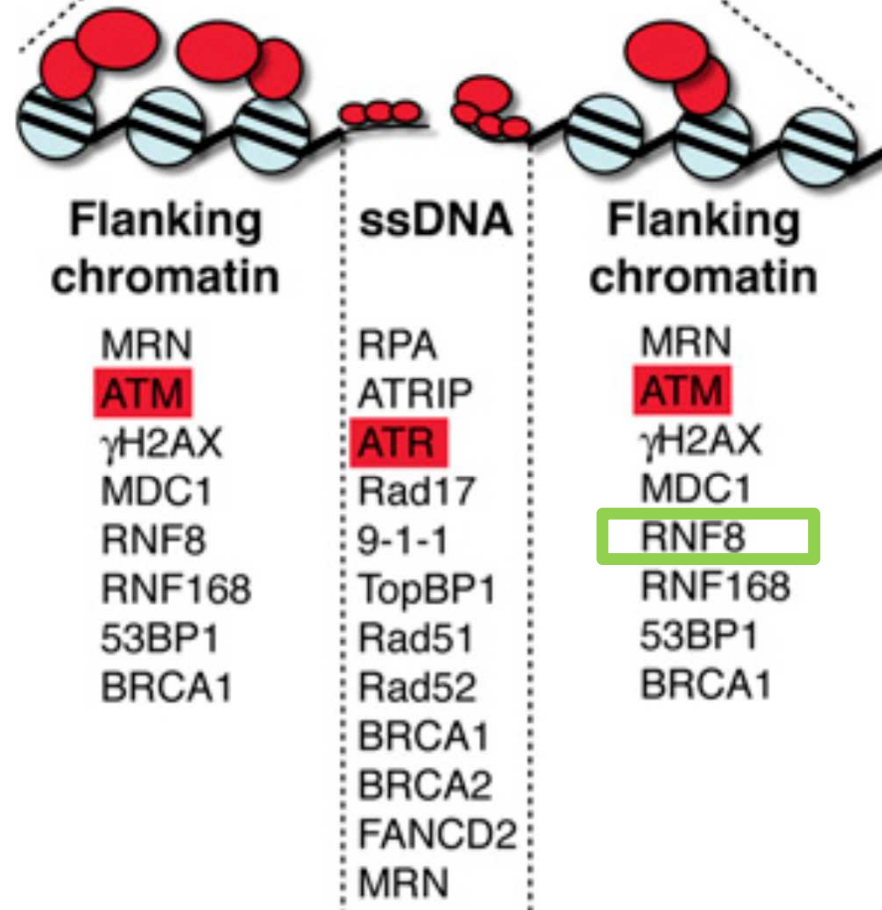
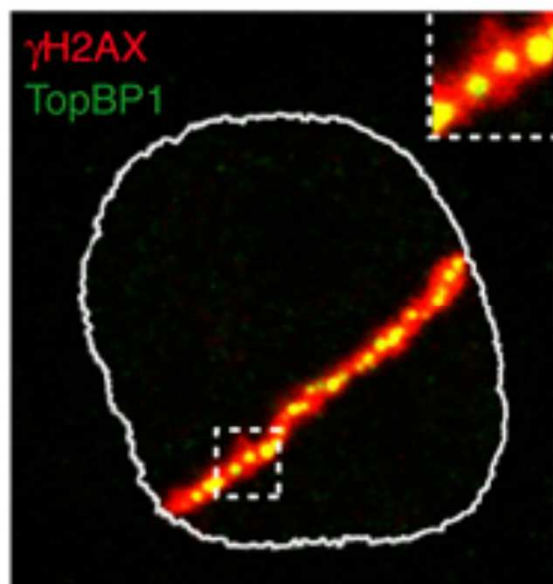


RNF8



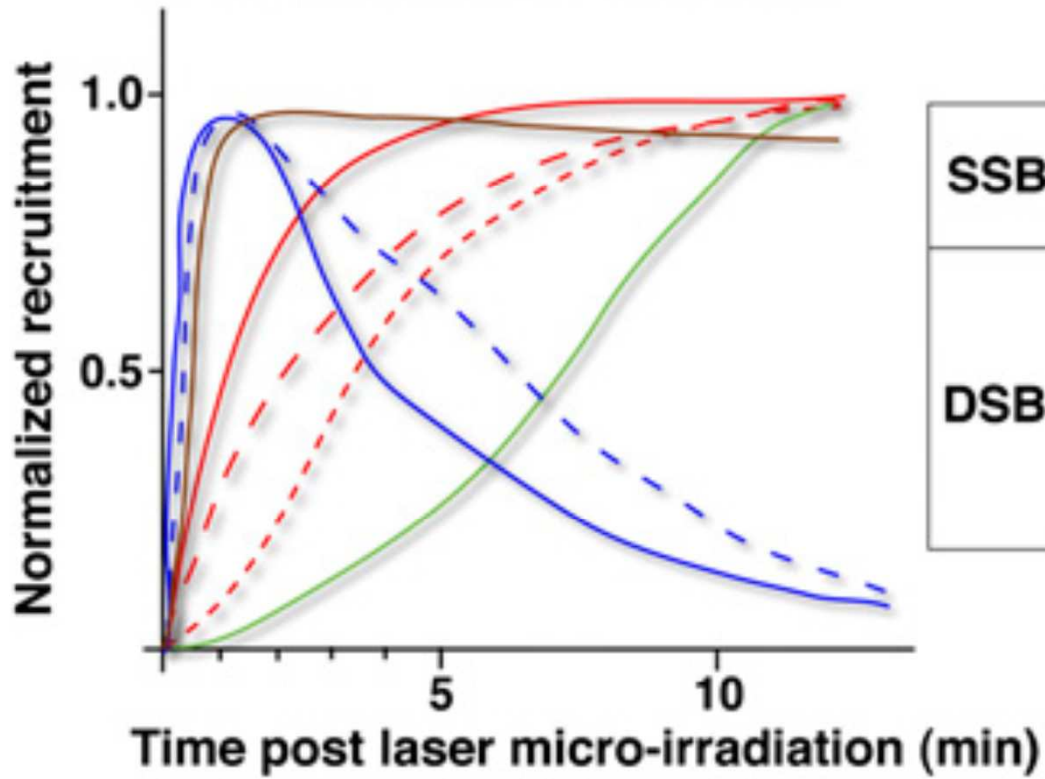
**B** REGIONAL DISTRIBUTION



# Temporal regulation of DDR protein accumulation at DNA breaks

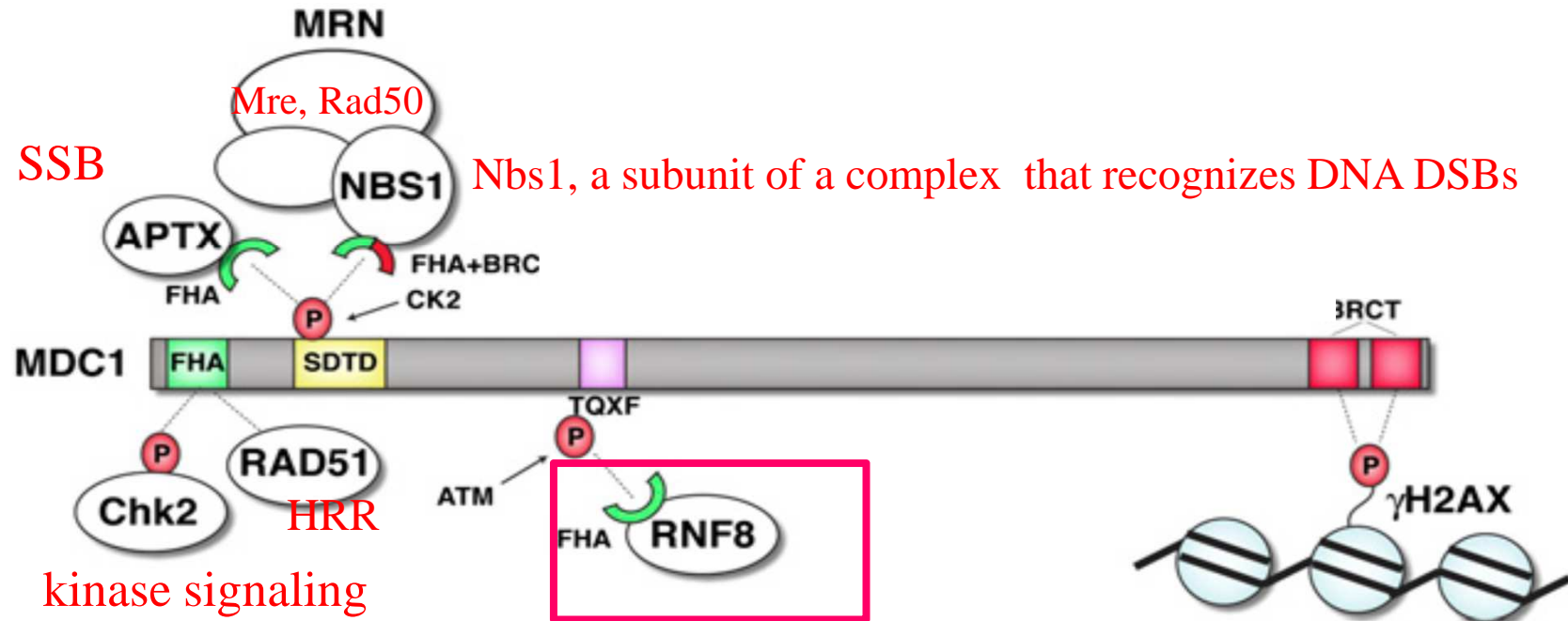
## A

### RECRUITMENT KINETICS



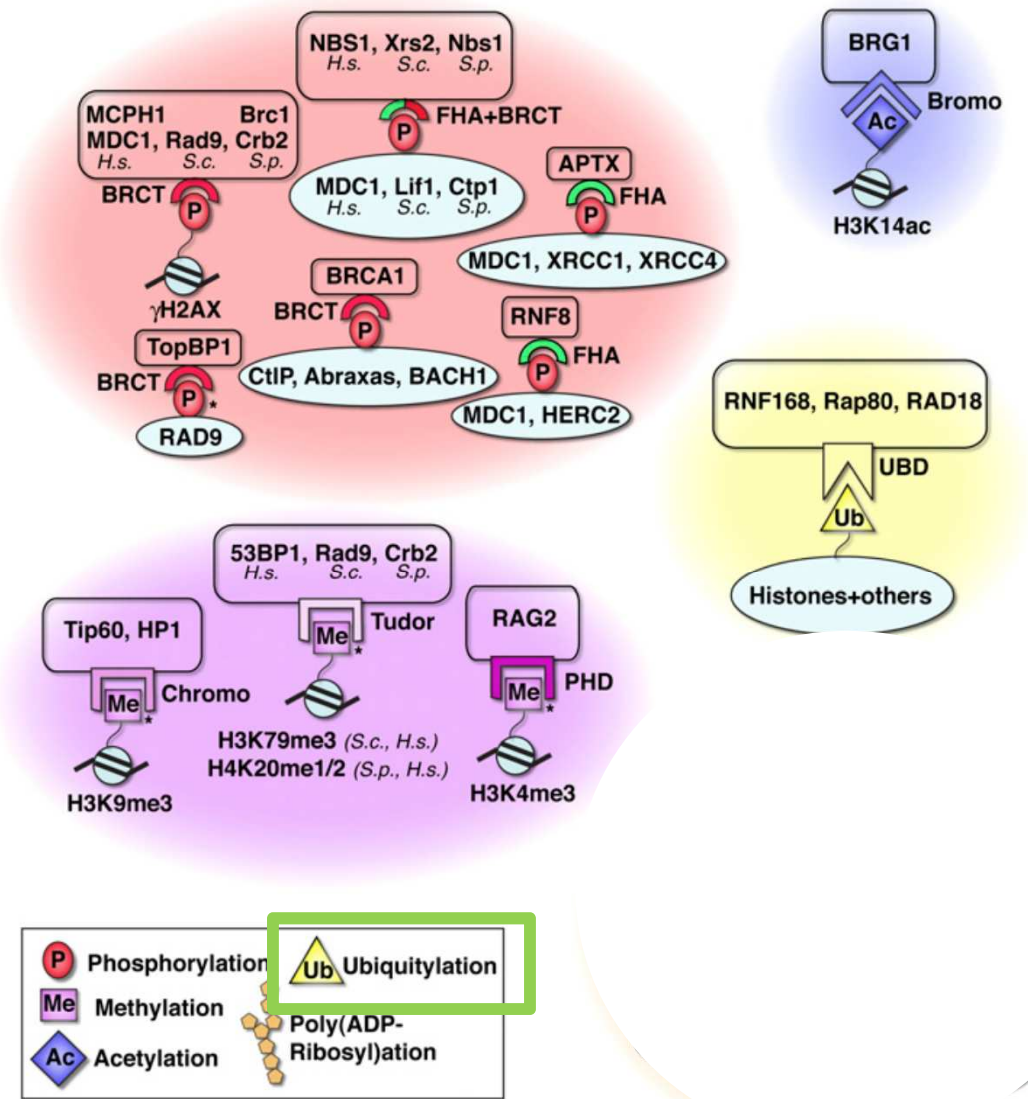
# Proteine piattaforma

## Damage signaling





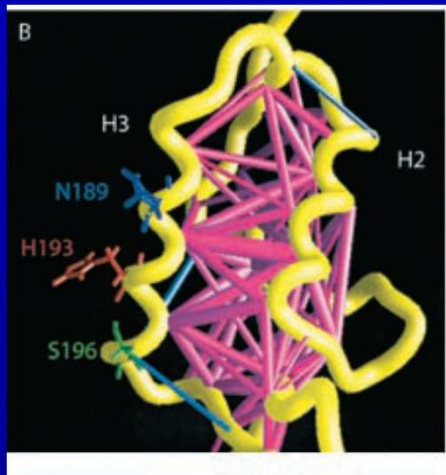
# Specialized binding modules for recognition of post-translational modifications (PTMs) at DNA breaks.



# Domain architecture of RNF8



Forkhead associated(FHA) domain  
bind phosphothreonine-bearing epitopes  
interaction with ATM-phosphorylated MDC1.



Forkhead domain

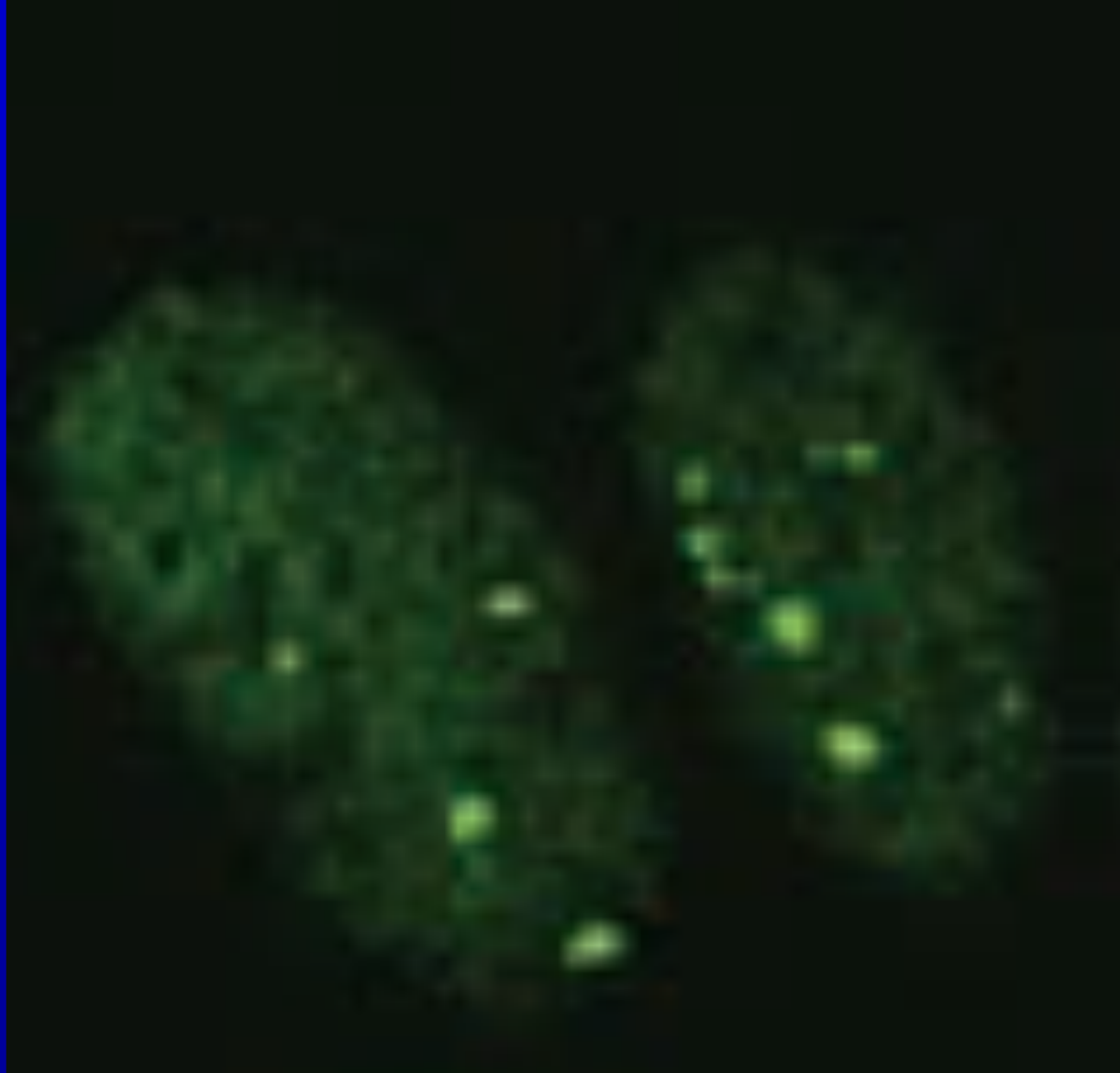
Ubiquitin ligase activity

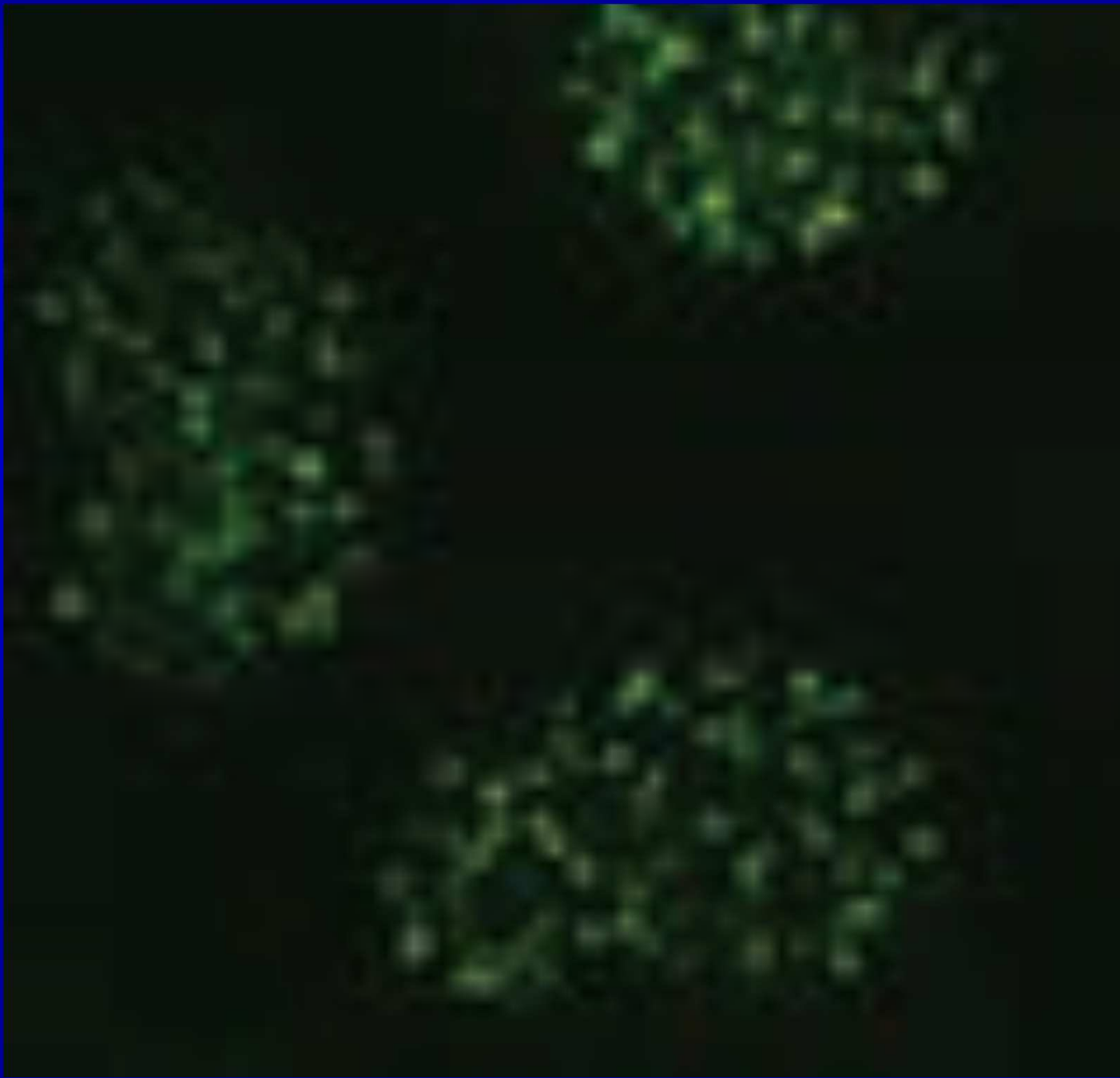
- FHA-(R42A) and
- RING finger (C406s) mutants.



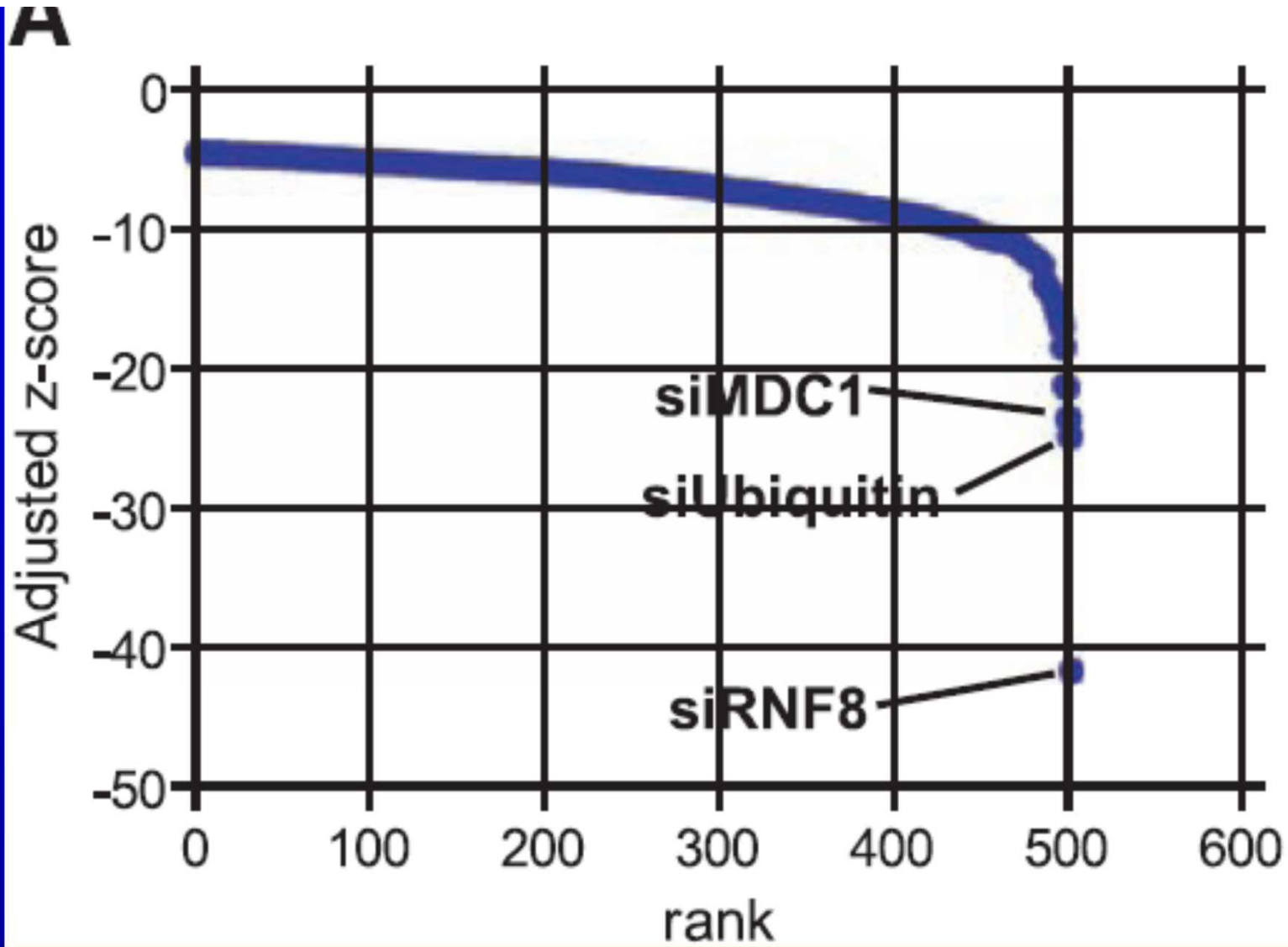
## Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase (Nadine Science Feb2008)

- Cells respond to DSBs by recruiting the DNA-damage mediator protein MDC1, the p53-binding protein 1 (53BP1) to sites of damaged DNA.
- 53BP1 is an established player- important role in modulating chromatin structure surrounding the break site- in the cellular response to DNA damage and is a canonical component of ionizing-radiation induced foci (IRIF)



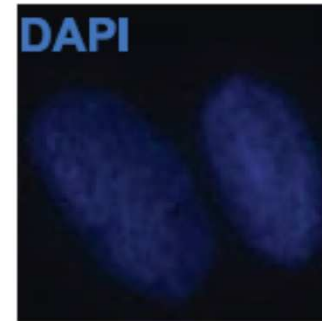
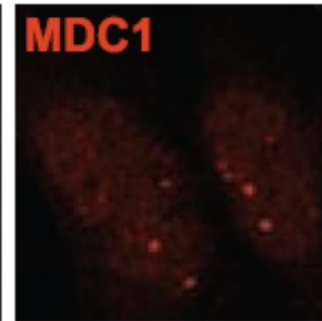
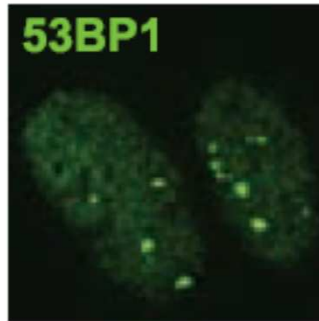


Ranking by z score of 500 siRNAs giving the least 53BP1 foci from a siRNA screen

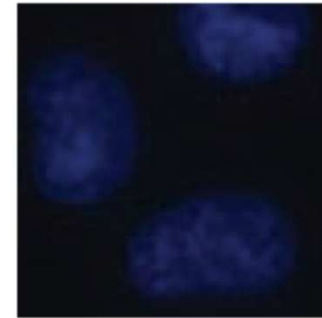
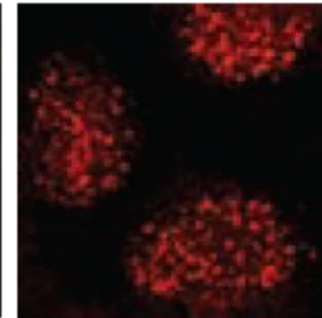
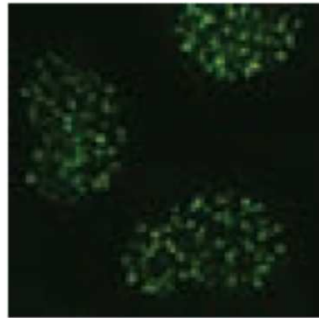


**B**

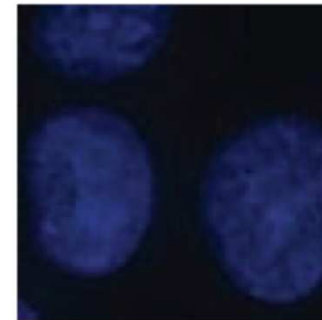
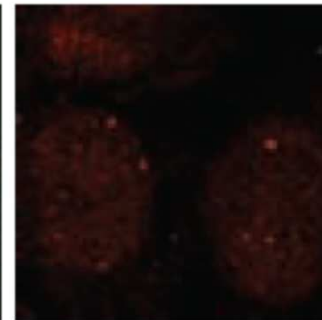
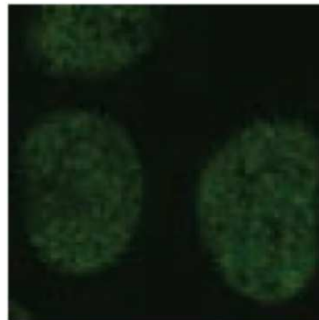
siCTRL  
No IR



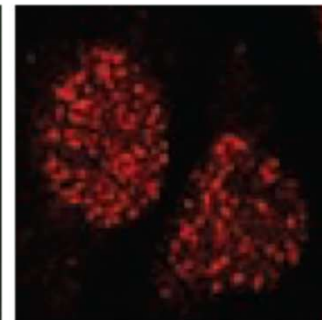
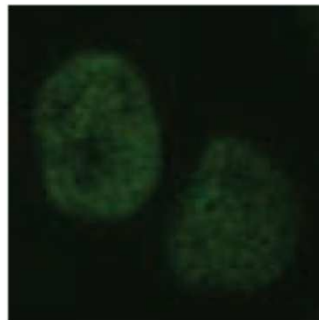
siCTRL  
10Gy

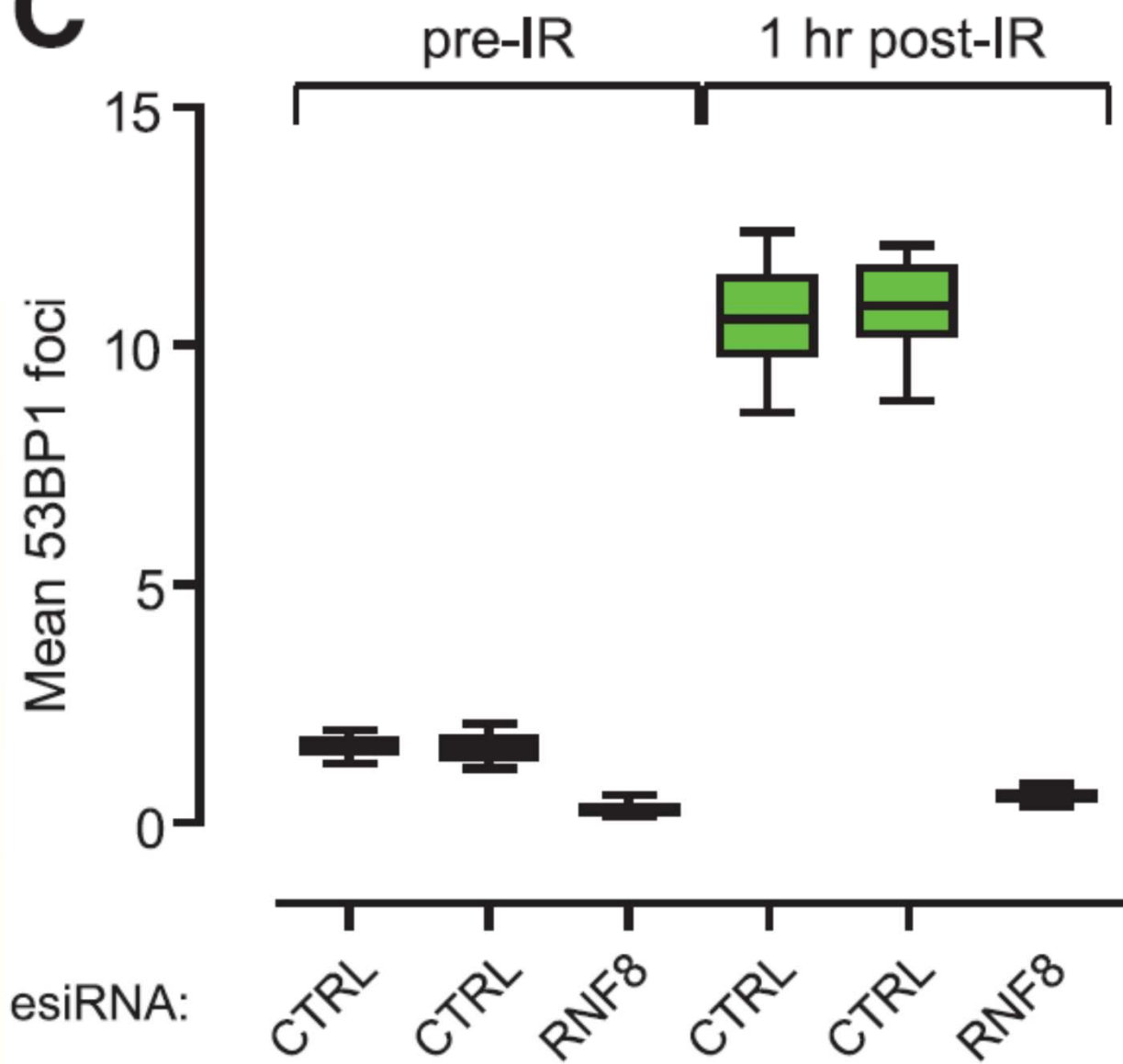


siRNF8  
No IR

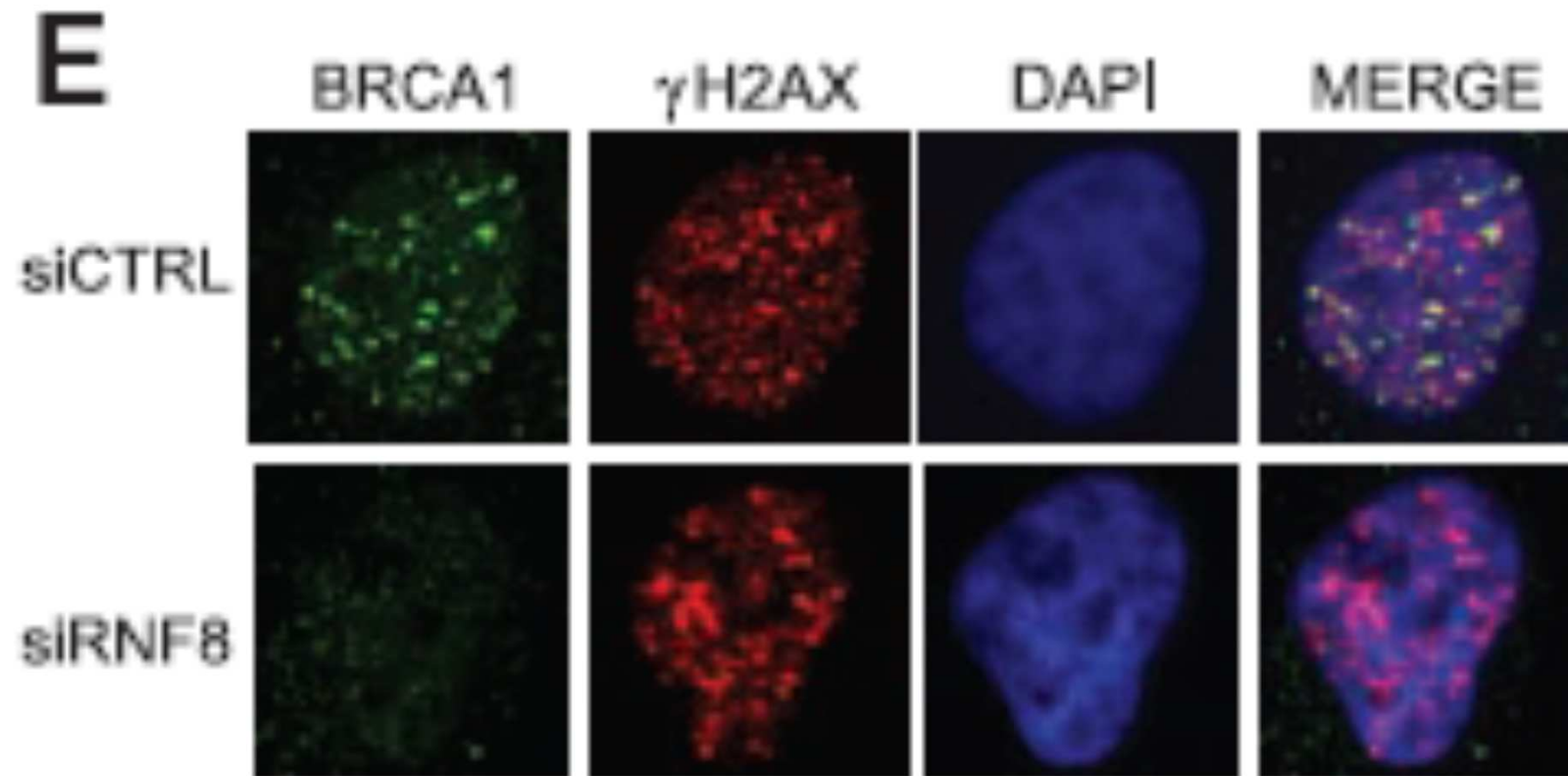


siRNF8  
10Gy



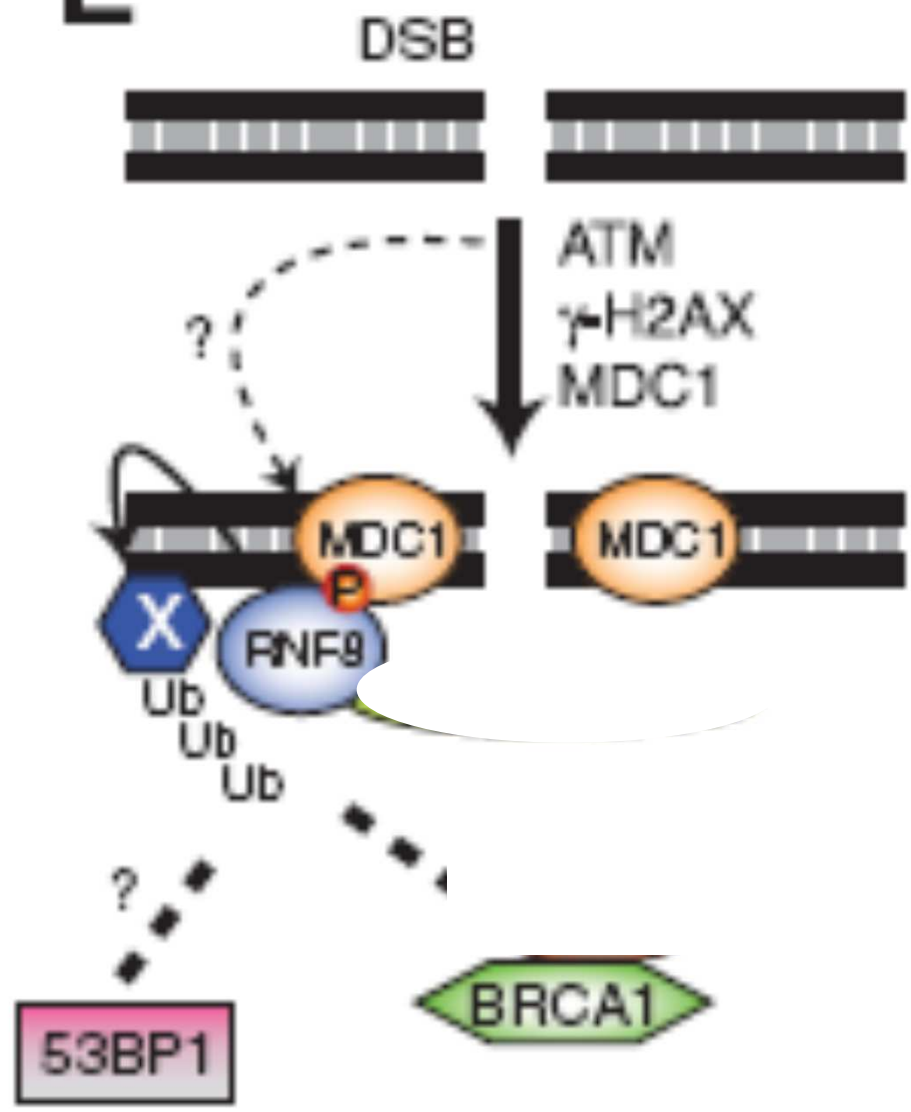
**C**





Irradiated (10 Gy) HeLa cells transfected with the indicated siRNAs were stained with antibodies to  $\gamma$ H2AX, BRCA1

E



# Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase (Nadine Science Feb2008)

- Cells respond to DSBs by recruiting the DNA-damage mediator protein MDC1, the p53-binding protein 1 (53BP1), and the breast cancer susceptibility protein BRCA1 to sites of damaged DNA.
- **The ubiquitin ligase RNF8 mediates ubiquitin conjugation and 53BP1 and BRCA1 focal accumulation at sites of DNA lesions.**

MDC1 recruits RNF8 through phosphodependent interactions between the RNF8 forkhead-associated domain and motifs in MDC1 that are phosphorylated by the DNA-damage activated protein kinase ataxia telangiectasia mutated (ATM).

Depletion of the E2 enzyme UBC13 impairs 53BP1 recruitment to sites of damage, which suggests that it cooperates with RNF8.

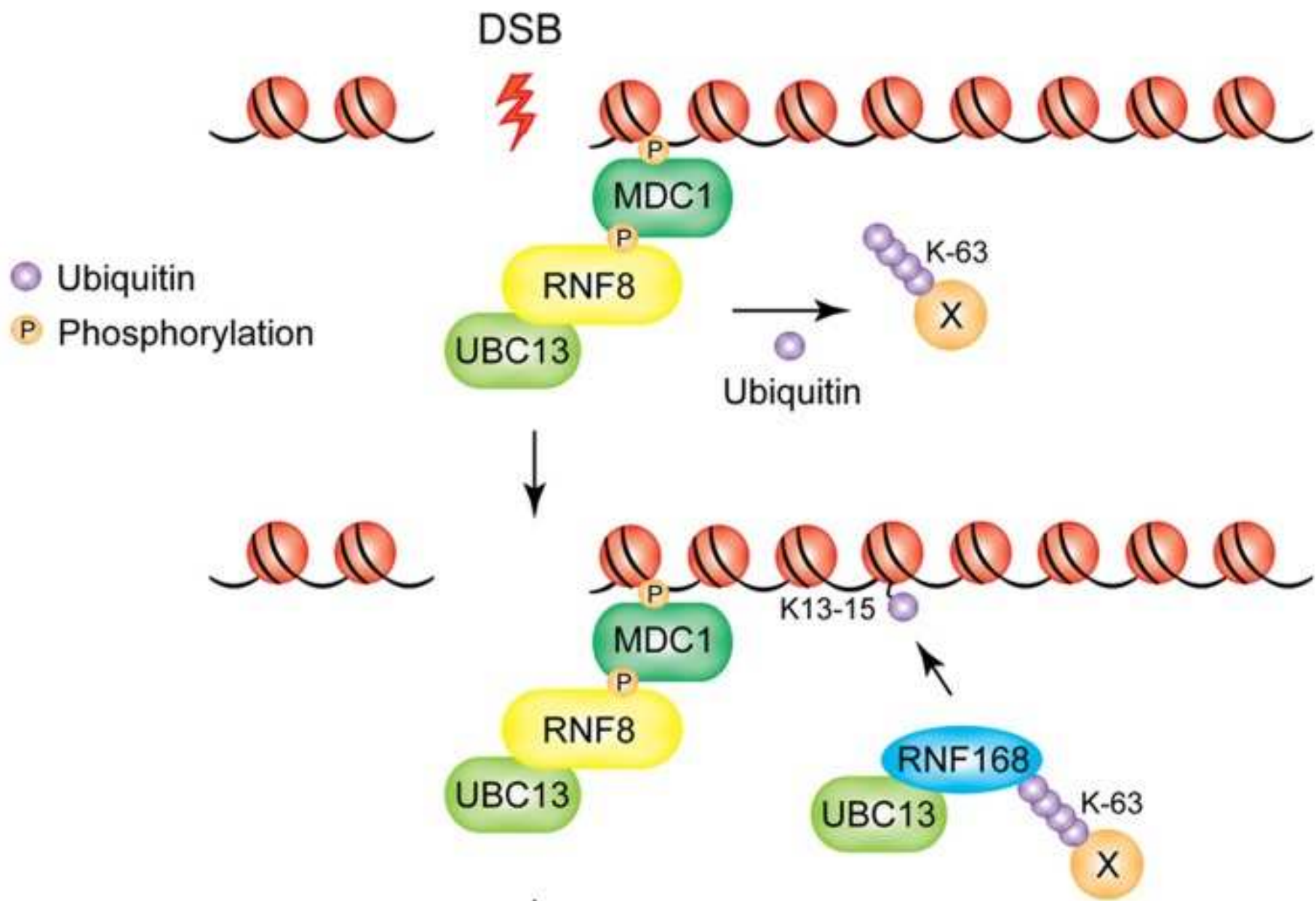
RNF8 promotes the G2/M DNA damage checkpoint and resistance to ionizing radiation.

the DNA-damage response is orchestrated by ATM-dependent phosphorylation of MDC1 and RNF8-mediated ubiquitination.

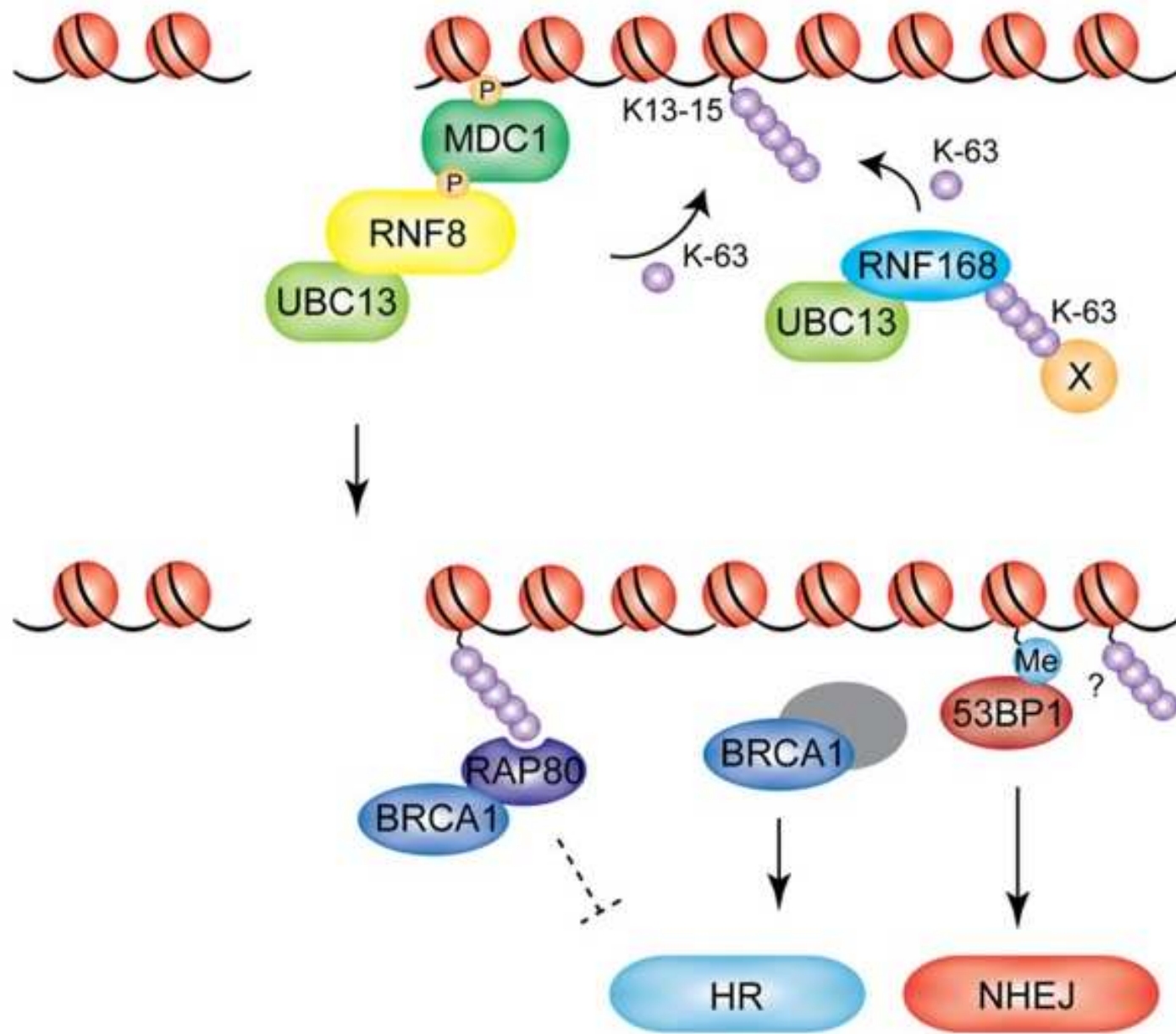
- It is possible to interfere with ubiquitin-dependent recruitment of DDR factors by expressing proteins containing ubiquitin binding domains (UBDs) that bind to lysine 63-linked polyubiquitin chains.

# RNF8 e RNF168

- The rapid ubiquitination of chromatin surrounding DNA double-stranded breaks (DSB) drives the formation of large structures called ionizing radiation-induced foci (IRIF), comprising many DNA damage response (DDR) proteins.
- This process is regulated by RNF8 and RNF168 ubiquitin ligases and is thought to be necessary for DNA repair and activation of signaling pathways involved in regulating cell cycle checkpoints.



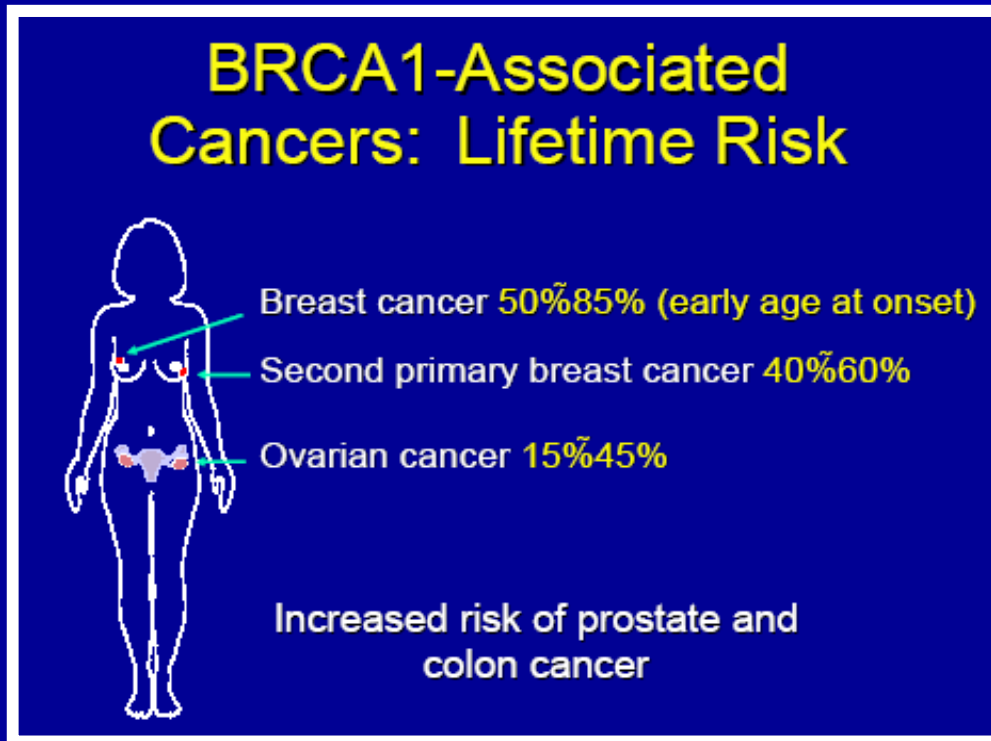




# BRCA 1

- impairment of homologous repair in Brca1-deficient mouse embryonic stem cells
- increase in the frequency of NHEJ in Brca1-deficient cells

Mutazioni in BRCA 1 o 2 → inattivazione meccanismo HRR  
→ predisposizione allo sviluppo di **Carcinoma mammario ereditario**, con insorgenza precoce tumore seno e ovaie



**BRCA 1:** 50% mutazioni tumore mammario familiare

**BRCA 2:** 35% mutazioni tumore mammario familiare

Eredità di un allele mutante → predisposizione al tumore, che insorge solo quando la seconda copia del gene è persa o mutata (**perdita di eterozigotità**)

# Riparazione per ricombinazione omologa (HRR)

Ripara le DSBs (Double-Strand Breaks) causate da radiazioni, stress ossidativo, farmaci

Replicazione e trascrizione vengono bloccate nel sito della DSB e le estremità esposte sono soggette a degradazione con perdita di materiale genetico → importanza HRR

HRR utilizza come stampo il cromatidio fratello → protezione dagli errori

HRR avviene in tarda fase S o in G<sub>2</sub>, quando i cromatidi fratelli sono vicini

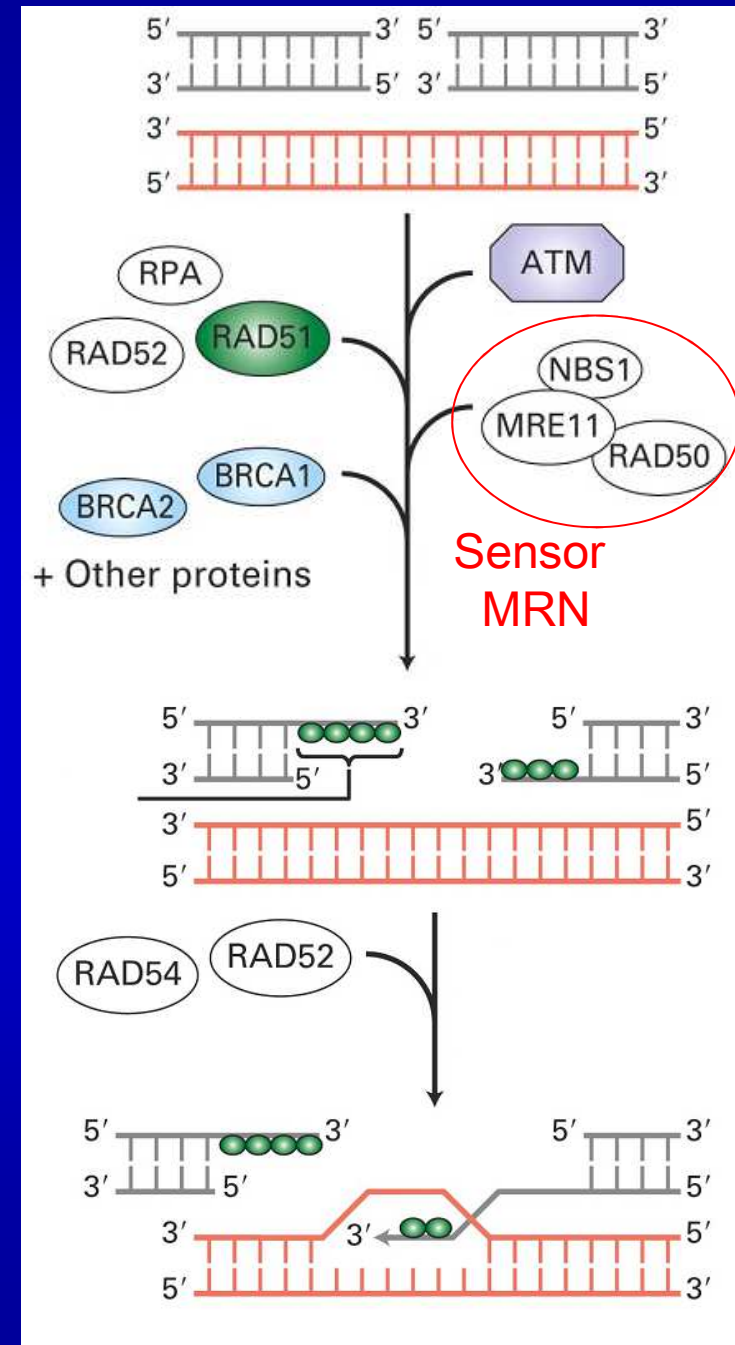
# RPA

(ss DNA replication binding protein)

la polimerizzazione di **RAD51** sul 3' libero è **BRCA1/2**-dipendente



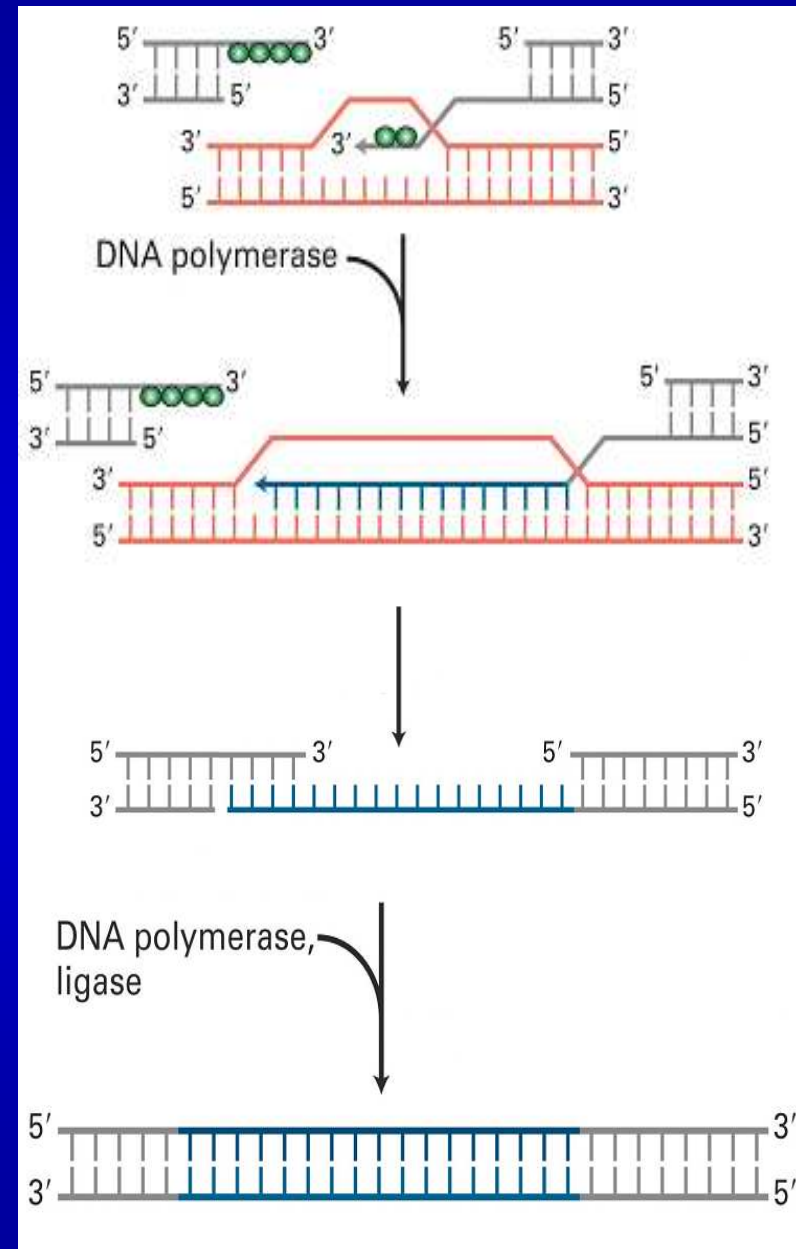
**RAD51** (aiutata dall'elicasi **RAD54**) cerca la sequenza omologa sul cromatidio fratello e invade la doppia elica; le regioni 3' a singola elica si appaiano con quelle complementari



La DNA polimerasi allunga l'estremità 3' libera (in cui si trova la nucleoproteina RAD51) utilizzando come stampo il cromatidio omologo non danneggiato

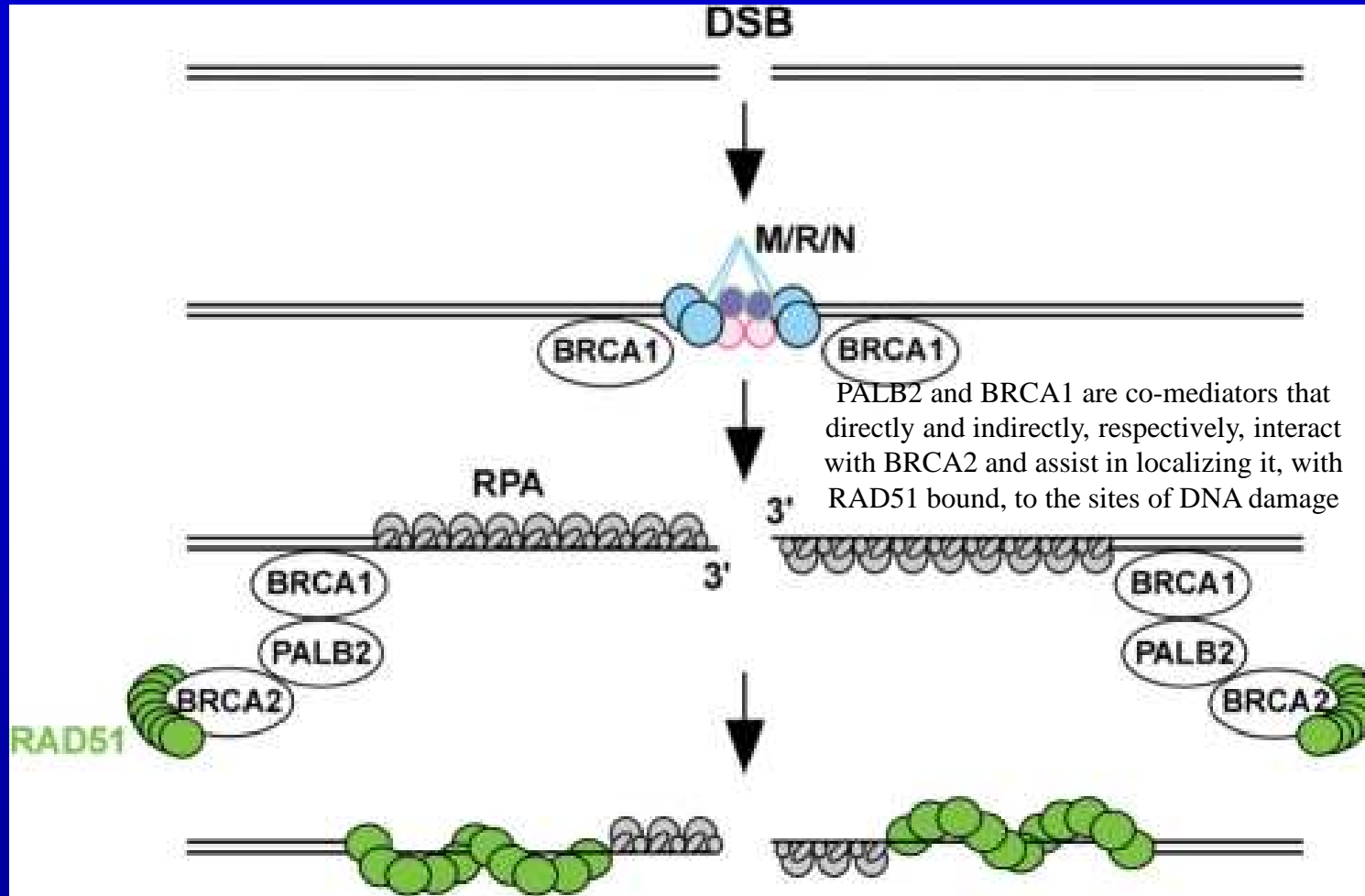


L'altra regione 3' a singola elica attorno alla zona danneggiata si appaia all'elica ormai corretta e gli eventuali gap sono riempiti da polimerasi e ligasi



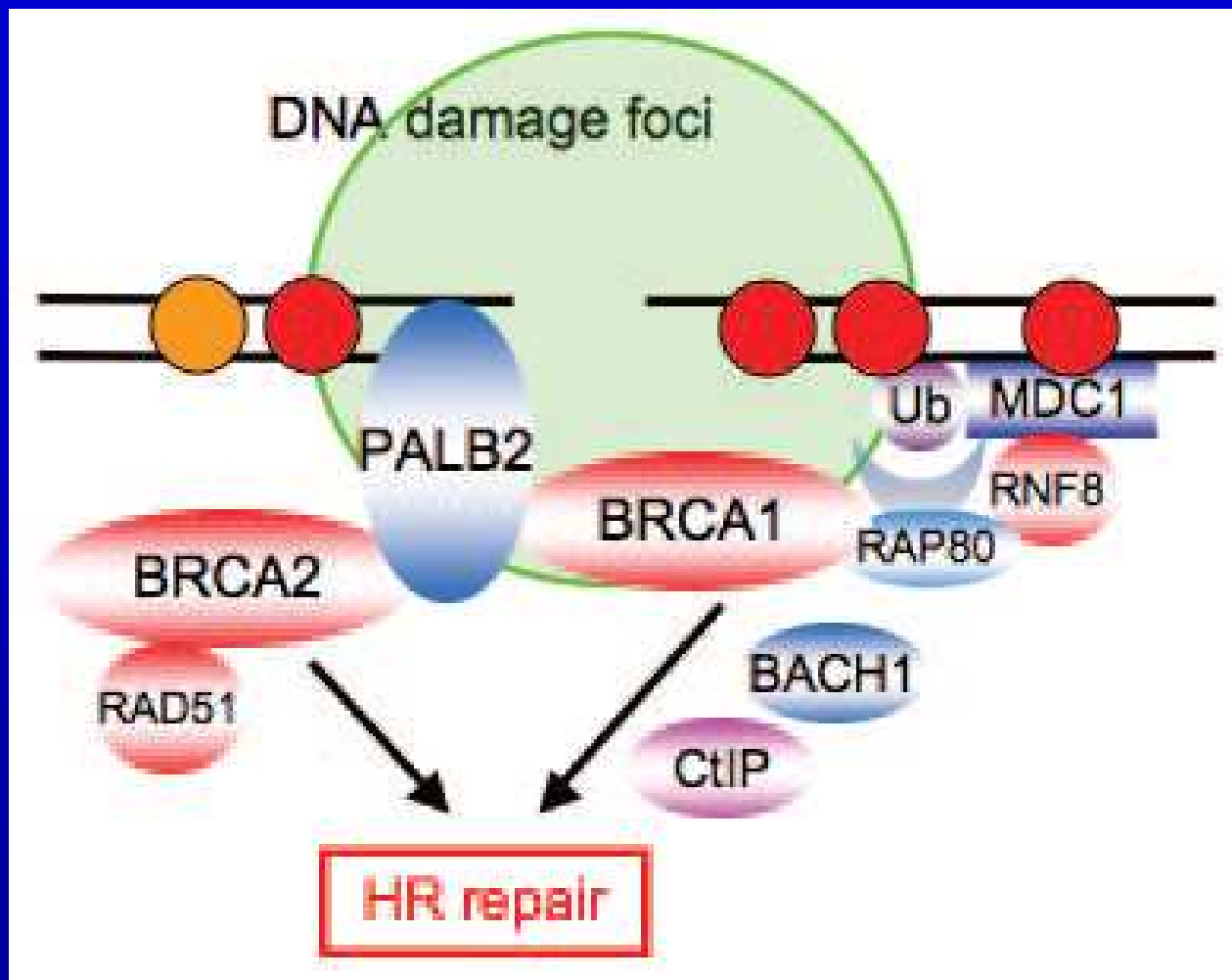


# BRCA1/BRCA2



Human BRCA2 is a mediator that interacts directly with approximately eight RAD51 molecules and transports them to the site of ss-DNA bound by RPA

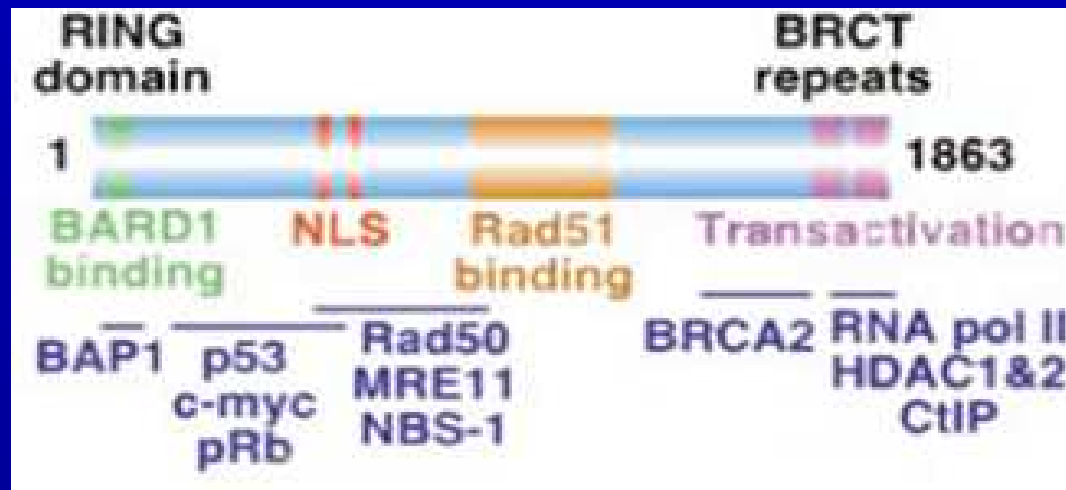




# BRCA 1

- Gene oncosoppressore, localizzato sul cromosoma 17
- Espressione nel nucleo di molti tipi cellulari

**N-ter:**  
dominio  
**RING  
FINGER**,  
coordina il  
legame con  
2 ioni zinco  
→ contatta  
DNA



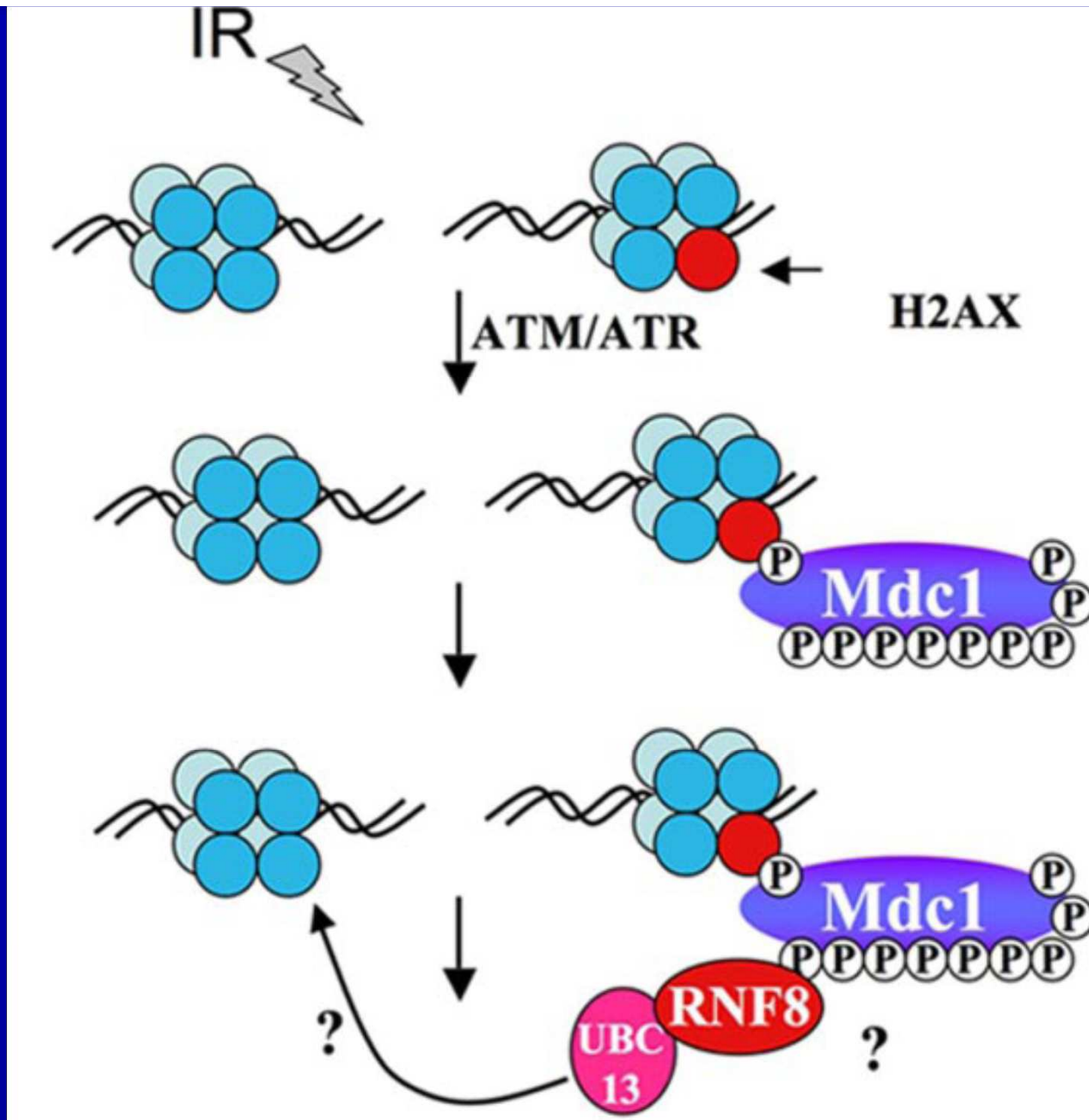
**C-ter:**  
dominio  
**BRCT** →  
induce /  
reprime  
geni;  
interazione  
con RNA  
pol II

# BRCA 1

- An E3 ubiquitin ligase mediates the transfer of activated ubiquitin from an E2 ubiquitin-conjugating enzyme to its substrate lysine residues.
- BRCA1 has the ability to direct the synthesis of specific polyubiquitin chain linkages, depending on the E2 bound to its RING.

# BRCA 1

- The BRCA1 tumor suppressor exists as a heterodimeric complex with BARD1, and this complex is thought to mediate many of the functions ascribed to BRCA1, including its role in tumor suppression.
- The two proteins share a common structural organization : an N-terminal RING domain and two C-terminal BRCT motifs
- 
- the BRCA1/BARD1 heterodimer enhances chromosome stability by promoting homology-directed repair (HDR) of double strand DNA breaks.

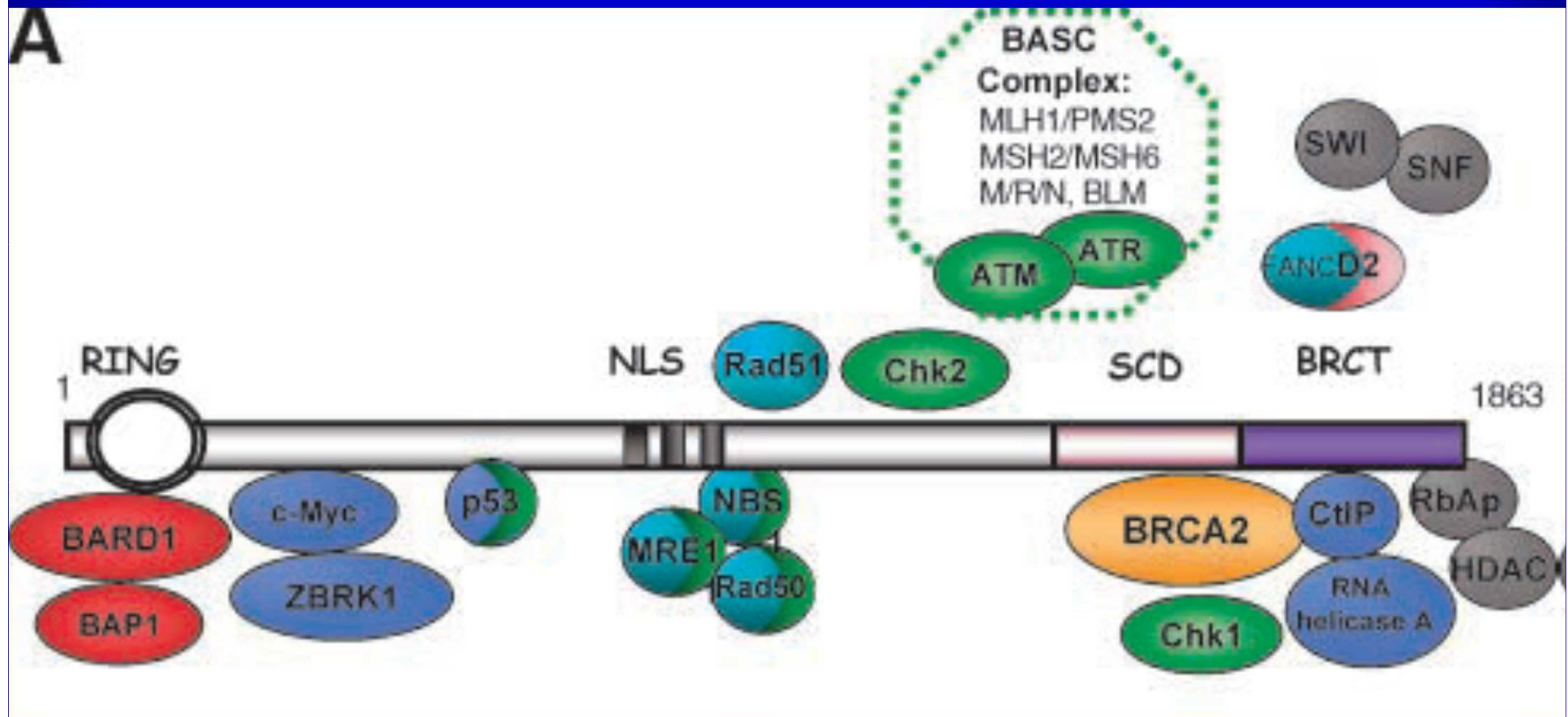


Ubc13 and Rnf8 are recruited to sites of DNA damage through DNA-damage-induced phosphorylation of a chromatin-associated protein

# BRCA MUTAZIONI ED INTERAZIONI

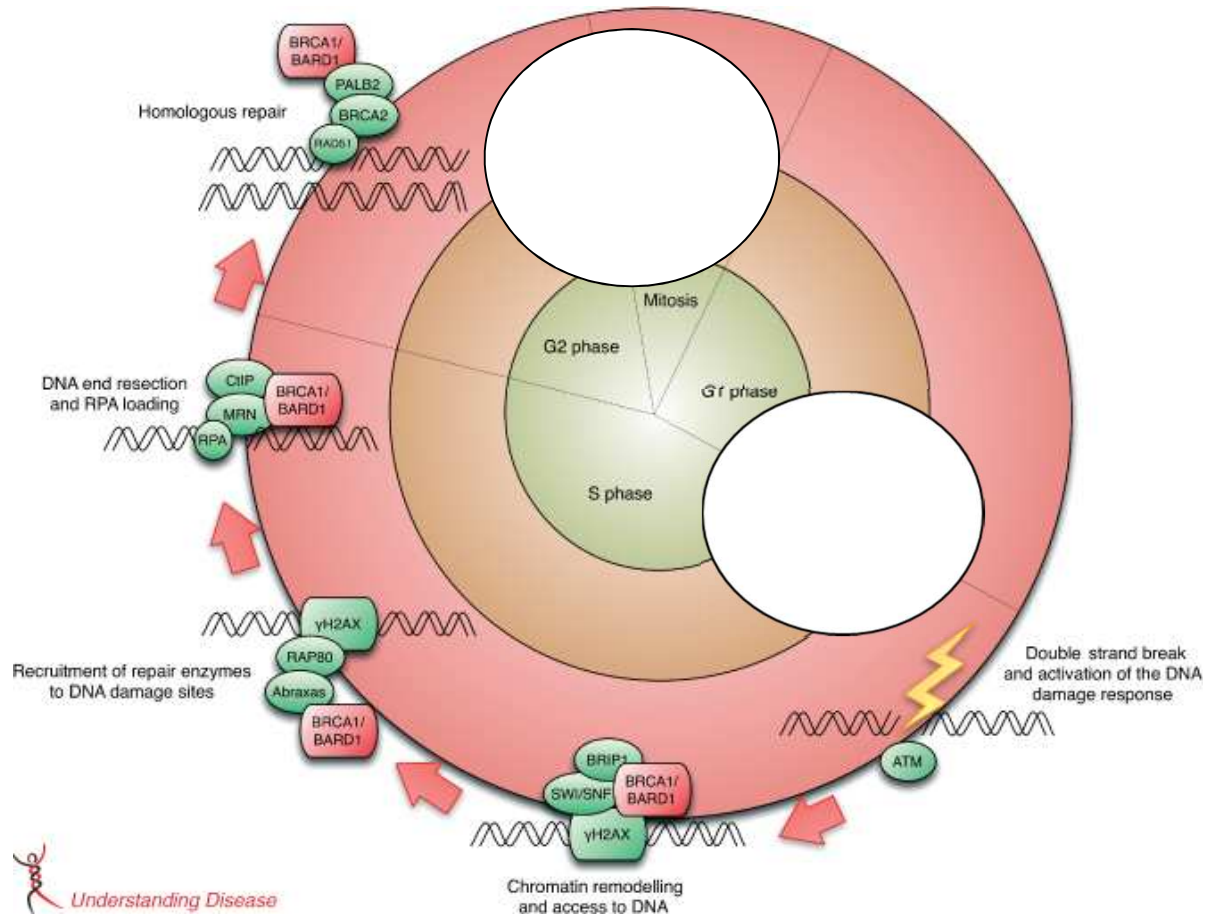


# BRCA1 is implicated in multiple cellular functions

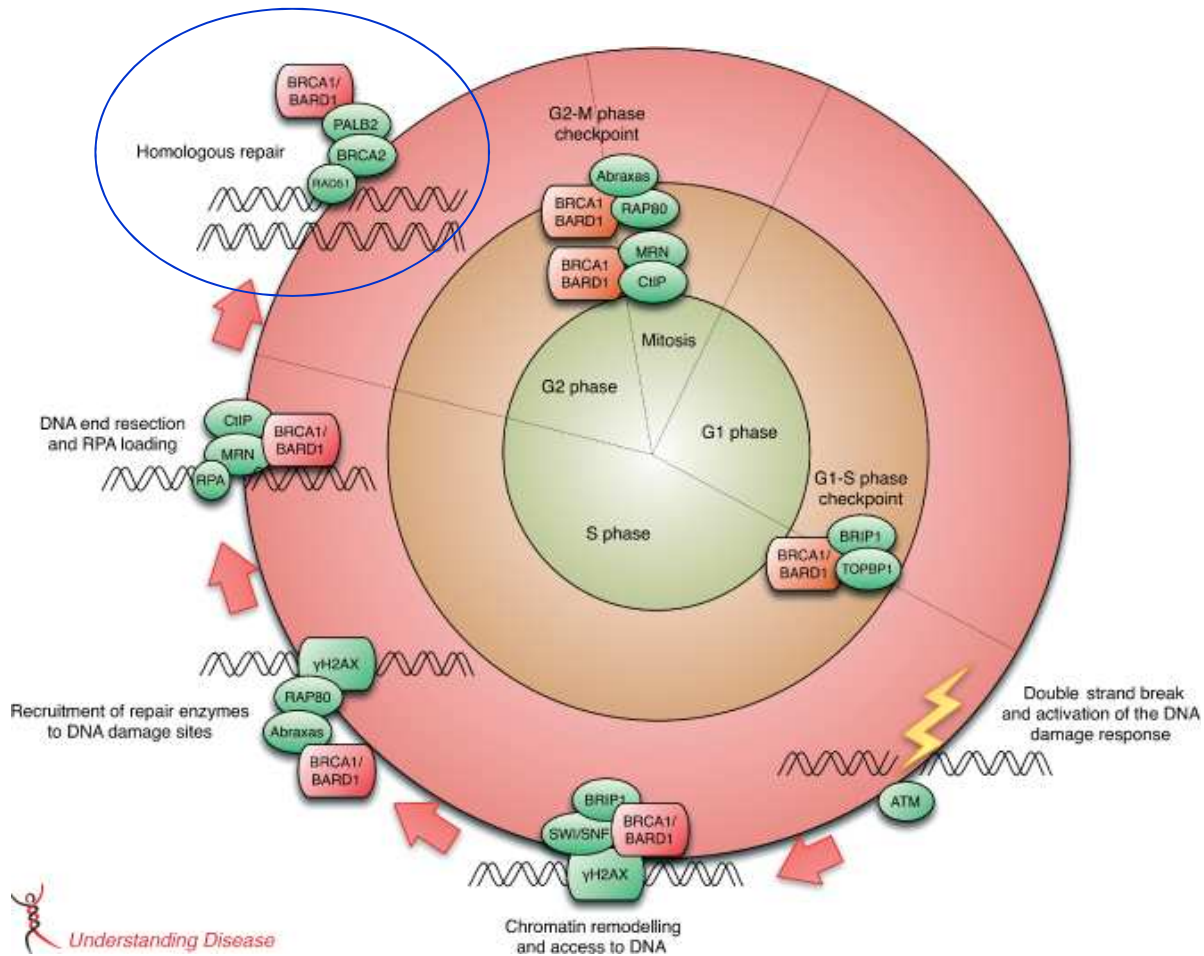


Red, ubiquitylation; Blue, transcription; Green, cell cycle checkpoint control; Light blue, DNA repair; Gray, chromatin modifications.

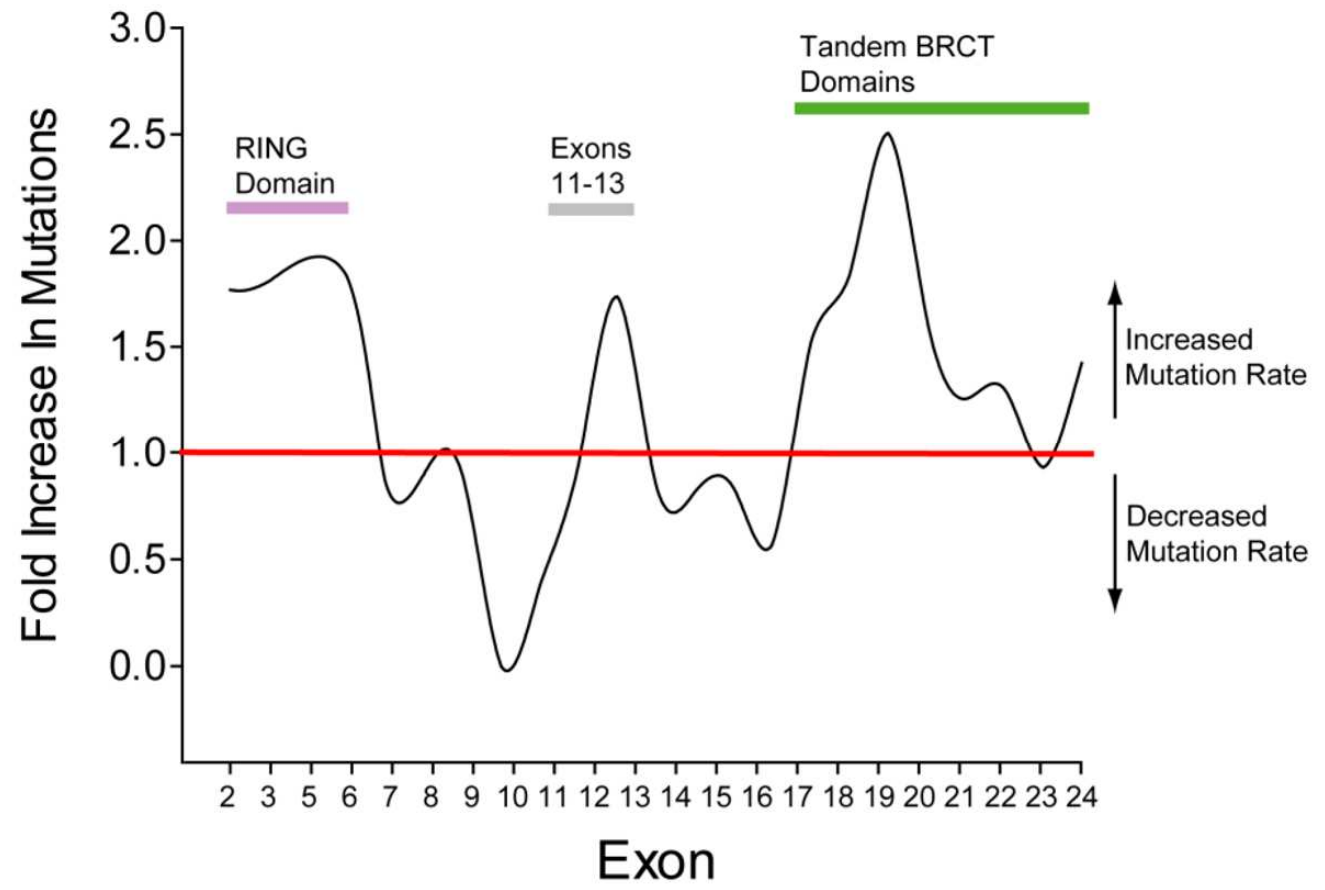
## In Brief: *BRCA1* and *BRCA2*



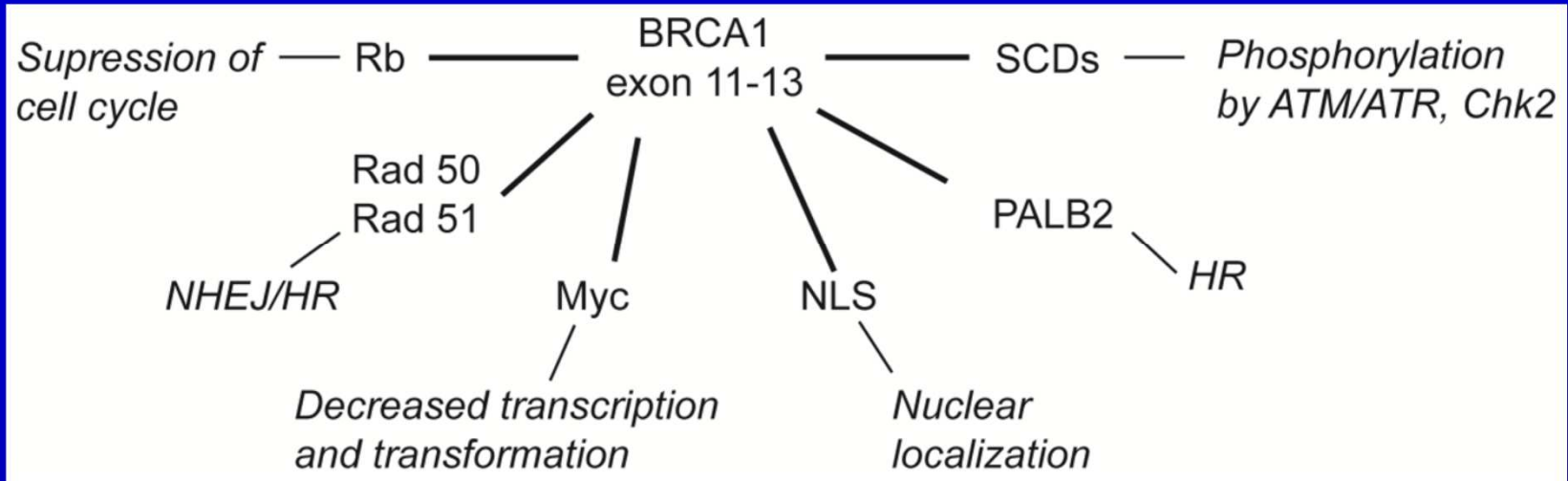
## In Brief: *BRCA1* and *BRCA2*



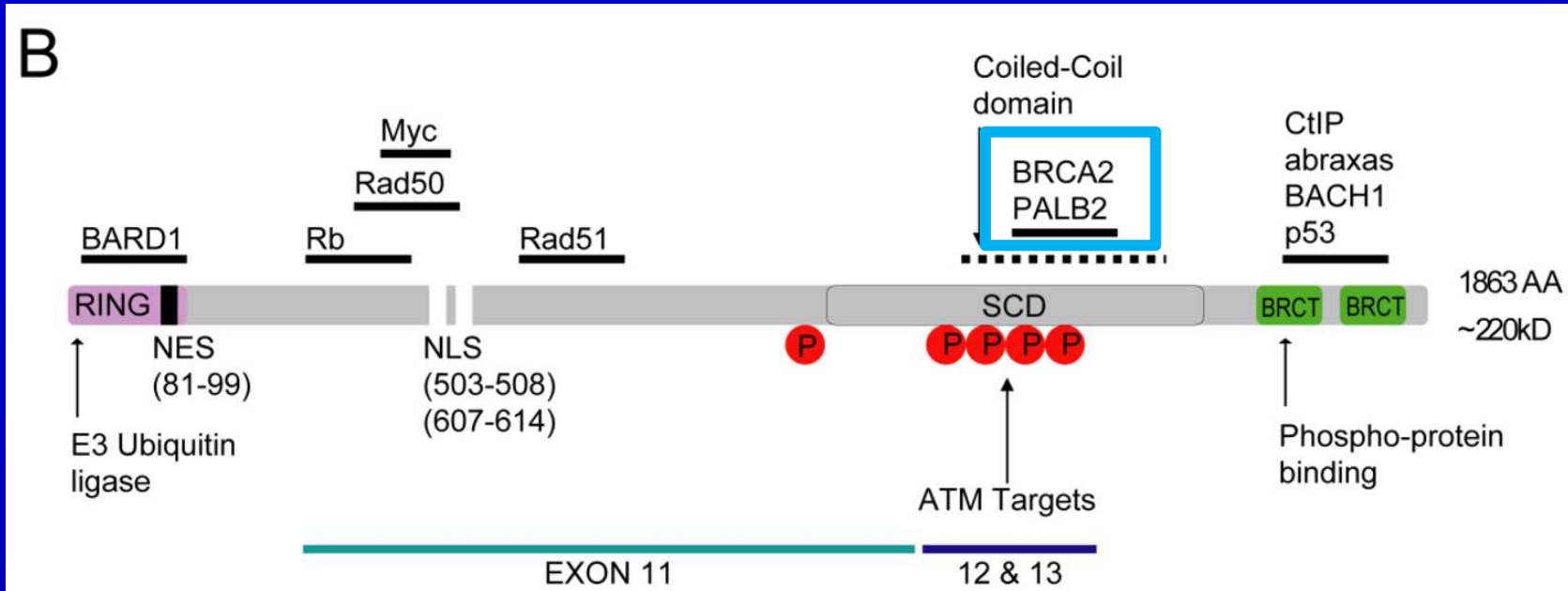
A



D

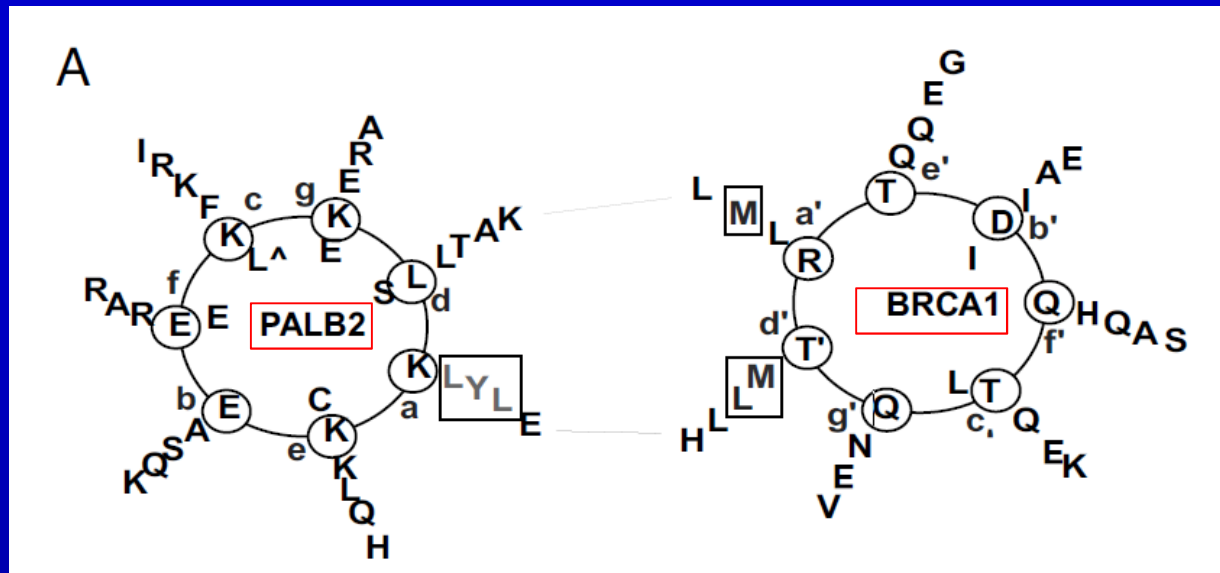


# BRCA1 is implicated in multiple cellular functions



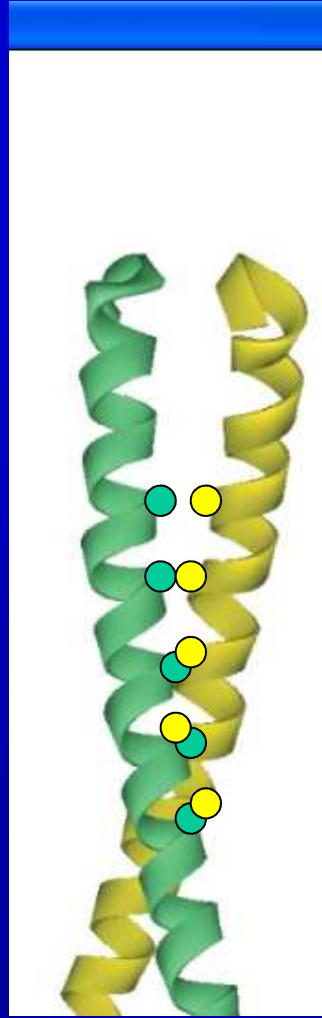
**PALB2 binds directly to BRCA1 and serves as the molecular scaffold in the formation of the BRCA1-PALB2-BRCA2 complex.**

regions required for the BRCA1-PALB2 interaction.



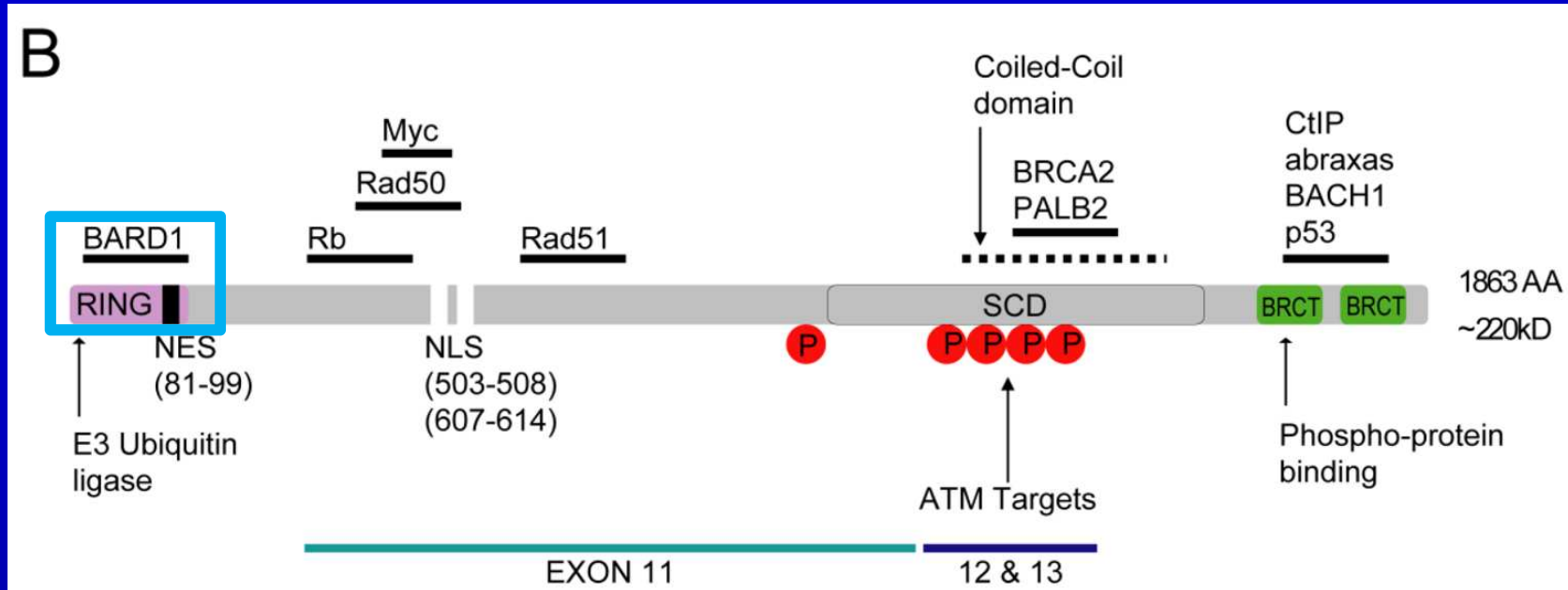
(A) Graphical projection of association between PALB2 (residues 9 – 42) and BRCA1 (residues 1393–1424) coiled-coil domains.

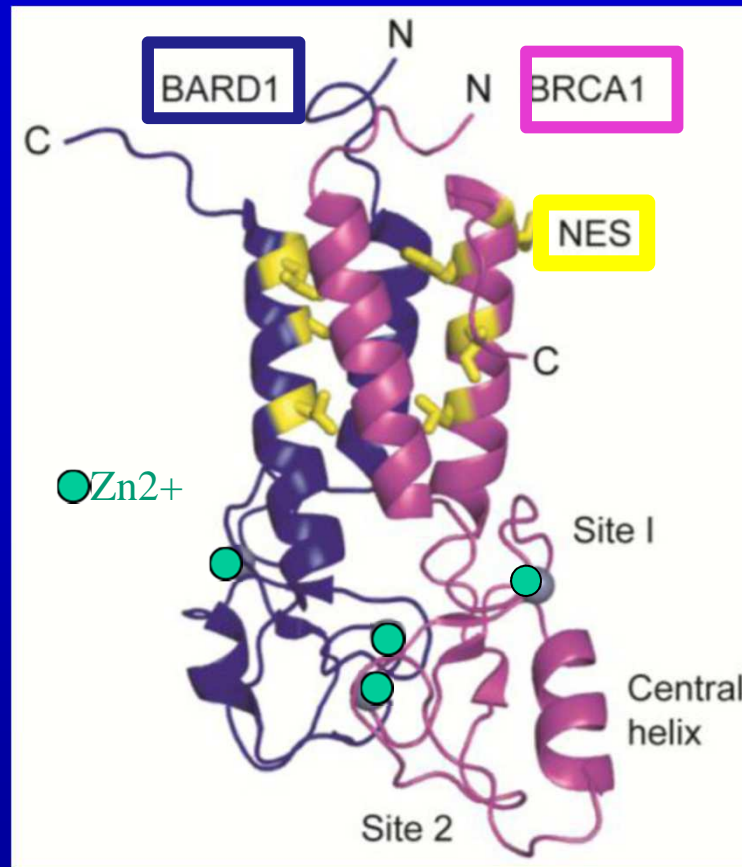
Positions of the heptad repeat (positions a to g) were predicted by the Coil program  
Boxed residues were experimentally demonstrated to be responsible for the hetero-oligomeric interaction between PALB2 and BRCA1.

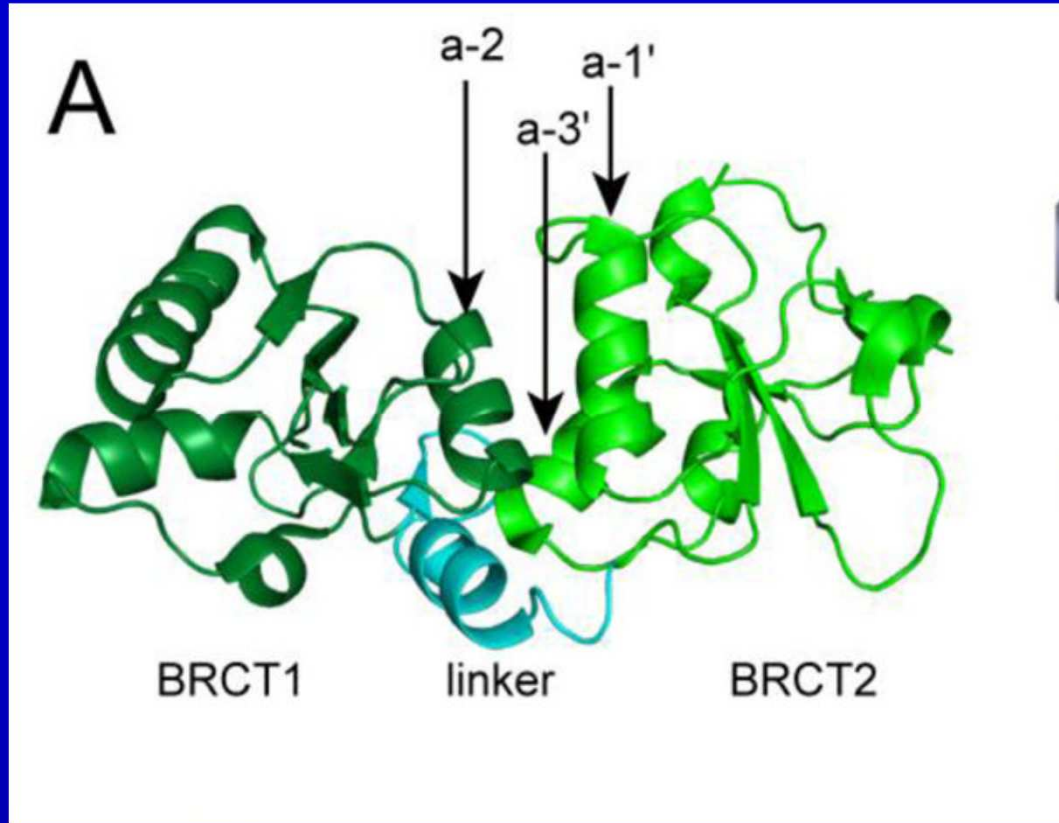




# BRCA1 is implicated in multiple cellular functions

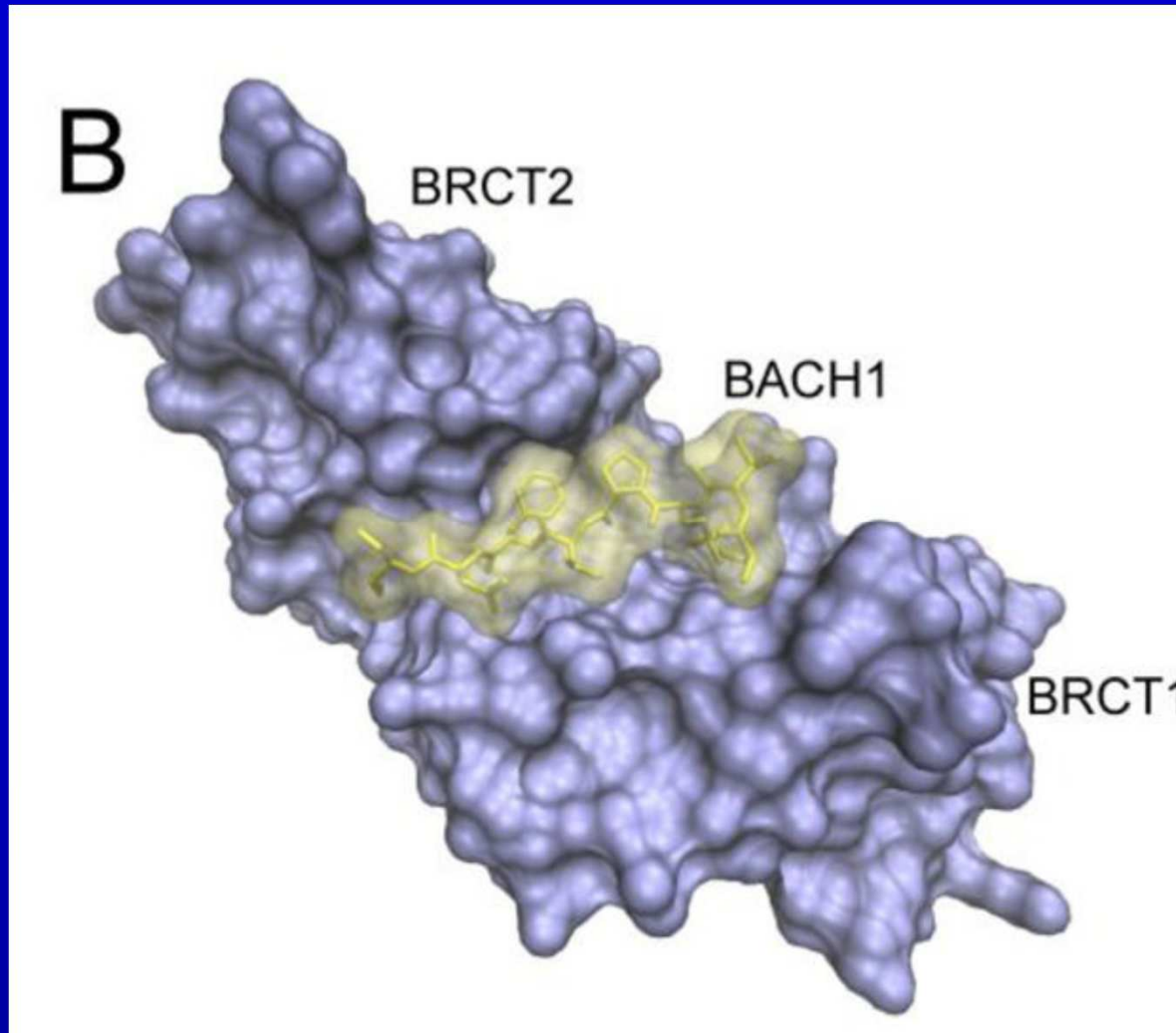






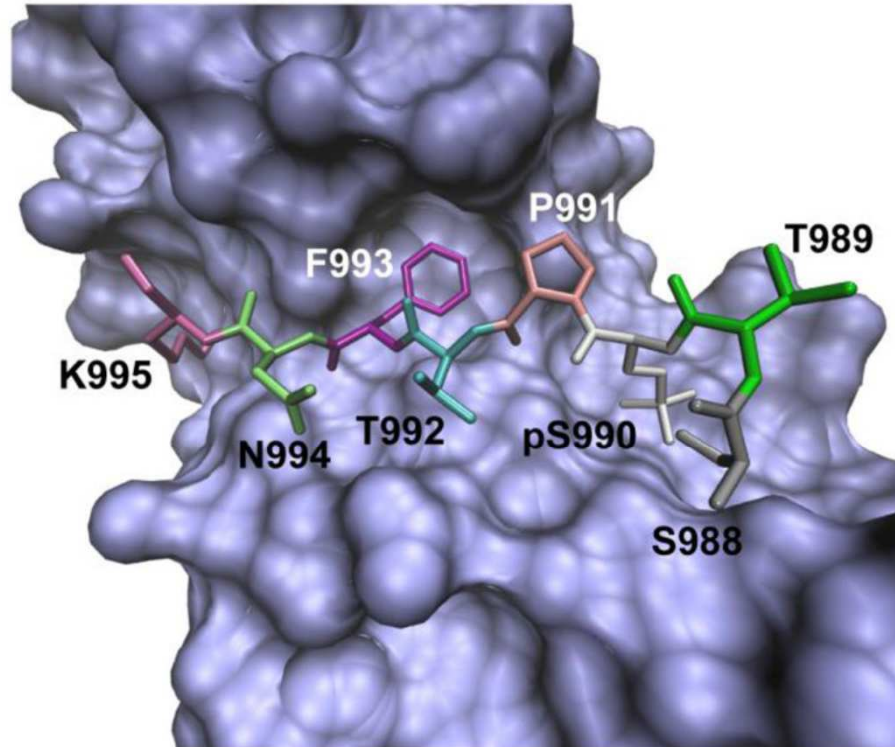
BRCT1 and BRCT2 pack together in a head-to-tail orientation and are connected by a linker helix.

Helix 2 from BRCT1 and helices 1 and 3 from BRCT2 form a hydrophobic core and stabilize the structure.



The cleft between BRCT1 and BRCT2 forms the binding pocket for proteins phosphorylated by ATM and ATR.

C



ST1

The consensus sequence for BRCA1 BRCT recognition of phosphoproteins is 990pSer-X-X-Phe993.

BRCA1 BRCT/BACH1 binding pocket.

•Lega p53 attivando p21



Regolazione delle proliferazione cellulare

•Attiva TNF $\alpha$

•Lega Rad50/51 e BRCA2

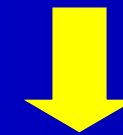
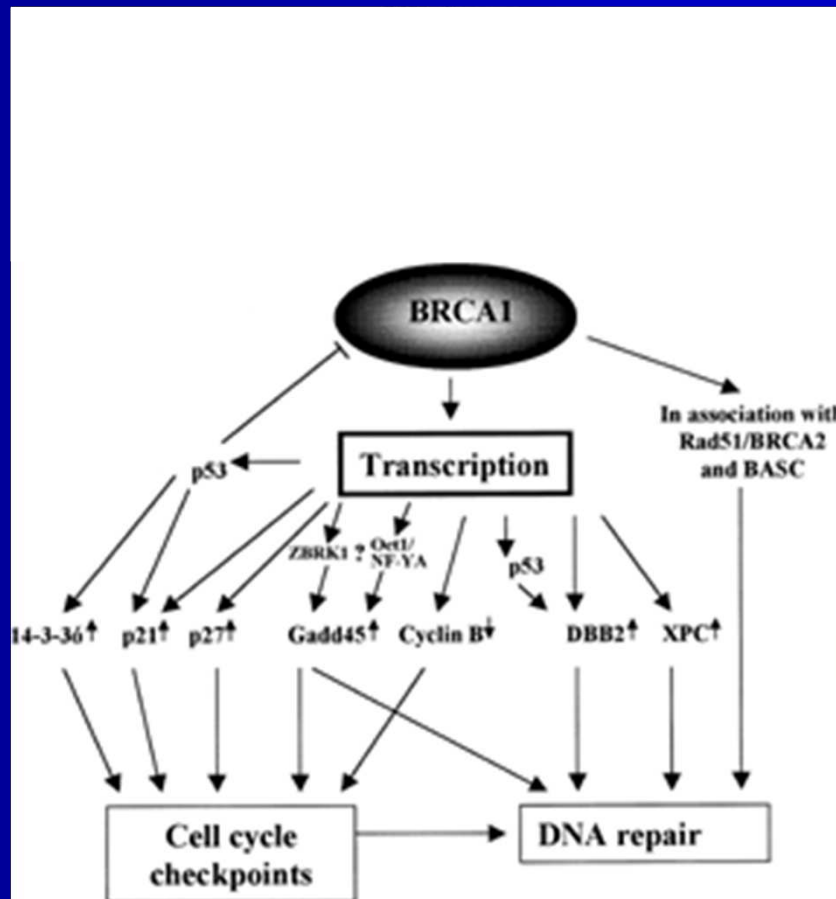


Riparazione HRR

•Inibisce la trasduzione del segnale da parte del recettore per l'estrogeno



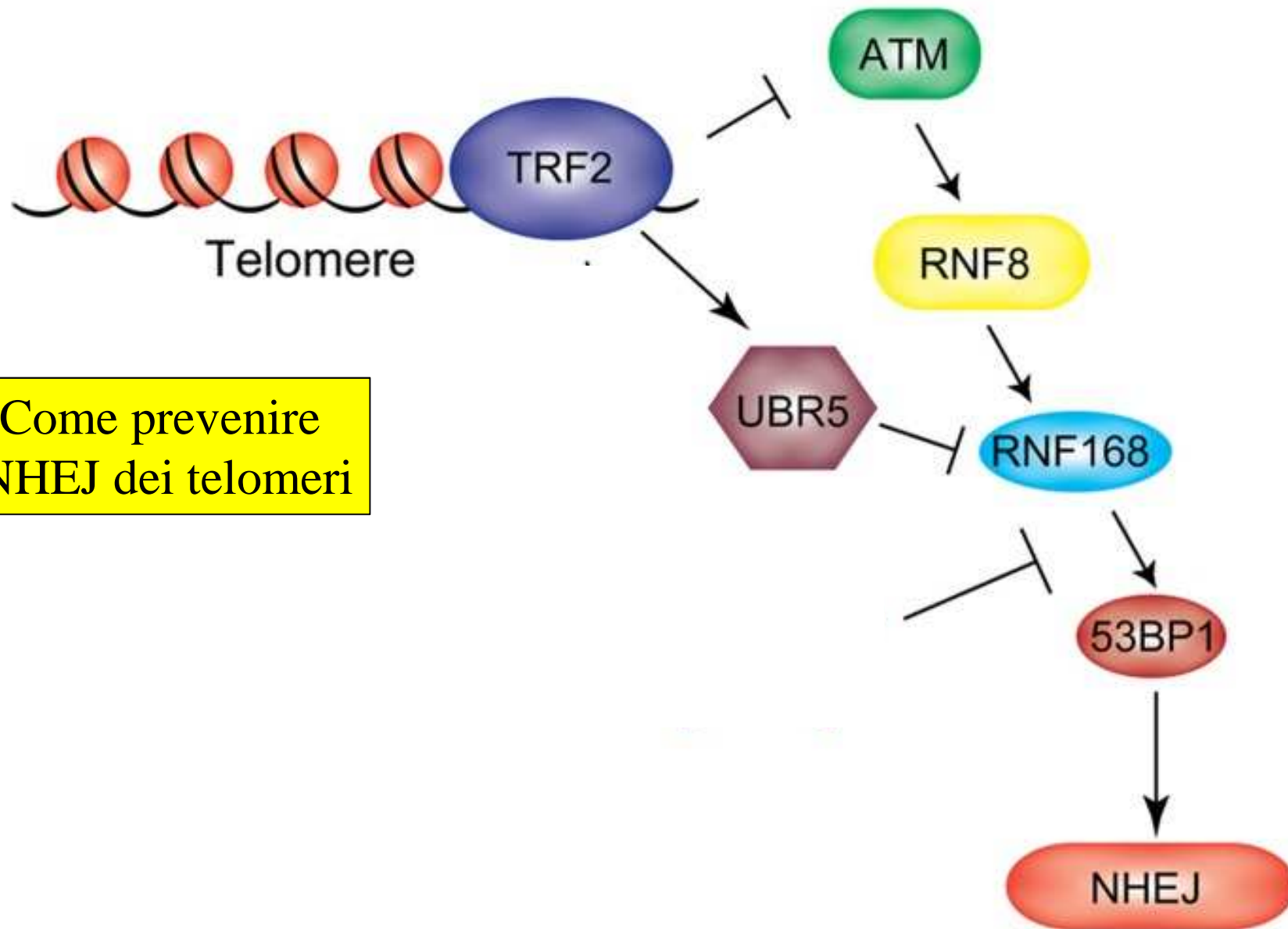
Reprime la proliferazione dell'epitelio mammario che è estrogeno-dipendente



**Mutazioni in BRCA1 possono determinare un'incontrollata proliferazione, in particolare delle cellule che rispondono all'estrogeno  $\rightarrow$  tumore**







Come prevenire  
NHEJ dei telomeri