

# **DSB**

**Double-Strand Breaks**

**causate da**

**radiazioni**

**stress ossidativo**

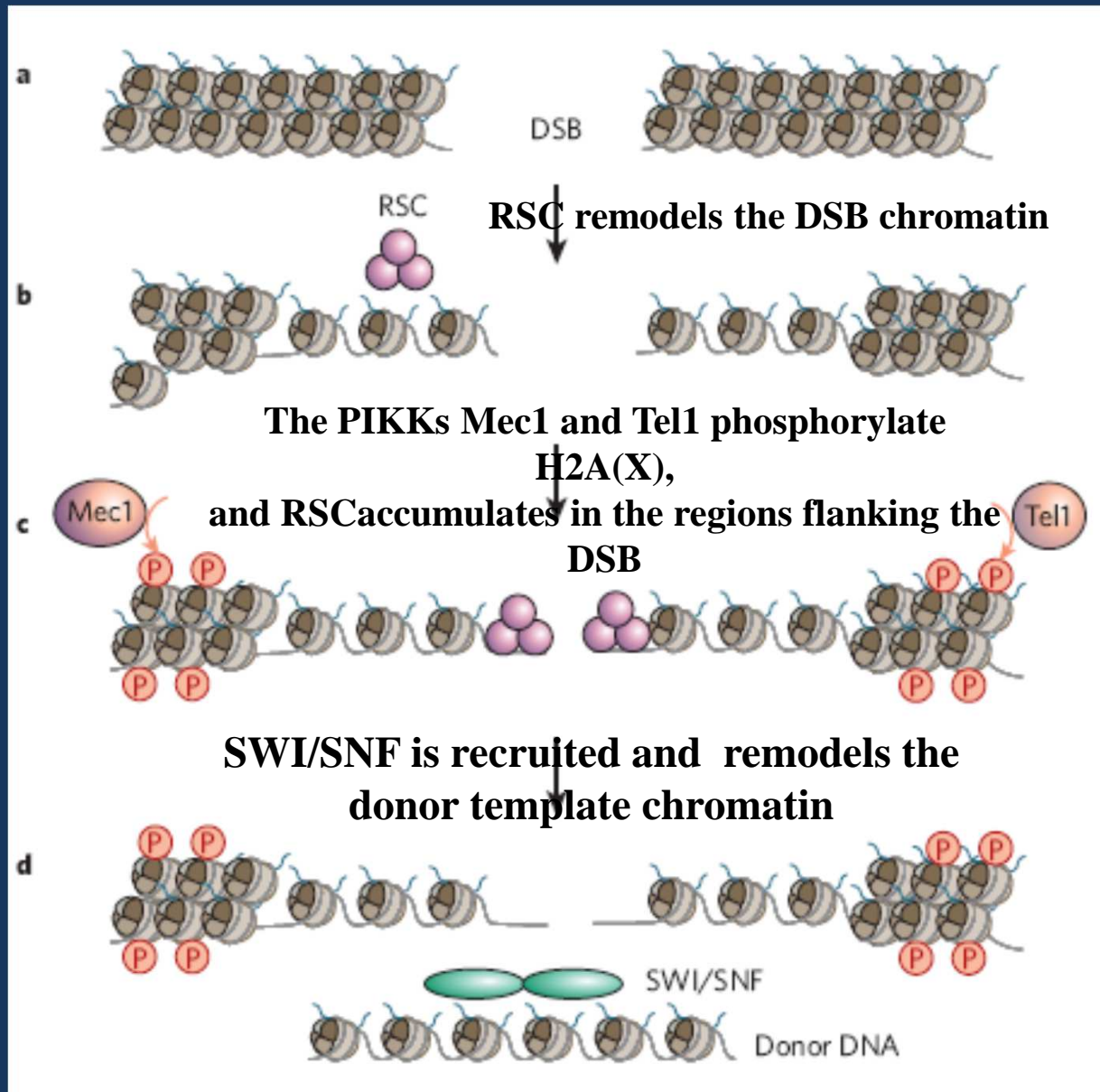
**farmaci**

# DSB

## e CROMATINA

- Higher-order chromatin packaging is a barrier to the detection and repair of DNA damage
- **DSBs induce a local decrease in the density of the chromatin fibre, in addition to altering the position of nucleosomes**
- DSBs also elicit post-translational modifications on the protruding histone tails

# Chromatin remodelling and DSBs



# RSC

complex RSC (remodels the structure of chromatin)

ATP-dependent chromatin-remodelling

RSC can mediate nucleosome sliding, alter histoneDNA contacts and remove histones from DNA.

The chromatin-remodelling activity of RSC is important for transcriptional regulation of genes that are involved in stress responses and cell-cycle progression

Transcription of Site-specific RNA to be matured by DICER and DROSHA???

POSIZIONAMENTO

NUCLEOSOMA

Rimodellamento

Cromatina

sito 1  
di legame  
alle proteine

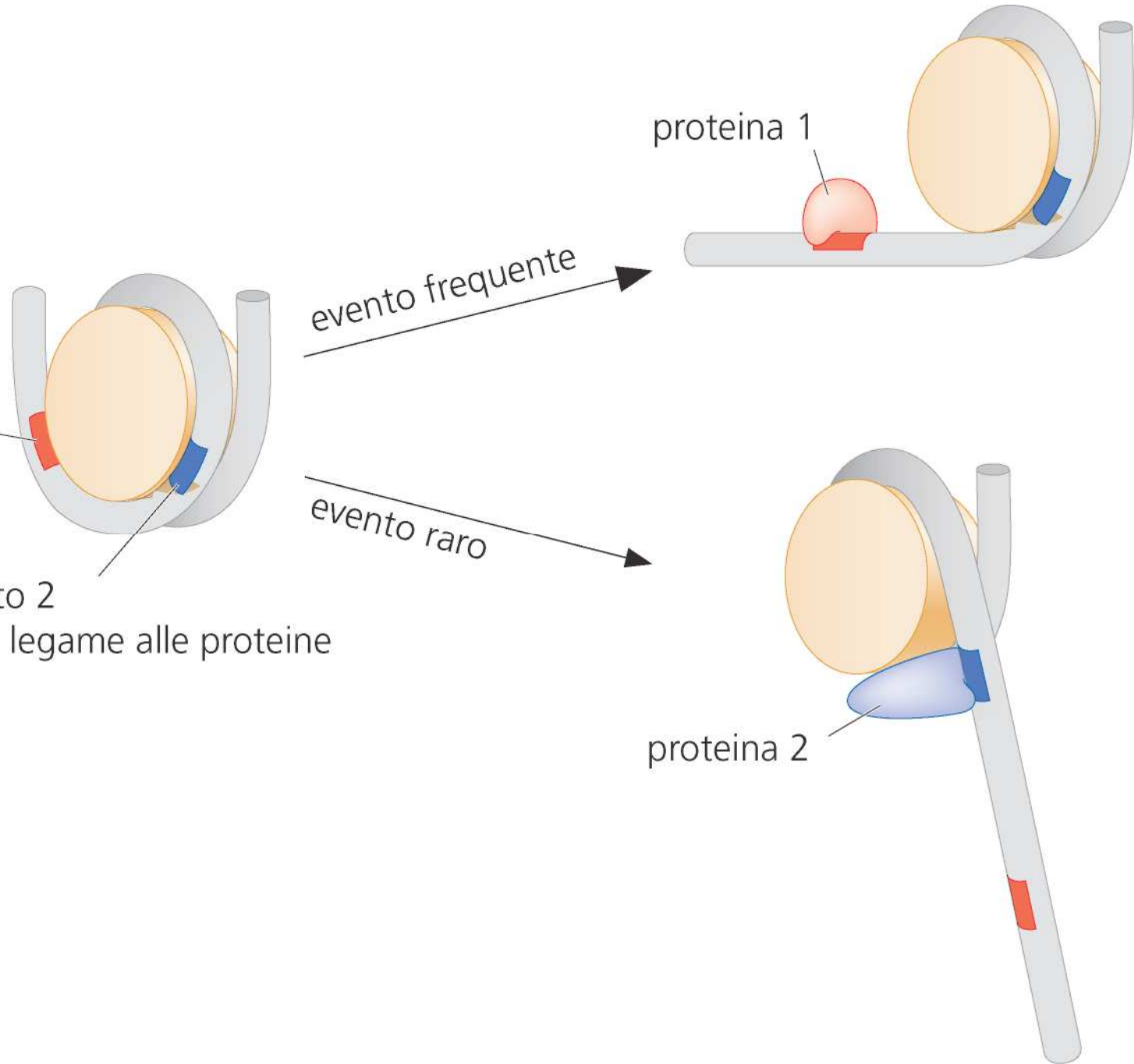
sito 2  
di legame alle proteine

evento frequente

evento raro

proteina 1

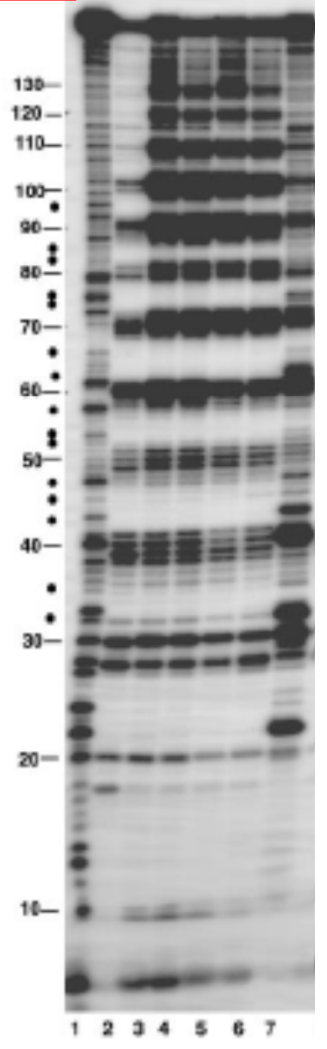
proteina 2



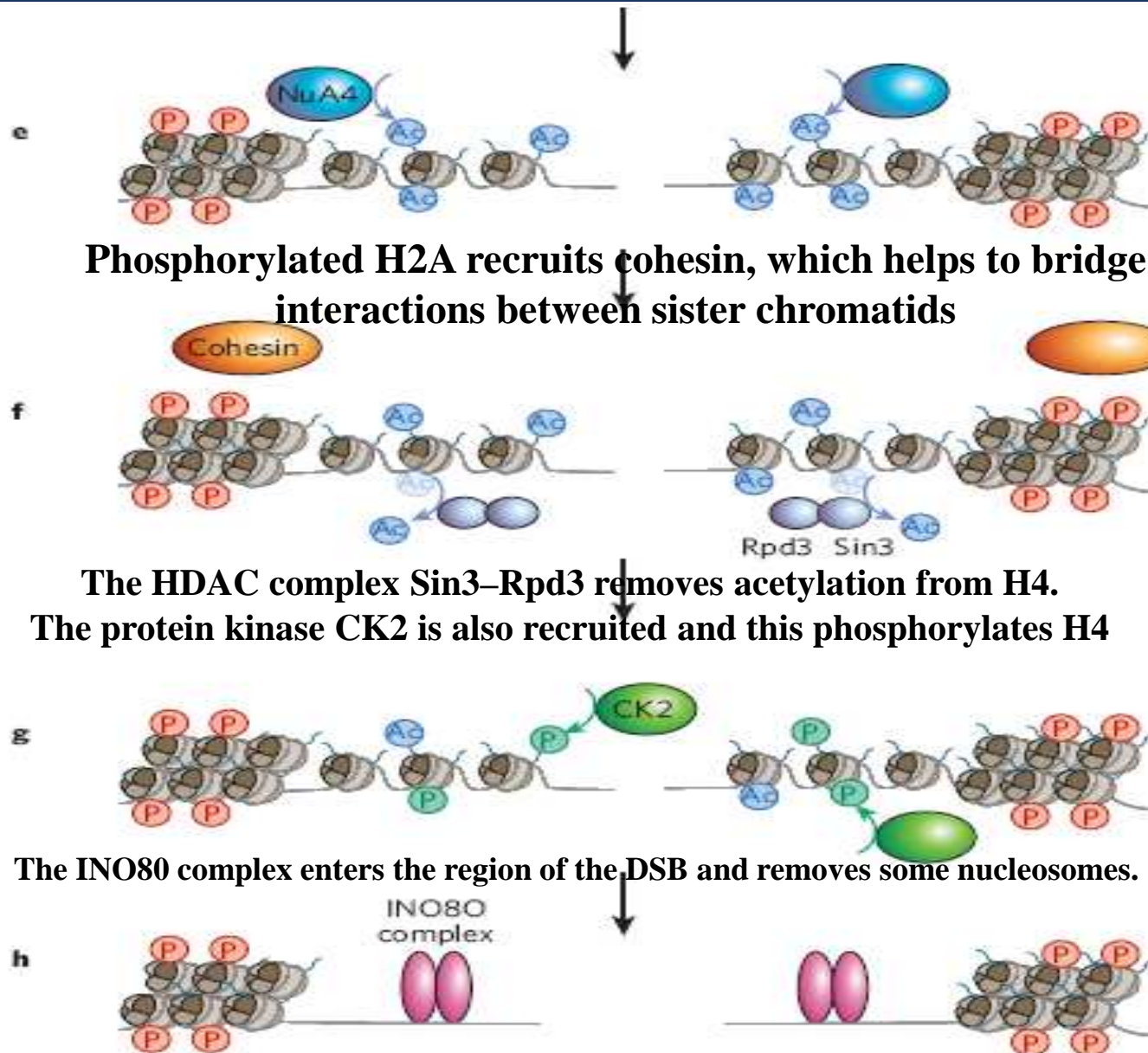


a

	Naked DNA		Nucleosomal Template			
			PnA Only		hSWI	
DNase I:	1	1	1	1	1	1
ATP:	A	A	-	A	-	γ A



# Chromatin remodelling and DSBs

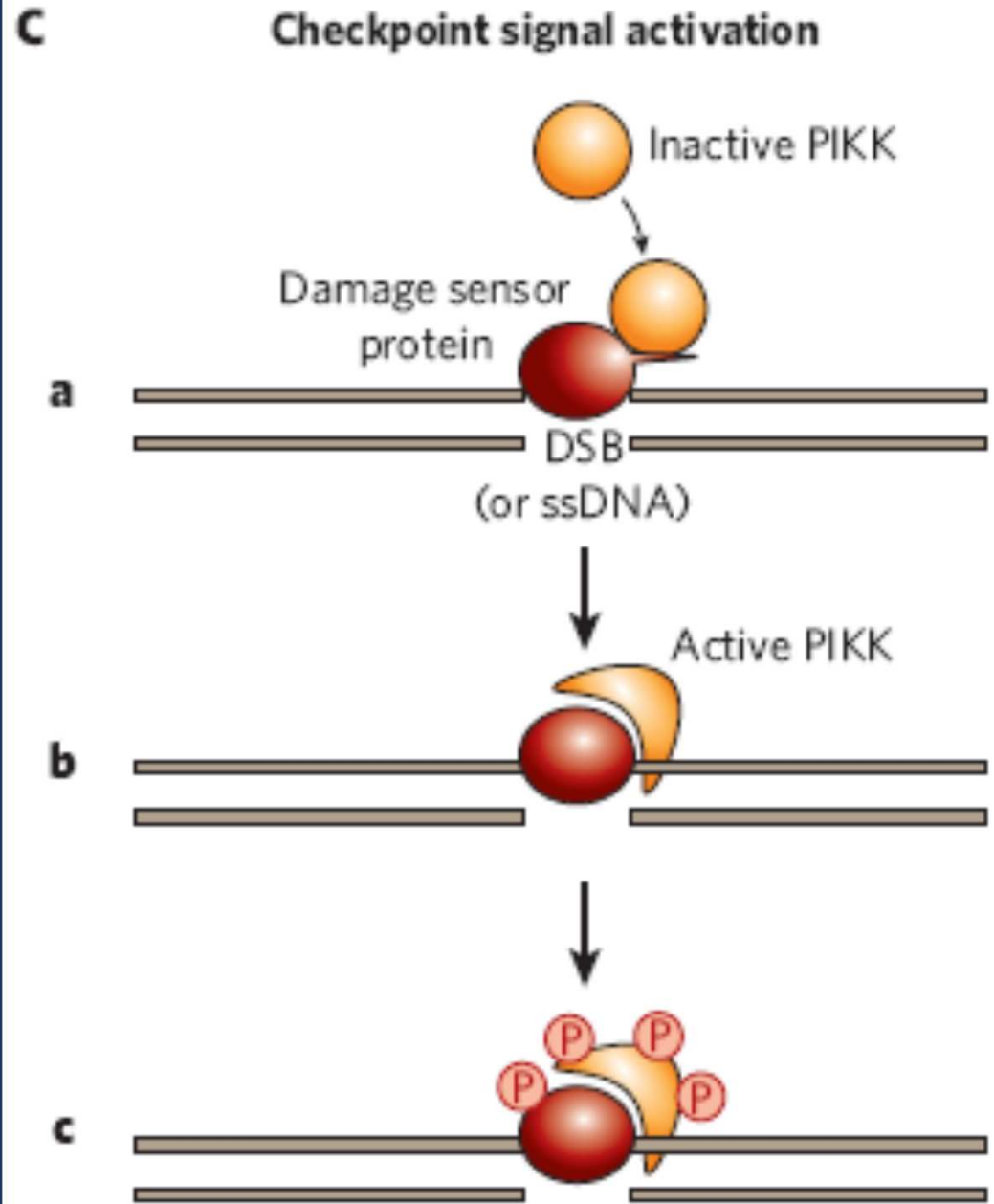


# MODIFICAZIONE ISTONI

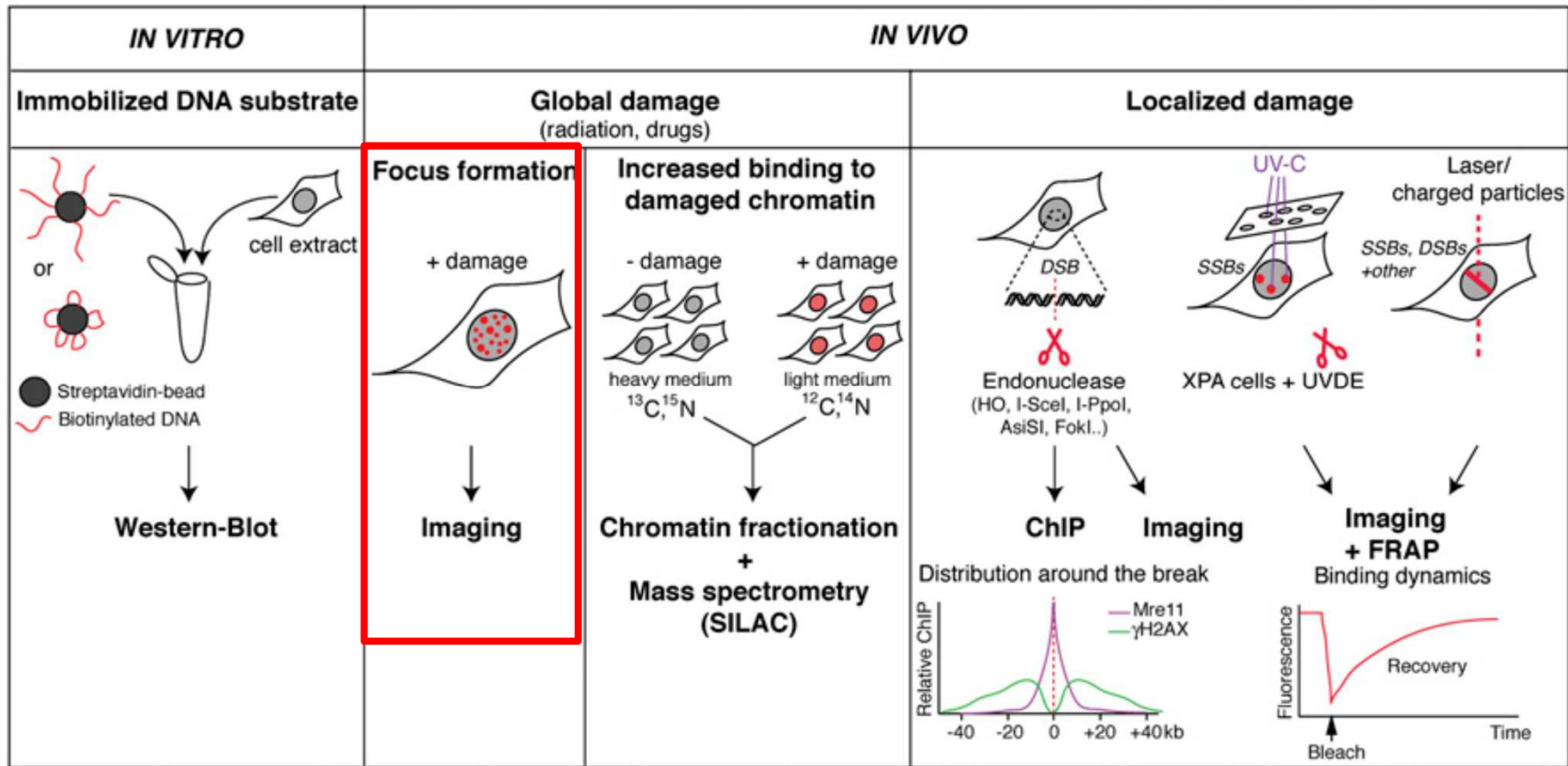
- Eukaryotes have several histone variants, which, as a result of their altered amino-acid composition, can affect both the structure of individual nucleosomes and the ability of nucleosomes to form higher order chromatin structure
- The earliest and most robust modification induced by DSB is phosphorylation of the histone H2A variant H2AX on its extended C-terminal tail.
- Within seconds, phosphorylated H2AX (known as  $\gamma$ -H2AX) spreads over a region spanning thousands to millions of bases surrounding a DSB

**PIKKs =  
phosphatidylinositol-3OH-kinase  
-like kinases**

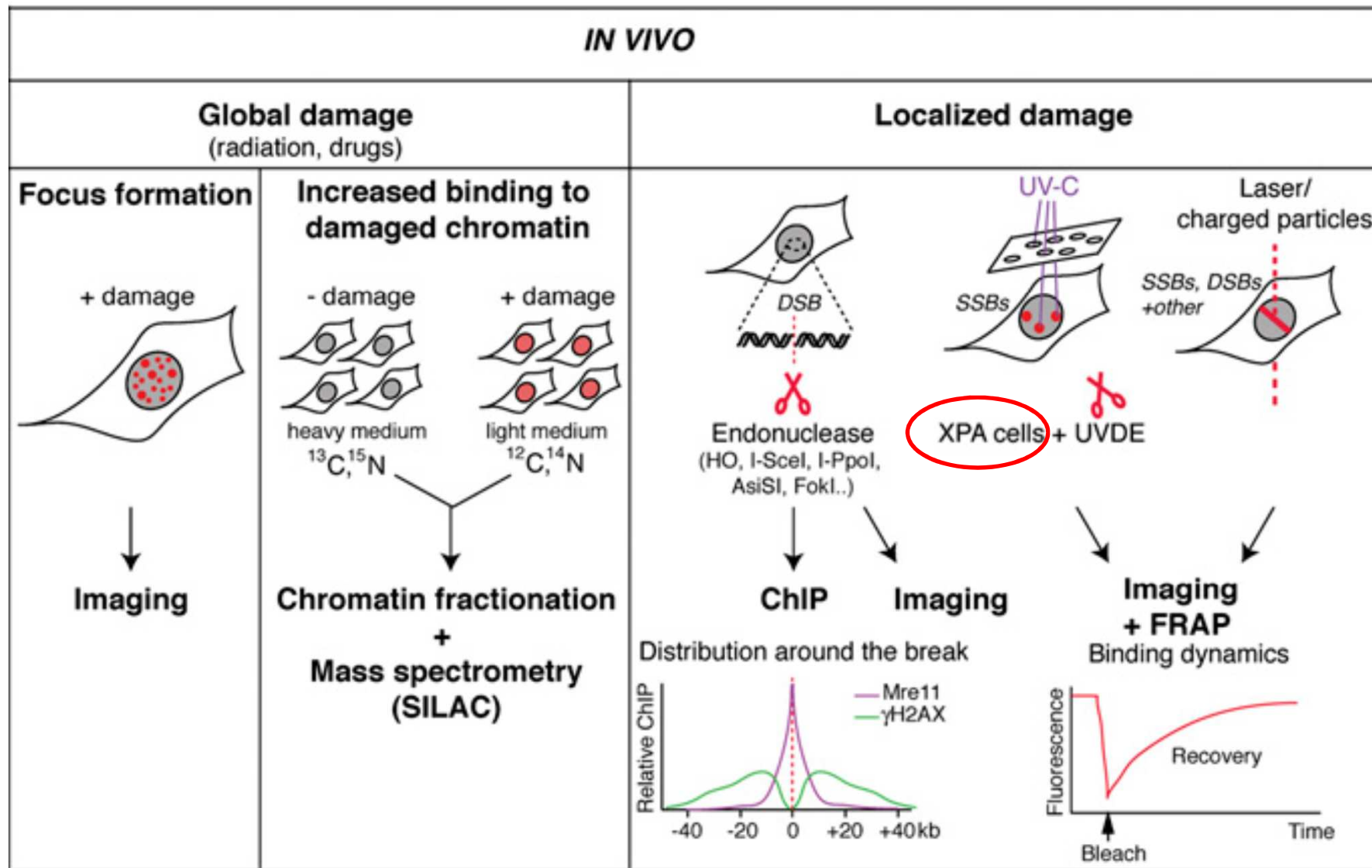
**DNA-damage sensor proteins  
Ku70–Ku80, MRN, RPA  
TOPBP1**



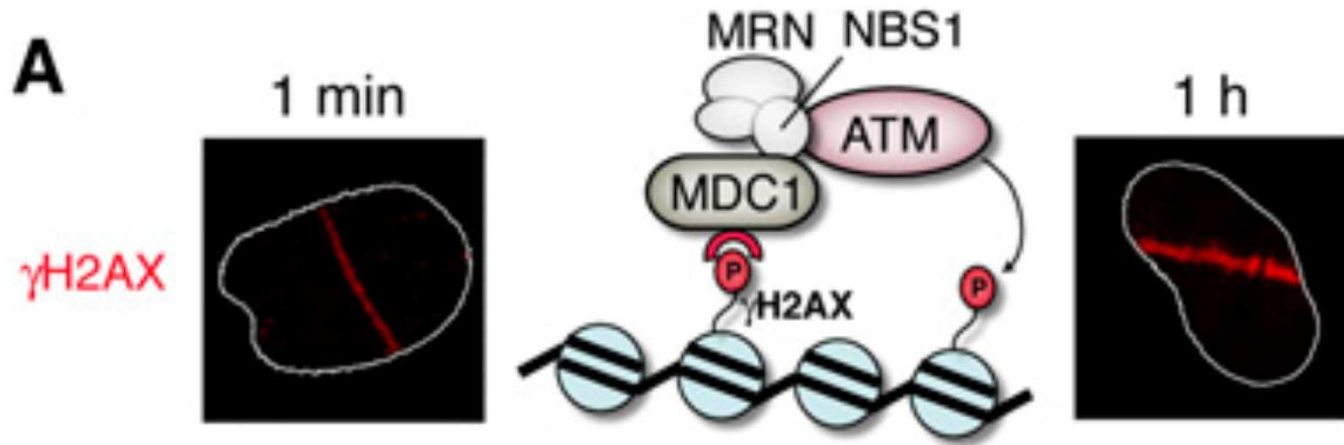
# METODI



# METODI

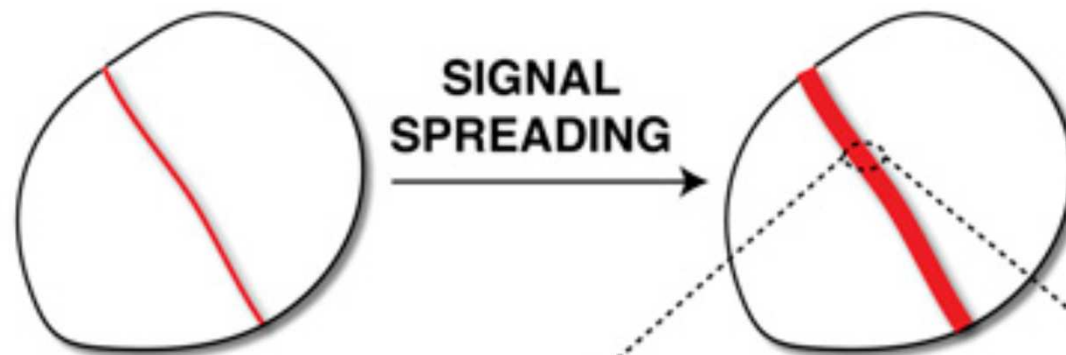


# DDR signal spreading

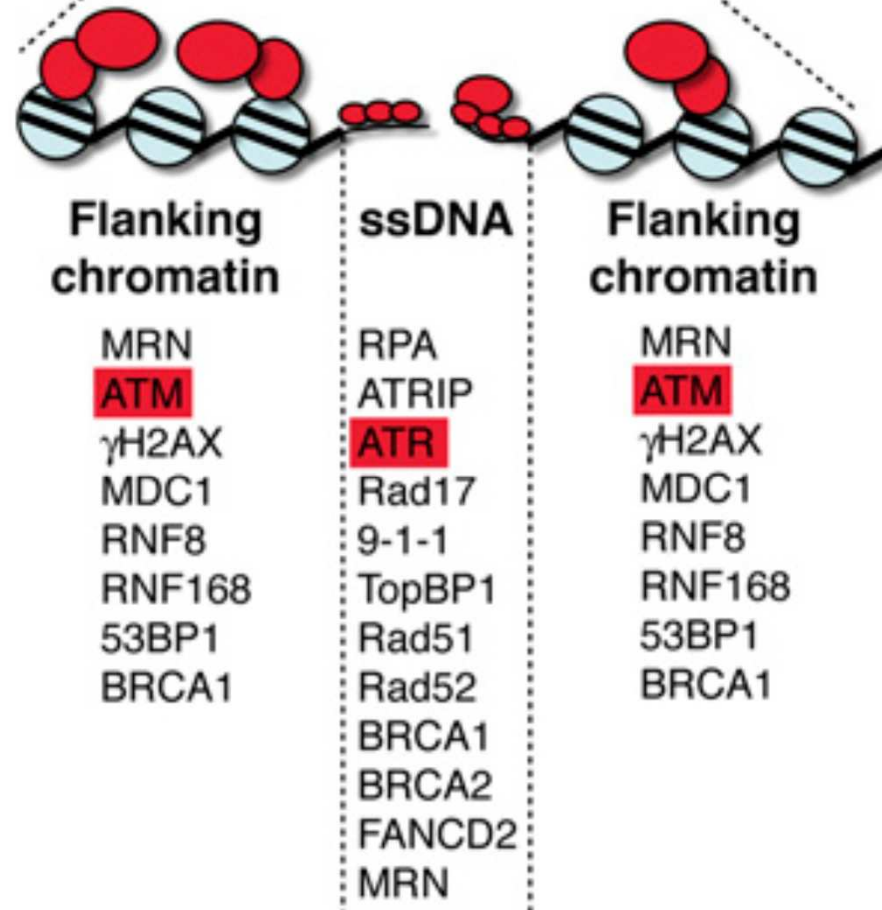
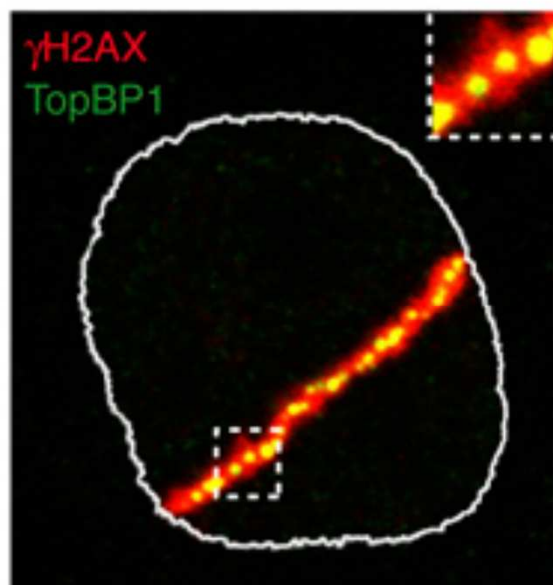


DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds  $\gamma$ H2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.





**B** REGIONAL DISTRIBUTION

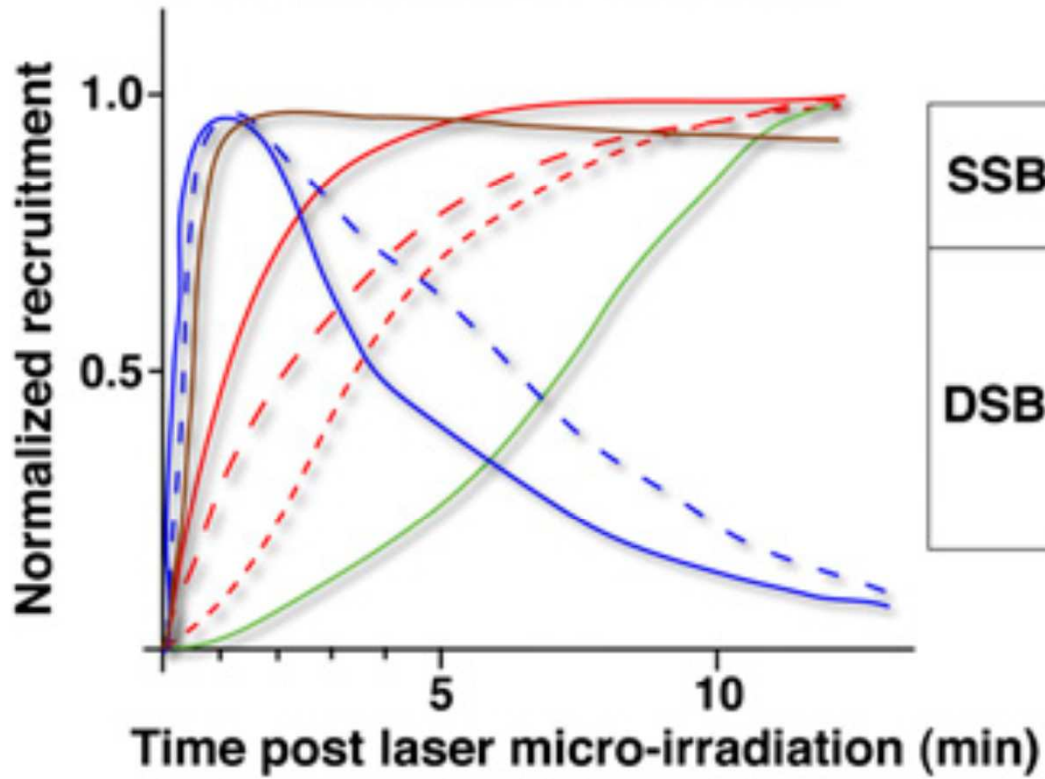




# Temporal regulation of DDR protein accumulation at DNA breaks

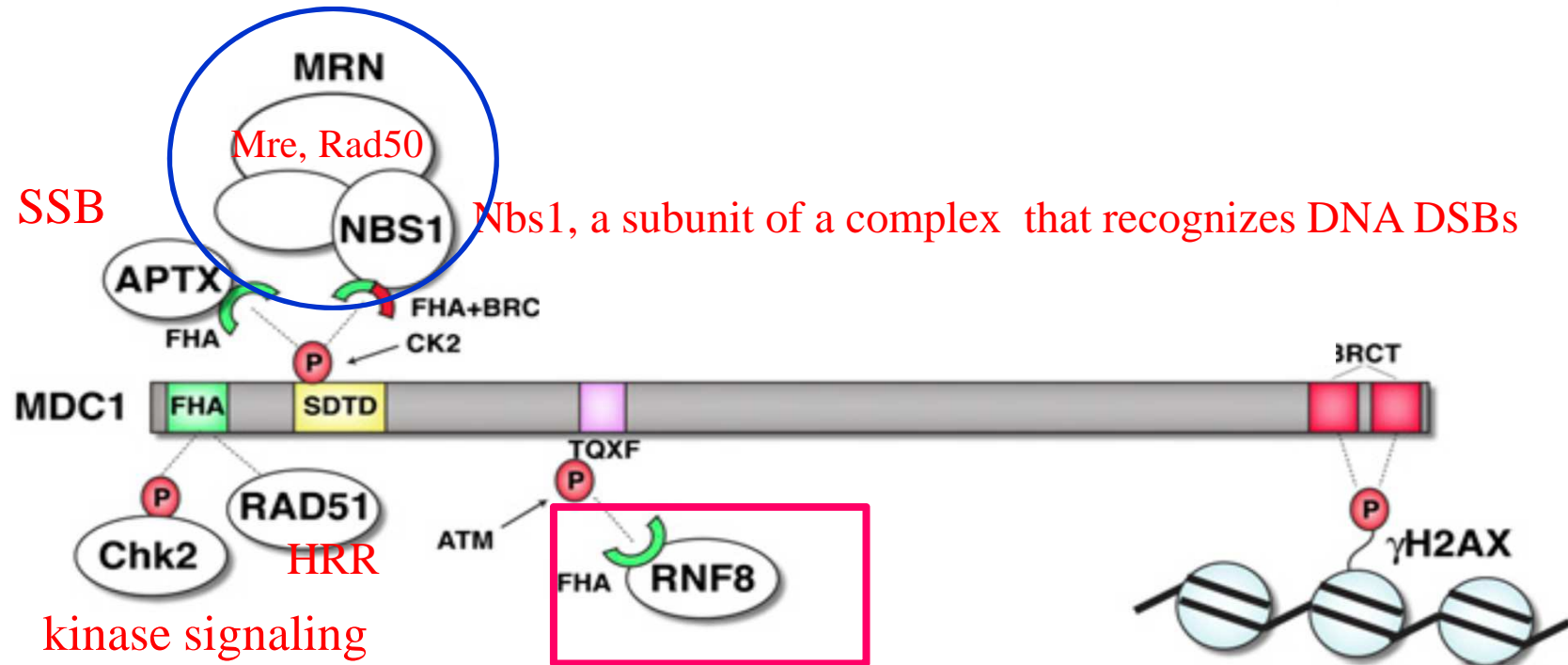
**A**

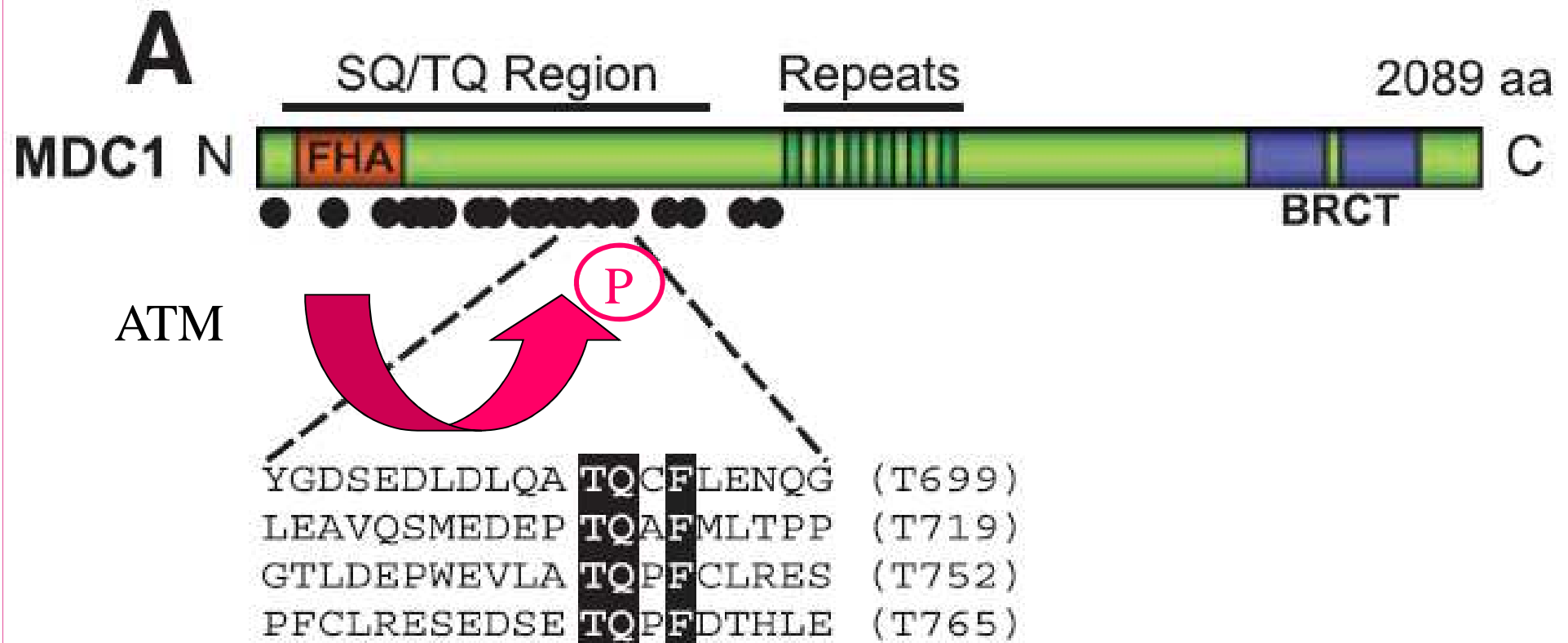
## RECRUITMENT KINETICS



# Proteine piattaforma

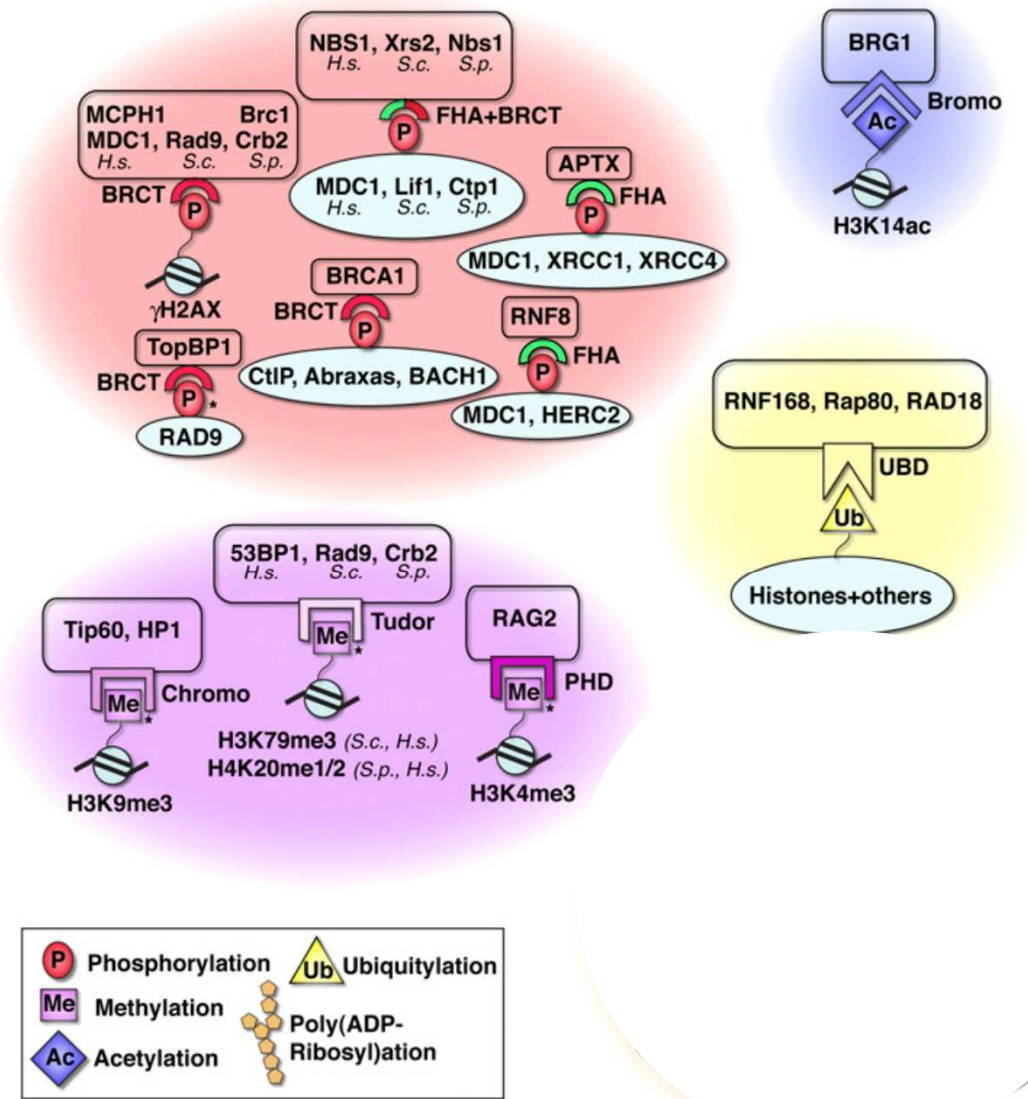
## Damage signaling



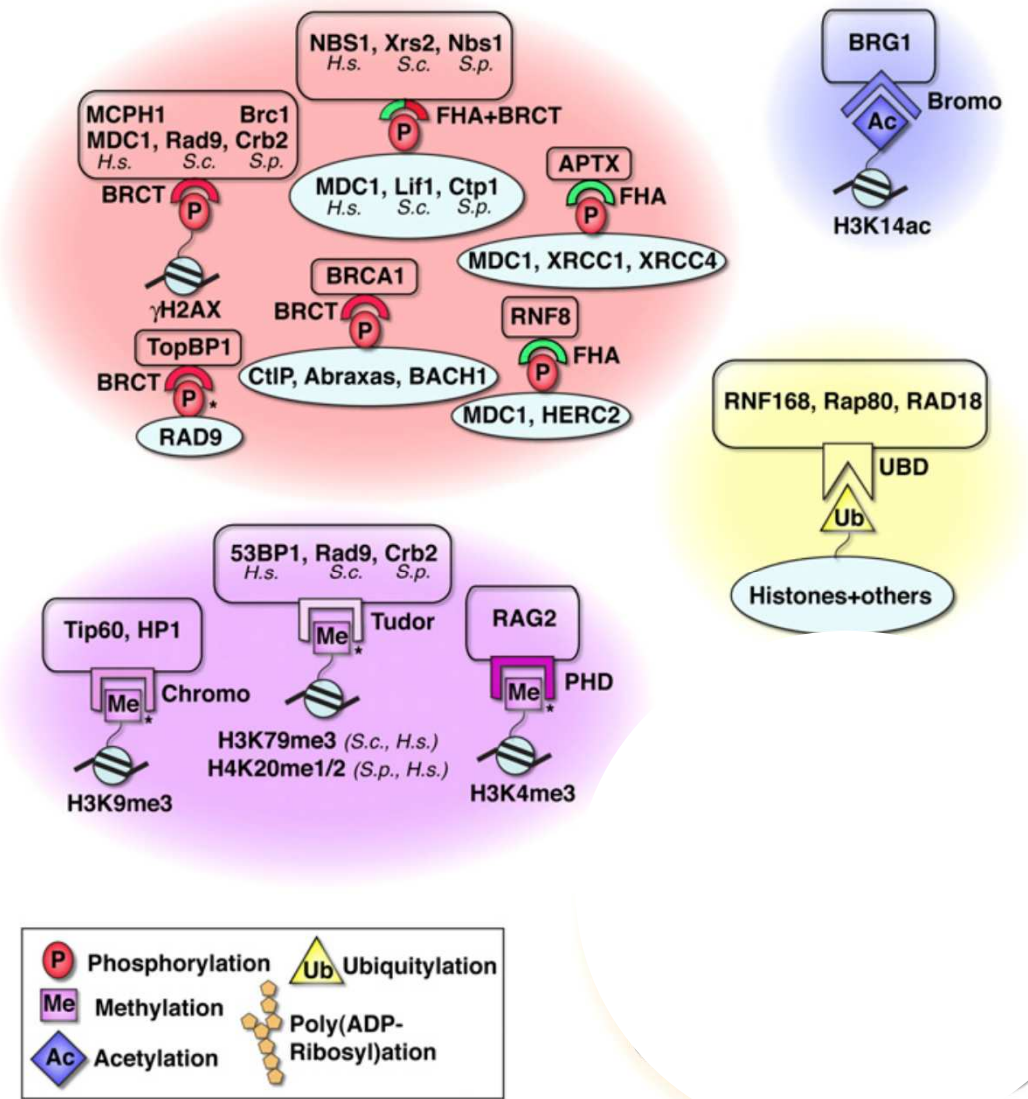


The MDC1 TQXF motifs are ATM targets required for 53BP1 IRIF. (A) Domain architecture of MDC1, with ATM consensus sites (dots).

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



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# RSC

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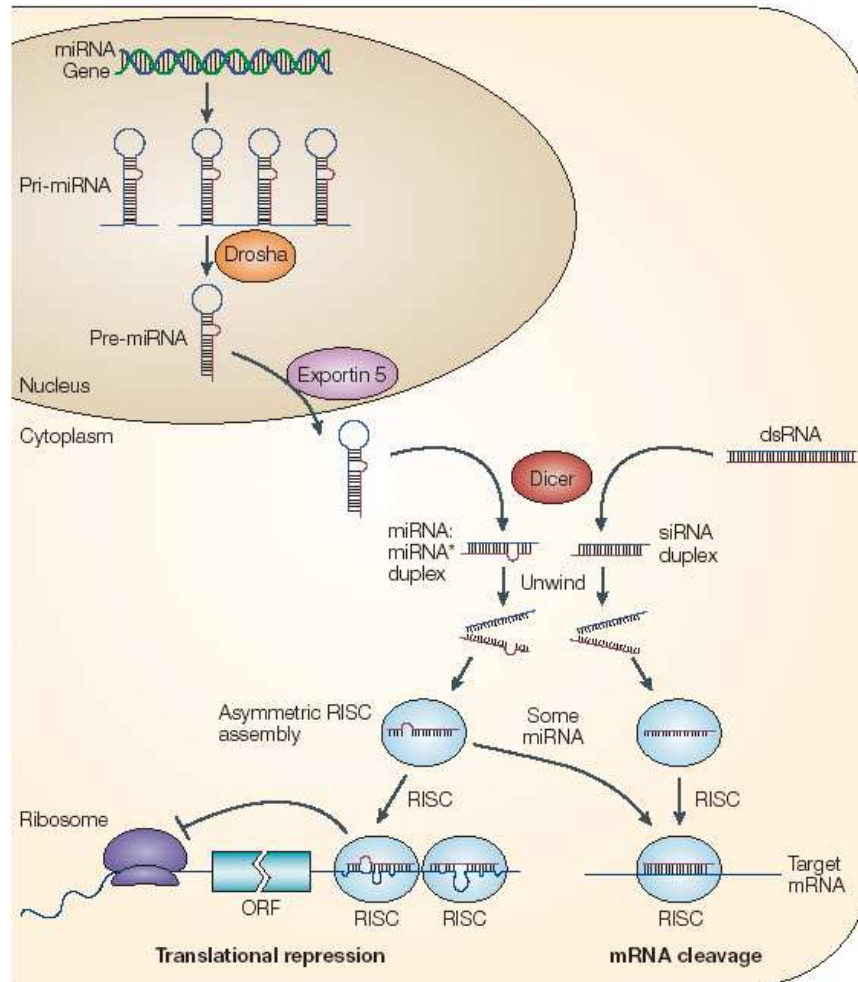
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# Biogenesis of miRNAs and siRNAs



**miRNAs are genomically encoded**

**siRNAs are produced exogenously or from bidirectionally transcribed RNAs**

**Drosha processes pri-miRNA to pre-miRNA in the nucleus**

**miRNA is selectively incorporated into the RISC for target recognition**

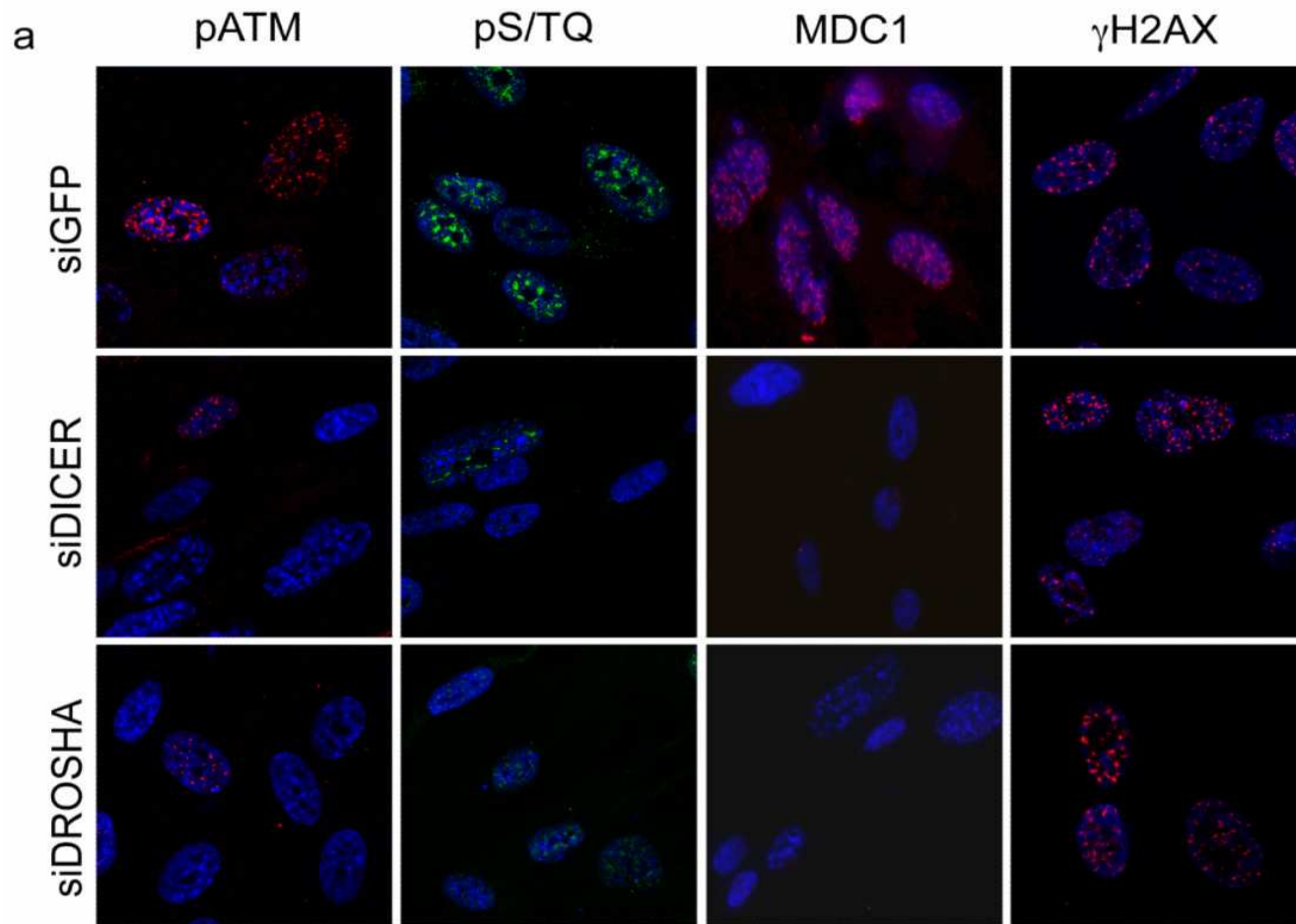
**Guide strand of siRNA is incorporated into the RISC for target recognition**

**miRNAs have imperfect complementarity to their target mRNA and inhibit translation**

**siRNAs form perfect duplex with their target mRNA and trigger mRNA degradation**

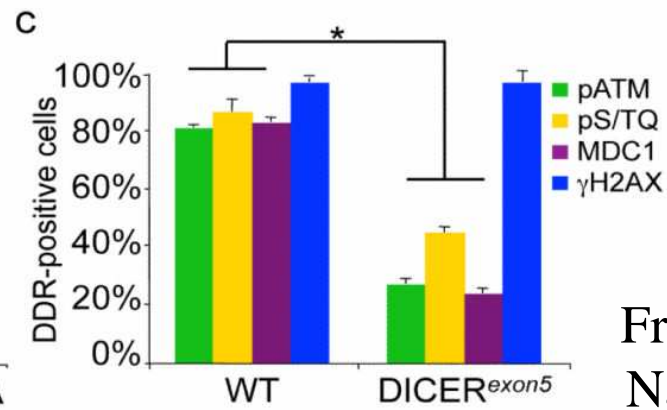
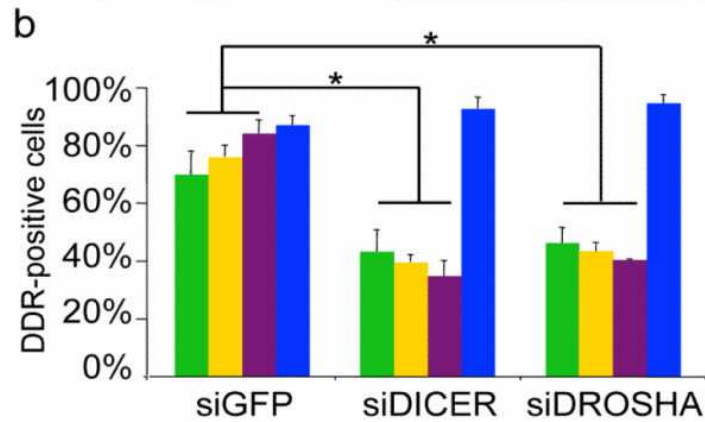
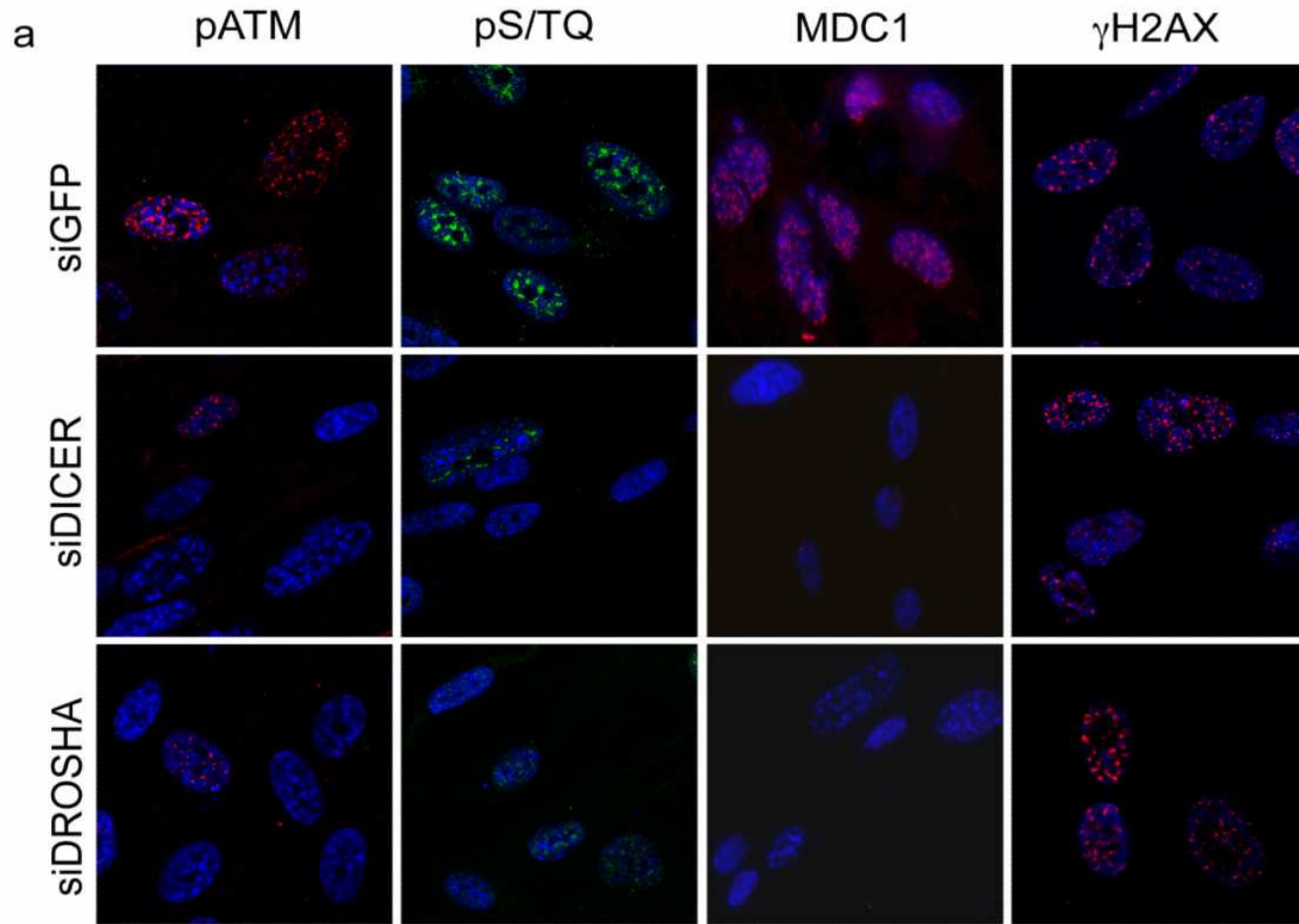
from Li and Hannon, *Nature Rev.Genet.* **5**, 522 (2004)





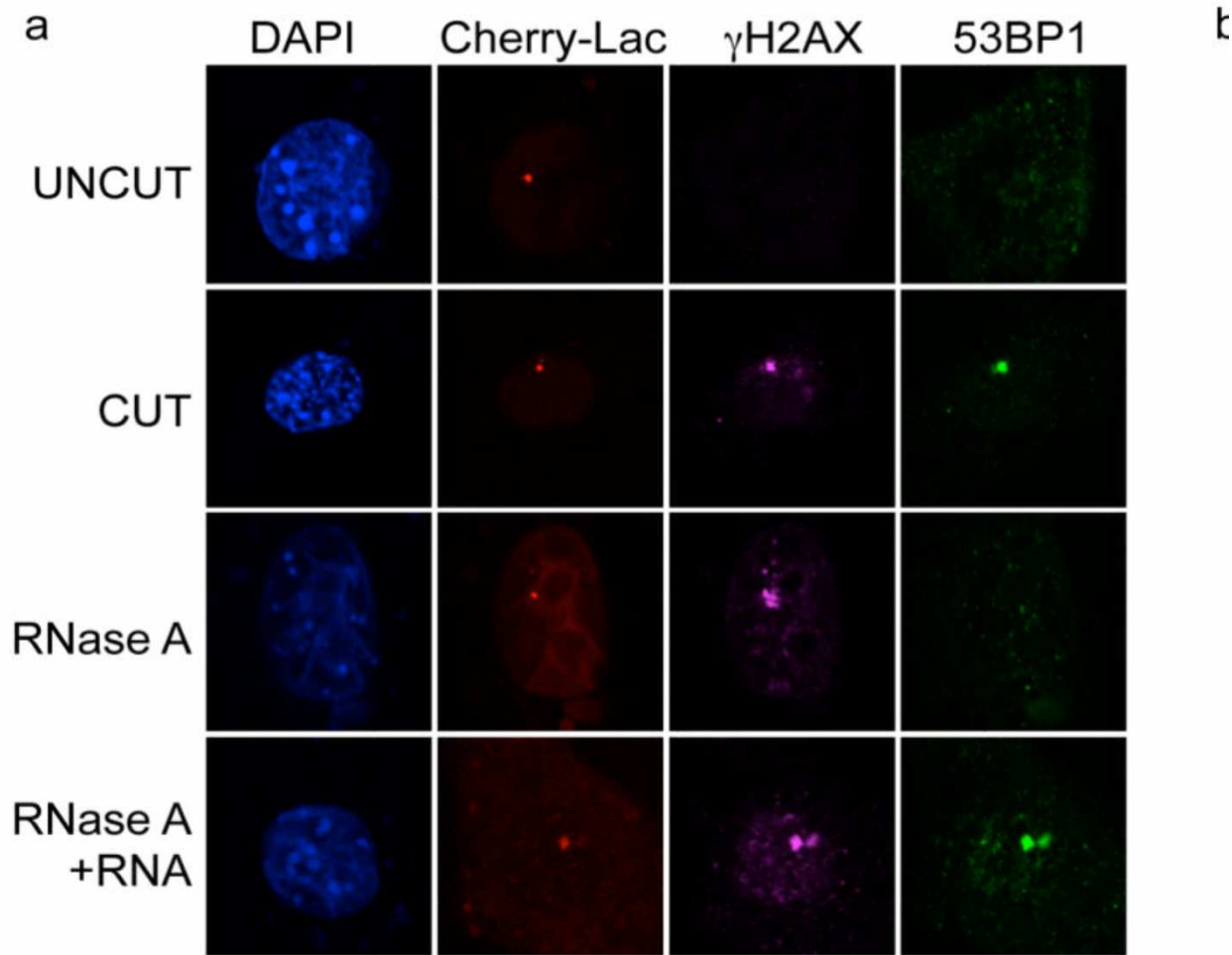
DICER or DROSHA inactivation impairs  
DDR foci formation in irradiated cells



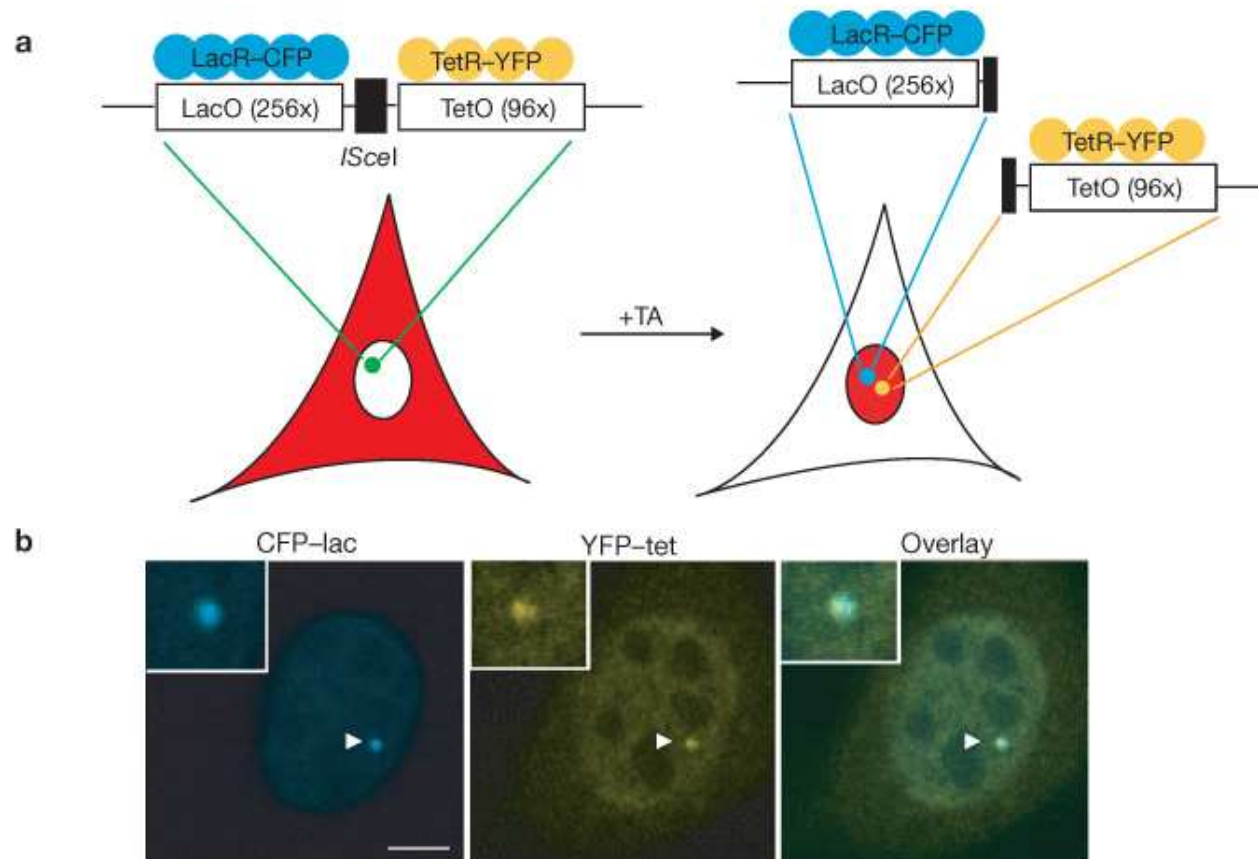


Francia et al  
Nature 2012

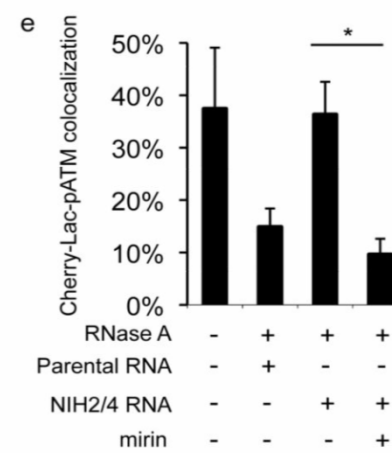
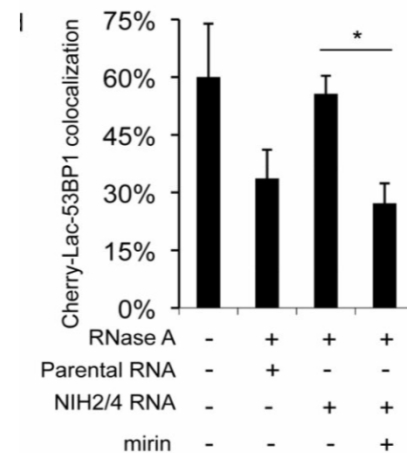
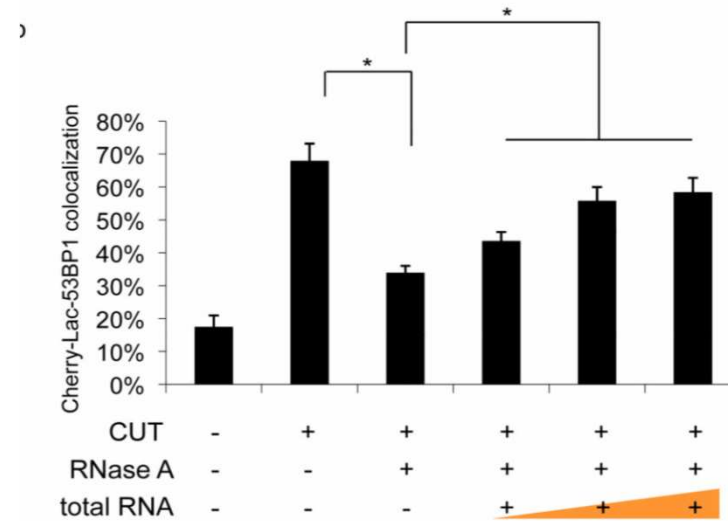
Site-specific DDR focus formation is RNase A-sensitive and can be restored by site specific RNA in a MRN-dependent manner



# A single inducible and detectable DSB



Site-specific DDR focus formation is RNase A-sensitive and can be restored by site specific RNA in a MRN-dependent manner



Mirin=MRN inhibitor

# Mirin prevents MRN-dependent activation of ATM

