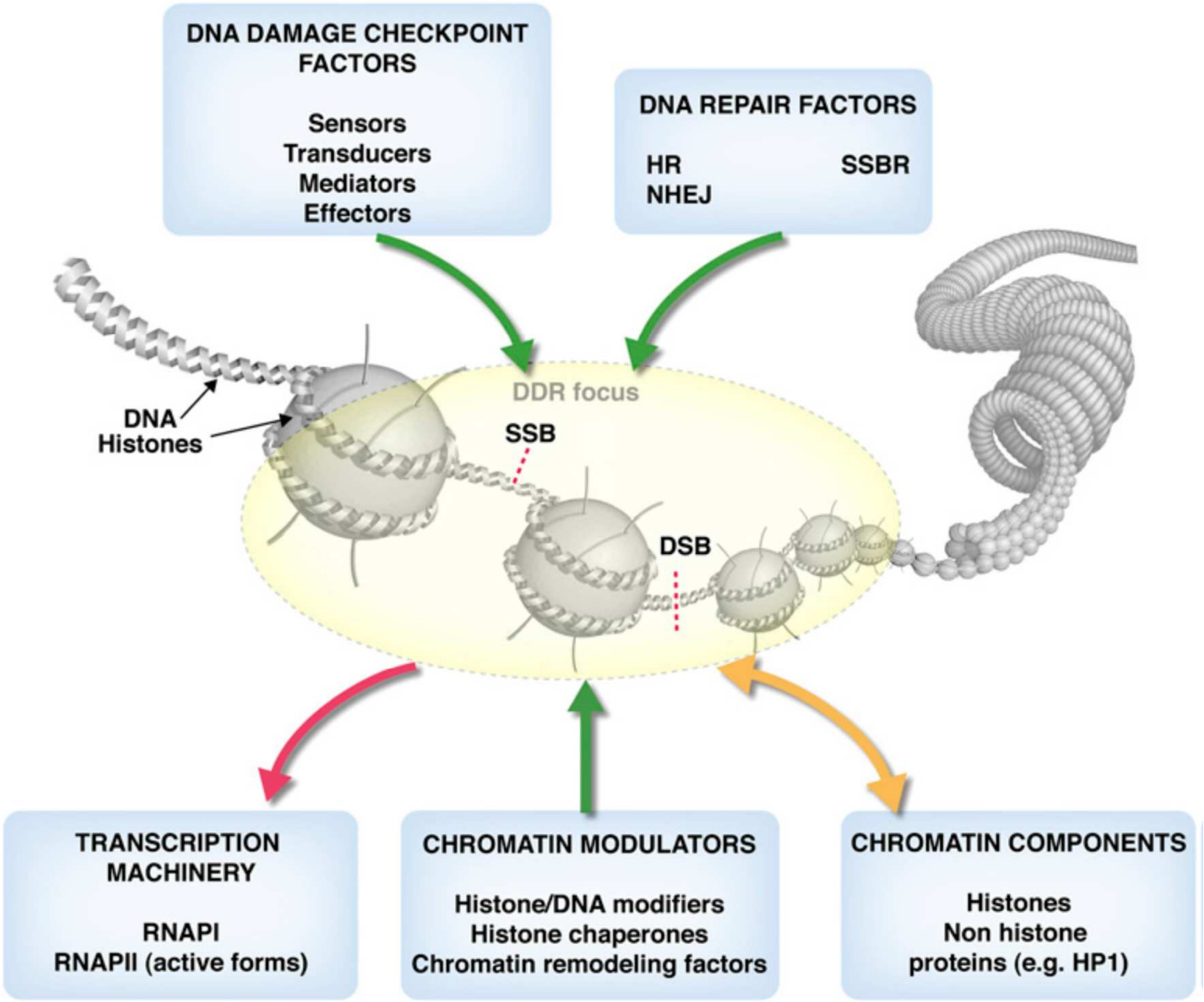
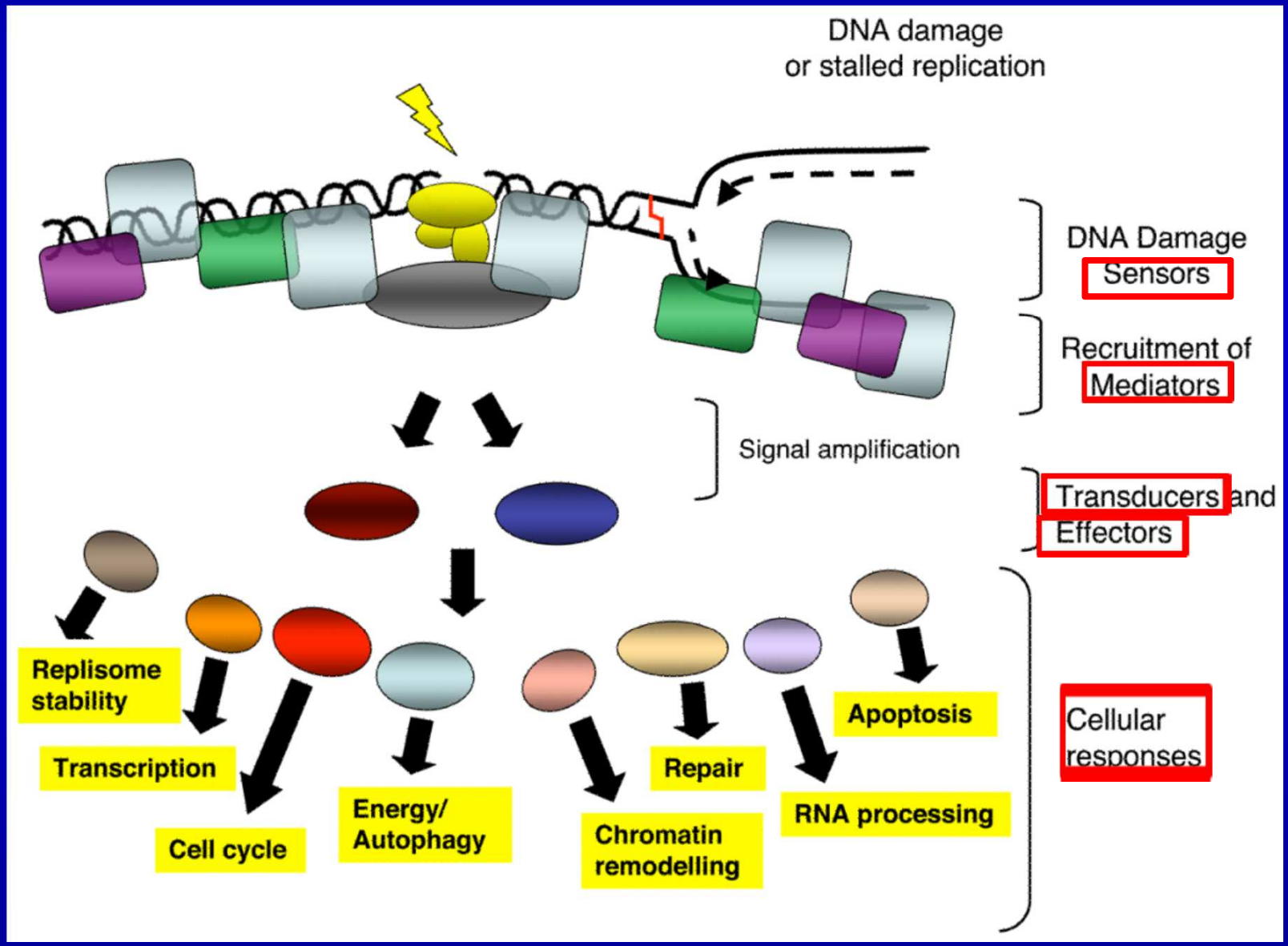
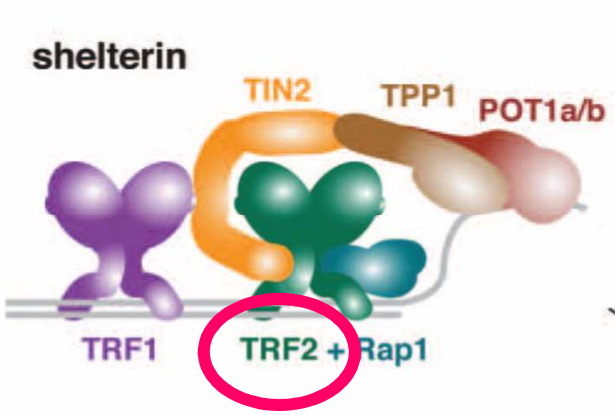

DNA DAMAGE CHECKPOINT FACTORS
Sensors
Transducers
Mediators
Effectors
DNA REPAIR FACTORS
HR
SSBR
NHEJ
DNA
Histones
DDR focus
SSB
DSB
TRANSCRIPTION MACHINERY
RNAPI
RNAPII (active forms)
CHROMATIN MODULATORS
Histone/DNA modifiers
Histone chaperones
Chromatin remodeling factors
CHROMATIN COMPONENTS
Histones
Non histone
proteins (e.g. HP1)


DNA damage
or stalled replication
DNA Damage
Sensors
Recruitment of
Mediators
Signal amplification
Transducers and
Effectors
Replisome
stability
Transcription
Cell cycle
Energy/
Autophagy
Chromatin
remodelling
Repair
RNA processing
Apoptosis
Cellular
responses

Removal of Shelterin Reveals the Telomere End-Protection Problem



ATM signaling	TRF2	-
ATR signaling	POT1a (b)	-
C-NHEJ	TRF2 (POT1a)	-
HDR	Rap1 + POT1a (or b)	Ku70/80
alt-NHEJ	redundant	Ku70/80
5' resection	redundant?	53BP1 (ATM/ATR dep)

DSB

Double-Strand Breaks

causate da

radiazioni

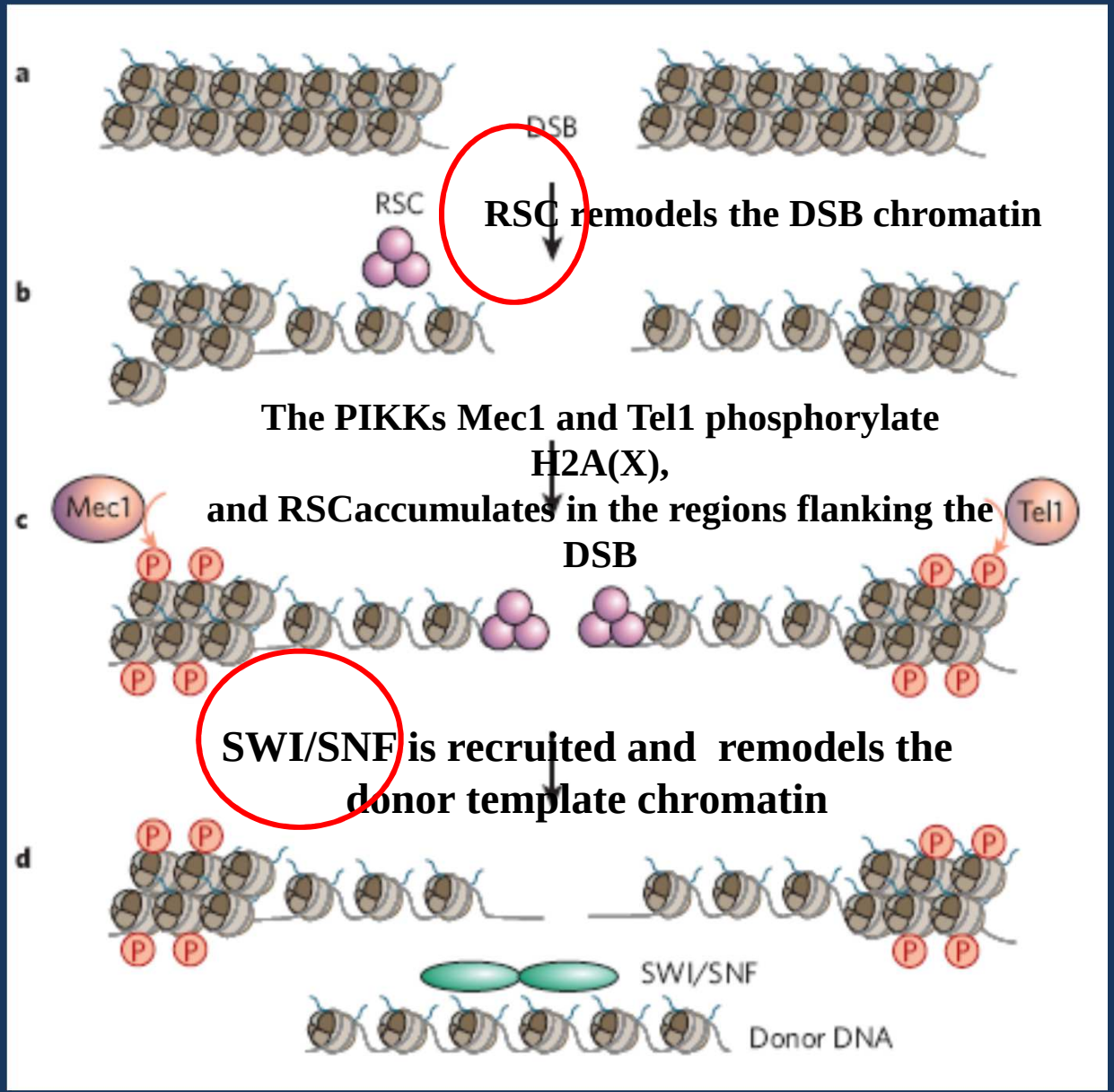
stress ossidativo

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

Chromating remodelling and DSBs



RSC

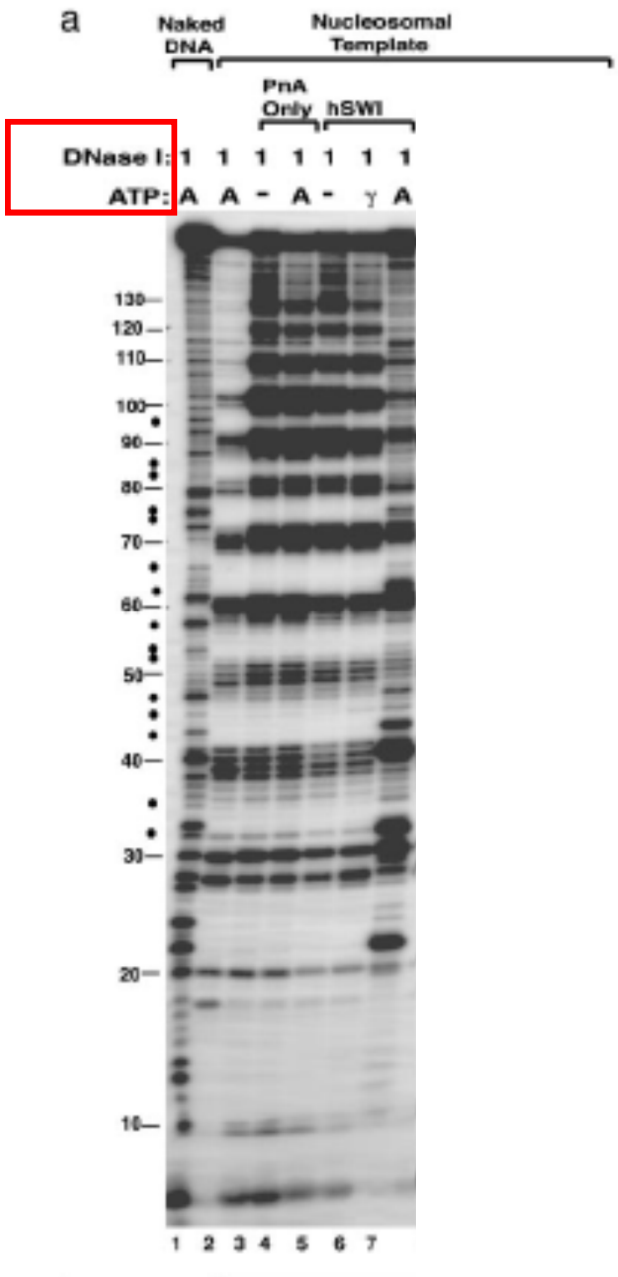
complex RSC (remodels the structure of chromatin)

ATP-dependent chromatin-remodelling

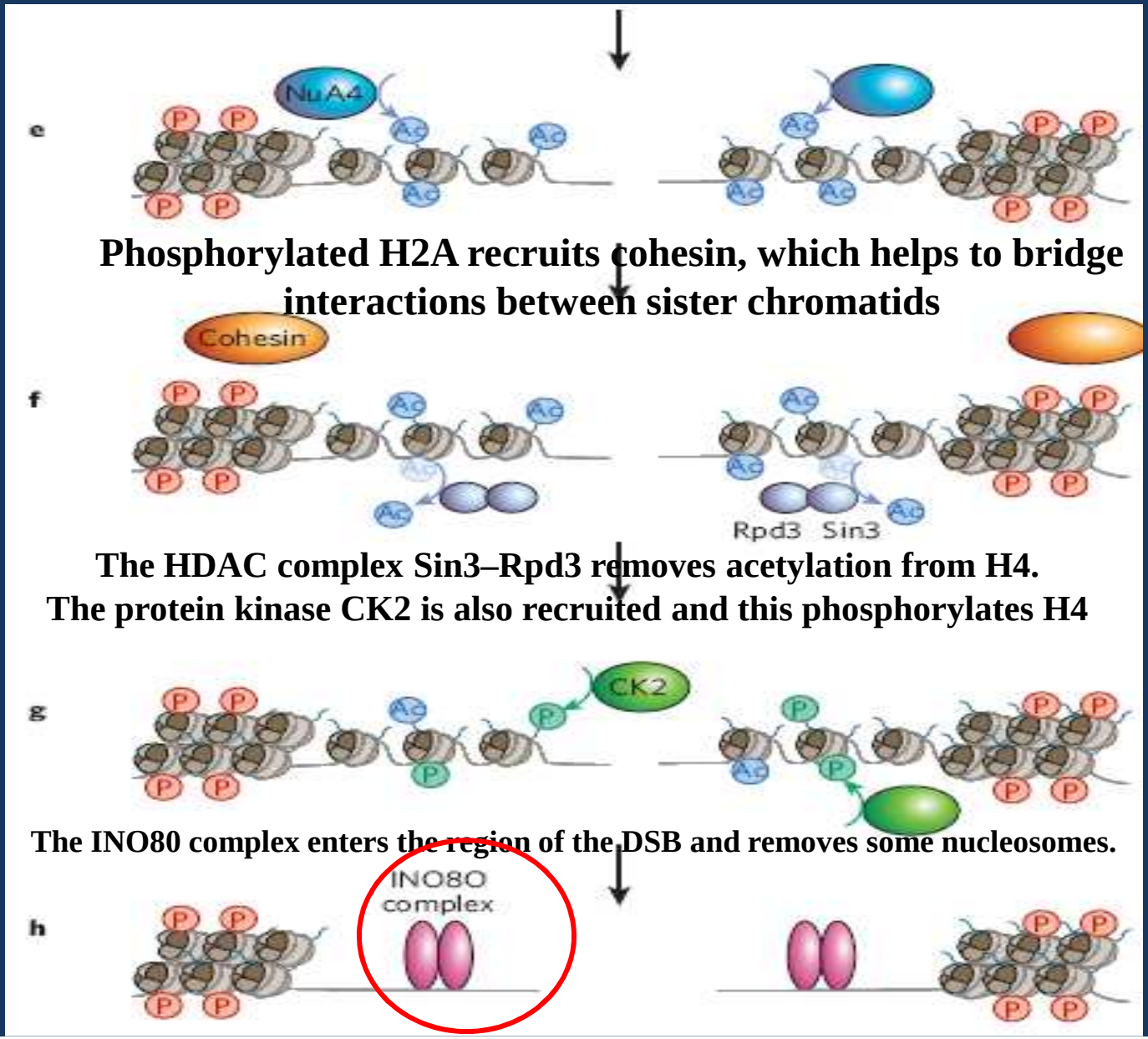
RSC can mediate nucleosome sliding, alter histoneDNA contacts and remove histones from DNA.

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???


a
Naked DNA
Nucleosomal Template
PnA Only
hSWI
DNase I: 1 1 1 1 1 1 1
ATP: A A - A - γ A
130
120
110
100
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7

Chromating remodelling and DSBs

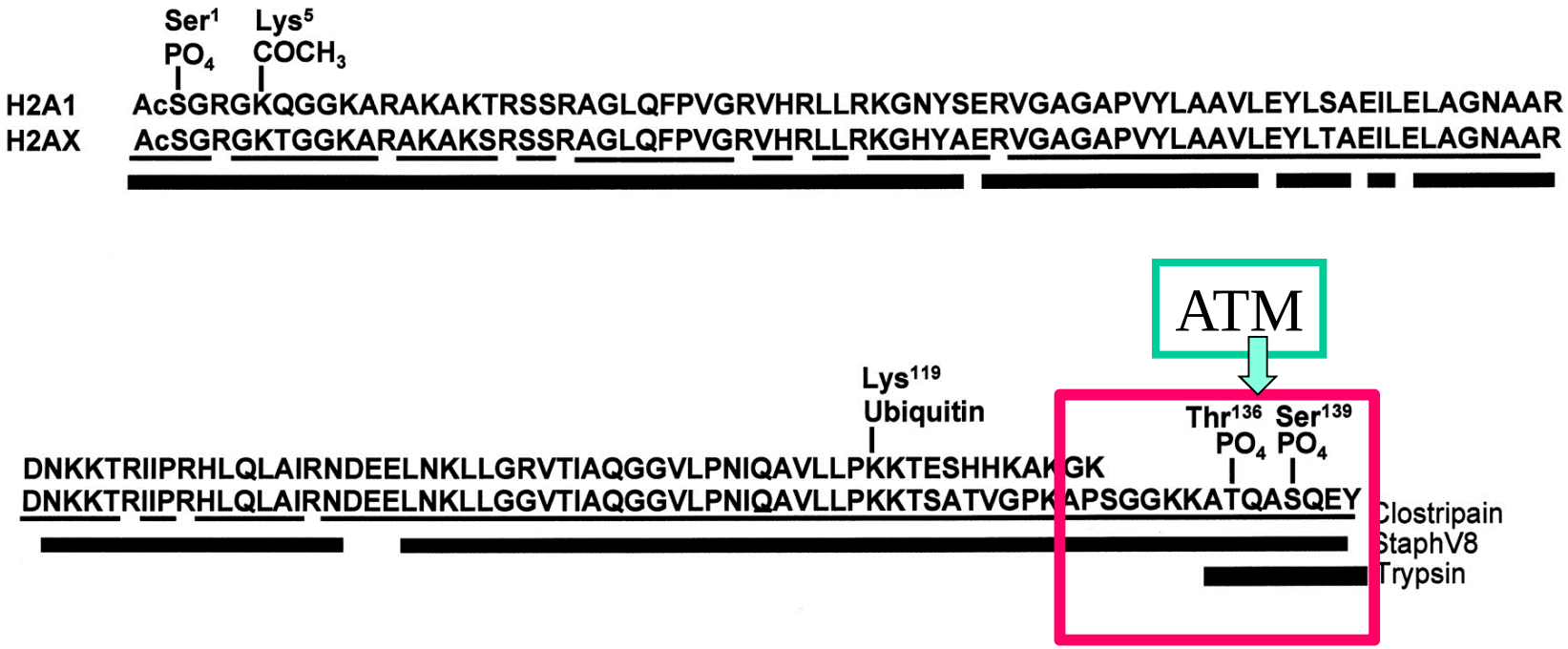


MODIFICAZIONE ISTONI

- 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 γ-H2AX) spreads over a region spanning thousands to millions of bases surrounding a DSB

Sequences of H2A1, H2AX, and recombinant H2AX constructs.



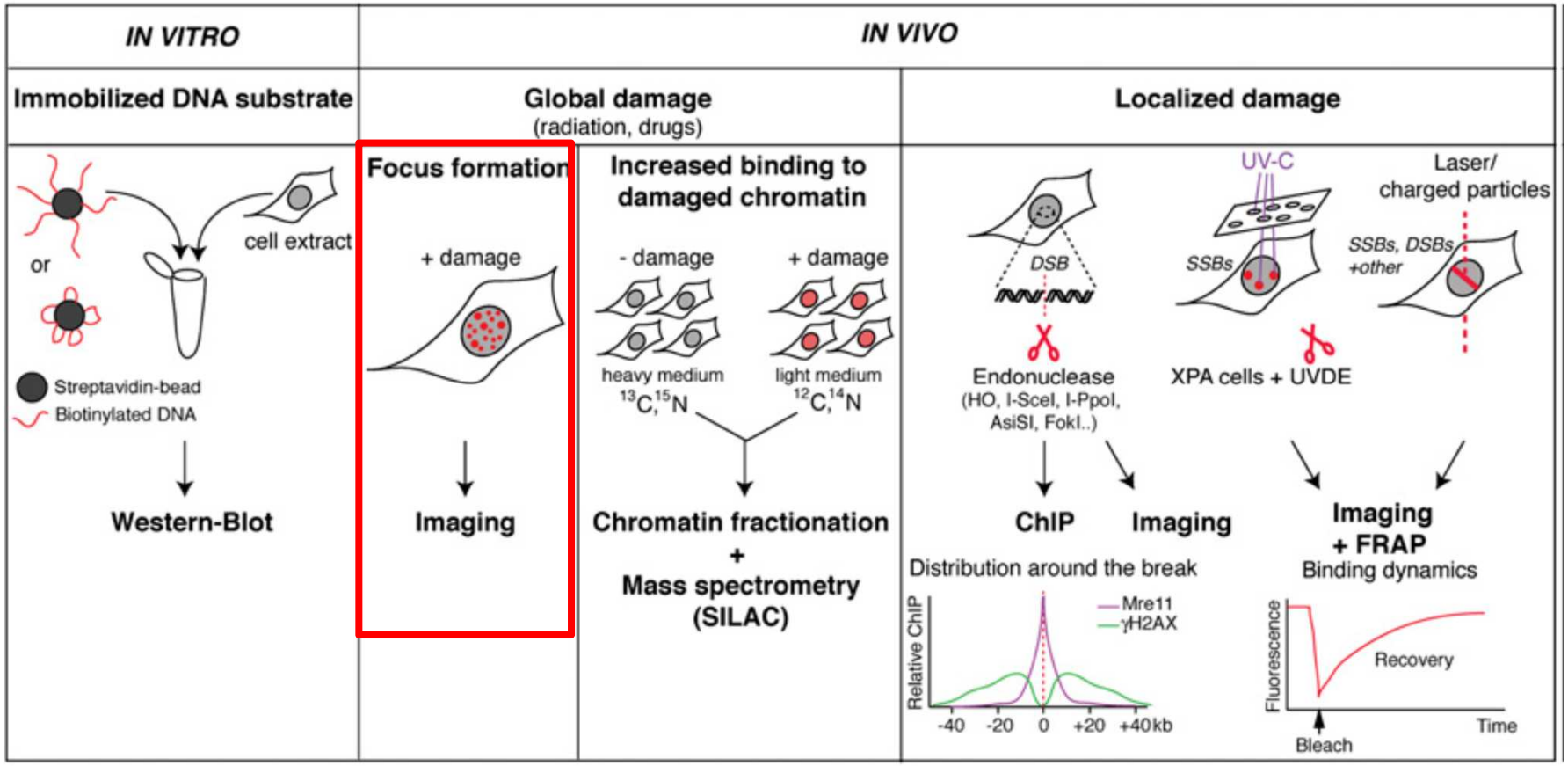
Emmy P. Rogakou et al. J. Biol. Chem. 1998;273:5858-5868

jbc

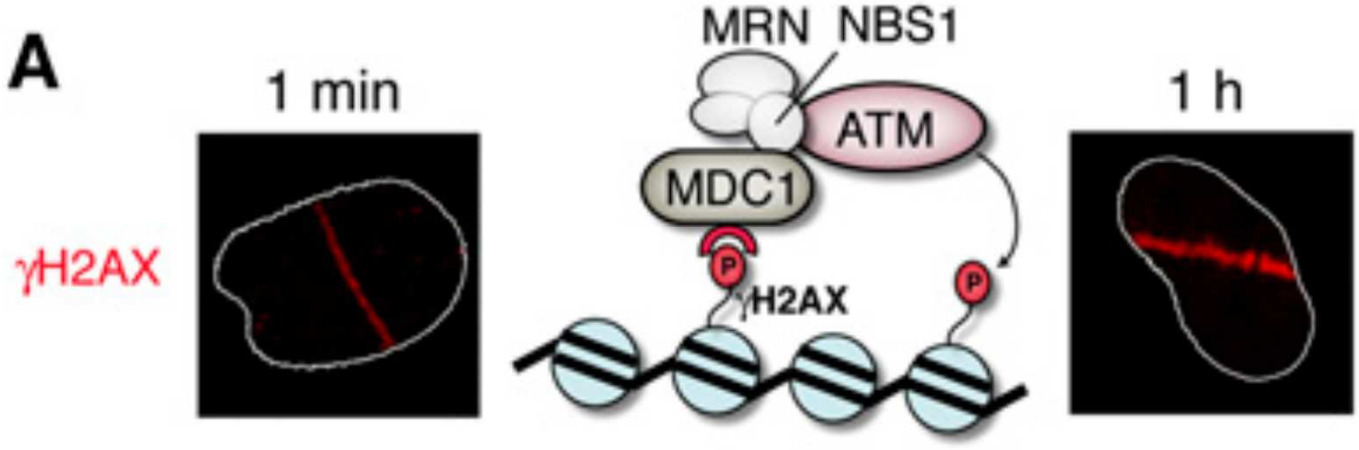
The determination of radiation exposure in diagnostic and interventional radiology

- γ-H2AX immunofluorescence microscopy is a reliable and sensitive method for the quantification of radiation induced DNA double-strand breaks (DSB) in blood lymphocytes.
- The detectable amount of these DNA damages correlates well with the dose received.

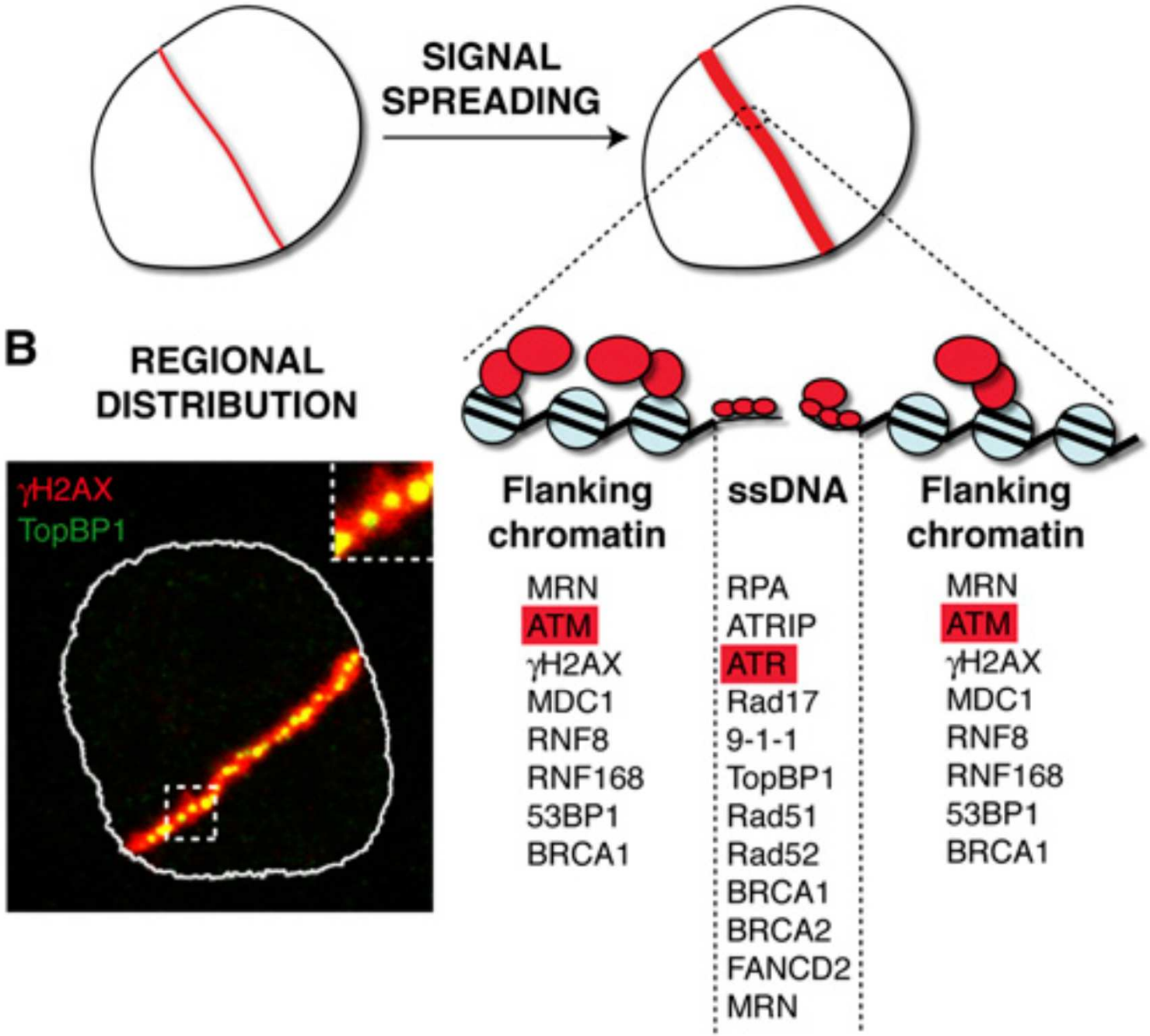
METODI


IN VITRO
IN VIVO
Immobilized DNA substrate
Global damage
(radiation, drugs)
Localized damage
cell extract
or
Streptavidin-bead
Biotinylated DNA
Western-Blot
Focus formation
+ damage
Imaging
Increased binding to
damaged chromatin
- damage
+ damage
heavy medium
$^{13}C,^{15}N$
light medium
$^{12}C,^{14}N$
Chromatin fractionation
+
Mass spectrometry
(SILAC)
DSB
Endonuclease
(HO, I-SceI, I-PpoI,
AsiSI, FokI..)
ChIP
Imaging
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb
UV-C
SSBs
XPA cells + UVDE
Laser/
charged particles
SSBs, DSBs
+other
Imaging
+ FRAP
Binding dynamics
Fluorescence
Recovery
Time
Bleach

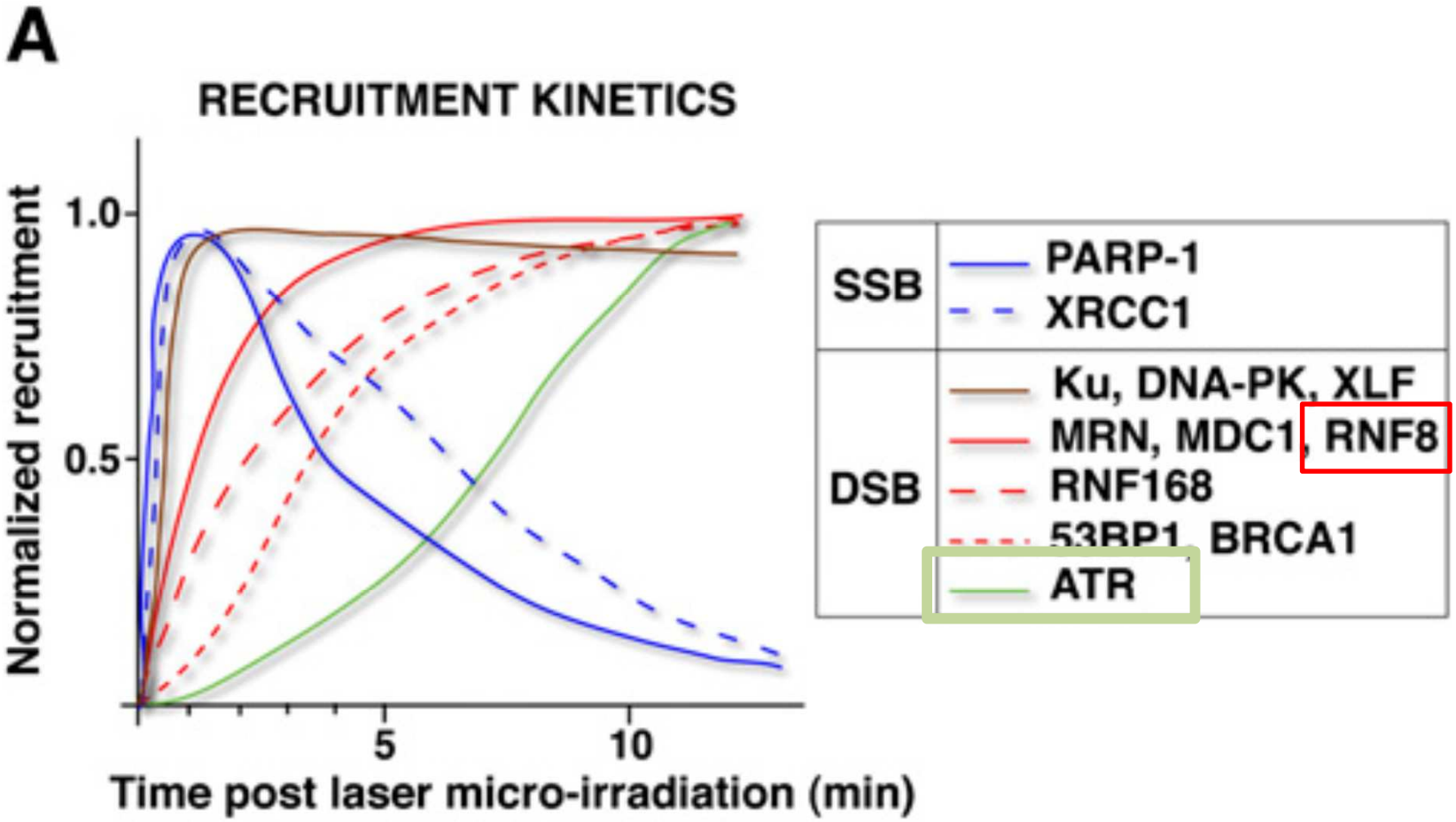
DDR signal spreading



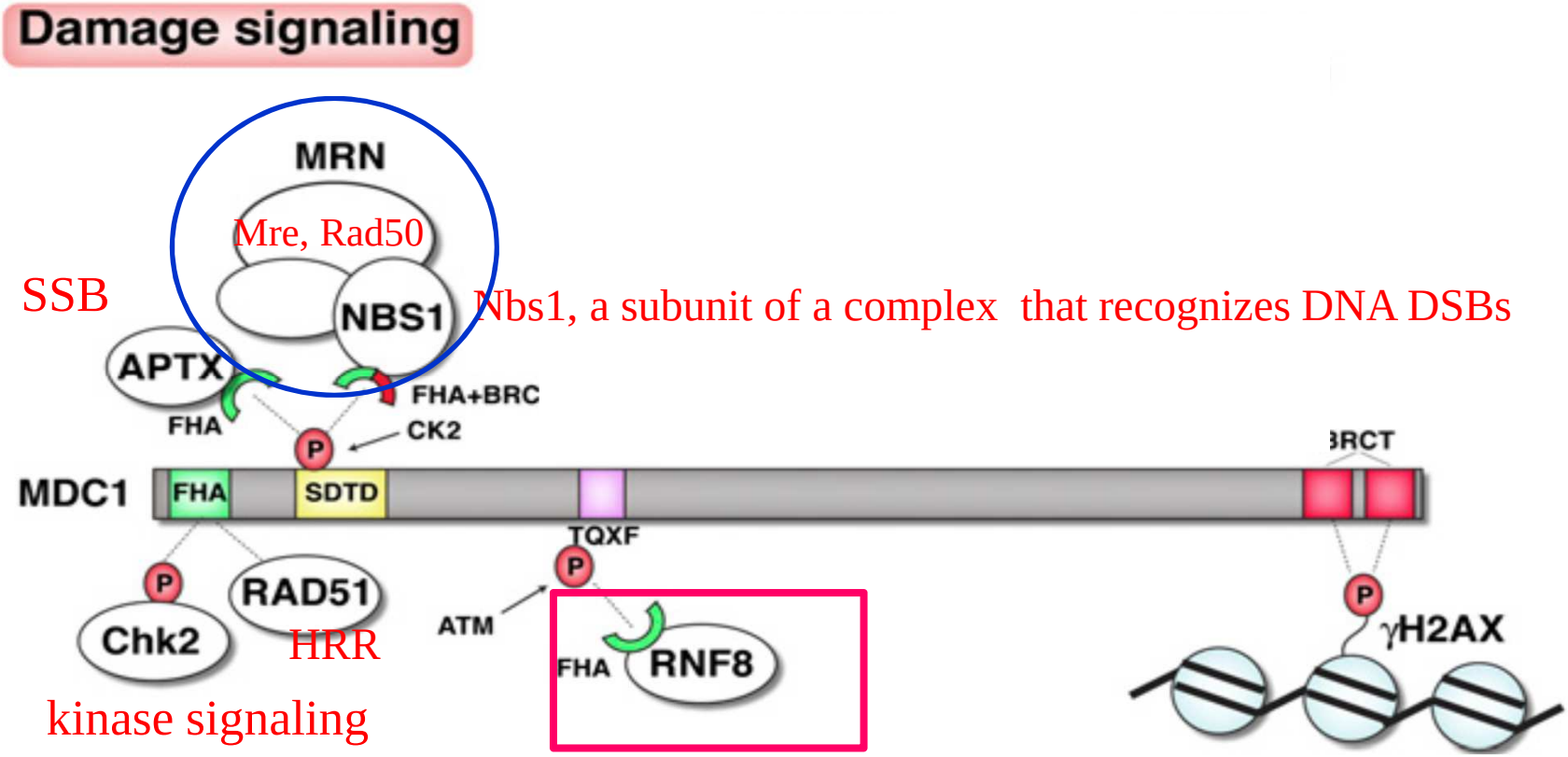
DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds gH2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.

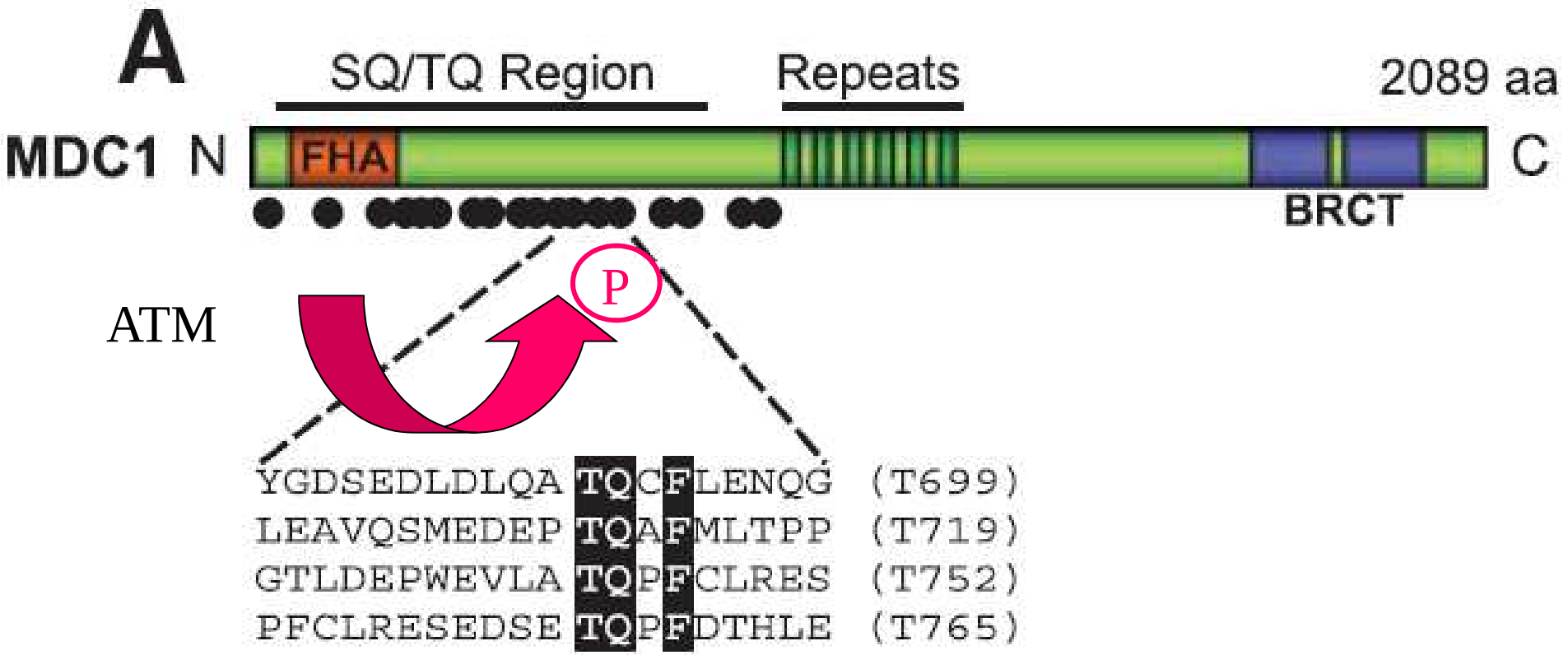

SIGNAL
SPREADING
B
REGIONAL
DISTRIBUTION
γH2AX
TopBP1
Flanking chromatin
MRN
ATM
γH2AX
MDC1
RNF8
RNF168
53BP1
BRCA1
ssDNA
RPA
ATRIP
ATR
Rad17
9-1-1
TopBP1
Rad51
Rad52
BRCA1
BRCA2
FANCD2
MRN
Flanking chromatin
MRN
ATM
γH2AX
MDC1
RNF8
RNF168
53BP1
BRCA1

Temporal regulation of DDR protein accumulation at DNA breaks



Proteine piattaforma

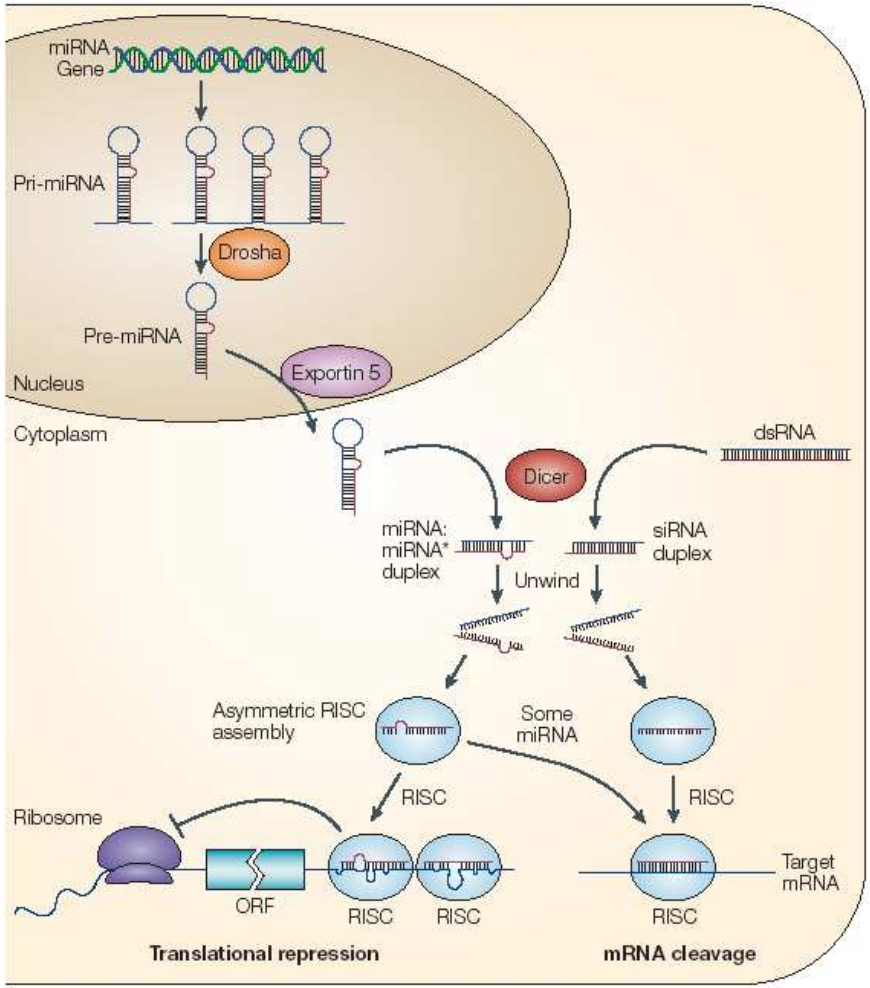




The MDC1 TQXF motifs are ATM targets required for 53BP1 IRIF. (A) Domain architecture of MDC1, with ATM consensus sites (dots).

DSB e piccoli RNA

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

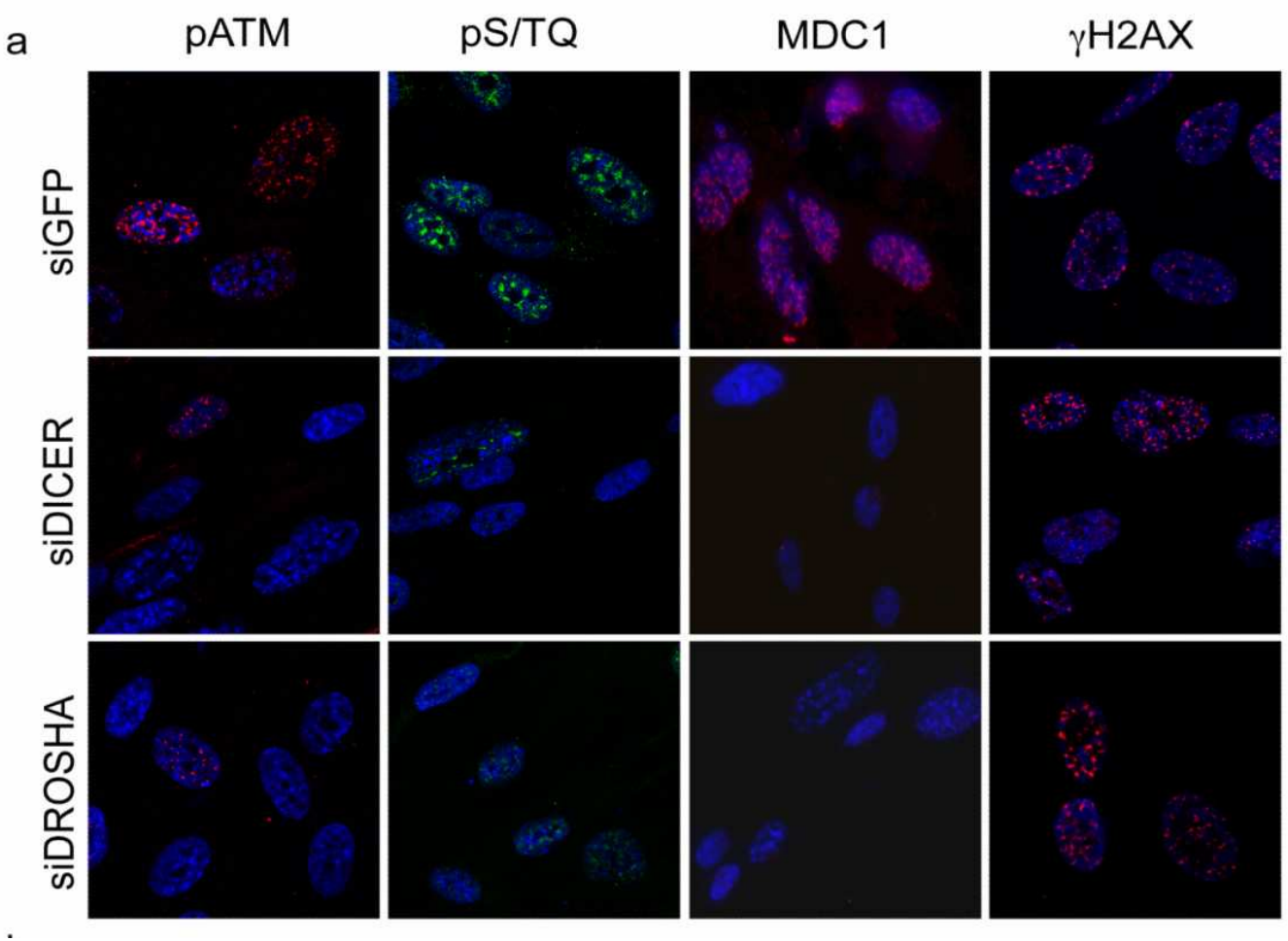
miRNA is selectively incorporated into the RISC for target recognition

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

miRNAs have imperfect complementarity to their target mRNA and inhibit translation

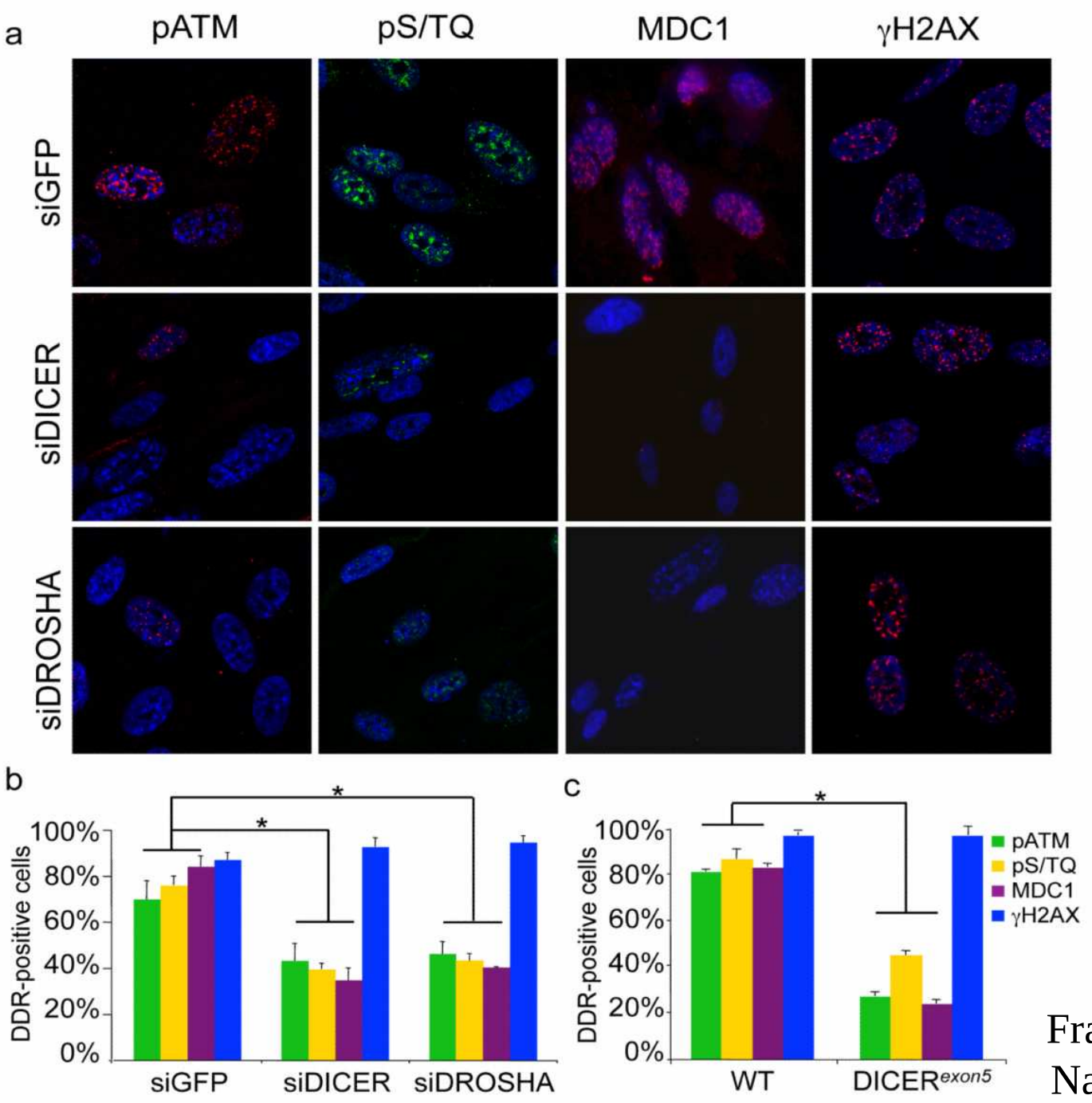
siRNAs form perfect duplex with their target mRNA and trigger mRNA degradation

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



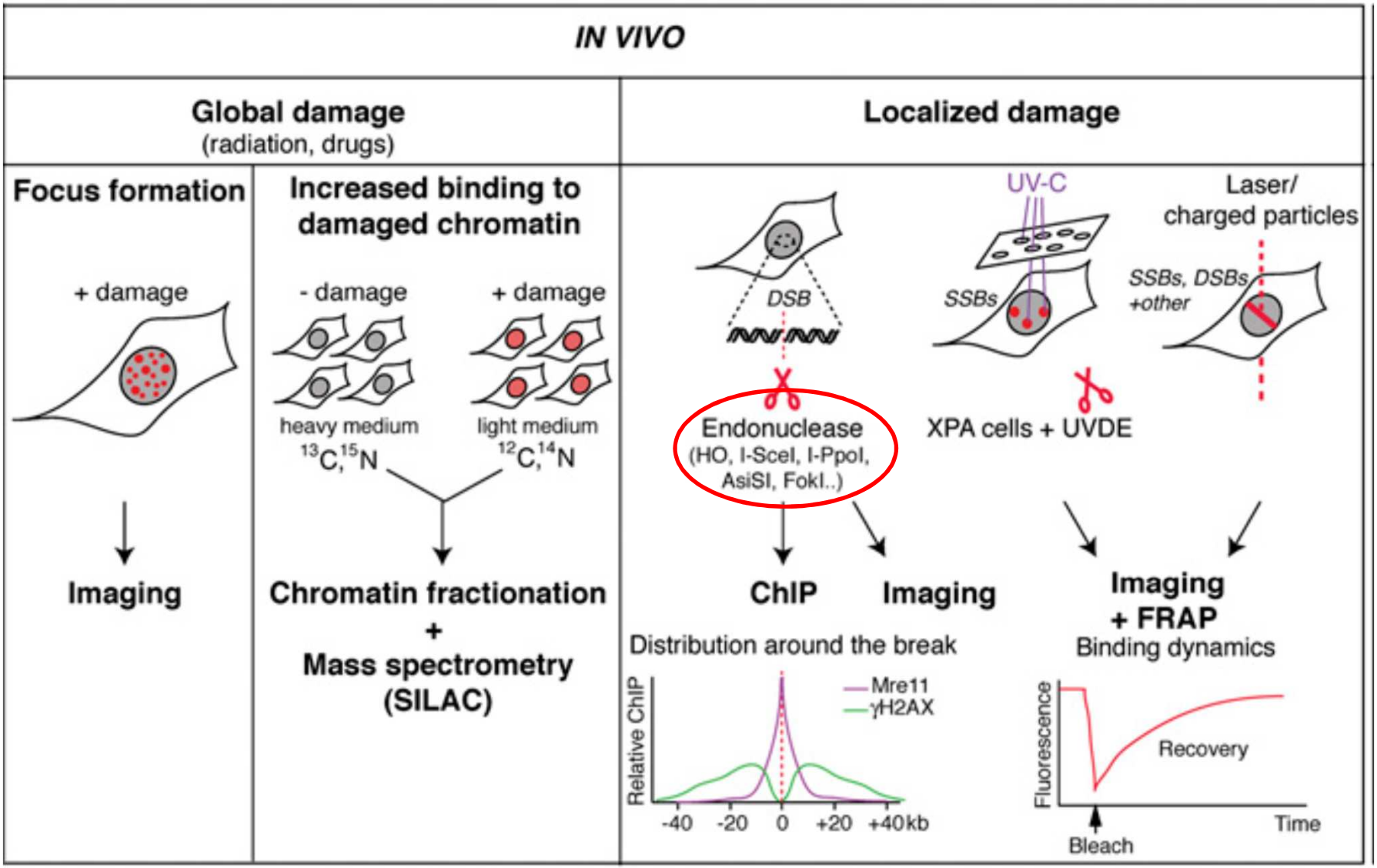
DICER or DROSHA inactivation impairs
DDR foci formation in irradiated cells

Francia et al
Nature 2012

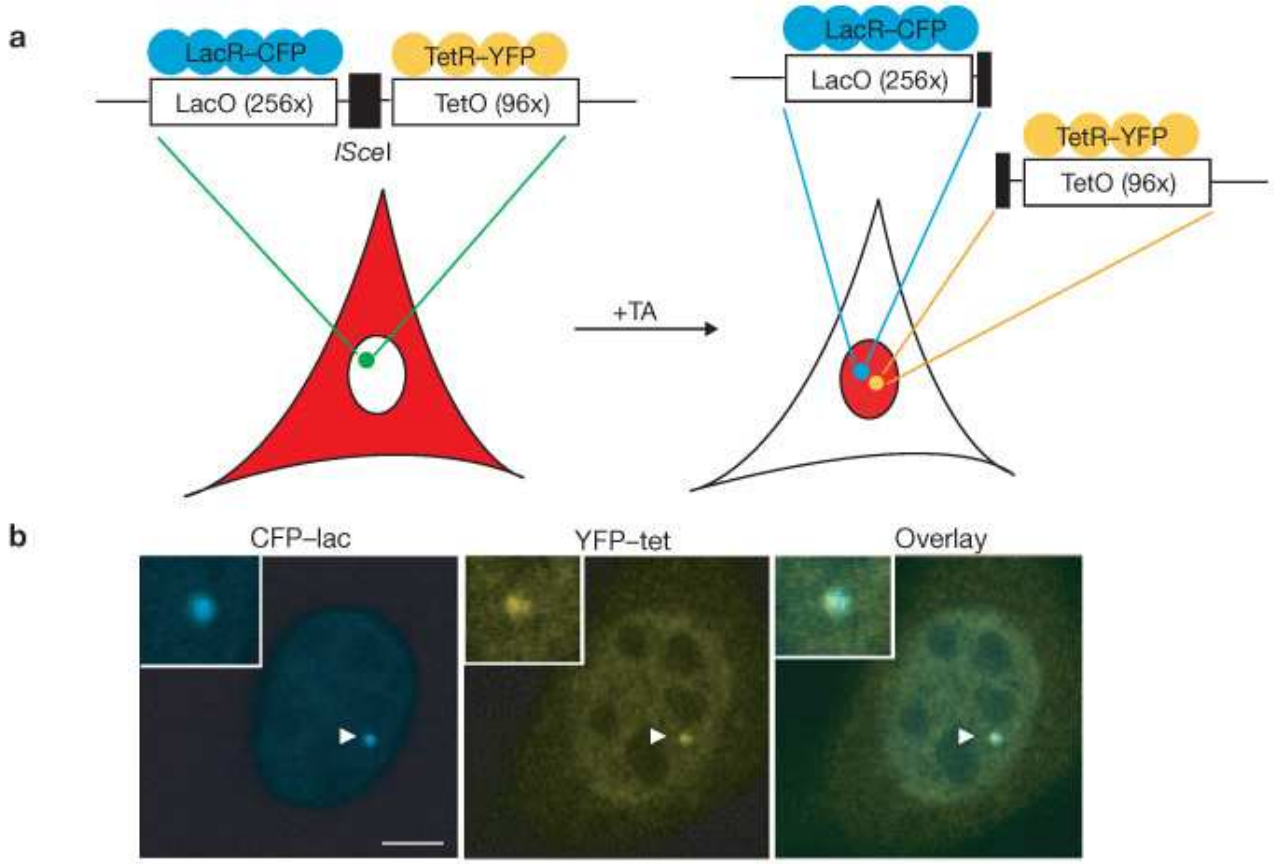


Francia et al
Nature 2012

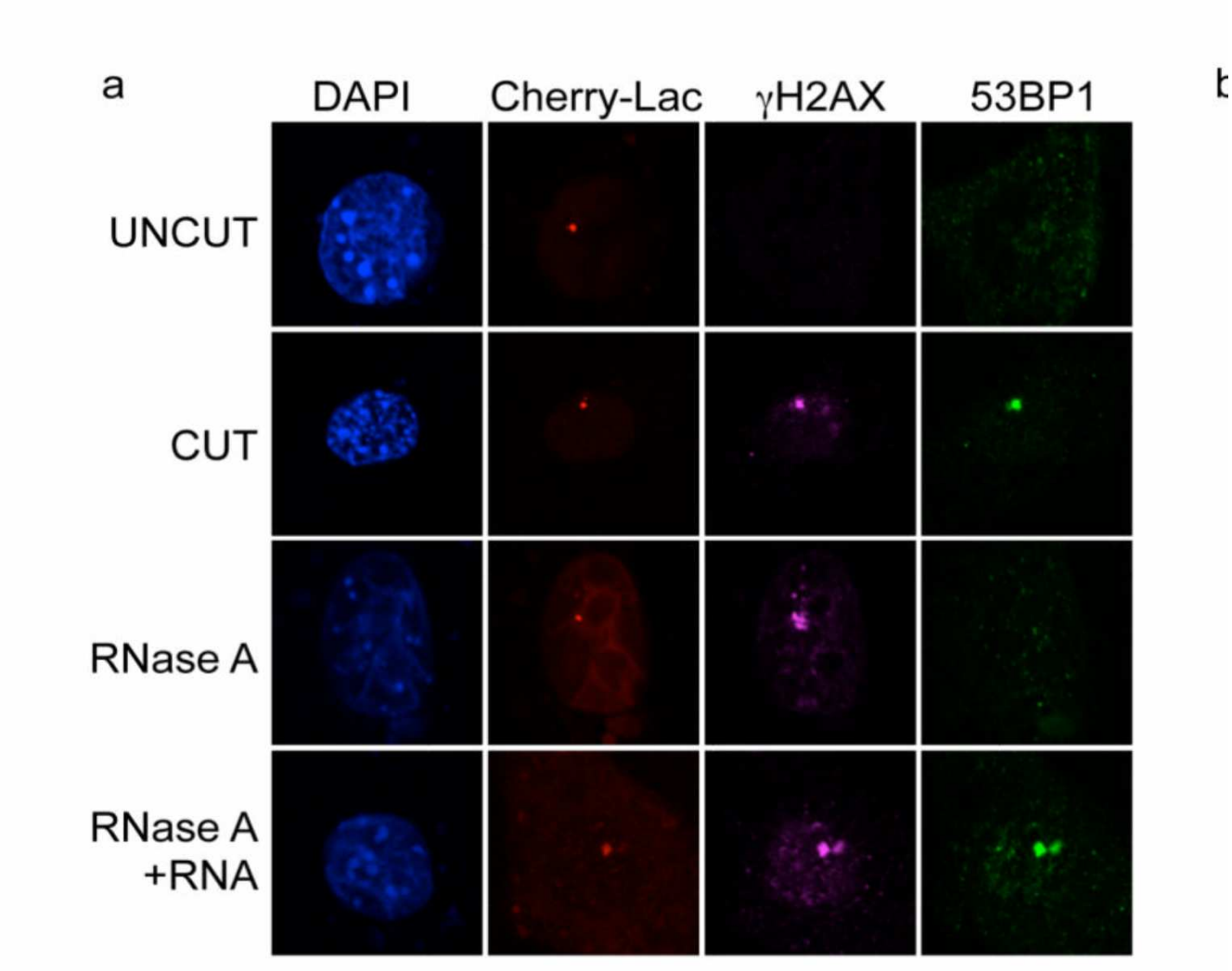
METODI


IN VIVO
Global damage
(radiation, drugs)
Localized damage
Focus formation
+ damage
Imaging
Increased binding to damaged chromatin
- damage
+ damage
heavy medium
$^{13}C,^{15}N$
light medium
$^{12}C,^{14}N$
Chromatin fractionation
+
Mass spectrometry
(SILAC)
DSB
Endonuclease
(HO, I-SceI, I-PpoI, AsiSI, FokI..)
ChIP
Imaging
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb
UV-C
SSBs
XPA cells + UVDE
Laser/
charged particles
SSBs, DSBs
+other
Imaging
+ FRAP
Binding dynamics
Fluorescence
Recovery
Time
Bleach

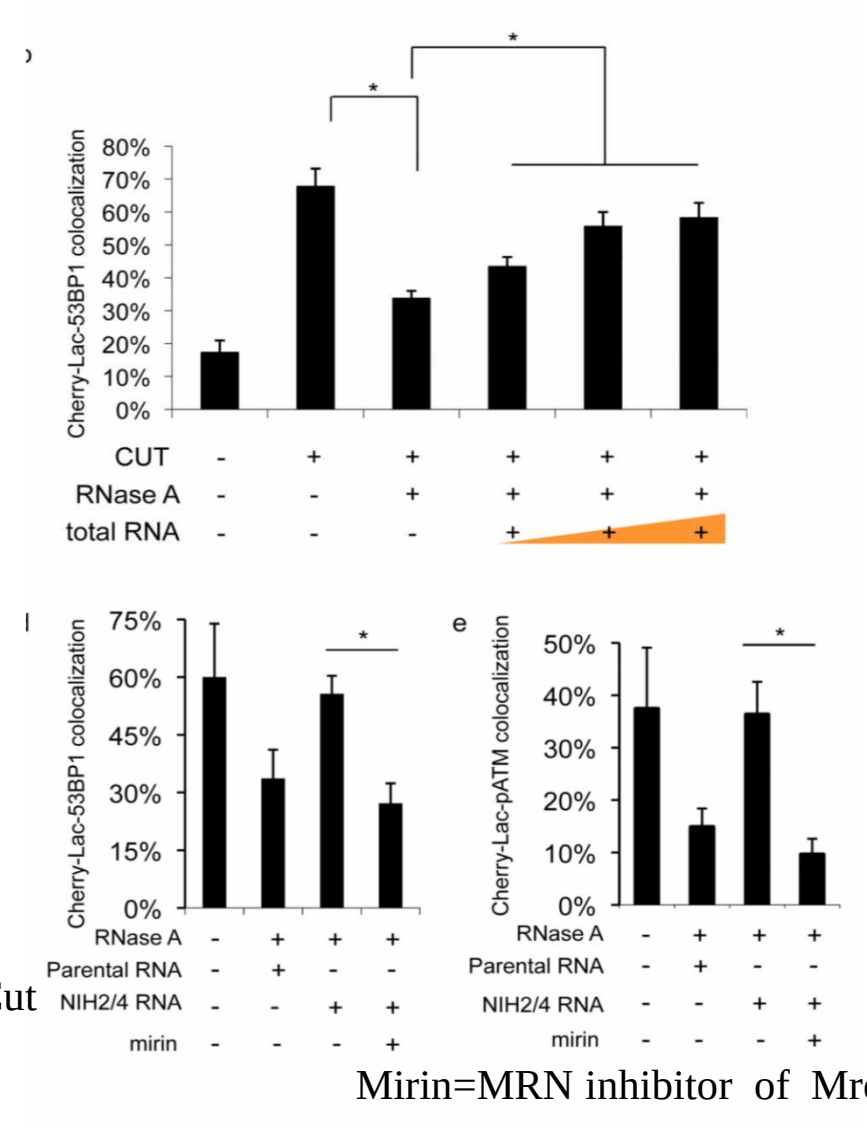
A single inducible and detectable DSB



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



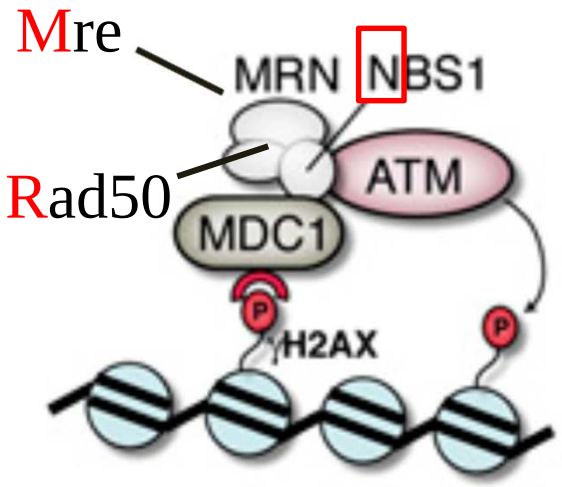
Site-specific DDR focus formation is RNase A-sensitive and can be restored by site specific RNA in a MRN-dependent manner



Cut

Mirin=MRN inhibitor of Mre11-associated exonuclease

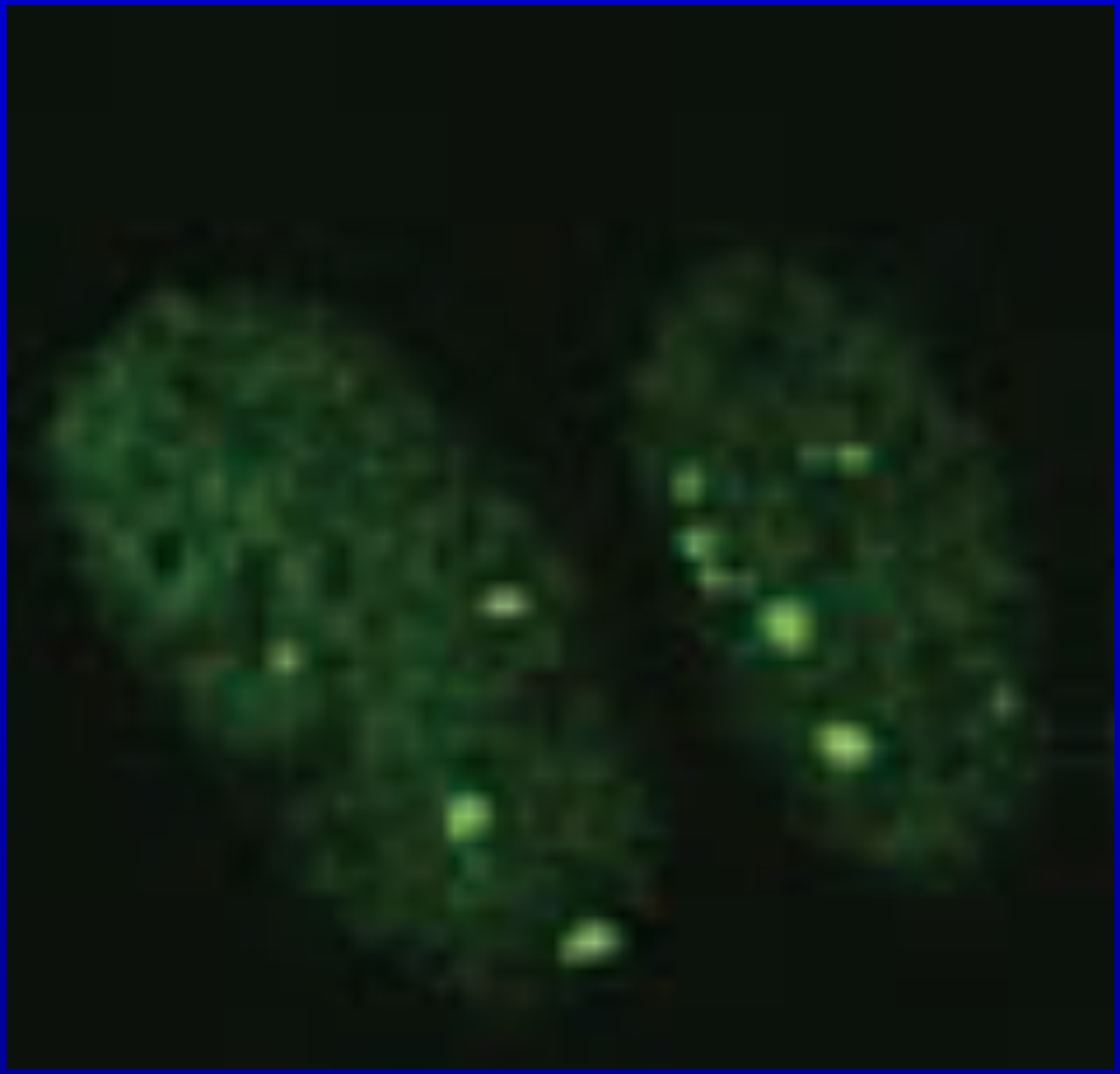
Mirin prevents MRN-dependent activation of ATM

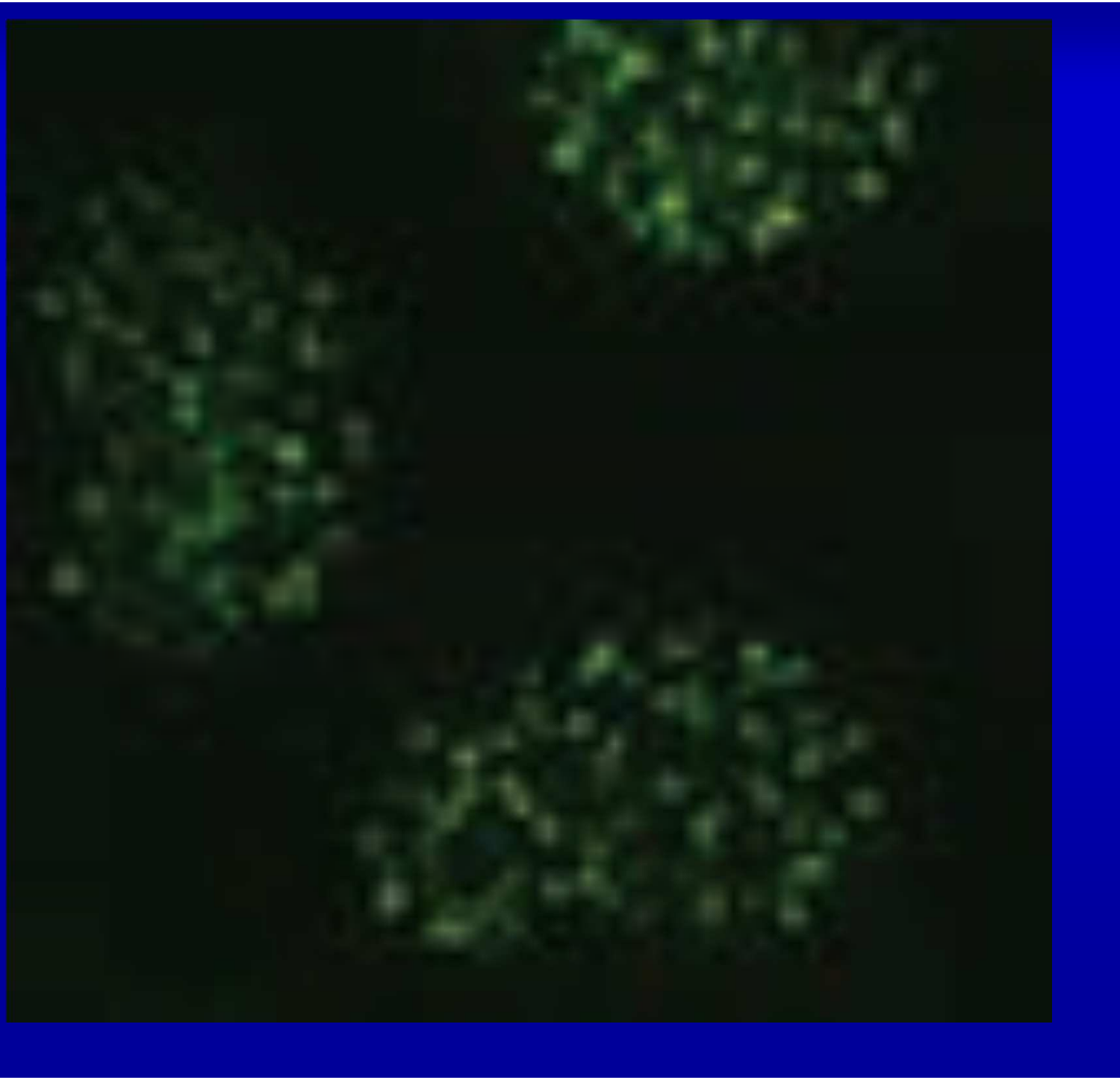


Orchestration of the DNA-Damage Response by the RNF8 Ubiquitin Ligase (Nadine Science Feb2008)

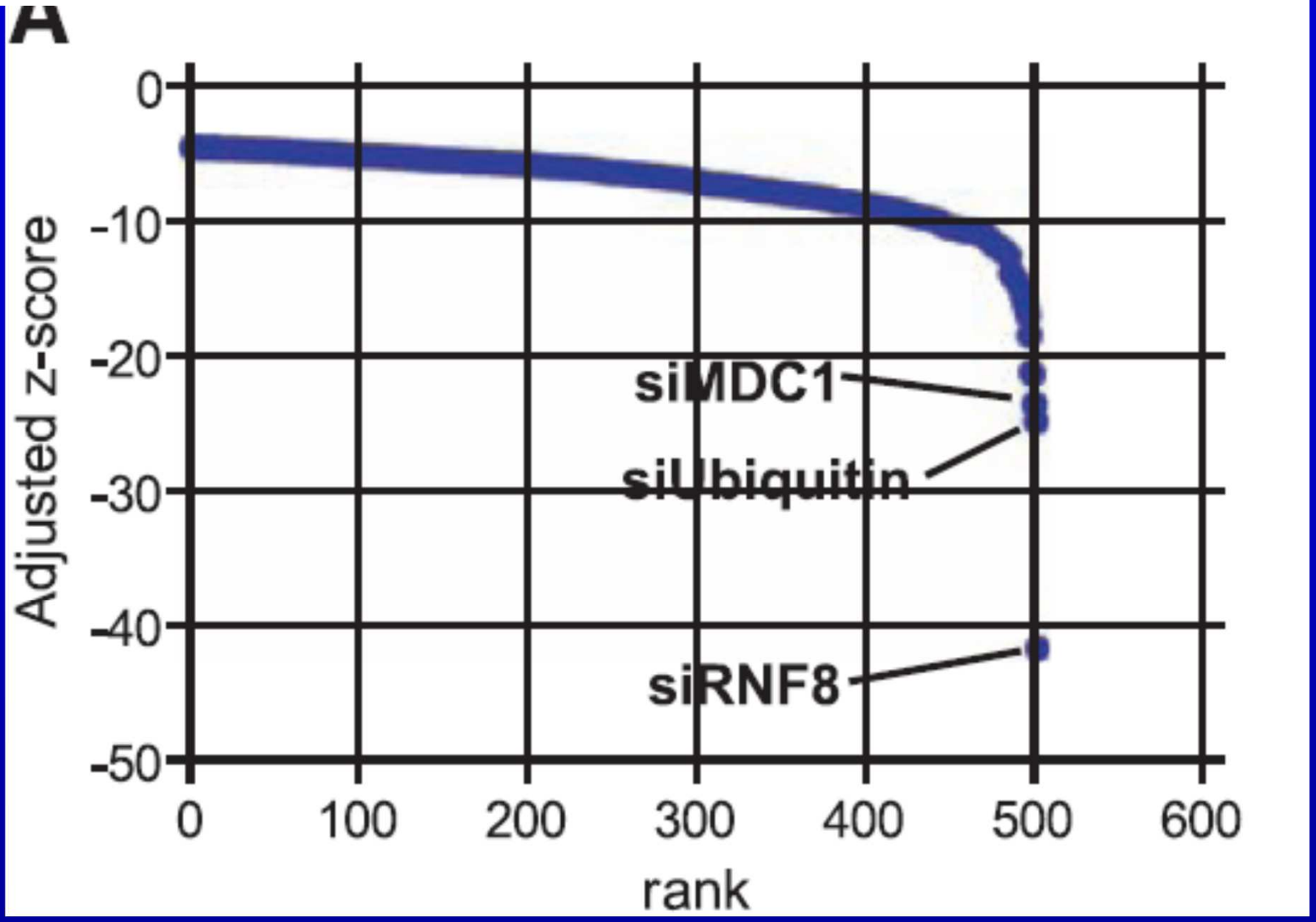
- Cells respond to DSBs by recruiting the DNA-damage mediator protein MDC1, the p53-binding protein 1 (53BP1) to sites of damaged DNA.

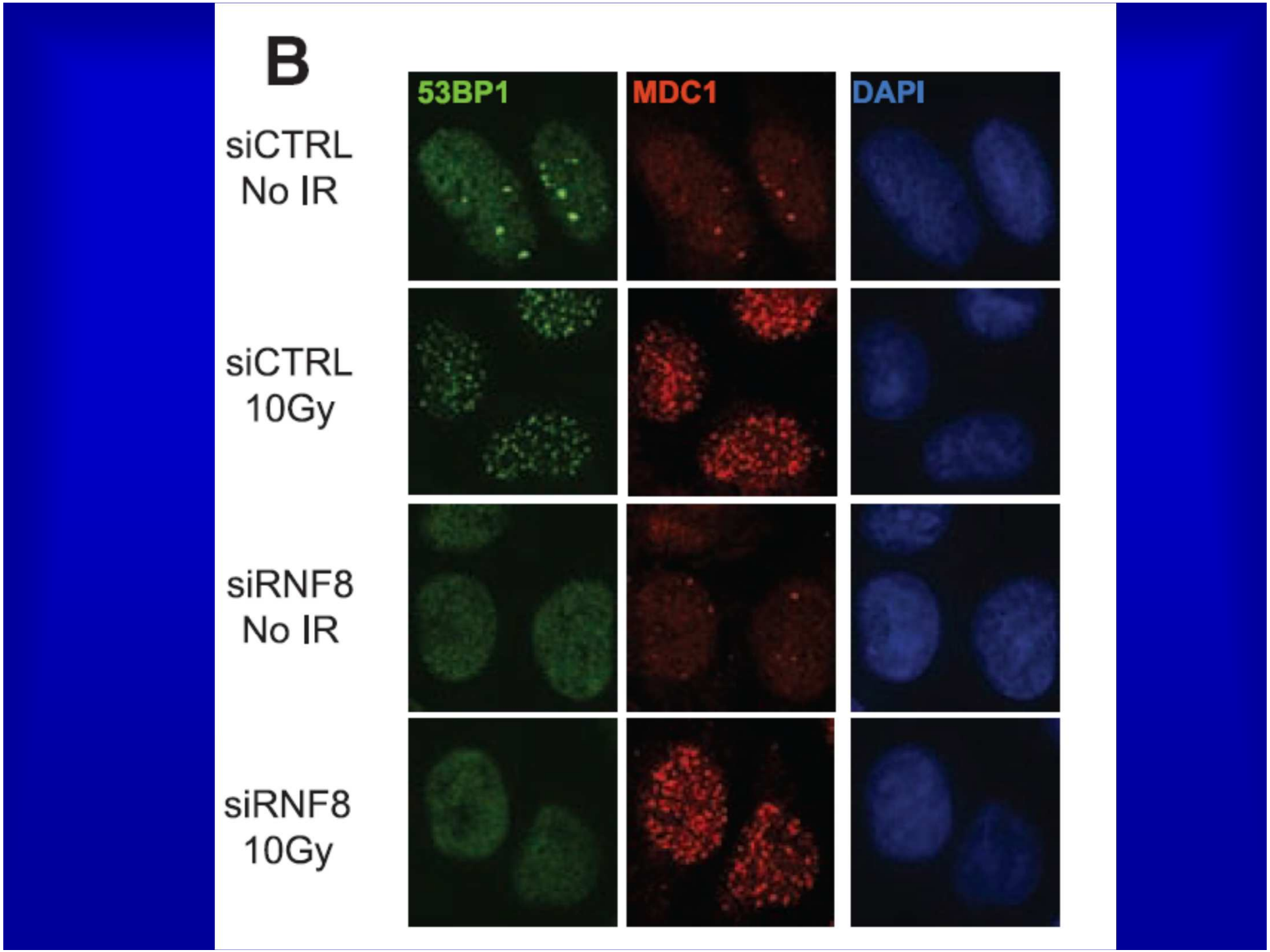
- 53BP1 is an established player- important role in modulating chromatin structure surrounding the break site- in the cellular response to DNA damage and is a canonical component of ionizing-radiation induced foci (IRIF)

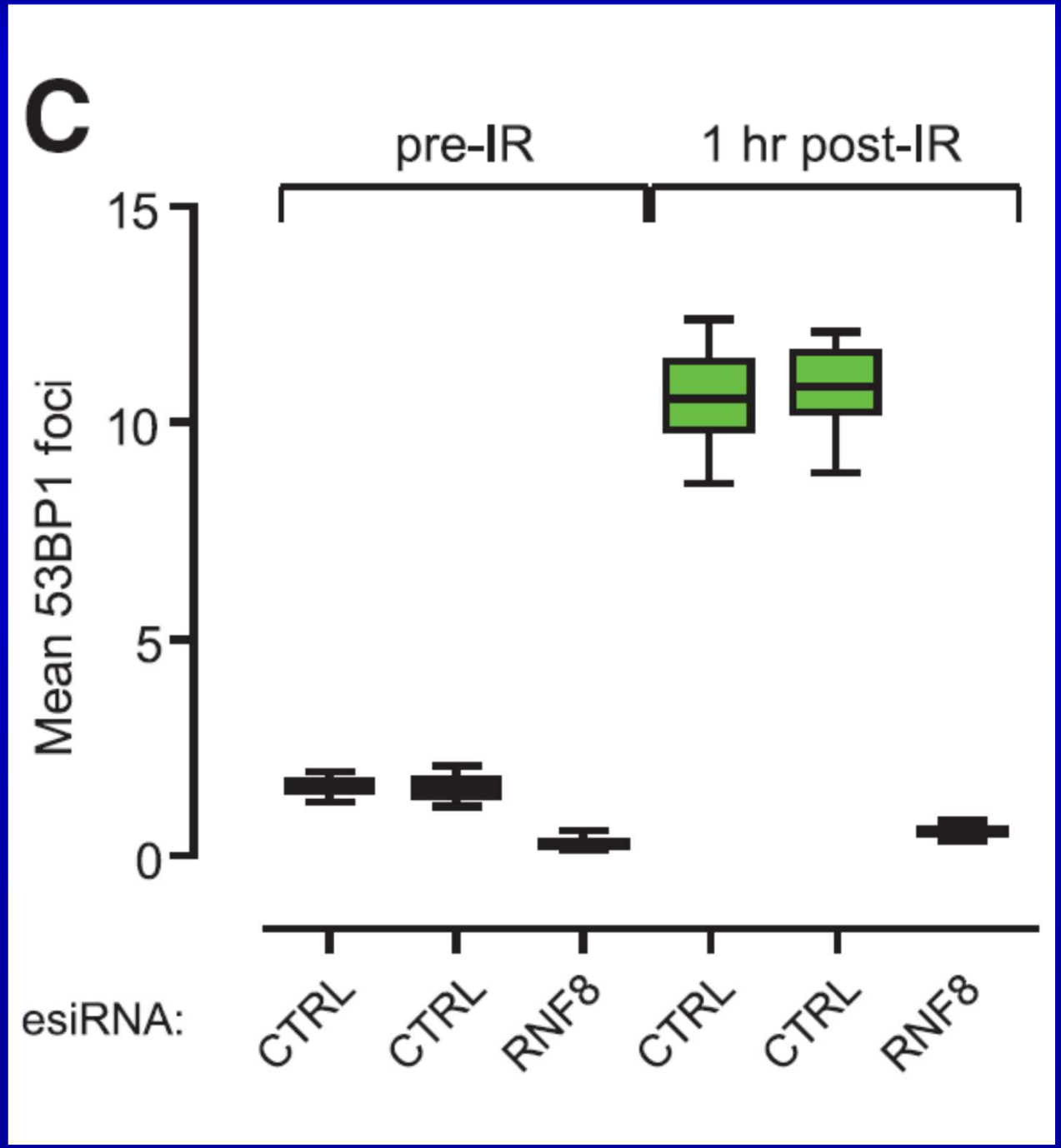




Ranking by z score of 500 siRNAs giving the least 53BP1 foci from a siRNA screen



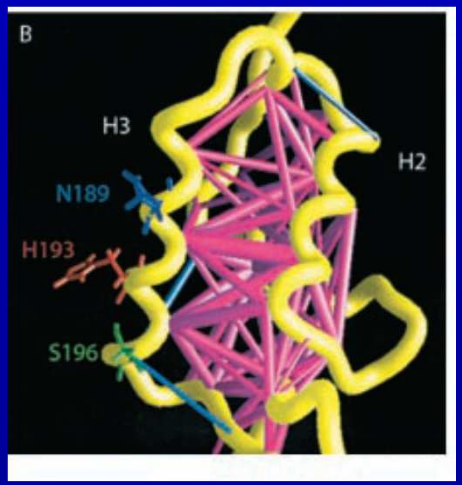

B
53BP1
MDC1
DAPI
siCTRL
No IR
siCTRL
10Gy
siRNF8
No IR
siRNF8
10Gy


C
pre-IR
1 hr post-IR
15
10
5
0
Mean 53BP1 foci
esiRNA:
CTRL
CTRL
RNF8
CTRL
CTRL
RNF8

Domain architecture of RNF8

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

Ubiquitin ligase activity



Forkhead domain

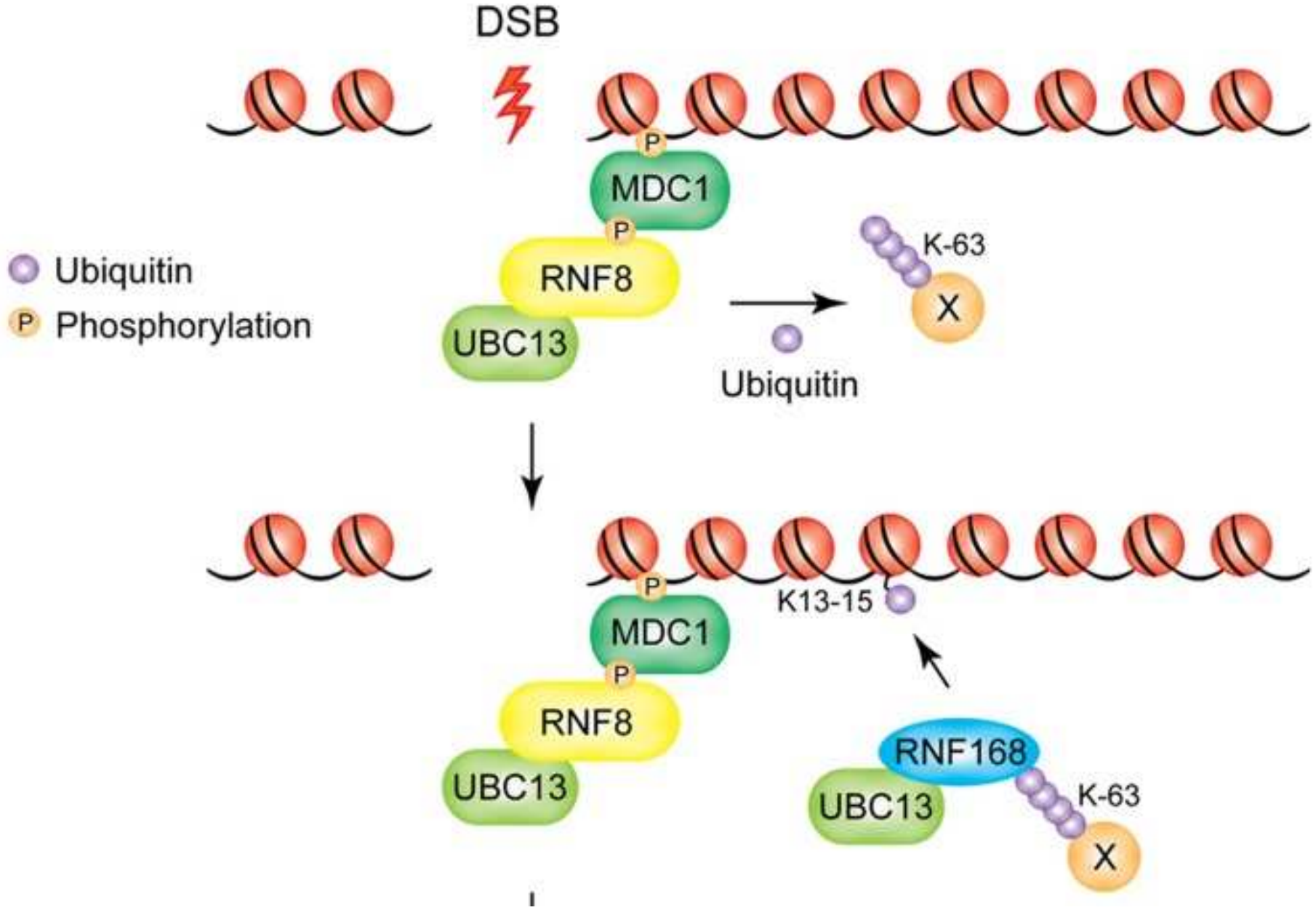
- FHA-(R42A) and
- RING finger (C406s) mutants.

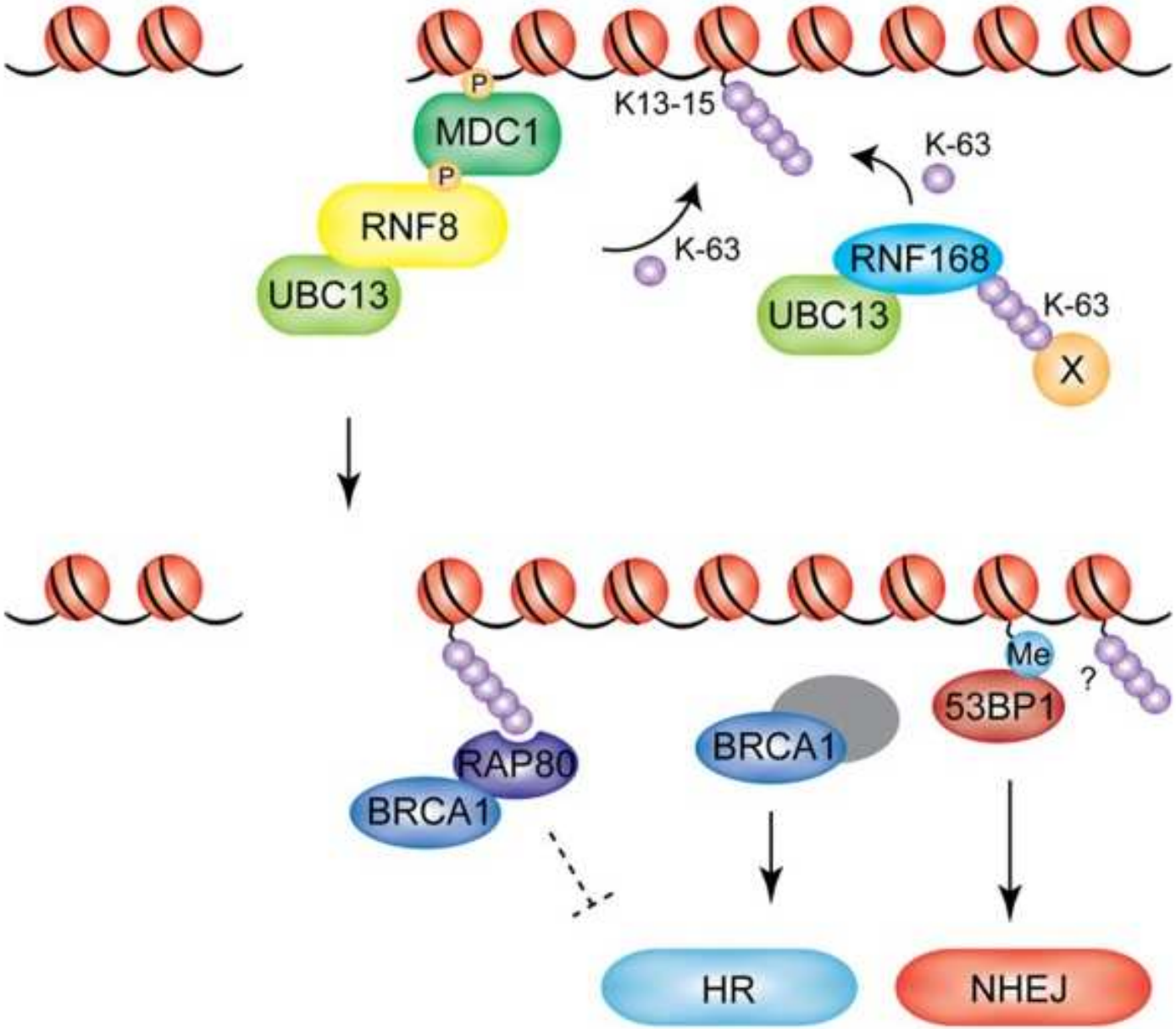
- It is possible to interfere with ubiquitin-dependent recruitment of DDR factors by expressing proteins containing ubiquitin binding domains (UBDs) that bind to lysine 63-linked polyubiquitin chains.

RNF8 e RNF168

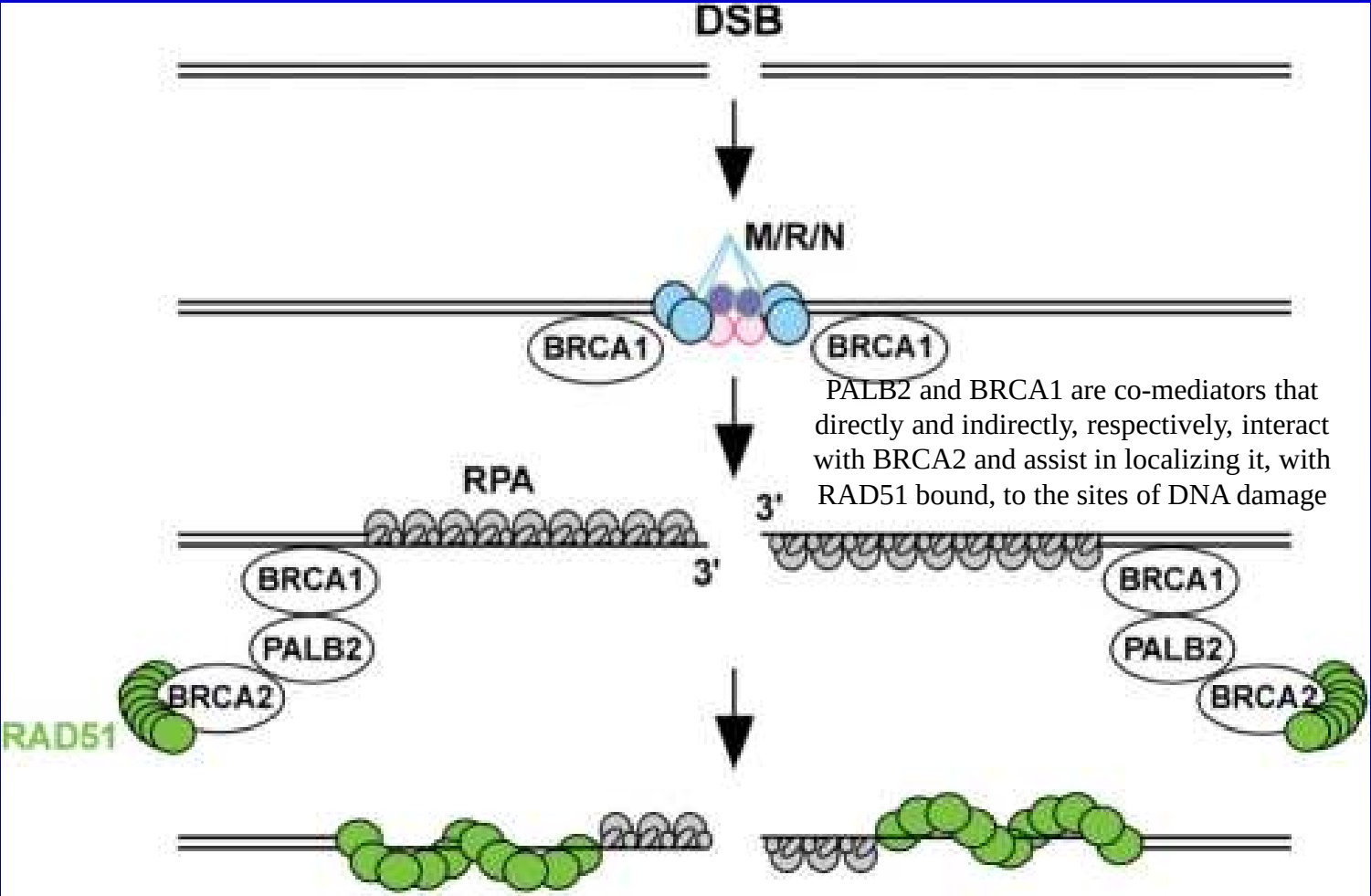
- The rapid ubiquitination of chromatin surrounding DNA double-stranded breaks (DSB) drives the formation of large structures called ionizing radiation-induced foci (IRIF), comprising many DNA damage response (DDR) proteins.

- 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.


DSB
Ubiquitin
Phosphorylation
P
MDC1
RNF8
UBC13
K-63
X
Ubiquitin
K13-15
RNF168


P
MDC1
P
RNF8
UBC13
K13-15
K-63
K-63
RNF168
UBC13
K-63
X
Me
?
53BP1
RAP80
BRCA1
BRCA1
HR
NHEJ

BRCA1/BRCA2

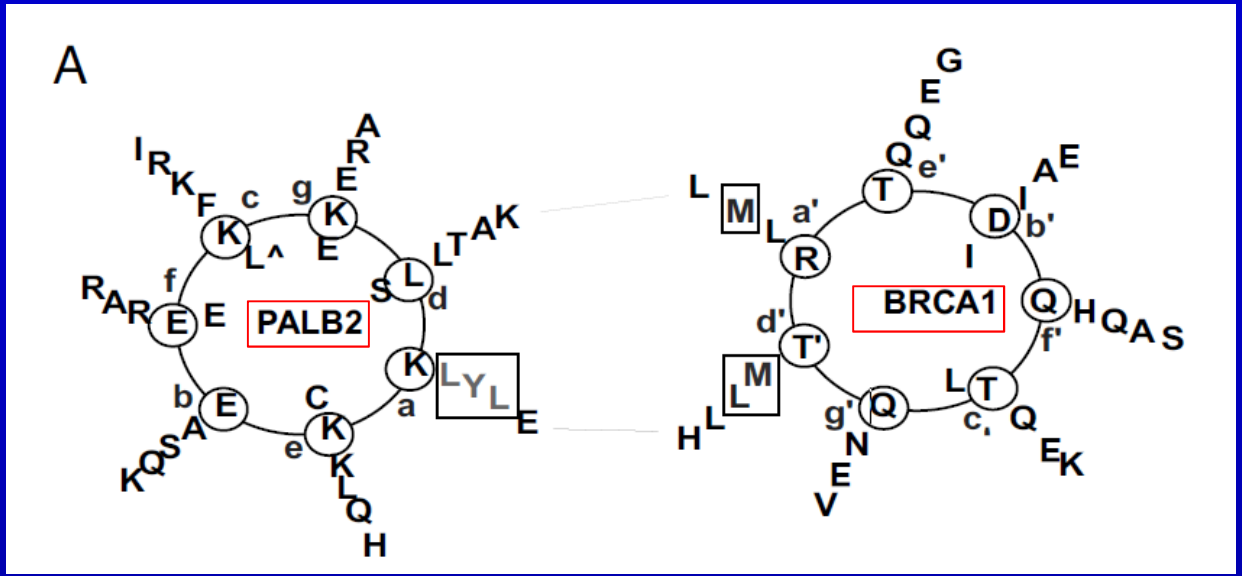


PALB2 and BRCA1 are co-mediators that directly and indirectly, respectively, interact with BRCA2 and assist in localizing it, with RAD51 bound, to the sites of DNA damage

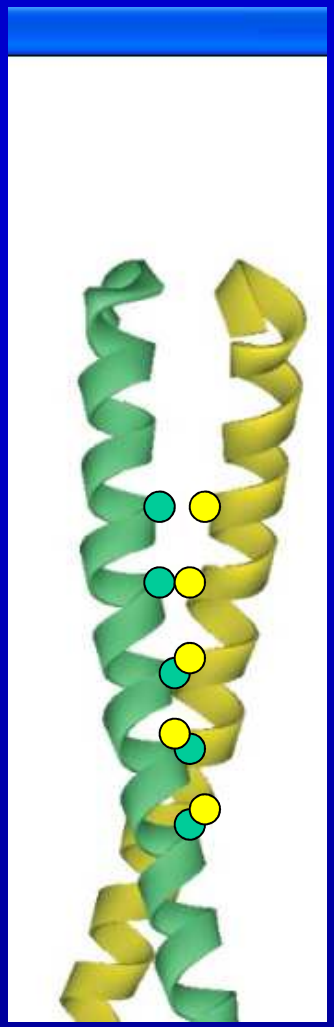
Human BRCA2 is a mediator that interacts directly with approximately eight RAD51 molecules and transports them to the site of ss-DNA bound by RPA

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

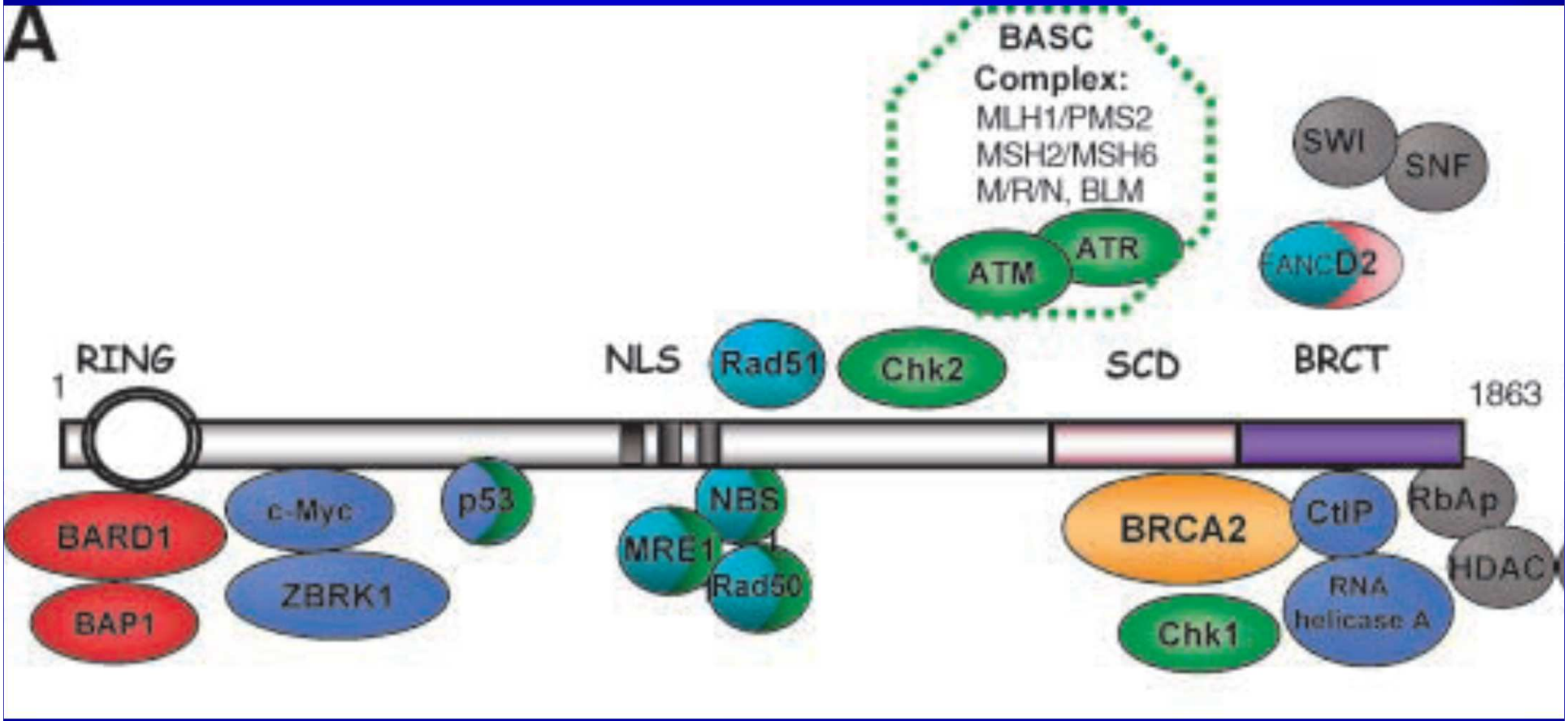
regions required for the BRCA1-PALB2 interaction.



(*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



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