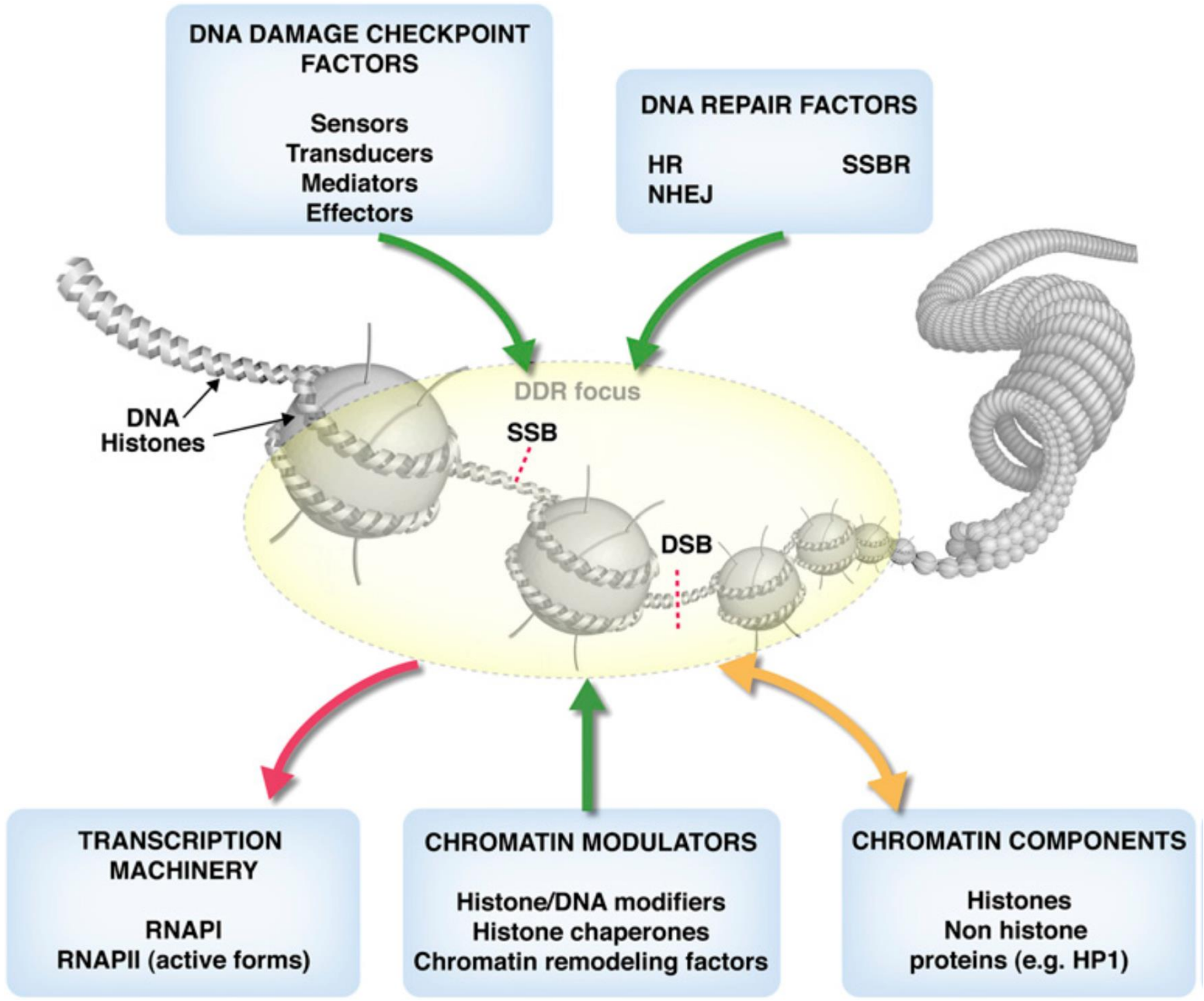
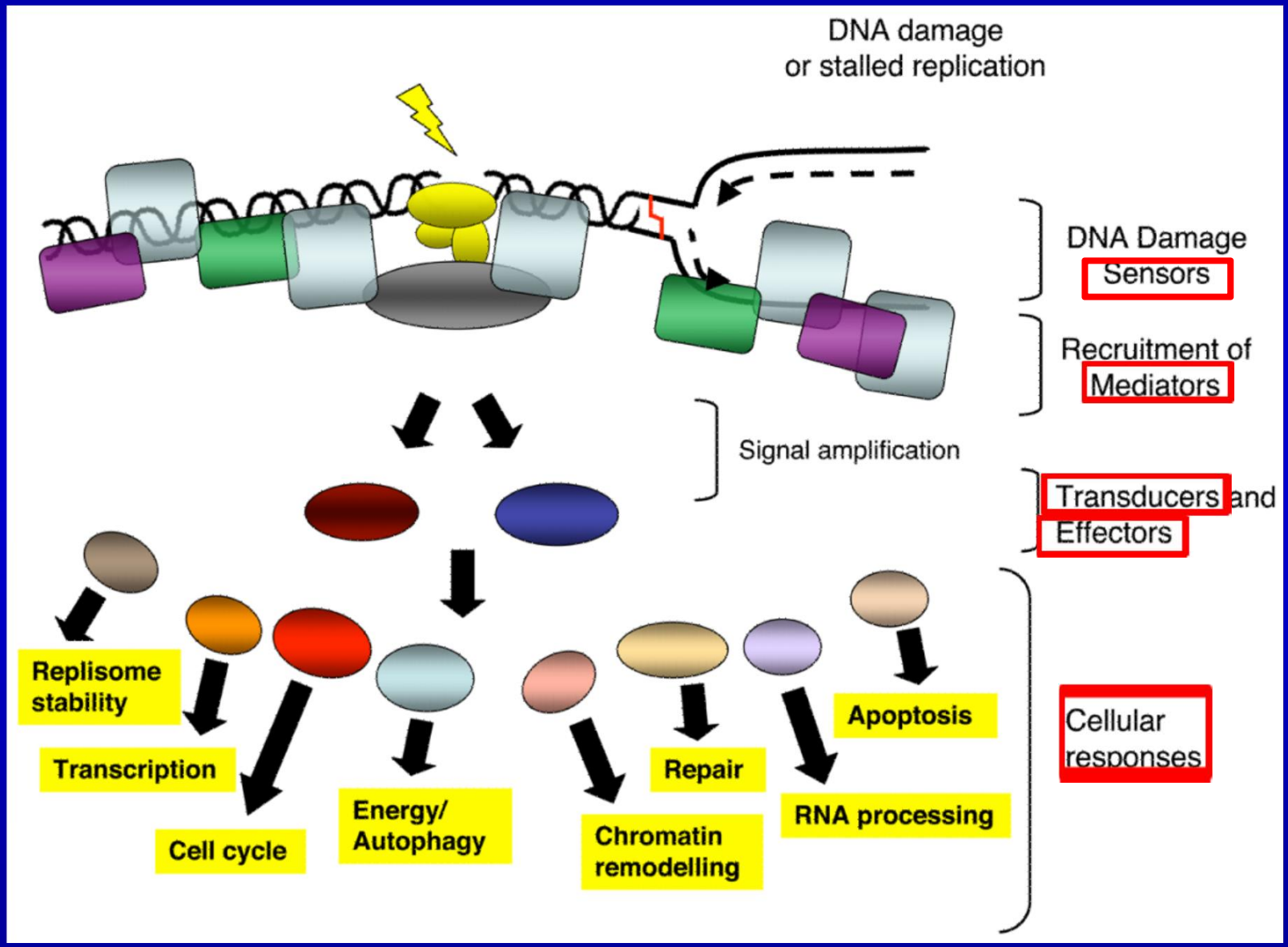
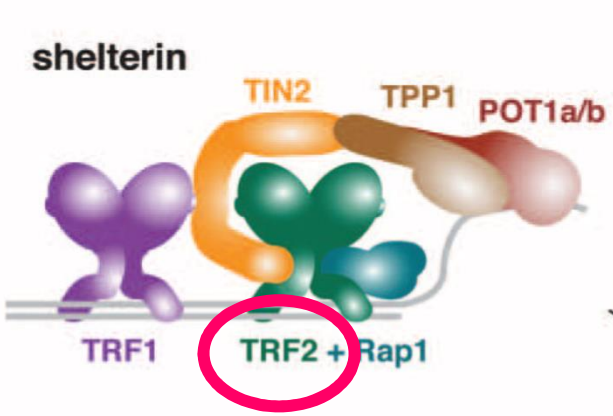

DNA DAMAGE CHECKPOINT FACTORS
Sensors
Transducers
Mediators
Effectors
DNA REPAIR FACTORS
HR
SSBR
NHEJ
DNA
Histones
DDR focus
SSB
DSB
TRANSCRIPTION MACHINERY
RNAPI
RNAPII (active forms)
CHROMATIN MODULATORS
Histone/DNA modifiers
Histone chaperones
Chromatin remodeling factors
CHROMATIN COMPONENTS
Histones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

six pathways!

DSB

Double-Strand Breaks

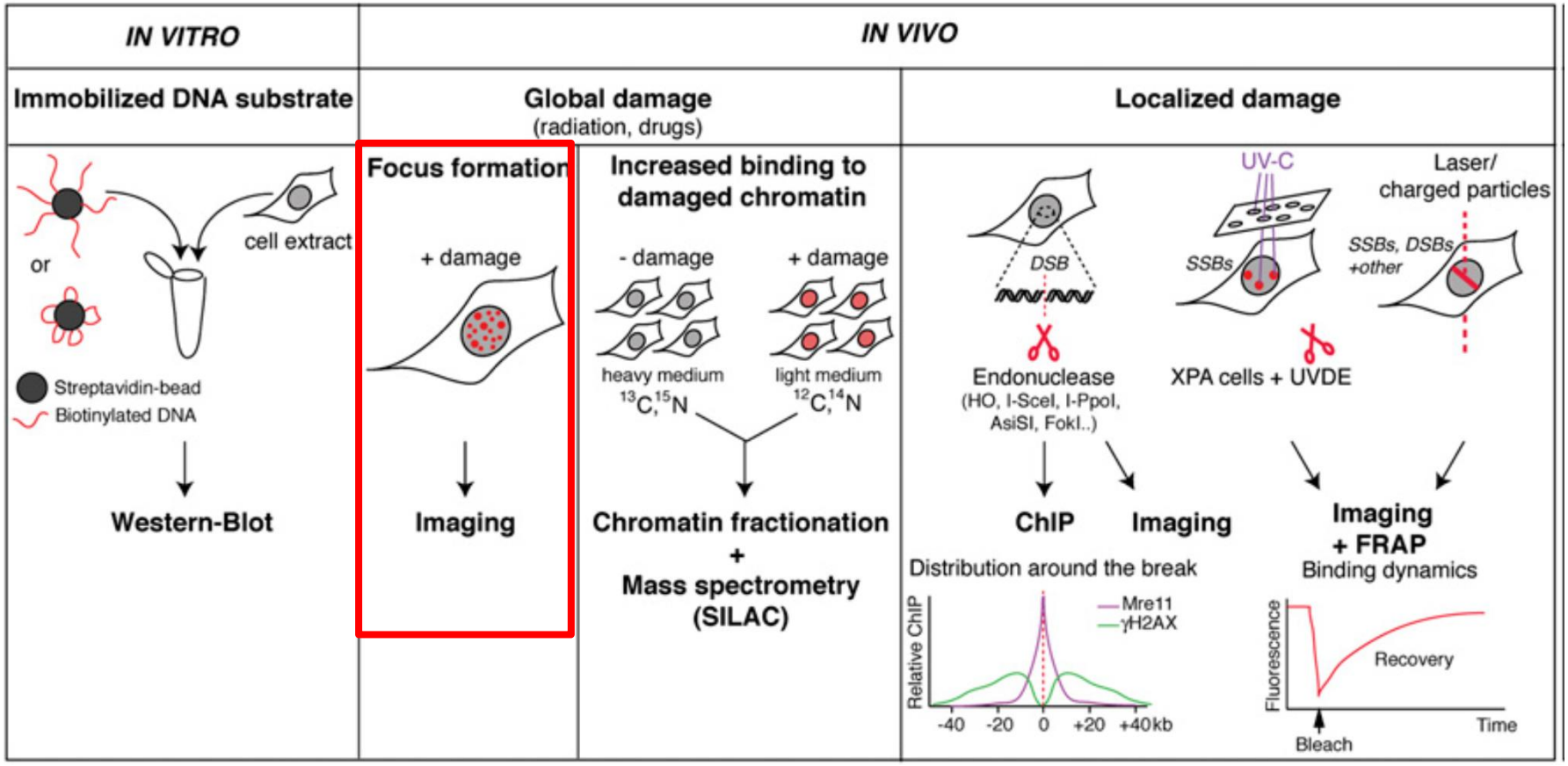
causate da

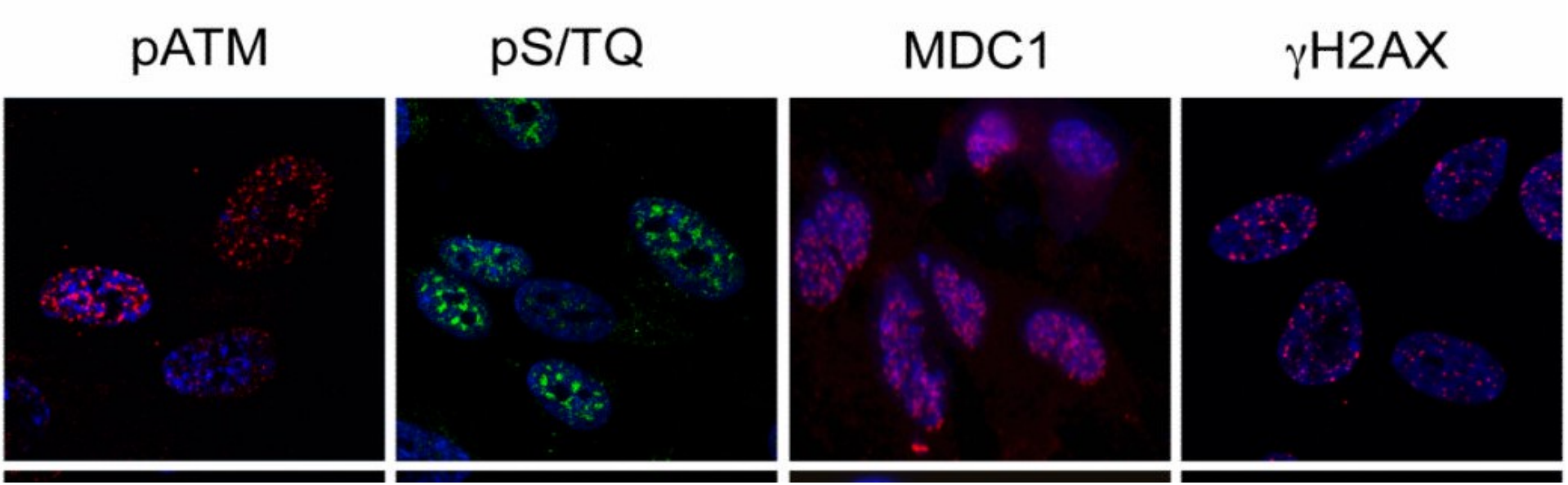
radiazioni

stress ossidativo

farmaci

METODI


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

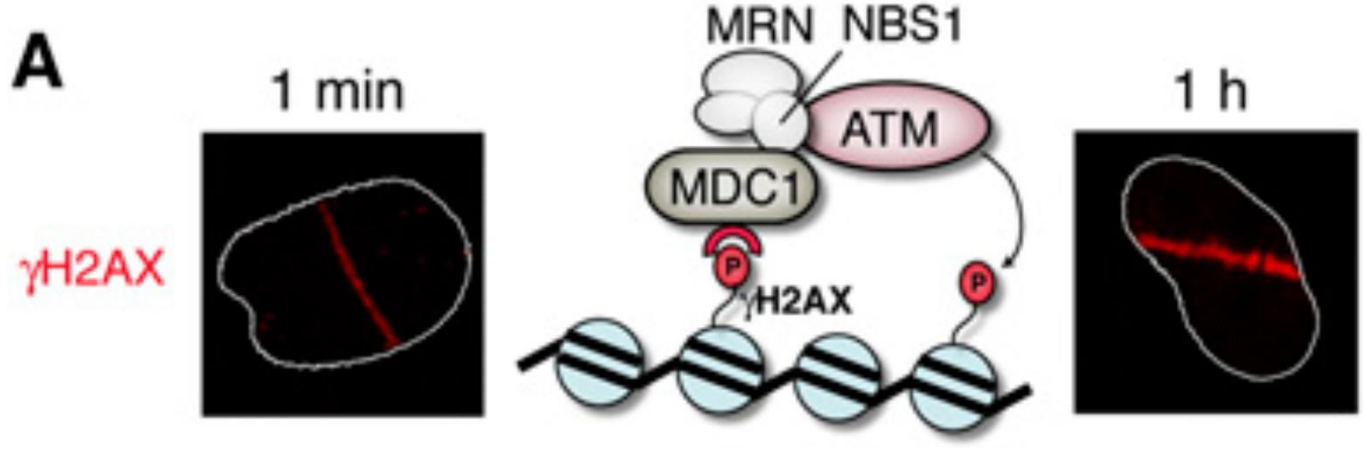


DDR foci formation in irradiated (2 Gy) cells
fixed 2 h later

IRIF IRradiation Induced Focus

DDR signal spreading

Laser micro-irradiation



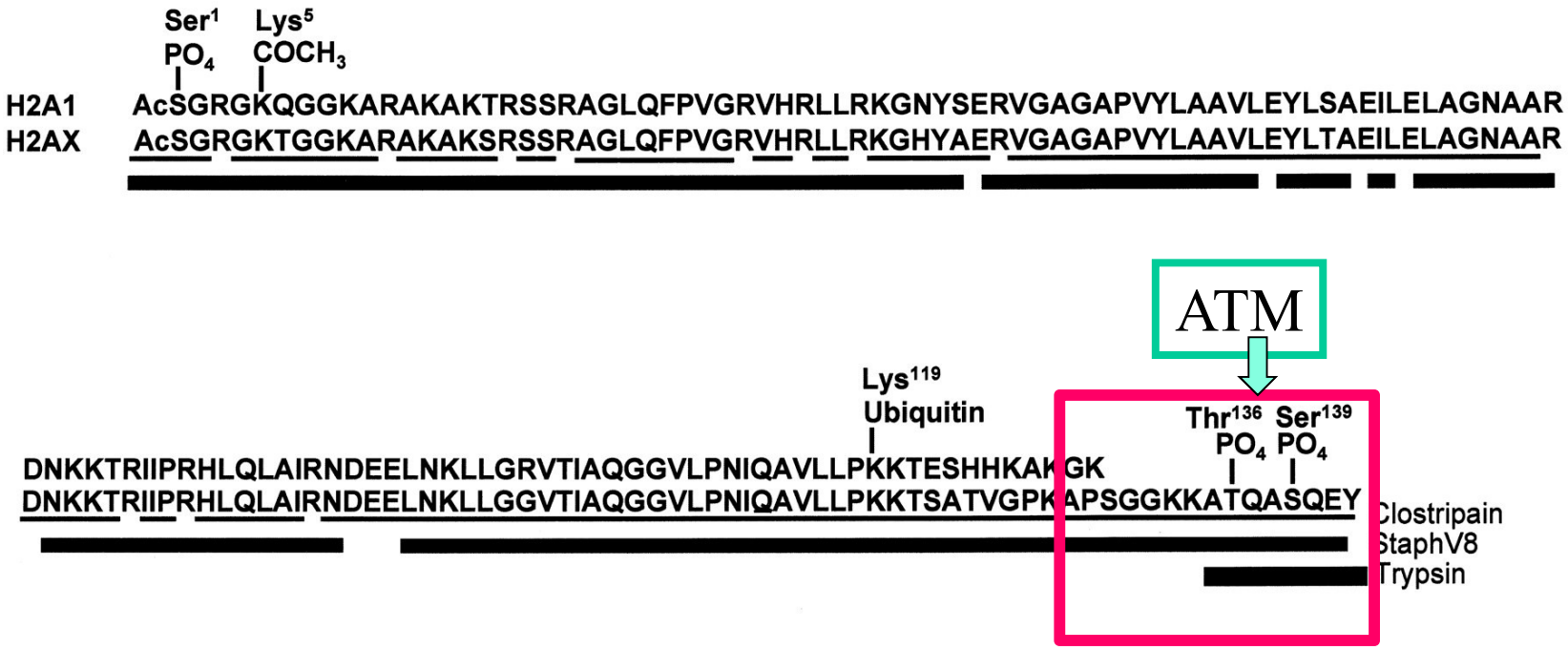
DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds gH2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.

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

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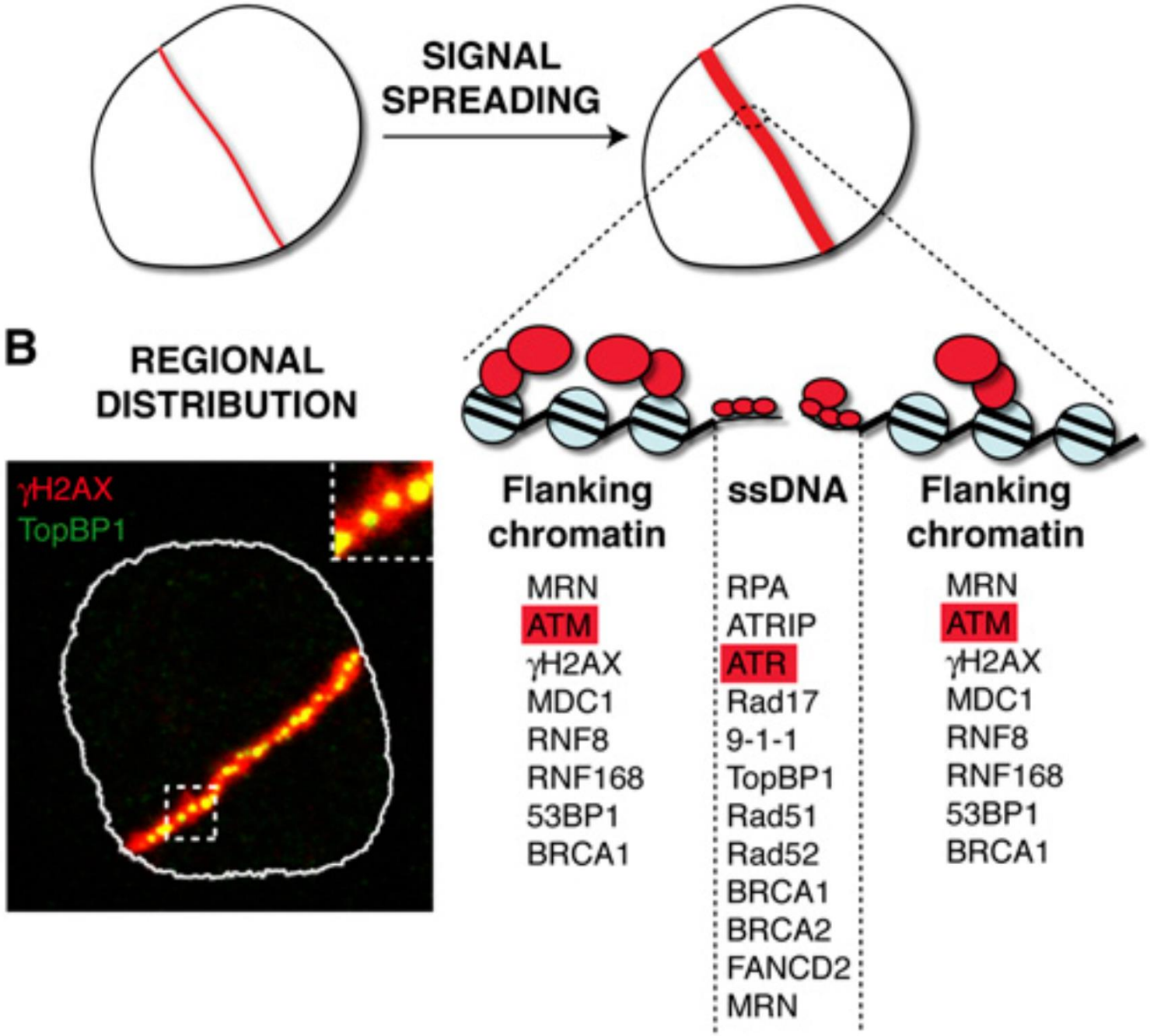
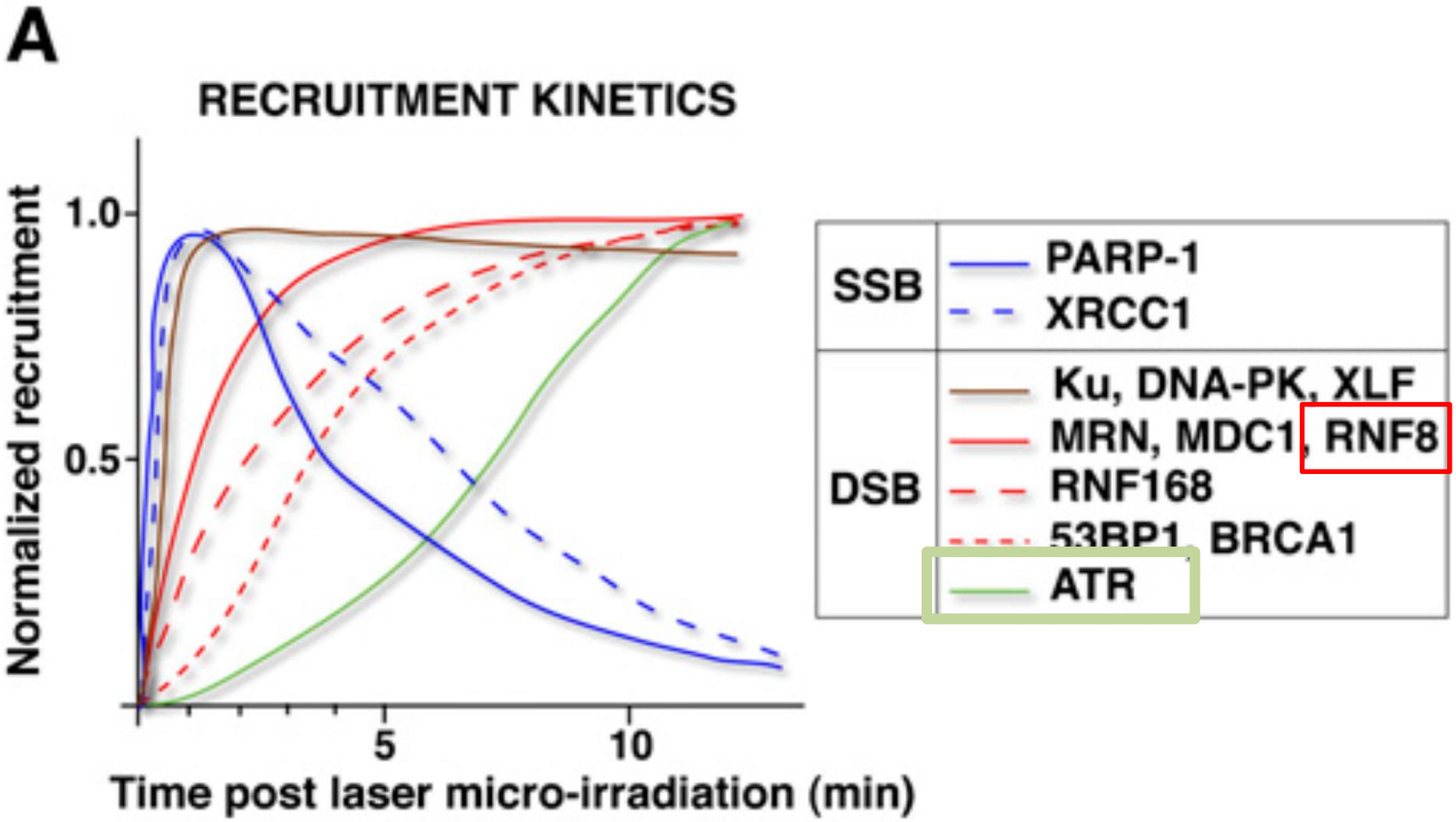
jbc

The determination of radiation exposure in diagnostic and interventional radiology

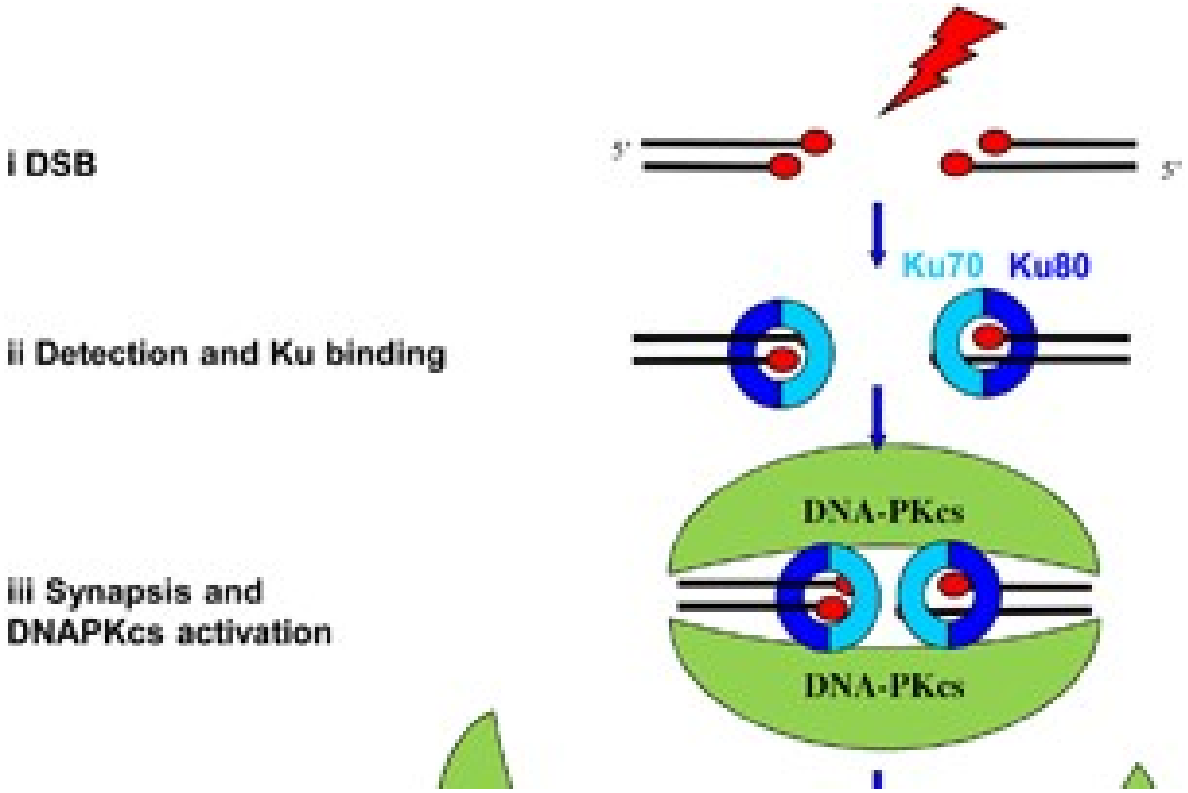
- γ-H2AX immunofluorescence microscopy is a reliable and sensitive method for the quantification of radiation induced DNA double-strand breaks (DSB) in blood lymphocytes.
- The detectable amount of these DNA damages correlates well with the dose received.


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

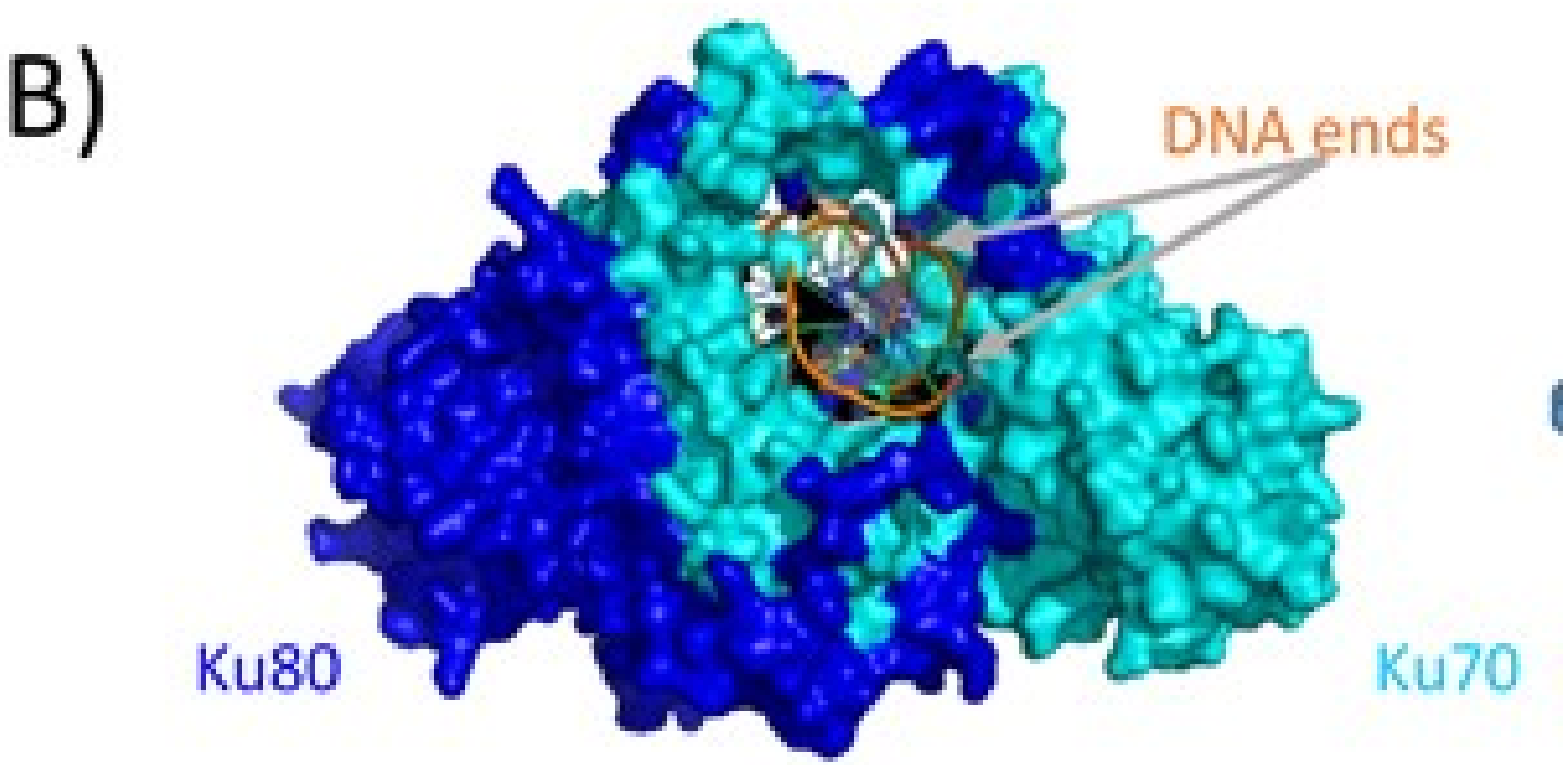


Non-homologous end joining: Common interaction sites and exchange of multiple factors in the DNA repair process



BioEssays
Volume 39, Issue 3, 30 JAN 2017 DOI: 10.1002/bies.201600209
http://onlinelibrary.wiley.com/doi/10.1002/bies.201600209/full#bies201600209-fig-0002

Non-homologous end joining: Common interaction sites and exchange of multiple factors in the DNA repair process



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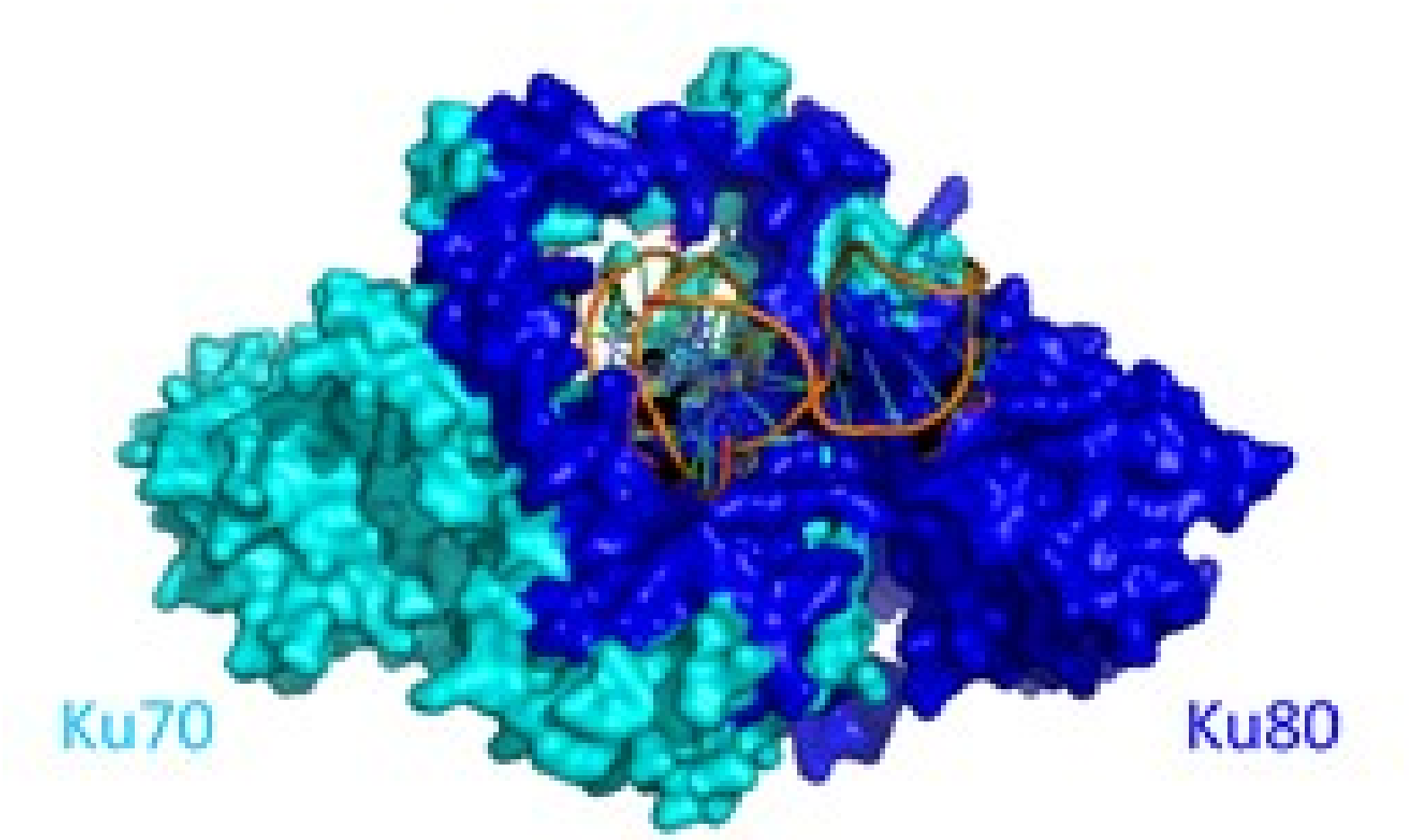

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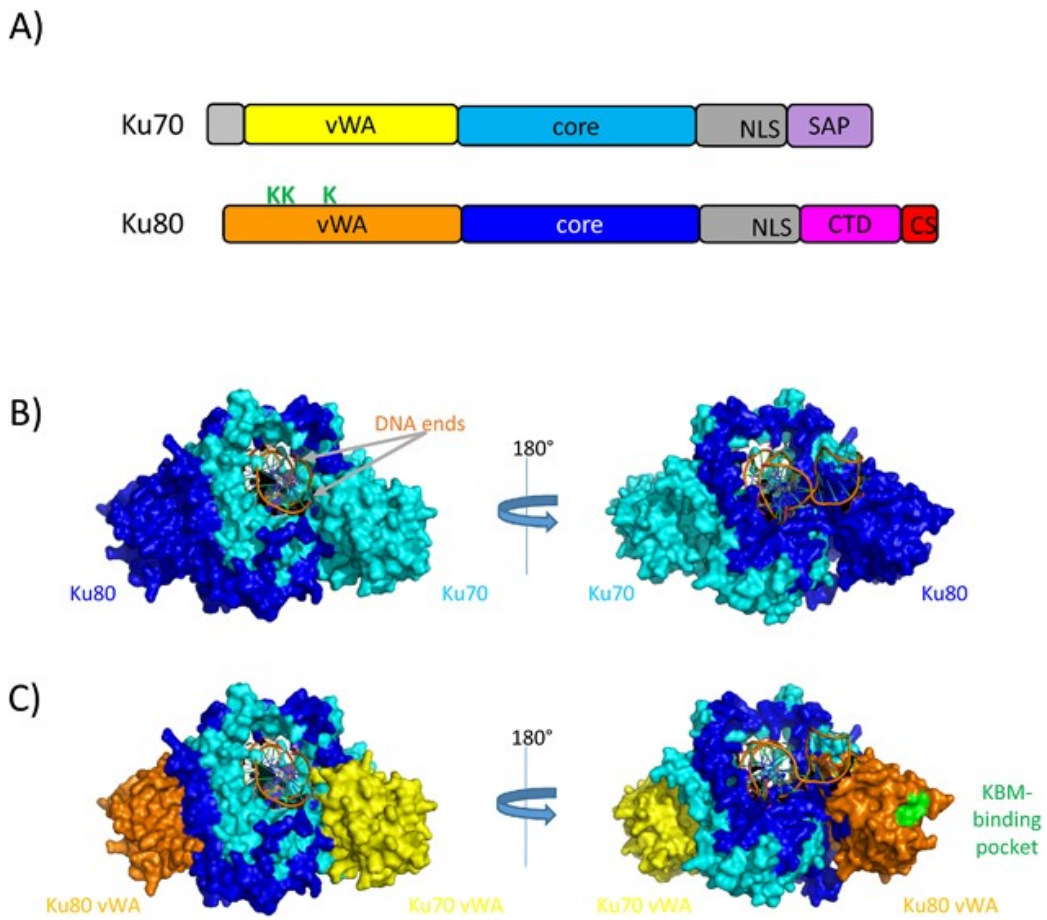

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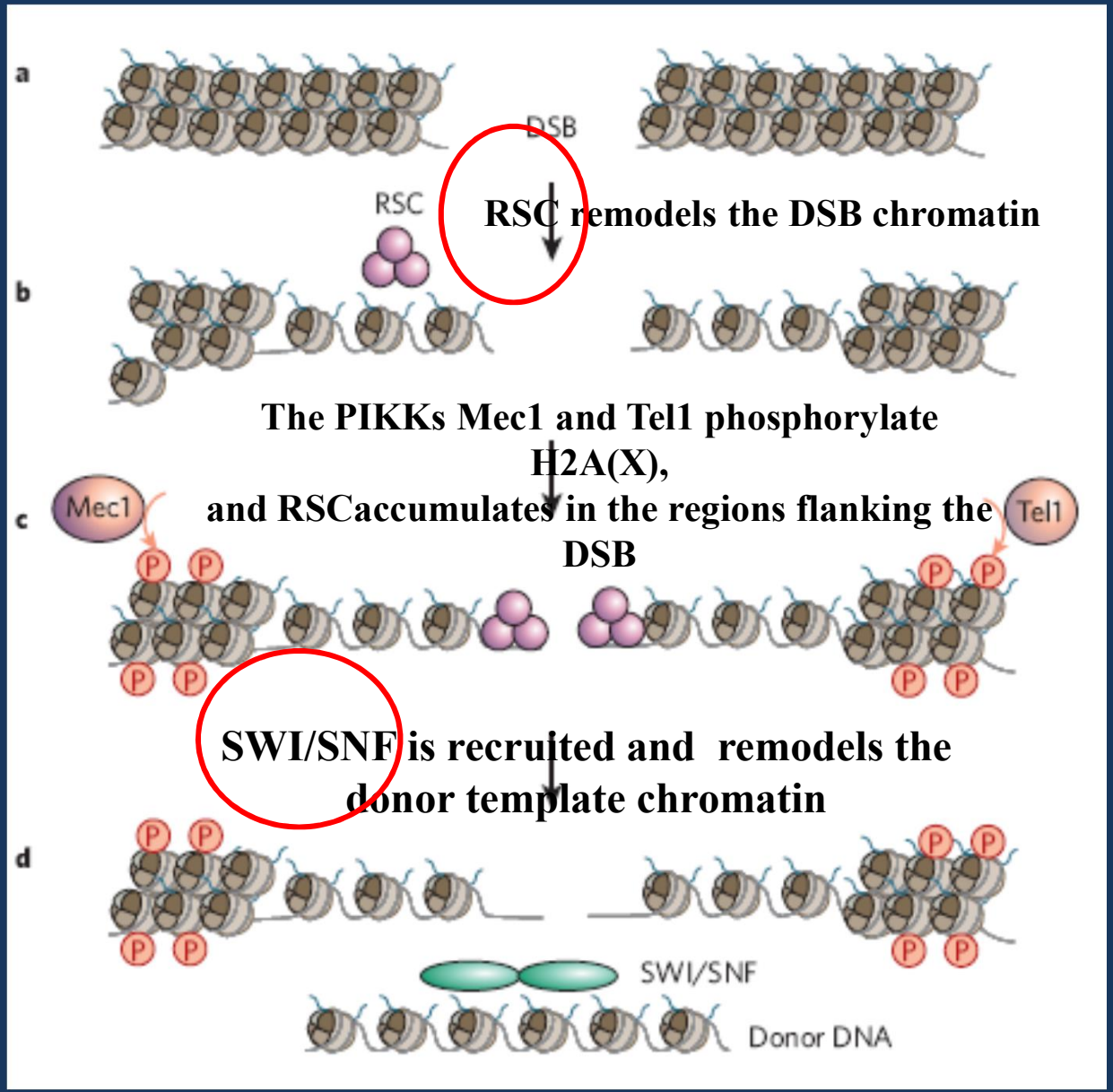
DSB
e CROMATINA

- 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



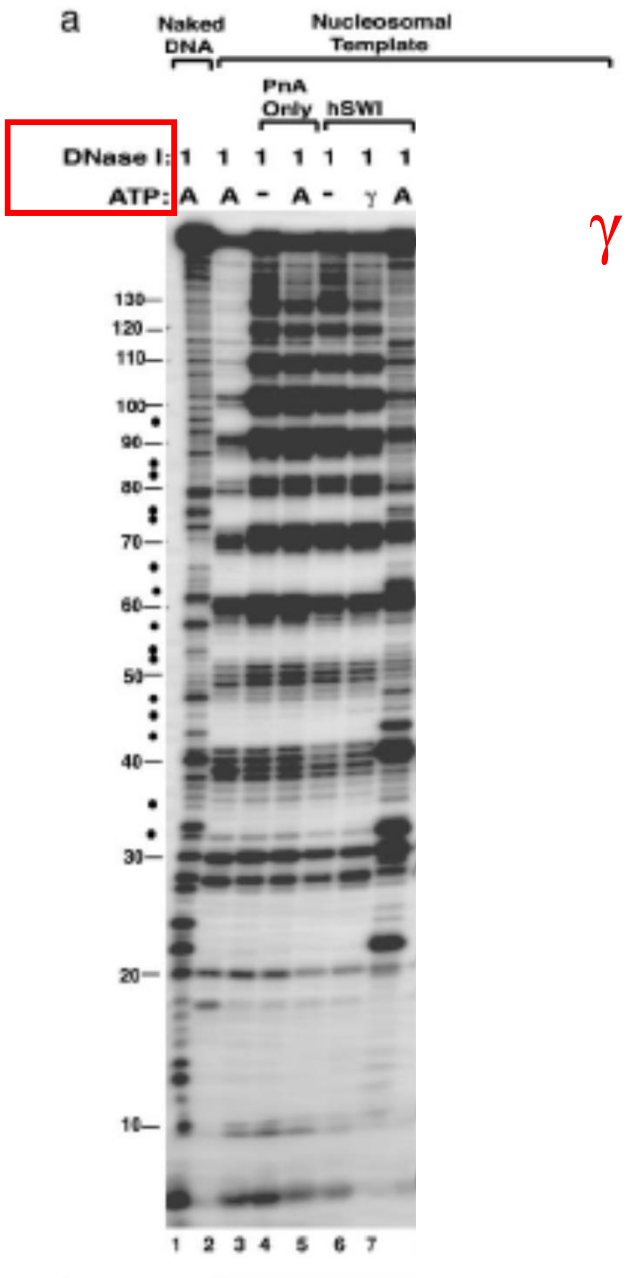
RSC

complex RSC (remodels the structure of chromatin)

ATP-dependent chromatin-remodelling

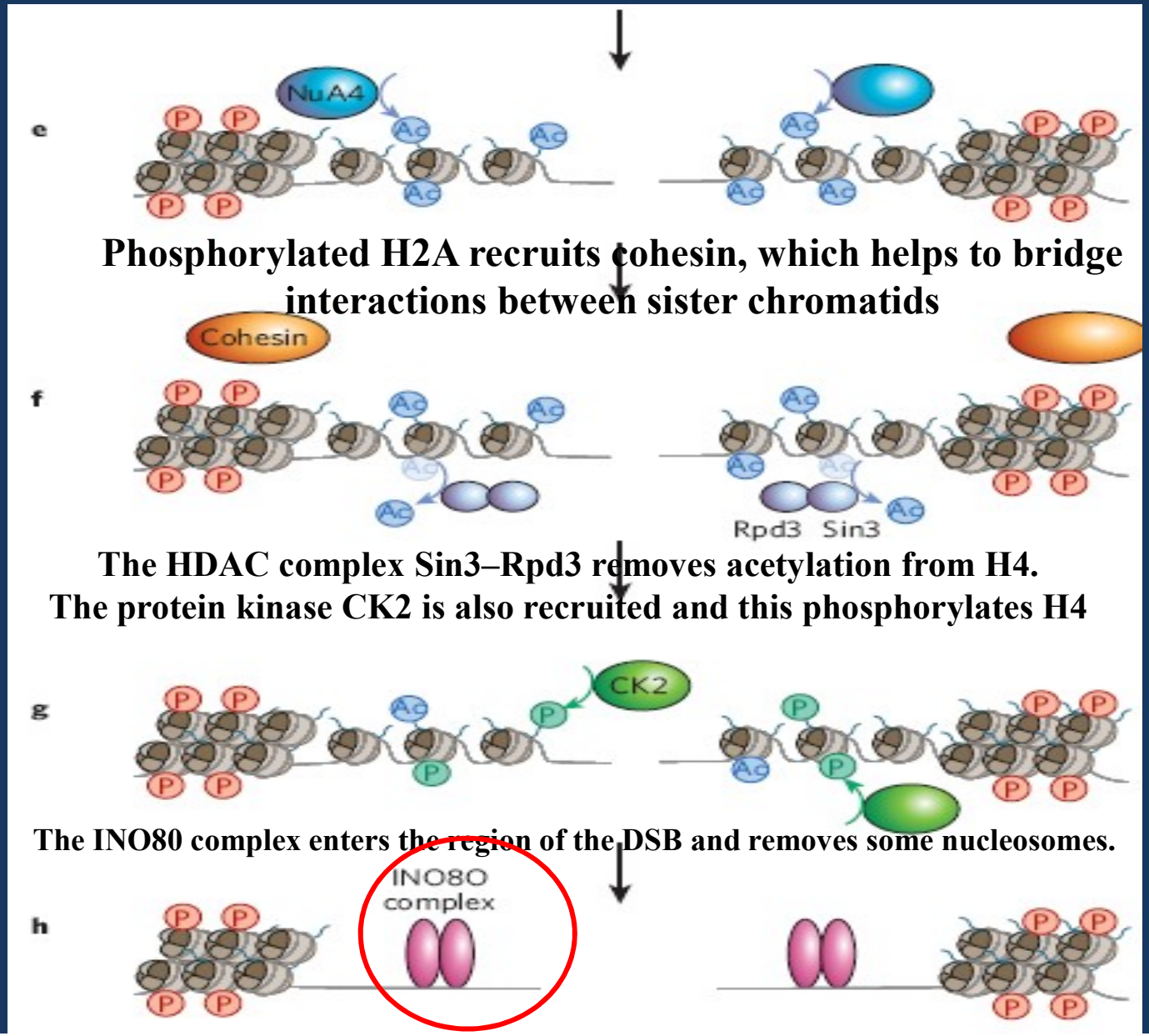
RSC can mediate nucleosome sliding, alter histoneDNA contacts and remove histones from DNA.

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

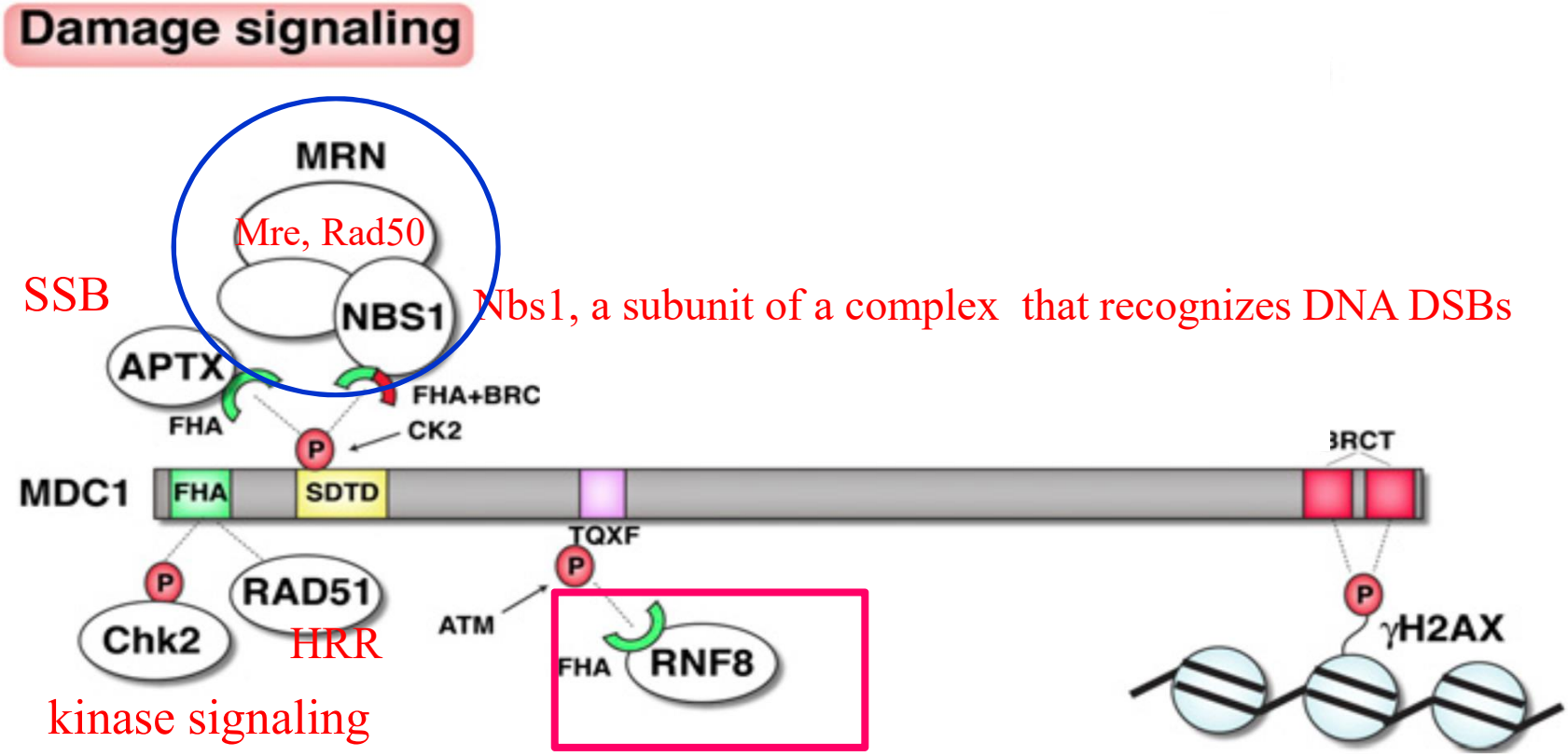


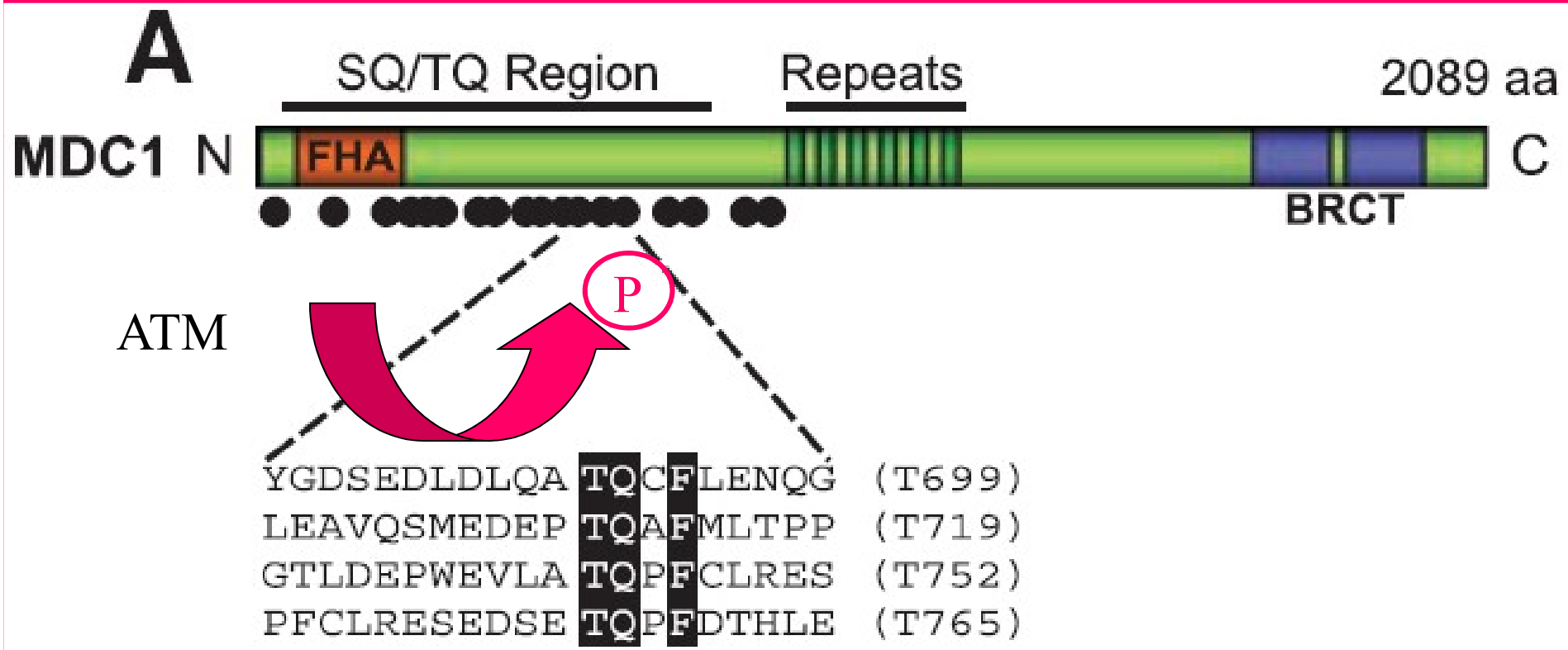
γ =Adenosine 5'-(gamma-thiotriphosphate)

Chromating remodelling and DSBs



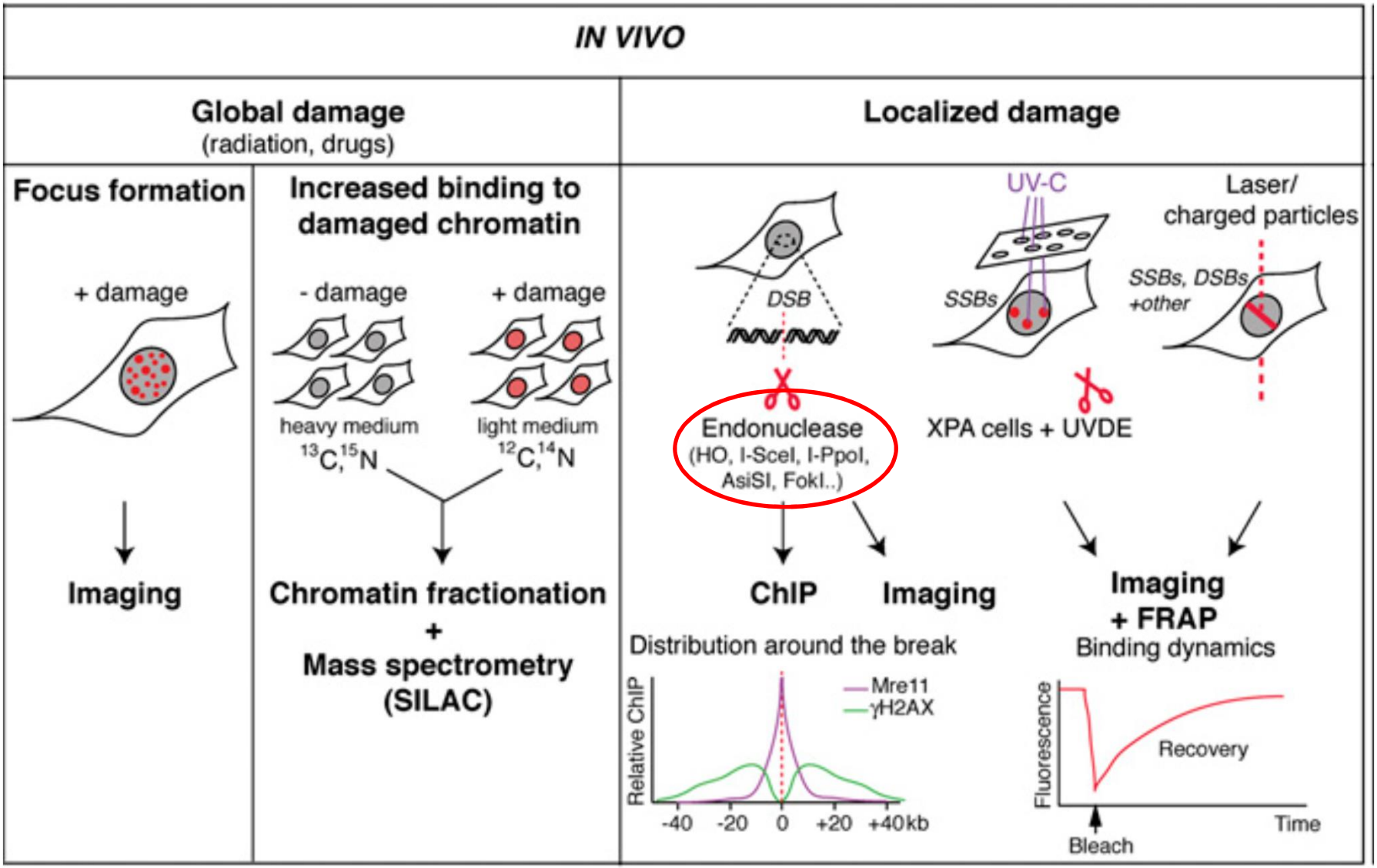
Proteine piattaforma





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

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