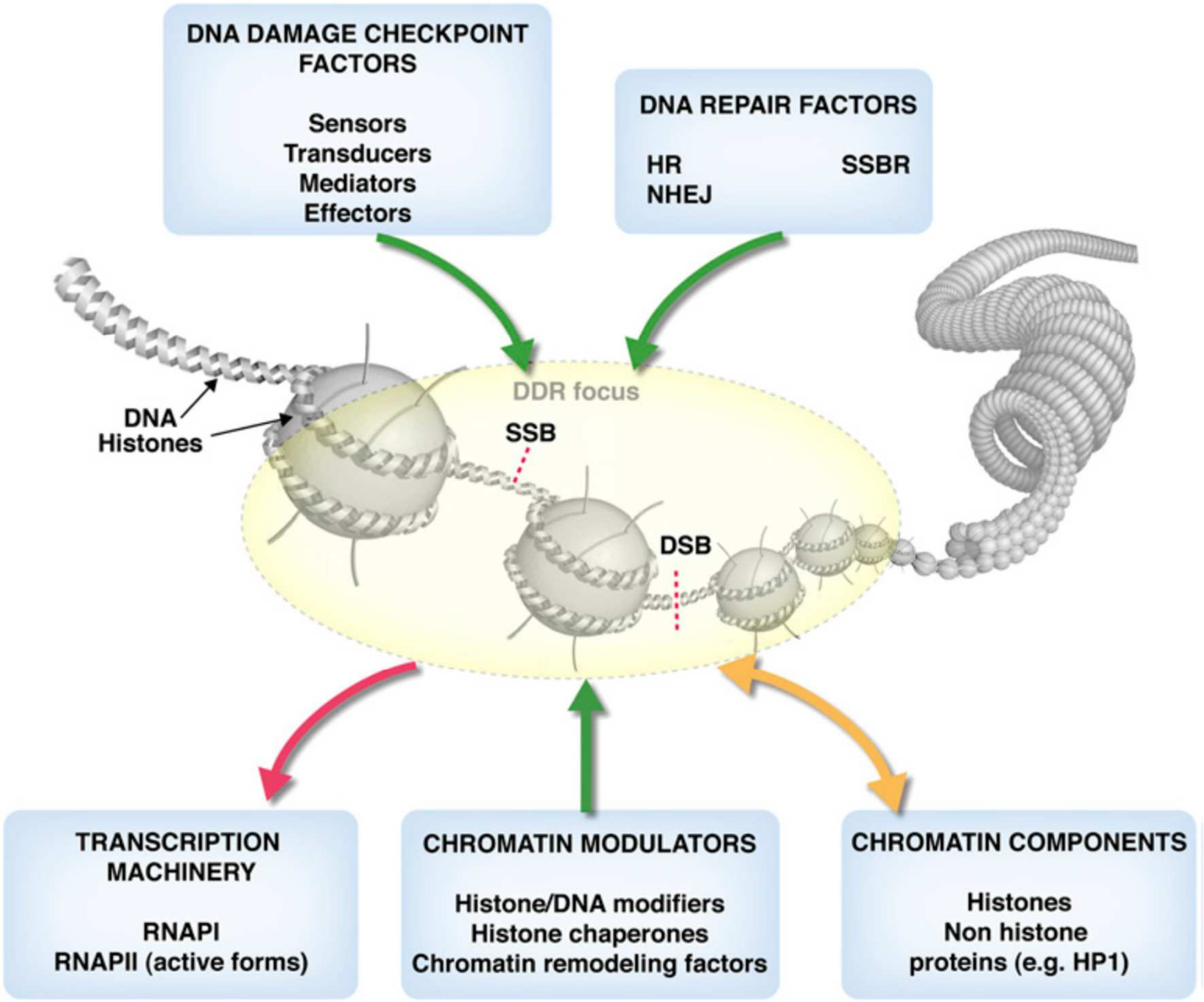

DNA DAMAGE CHECKPOINT FACTORS
Sensors
Transducers
Mediators
Effectors
DNA REPAIR FACTORS
HR
NHEJ
SSBR
DNA
Histones
DDR focus
SSB
DSB
TRANSCRIPTION MACHINERY
RNAPI
RNAPII (active forms)
CHROMATIN MODULATORS
Histone/DNA modifiers
Histone chaperones
Chromatin remodeling factors
CHROMATIN COMPONENTS
Histones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

DSB

Double-Strand Breaks

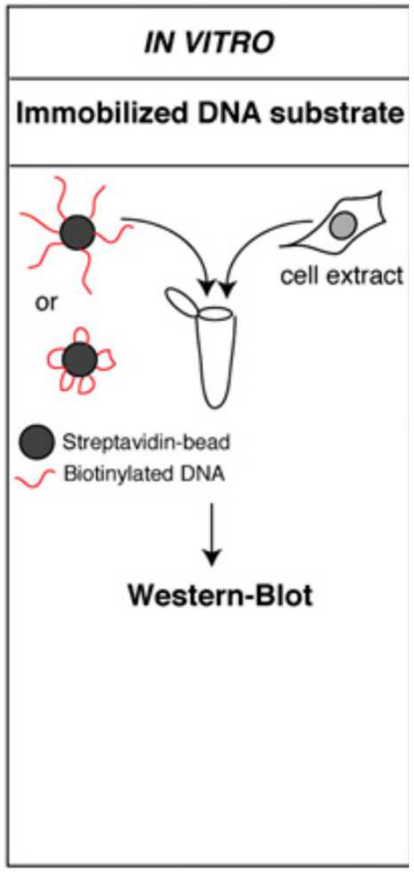
causate da

radiazioni

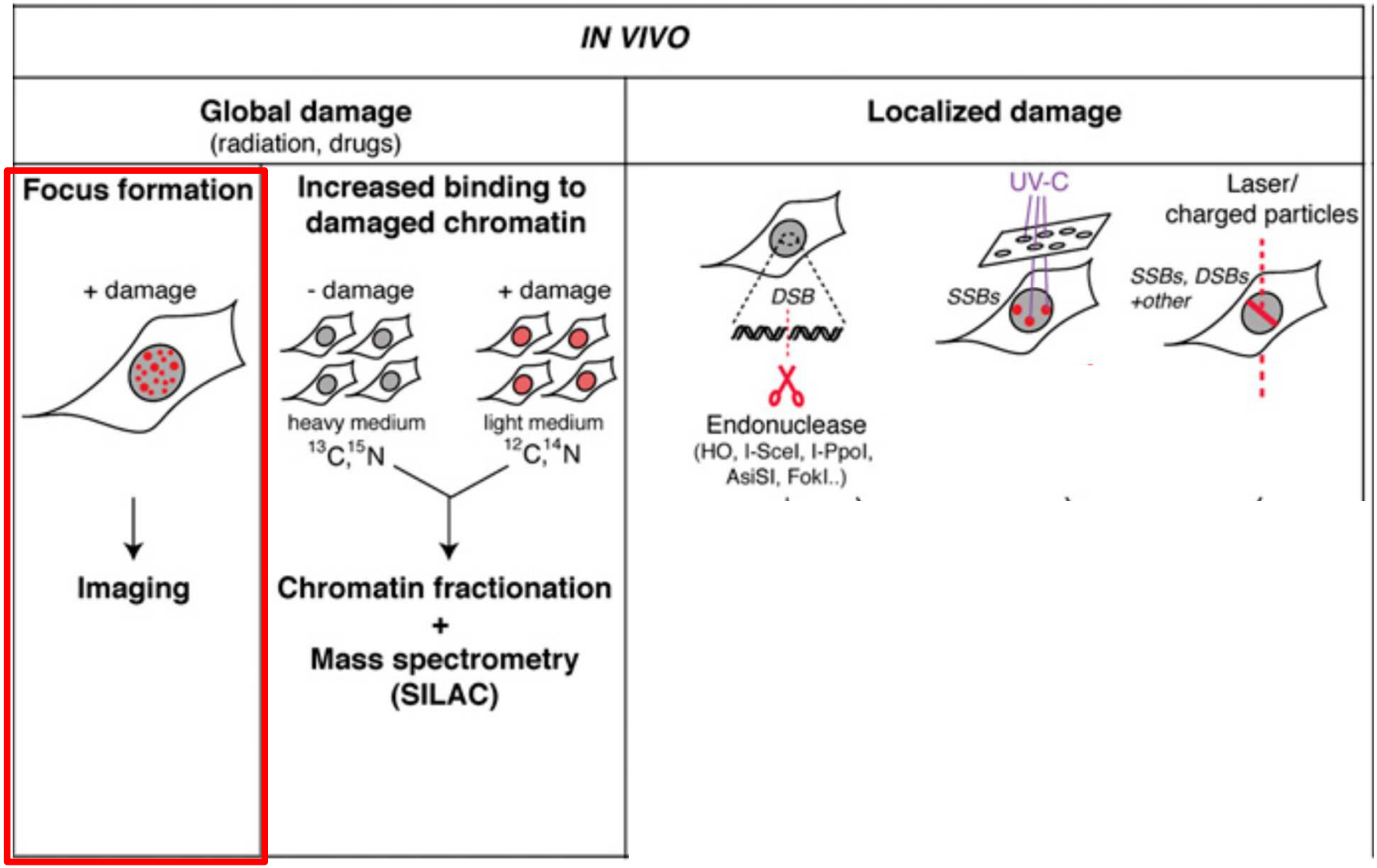
stress ossidativo

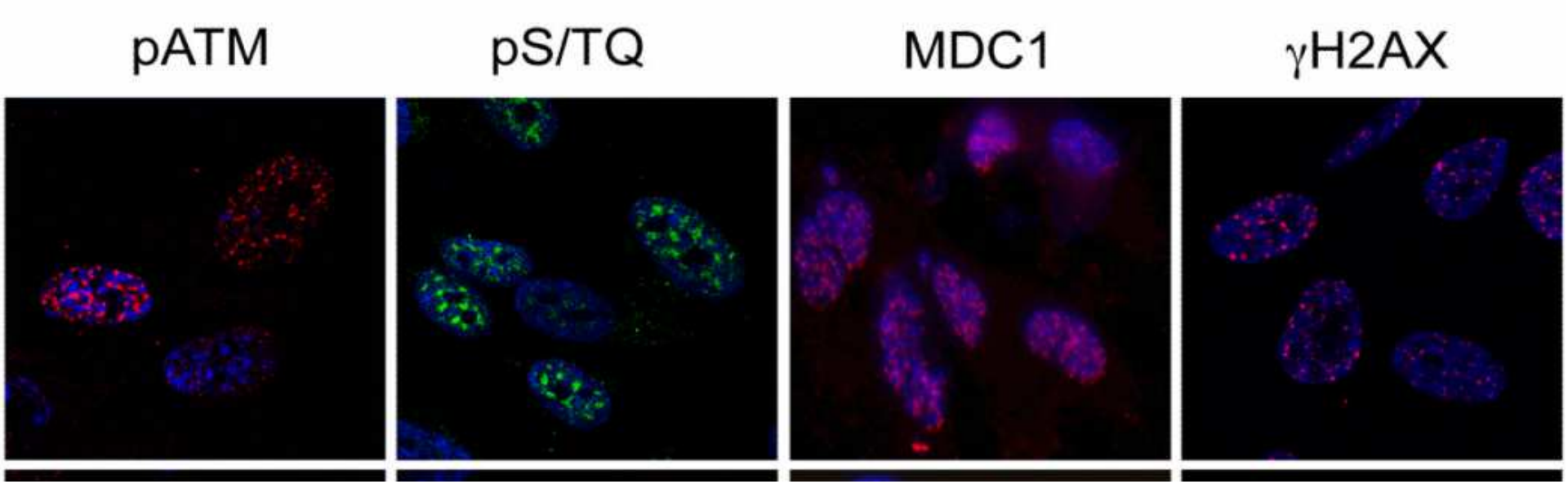
farmaci

METODI


IN VITRO
Immobilized DNA substrate
cell extract
or
Streptavidin-bead
Biotinylated DNA
Western-Blot

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..)
UV-C
SSBs
Laser/
charged particles
SSBs, DSBs
+other

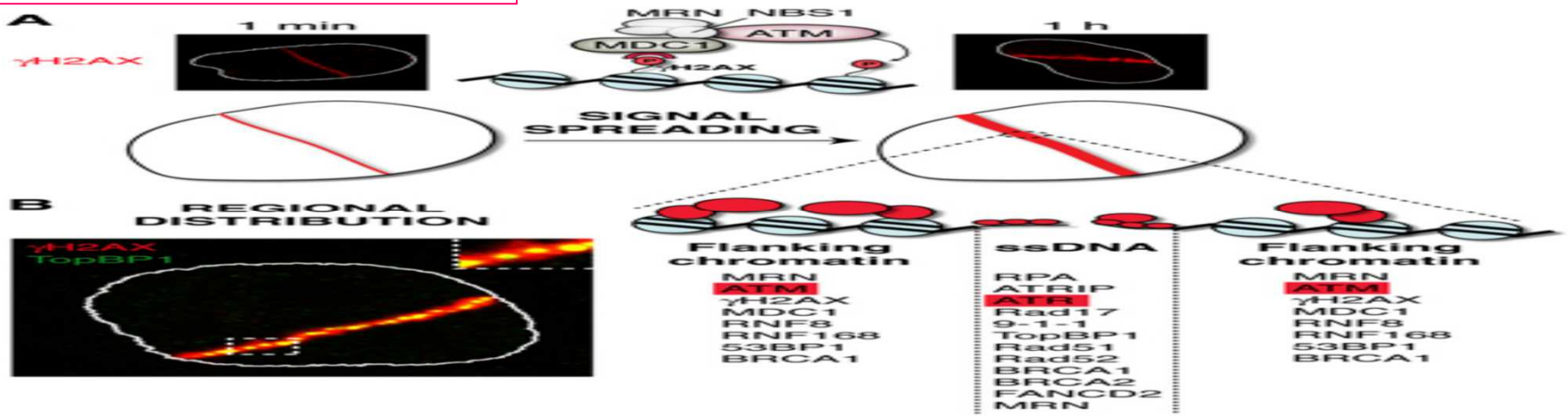


DDR foci formation in irradiated (2 Gy) cells
fixed 2 h later

IRIF IRradiation Induced Focus

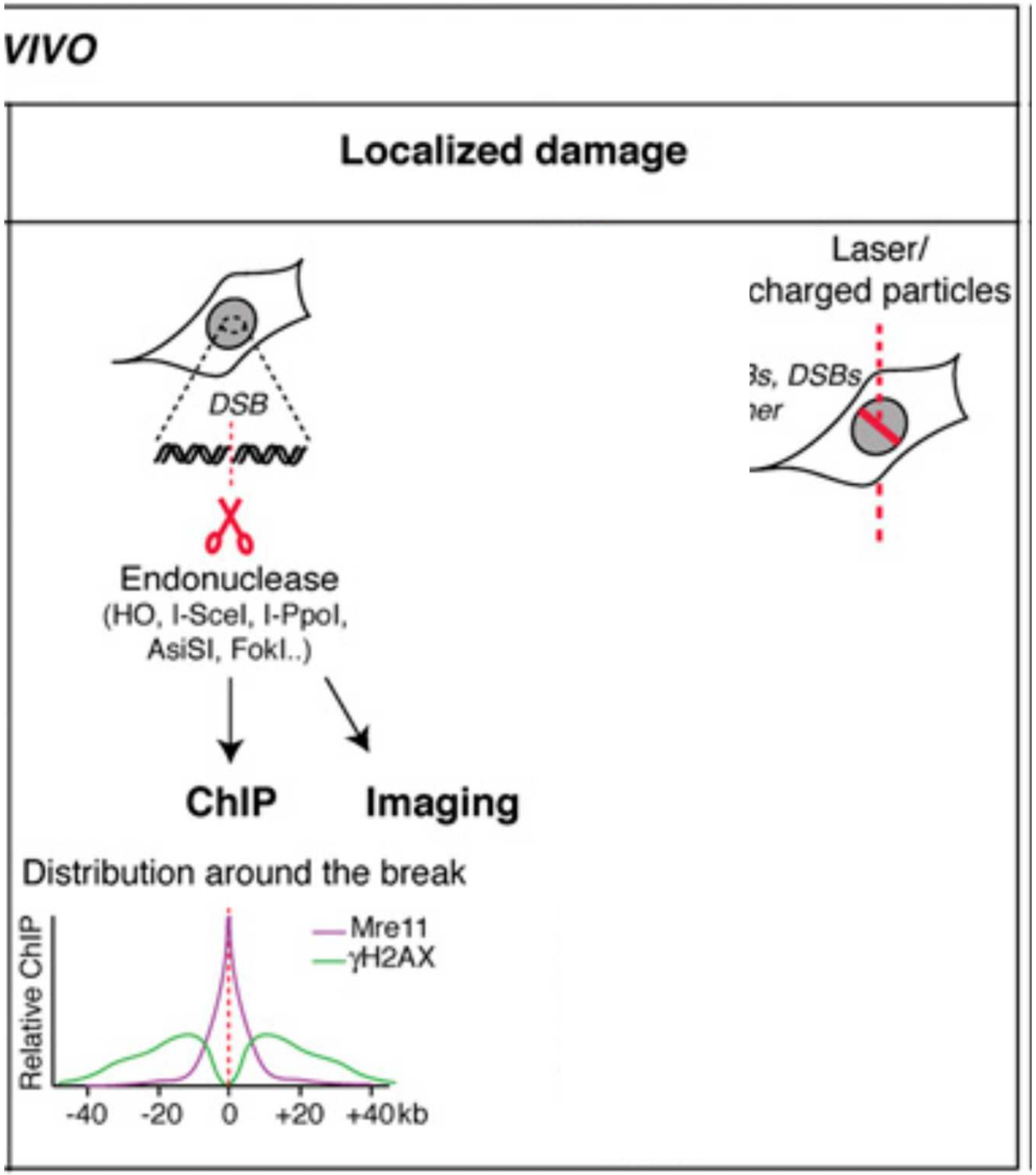
DDR signal spreading

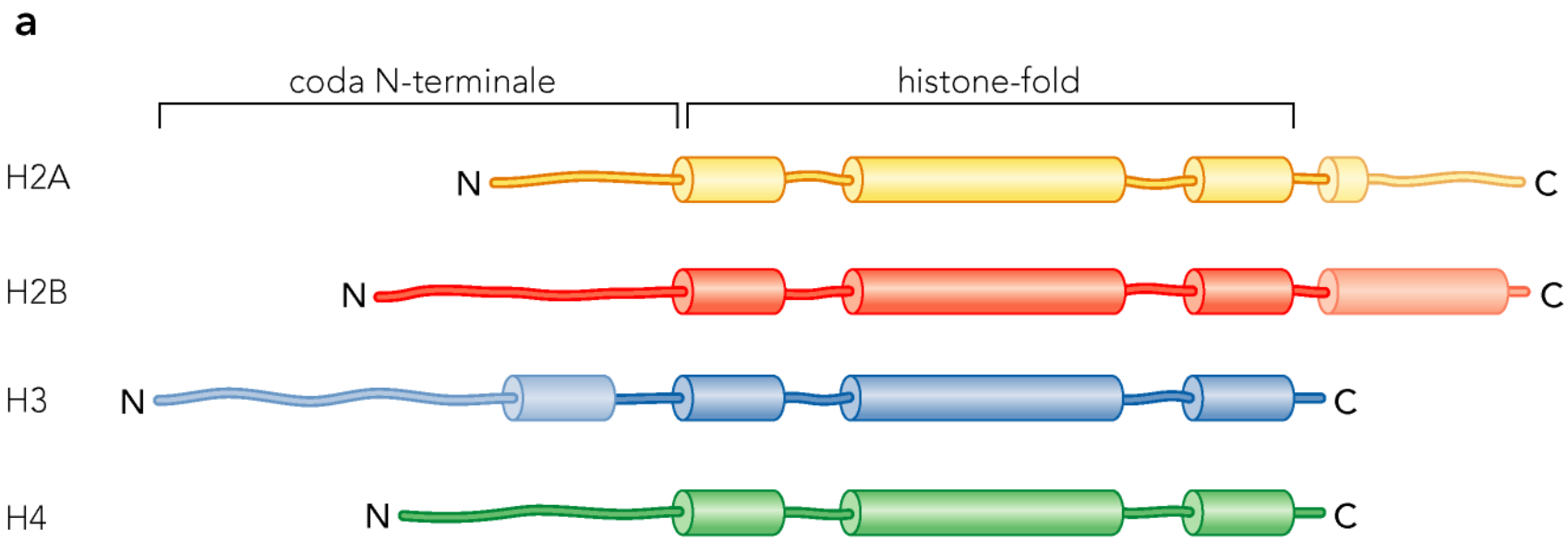
Laser micro-irradiation

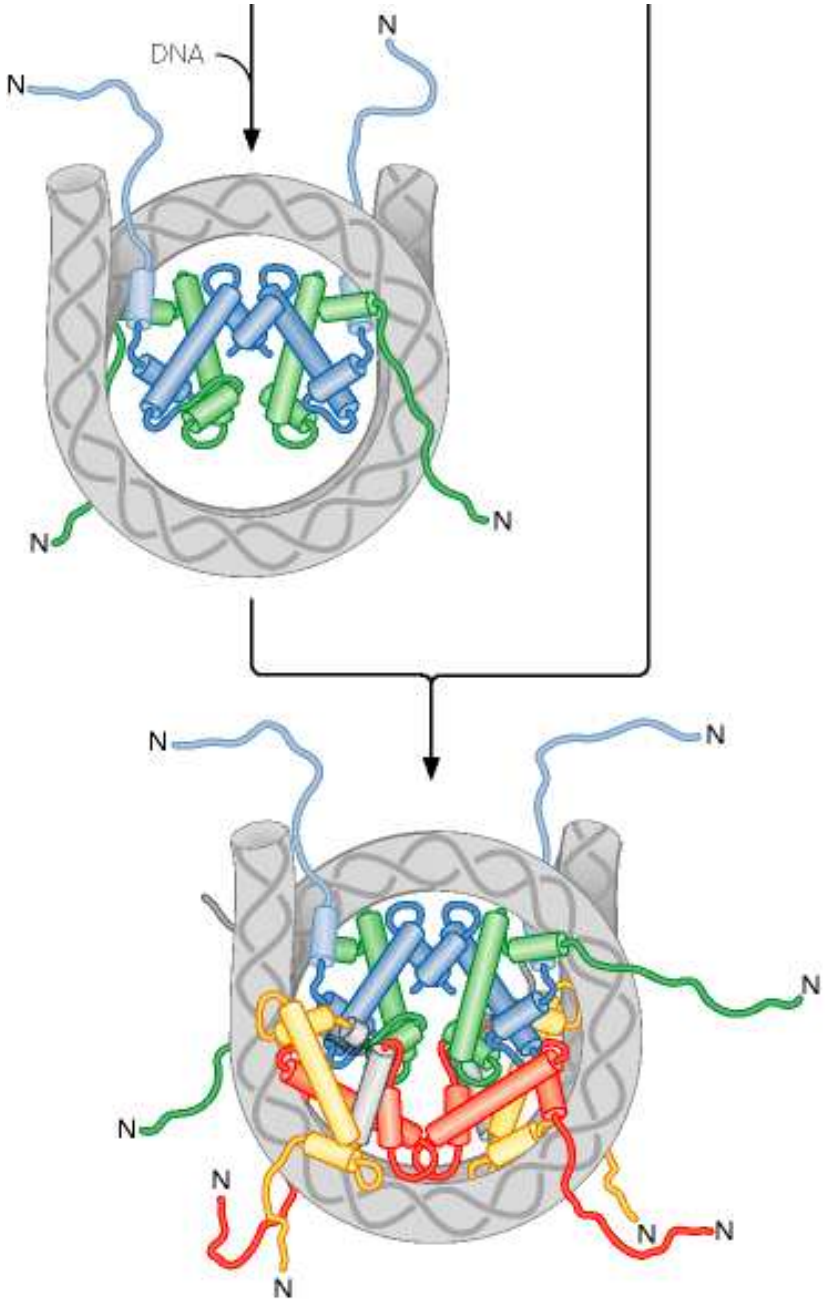


DDR proteins initially accumulate at DSB sites and then spread at distance via a positive feedback loop involving MDC1, which binds gH2AX, the MRN complex, and ATM kinase, which phosphorylates additional H2AX molecules further away from the break site.

METODI


VIVO
Localized damage
Laser/
charged particles
DSB
Endonuclease
(HO, I-SceI, I-PpoI,
AsiSI, FokI..)
ChIP
Imaging
Distribution around the break
Relative ChIP
Mre11
γH2AX
-40
-20
0
+20
+40kb



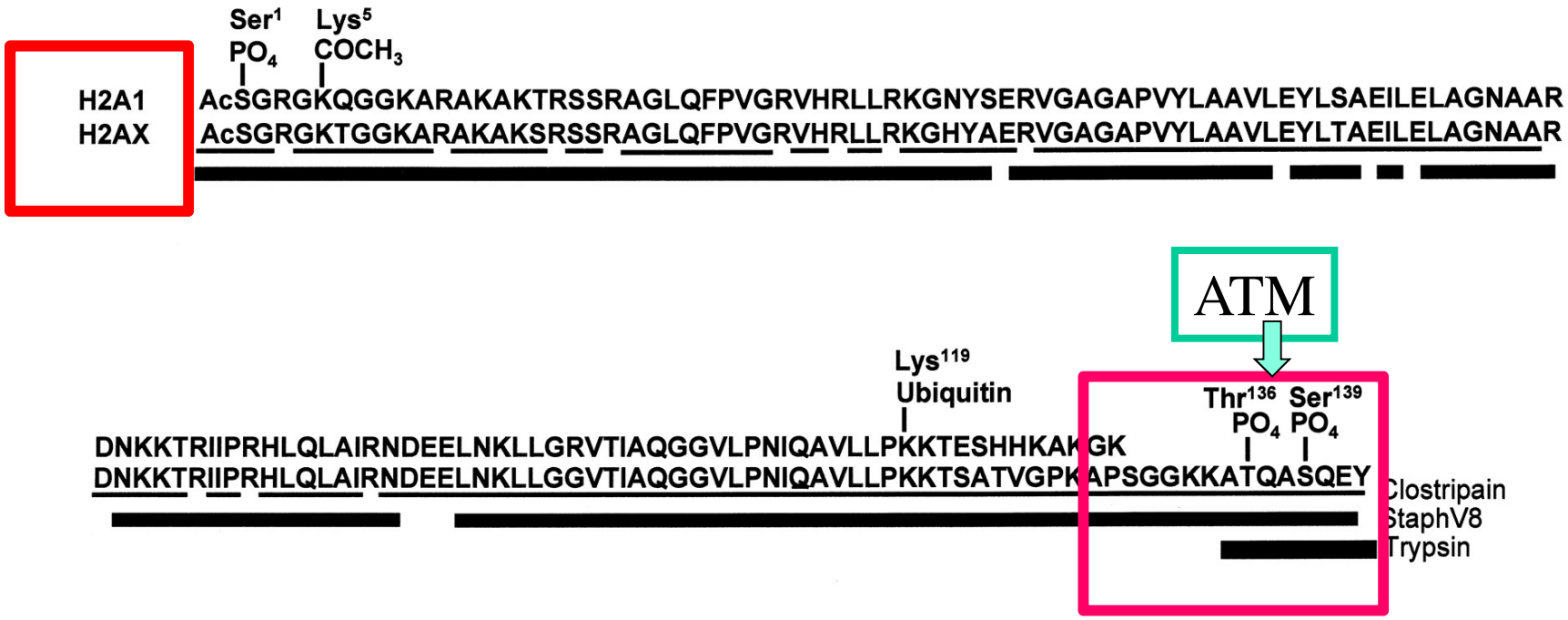


MODIFICAZIONE ISTONI

- 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

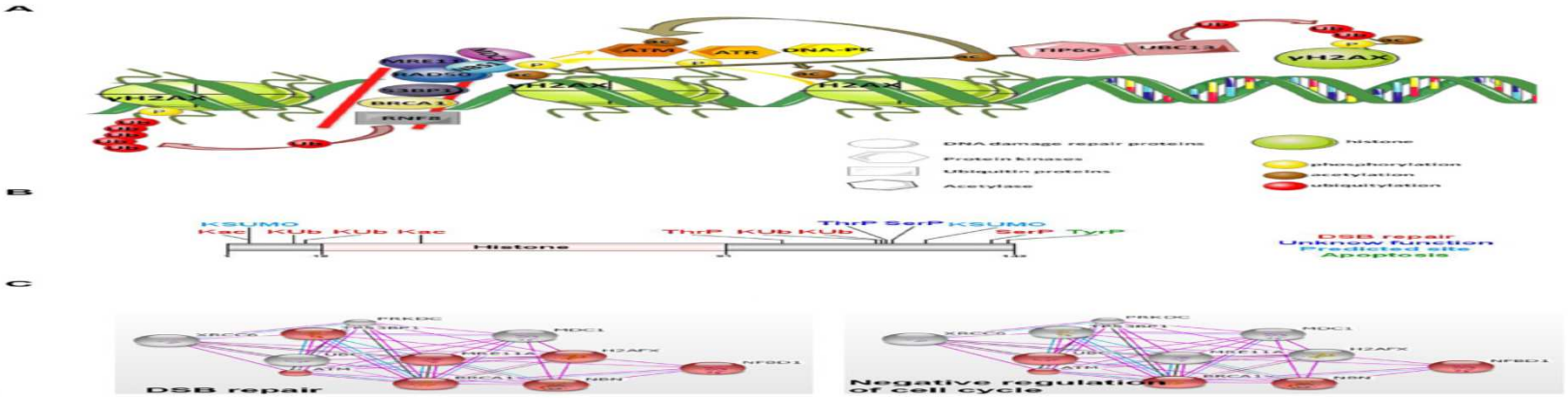
- The earliest and most robust modification induced by DSB is phosphorylation of the histone H2A variant H2AX on its extended C-terminal tail.
- Within seconds, phosphorylated H2AX (known as γ-H2AX) spreads over a region spanning thousands to millions of bases surrounding a DSB

Sequences of H2A1, H2AX, and recombinant H2AX constructs.



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

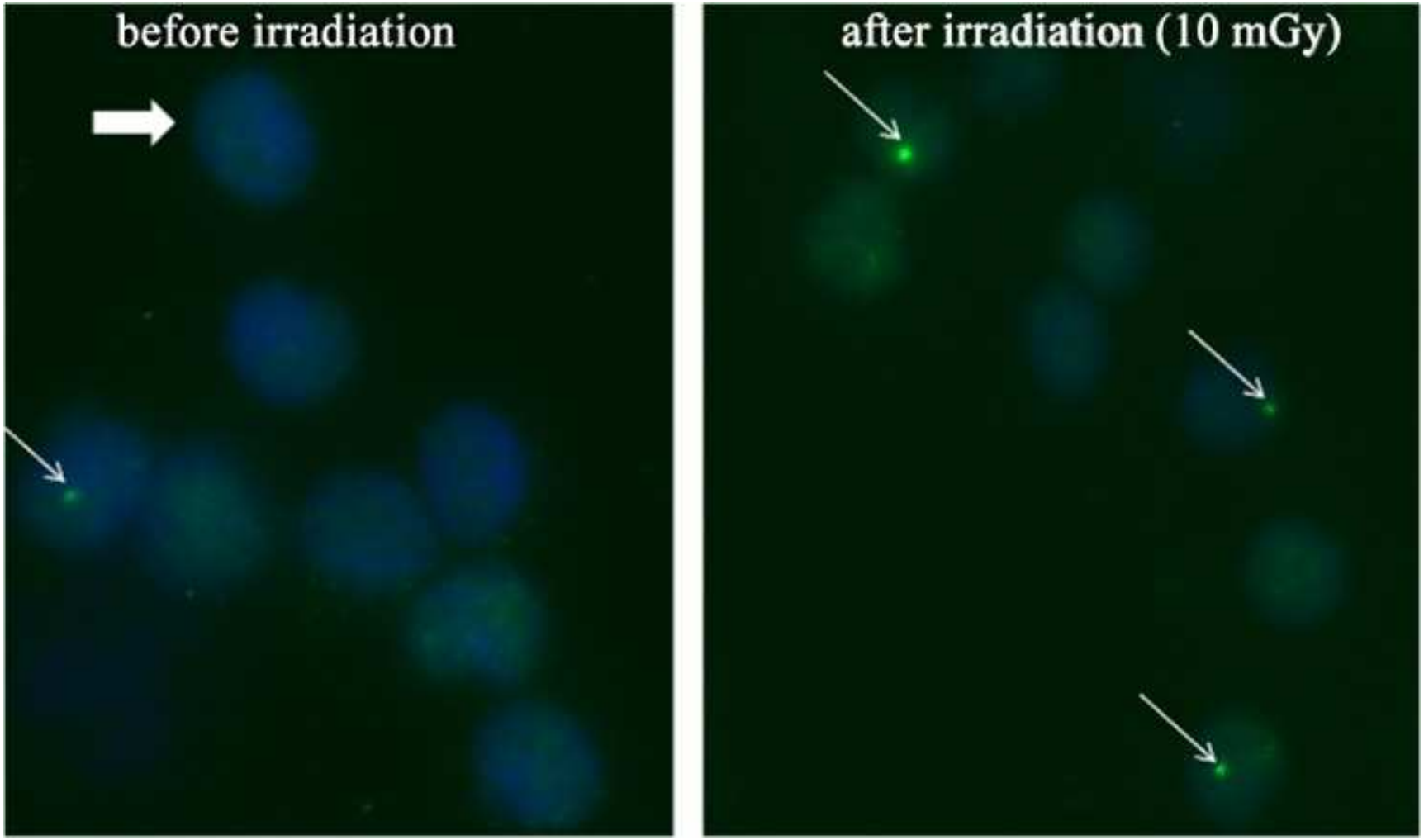
jbc



H2AX protein domain and
the multiple regulatory PTMs

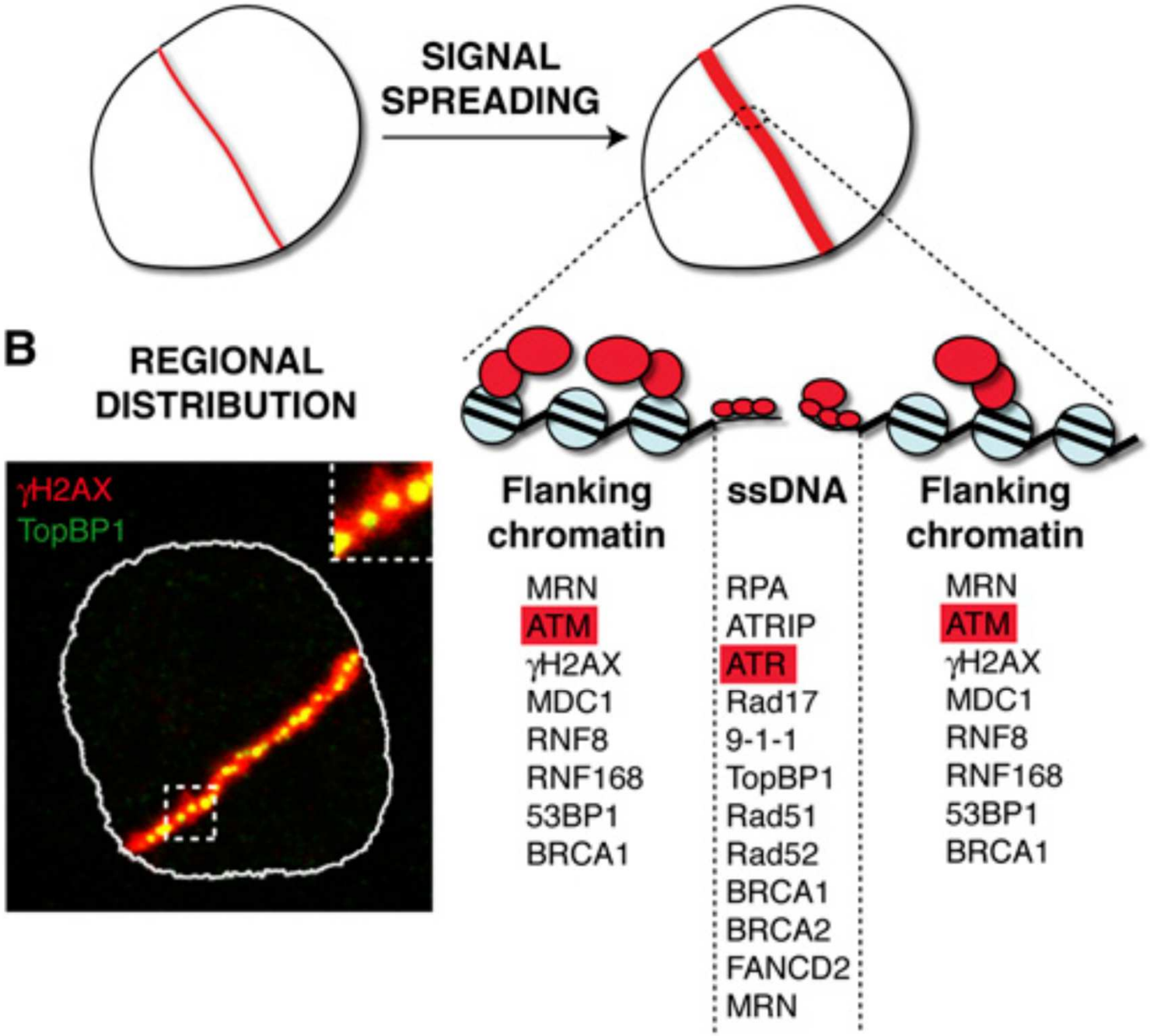
The determination of radiation exposure in diagnostic and interventional radiology

- 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.

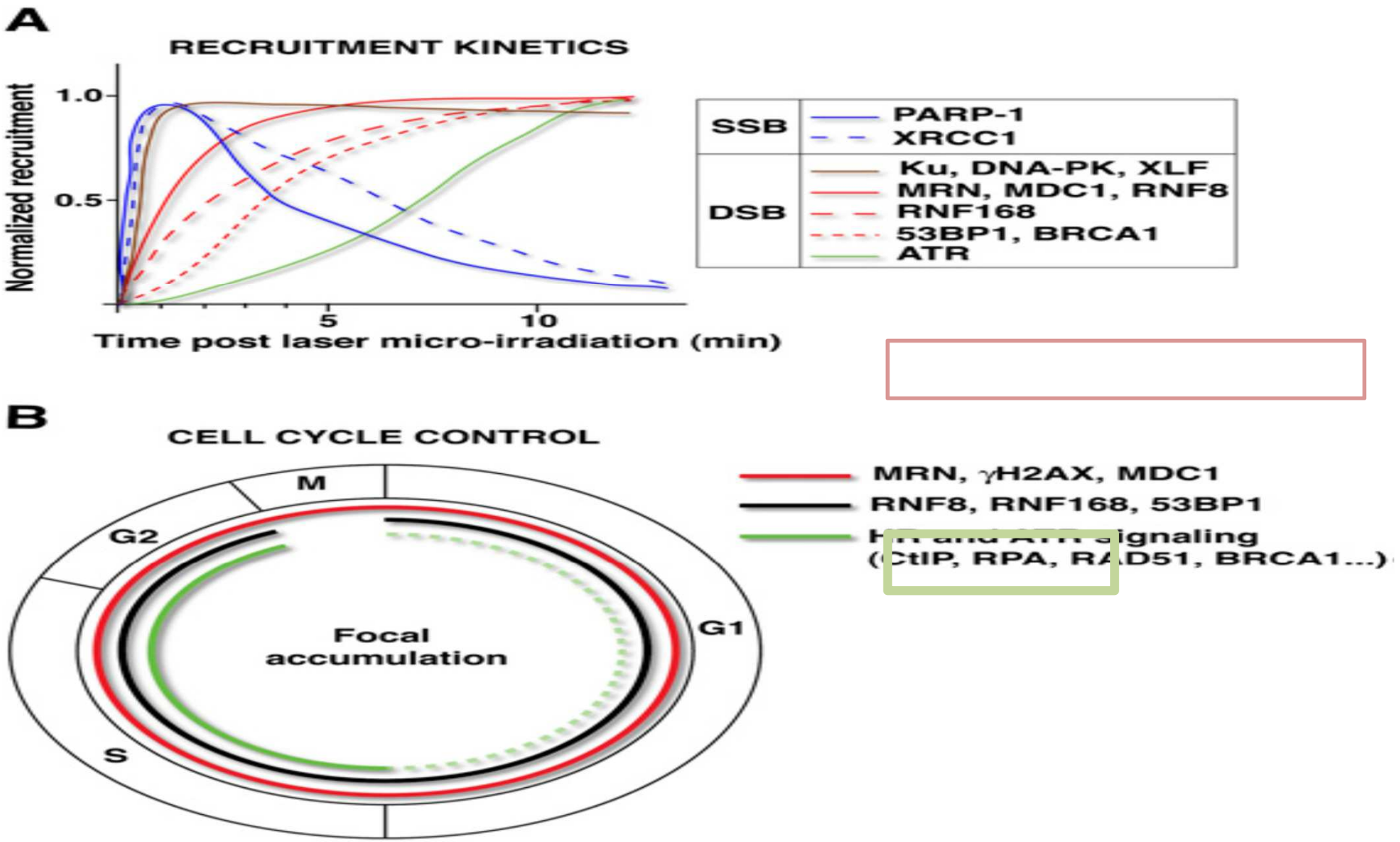


Microscopic image of γ-H2AX foci in human blood lymphocytes before and after irradiation with 10 mGy

specific γ-H2AX antibody (Anti-H2A.X-Phosphorylated (Ser 139)


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



DSB
e CROMATINA

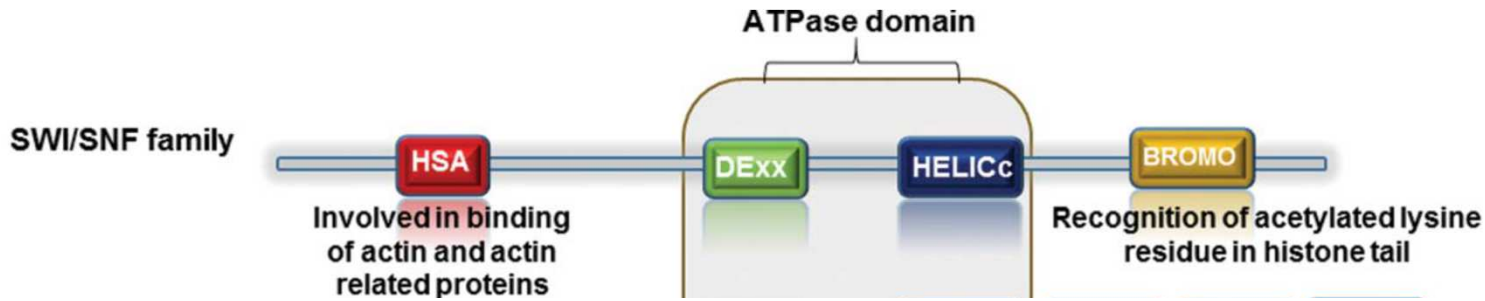
- Higher-order chromatin packaging is a barrier to the detection and repair of DNA damage

DSB
e CROMATINA 2

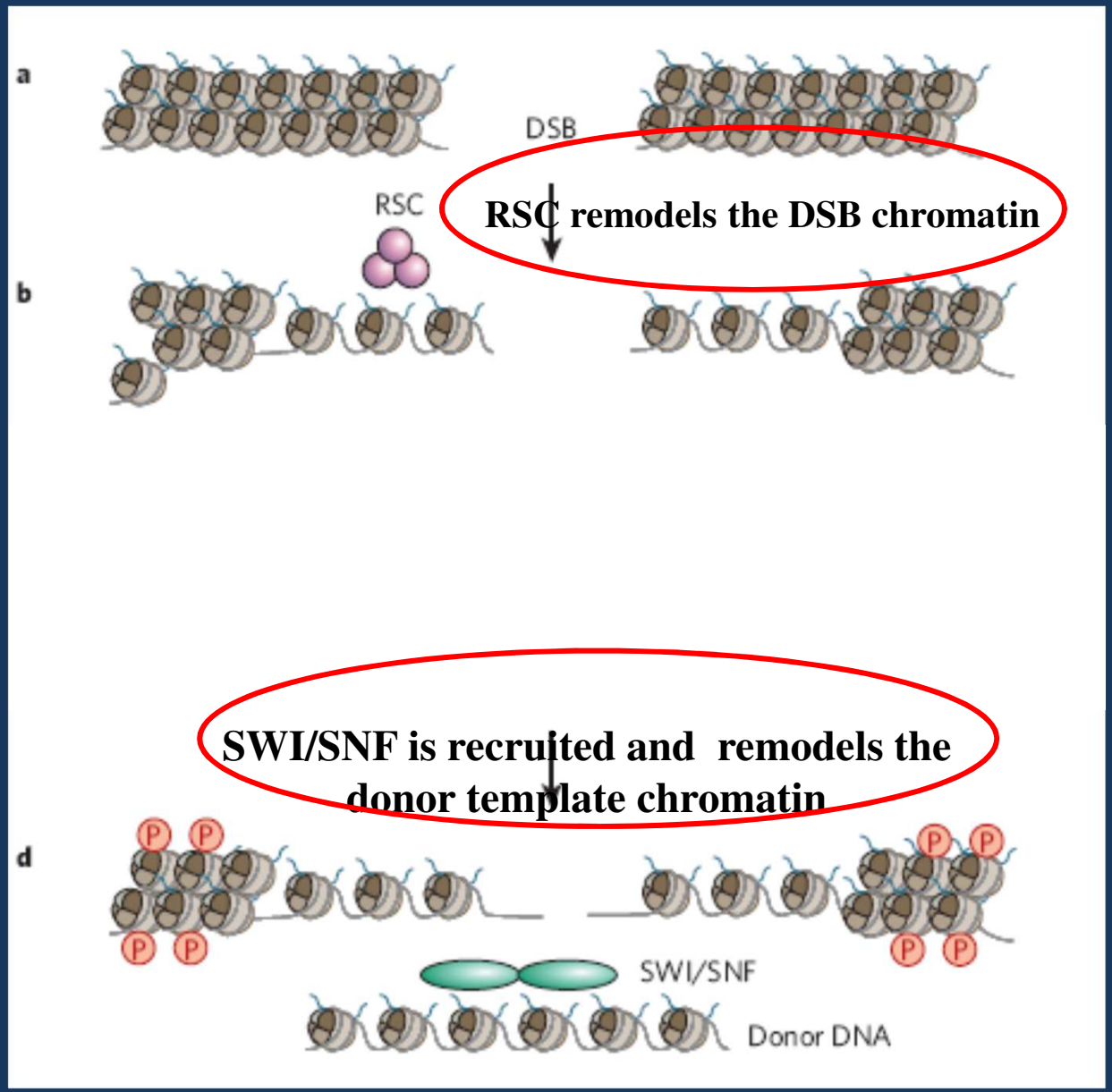
- **DSBs induce a local decrease in the density of the chromatin fibre, in addition to altering the position of nucleosomes**

- DSBs elicit post-translational modifications on the protruding histone tails

chromatin remodeler family



Chromating remodelling and DSBs



RSC

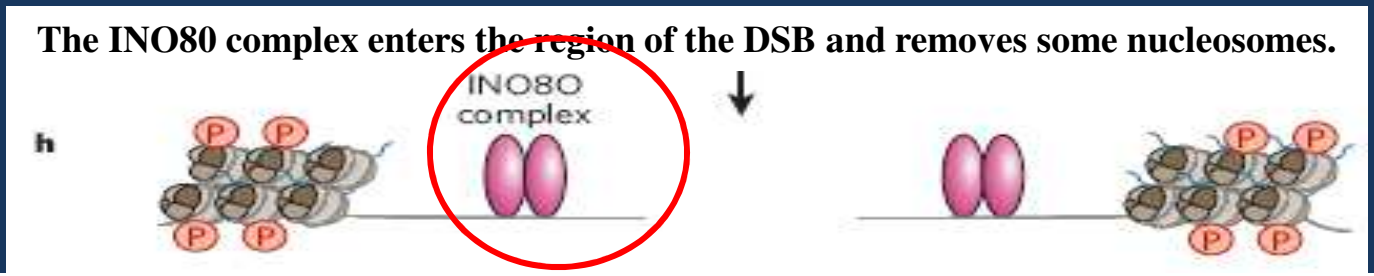
complex RSC (remodels the structure of chromatin)

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

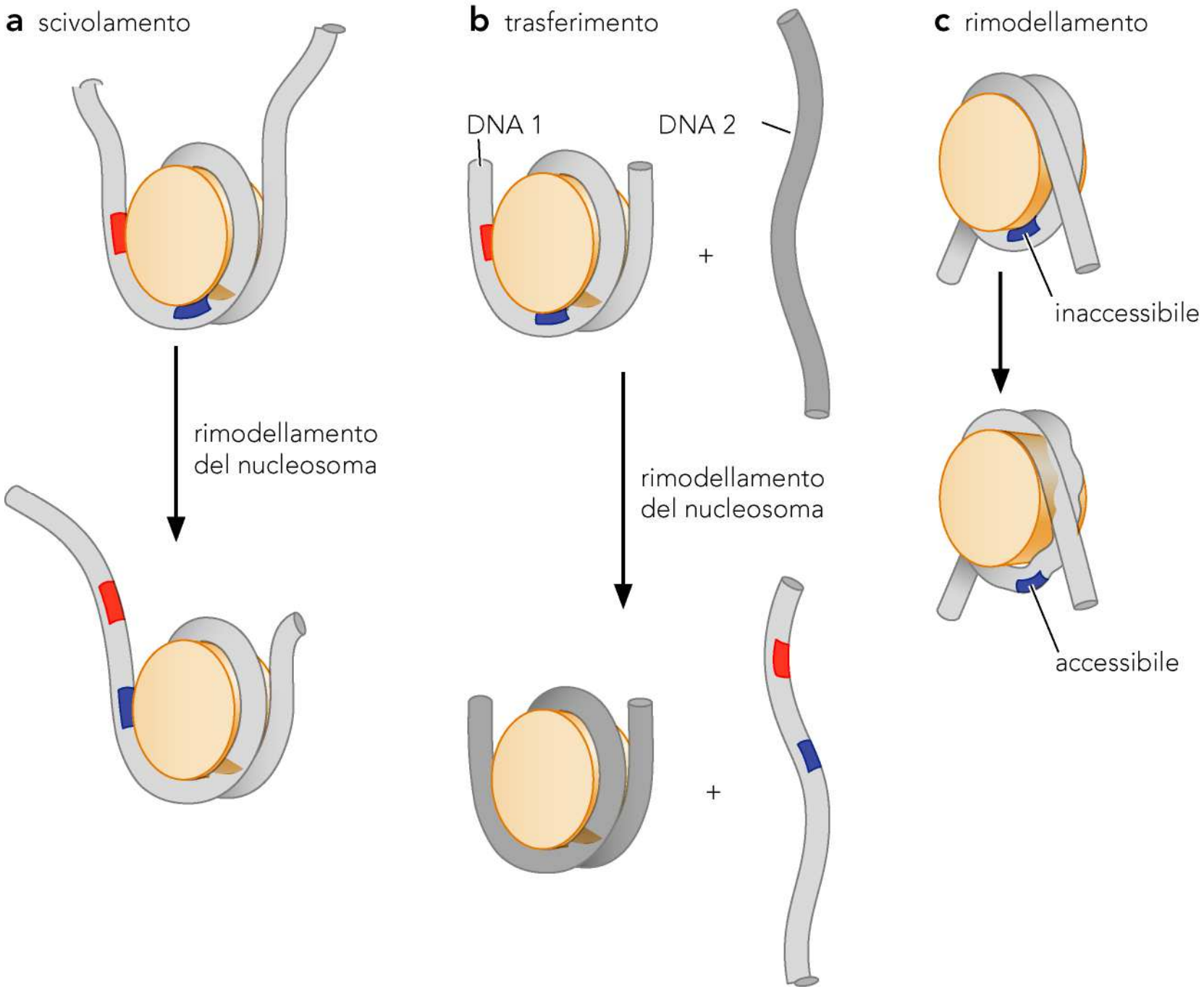
Chromating remodelling and DSBs

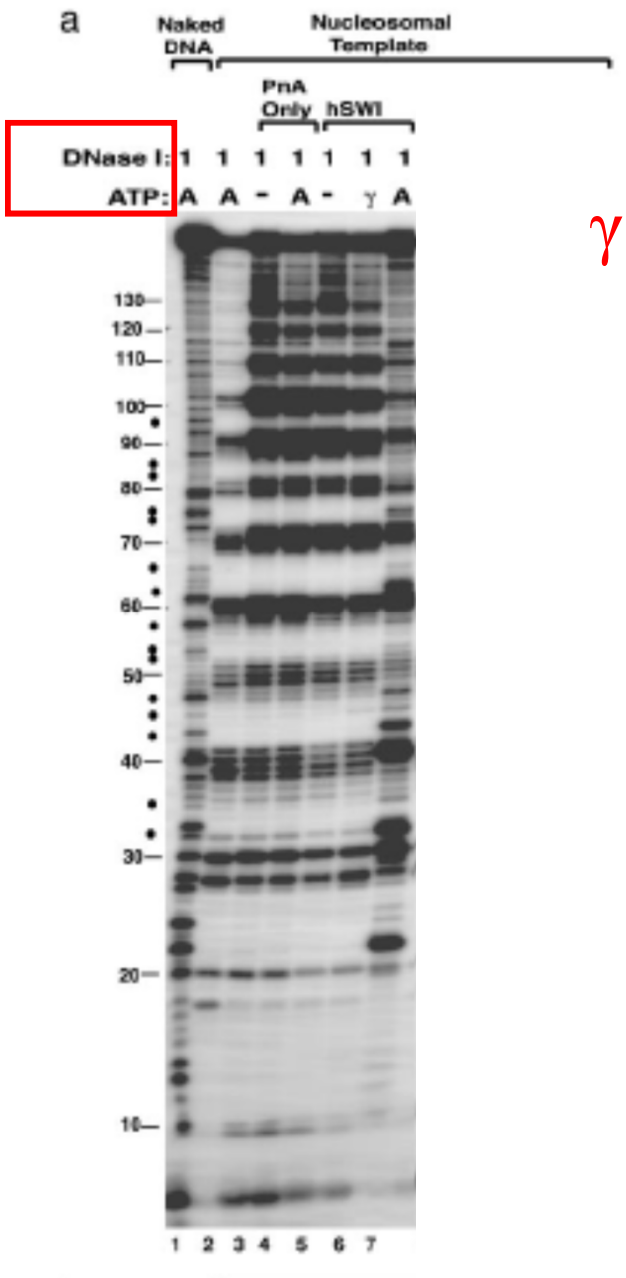


INO80 and Disease

Am J Hum Genet. 2017 Jan 5; 100(1): 21–30

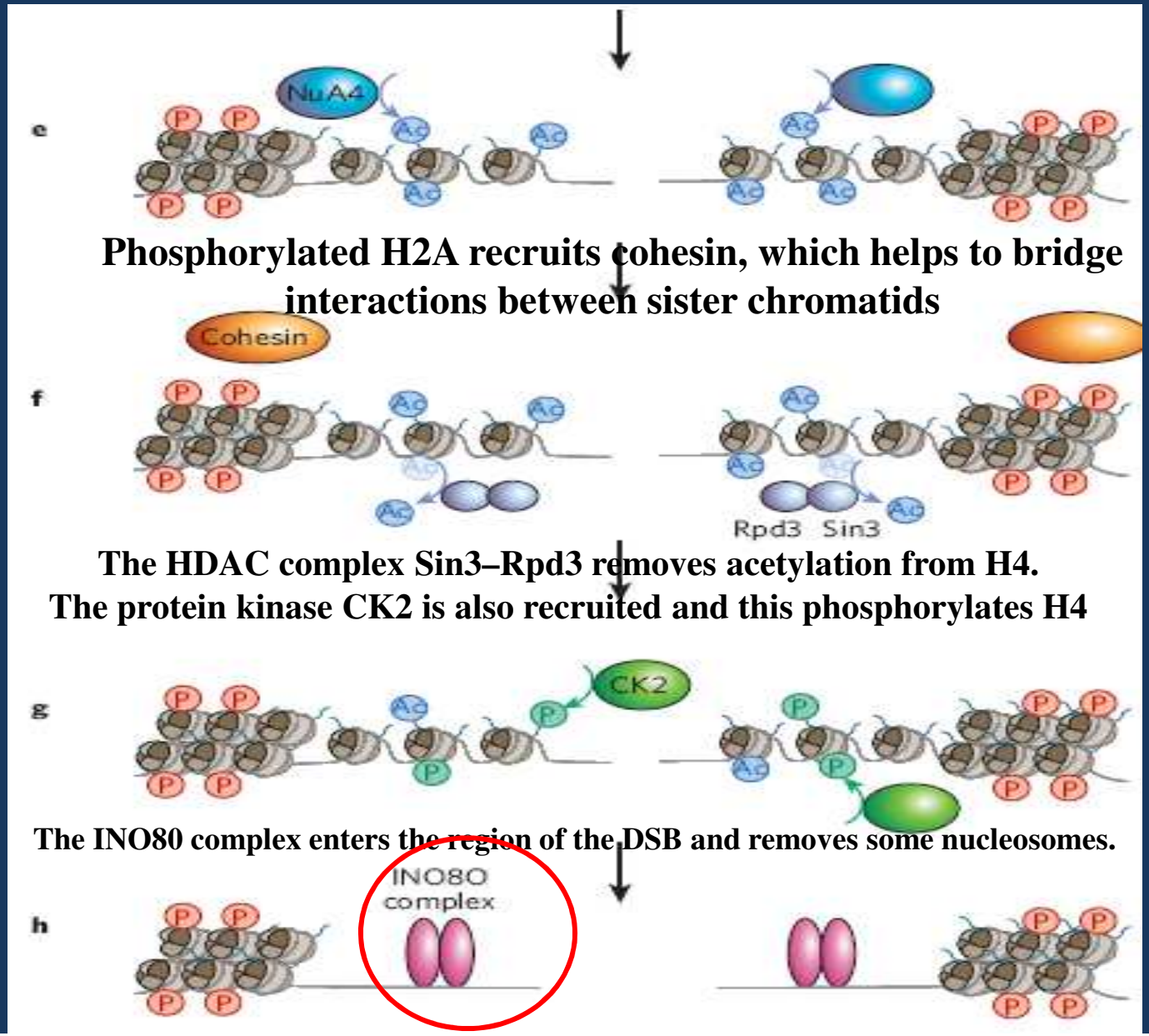
- YY1AP1 is a component of the nuclear INO80 chromatin remodeling complex.
- Homozygous YY1AP1 mutations predispose to vascular lesions of Fibromuscular dysplasia (FMD) - arterial diseases that involves the renal and cerebrovascular arteries (Grange syndrome)
- YY1AP1 deficiency profoundly alters Smooth Muscle Cell SMC phenotype.
- Grange syndrome is also associated with intellectual disability and bone abnormalities.





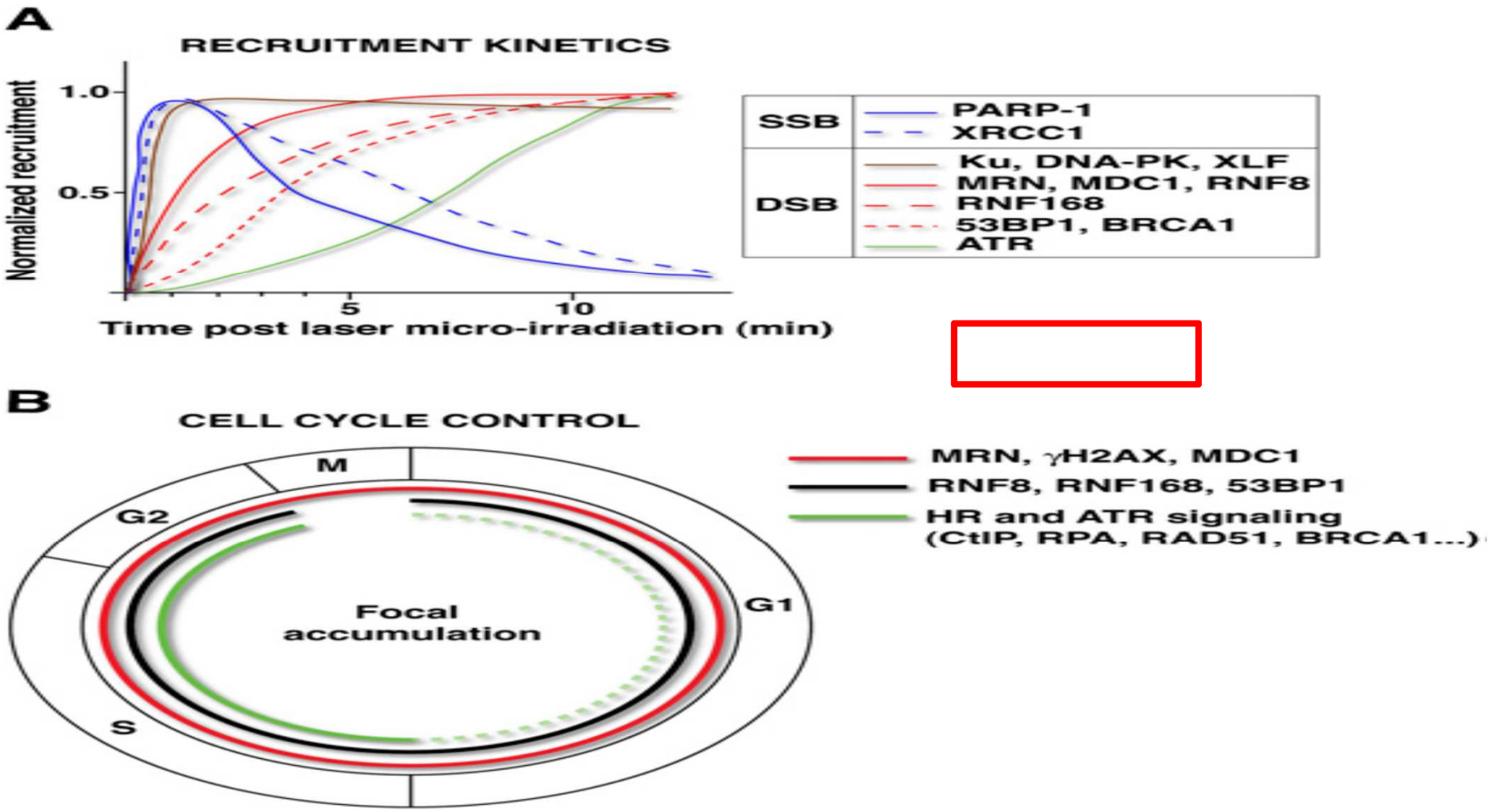
γ =Adenosine 5'-(gamma-thiotriphosphate)

Chromating remodelling and DSBs



I SENSORI ED I LORO COMPLESSI

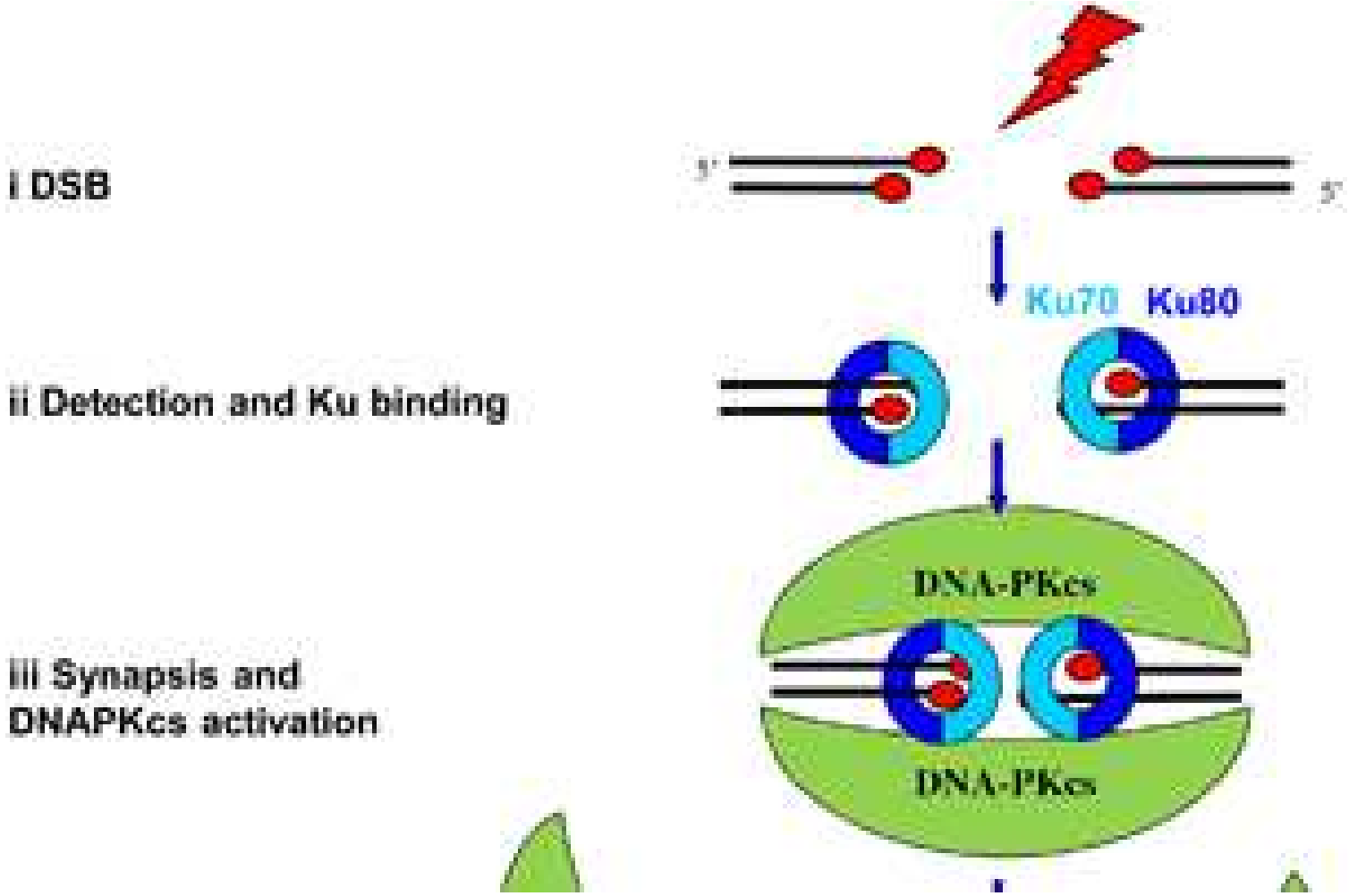
Temporal regulation of DDR protein accumulation at DNA breaks



NHEJ

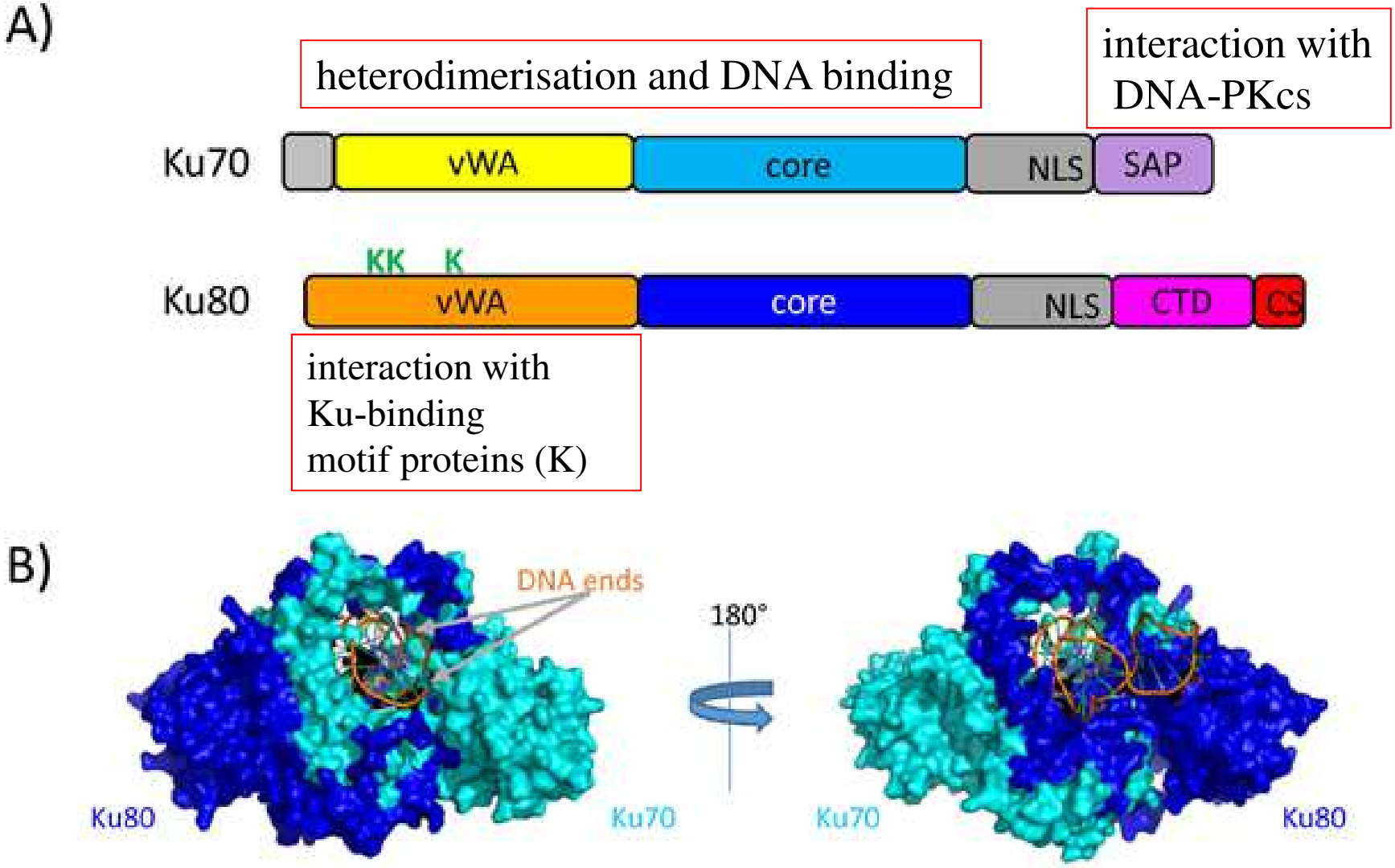
- Non-homologous end-joining (NHEJ) is the dominant means of repairing chromosomal DNA double strand breaks (DSBs), and is essential in human cells.
- 15 or more proteins can be involved in the detection, signalling, synapsis, end-processing and ligation events required to repair a DSB, and must be assembled in the confined space around the DNA ends.

Non-homologous end joining: multiple factors in the DNA repair process



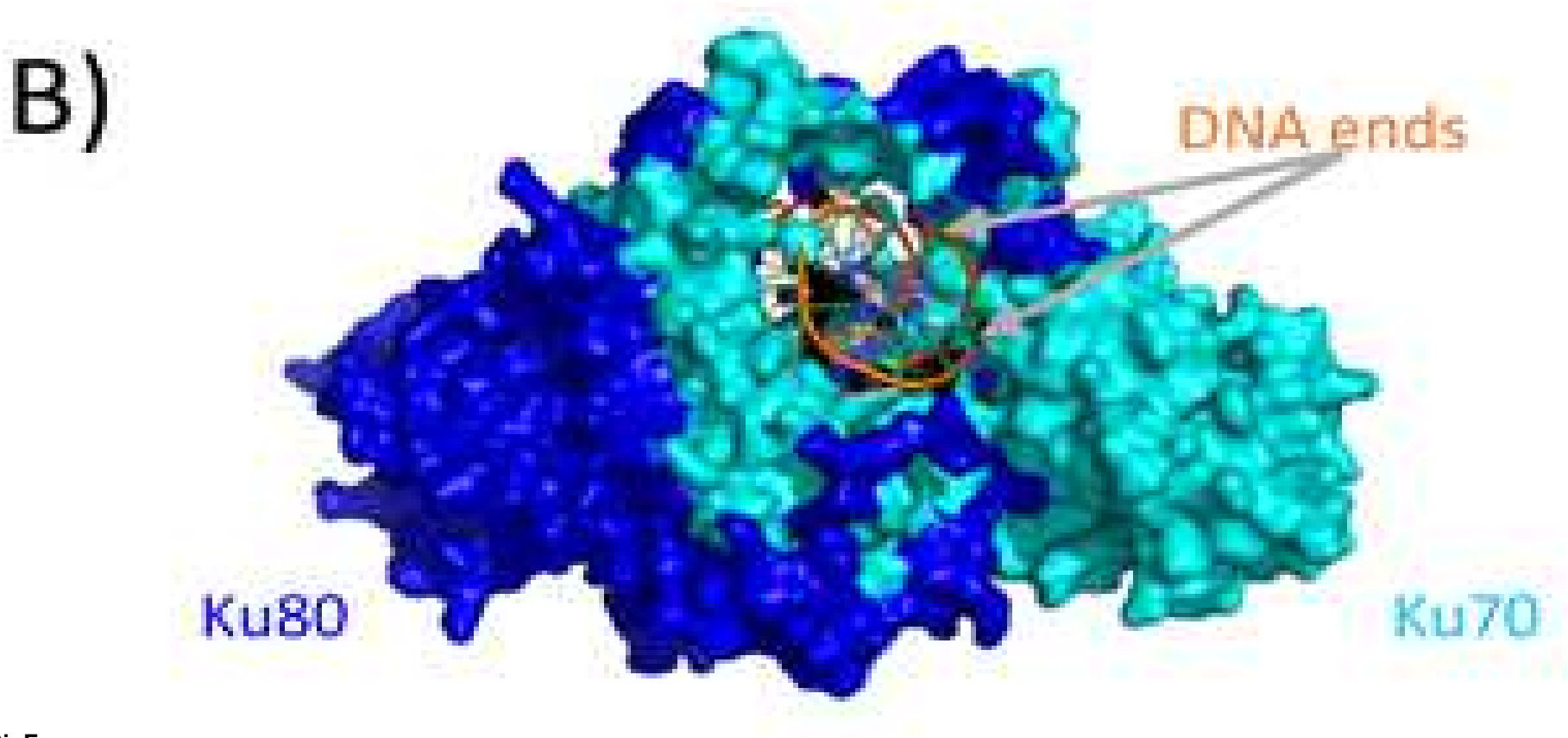
BioEssays
Volume 39, Issue 3, 30 JAN 2017 DOI: 10.1002/bies.201600209
http://onlinelibrary.wiley.com/doi/10.1002/bies.201600209/full#bies201600209-fig-0002

Ku proteins are central to DNA end recognition and recruitment of NHEJ factors



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-0001

Non-homologous end joining Structures of Ku heterodimer (PDB:1JEY)
Ku70 and Ku80 encircle the DNA.
The DNA ends thread through the Ku80 side so the DNA ends are located on the Ku70 side of the heterodimer.



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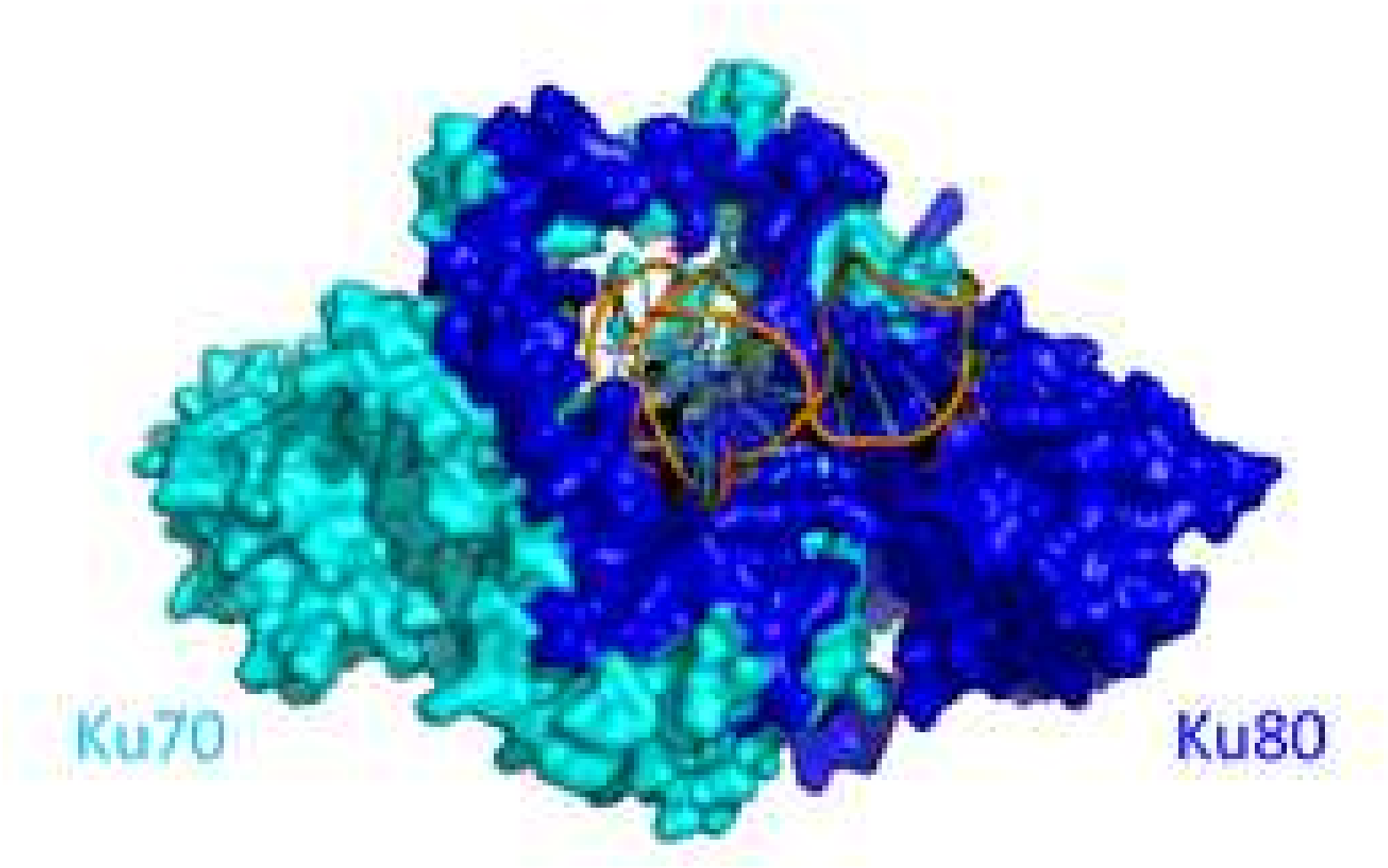

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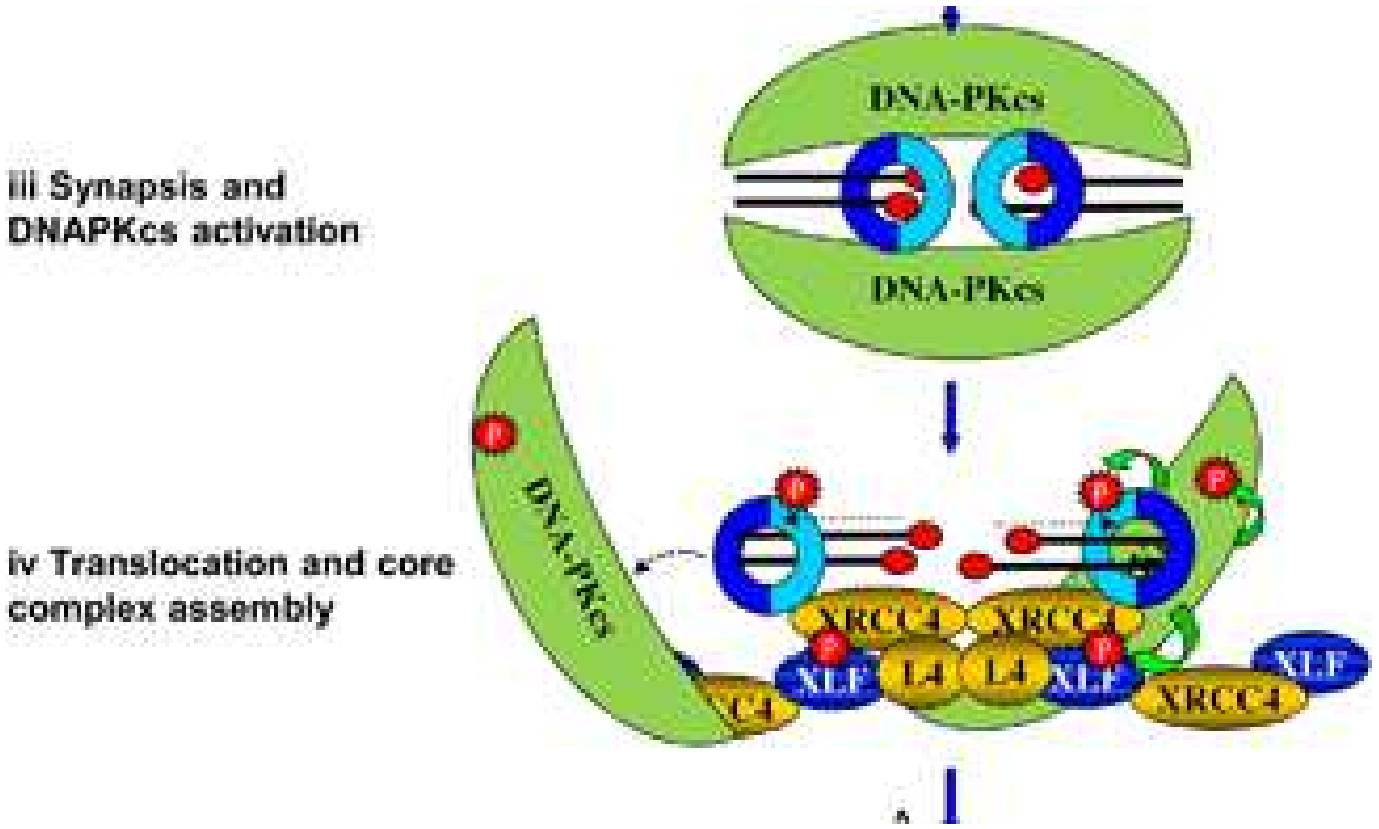
Volume 39, Issue 3, 30 JAN 2017 DOI: 10.1002/bies.201600209
http://onlinelibrary.wiley.com/doi/10.1002/bies.201600209/full#bies201600209-fig-0001

NHEJ Stages

- i: The DNA ends are chemically modified or resected (red circles) and or require processing before the break can be re-ligated

- ii: The ring-like Ku70/Ku80 heterodimer slides over the broken DNA ends. The inner core of the heterodimer binds tightly

- iii: DNA-PKcs is recruited to Ku and the dimerization forms a synapse between the DNA ends.



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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

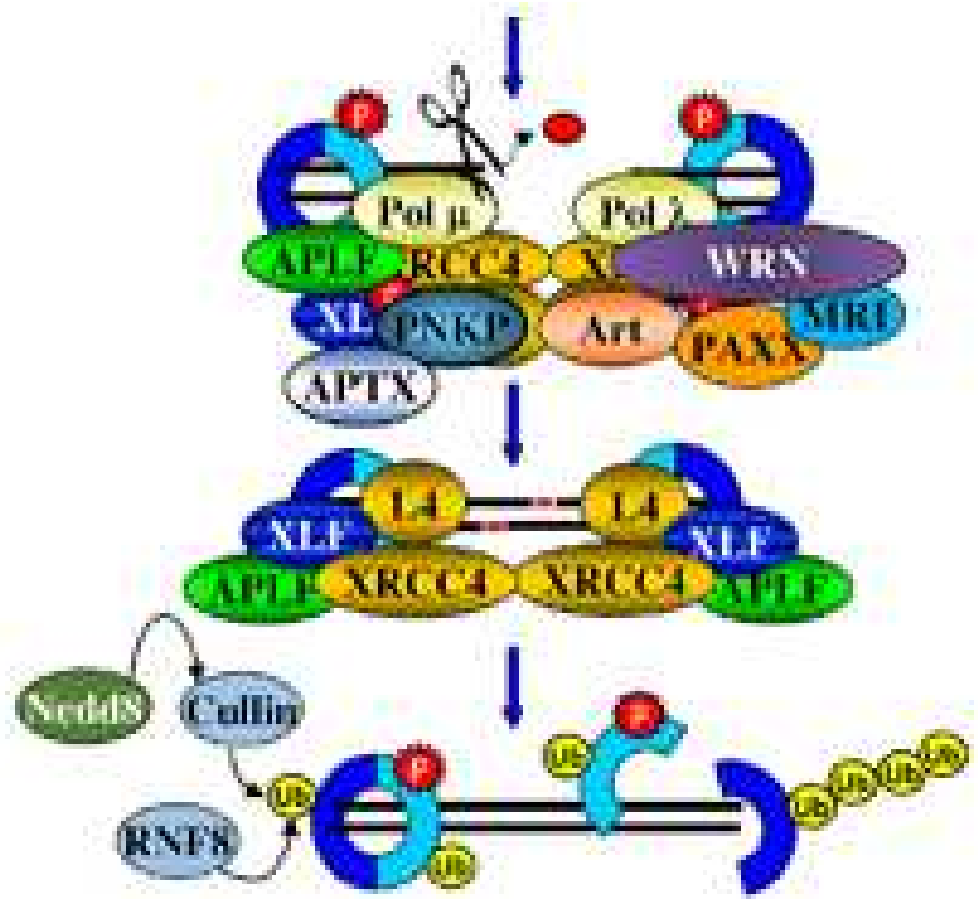
NHEJ Stages

- iv: Bridging contacts between the ends requires the presence of the other complex components, XRCC4-LIG4-XLF and DNA-PKcs autophosphorylation.

v Accessory factors and DNA processing

vi Ligation

vii Complex dissassembly and removal



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http://onlinelibrary.wiley.com/doi/10.1002/bies.201600209/full#bies201600209-fig-0002

NHEJ Stages

- v: Accessory factors are recruited to the core complex (enzymes for end-processing: PolX polymerases, PNKP, APTX and Artemis (Art)

- vi: LIG4 catalyses the ligation of canonical ends

- vii: The Ku complex must be removed from intact DNA by proteasomal degradation mediated by the ubiquitination of the Ku80 and Ku70 subunits by Cullin and RNF8

NHEJ

- A number of interaction between the core NHEJ components (Ku70, Ku80, DNA-PKcs, XRCC4 and Ligase IV) and kinases, phosphatases, polymerases and structural proteins.
- Different proteins compete for the same binding sites on the core machinery, and must be spatially and temporally regulated.
- Post-translational modifications such as phosphorylation, ADP-ribosylation and ubiquitinylation regulate sequential steps in the NHEJ pathway or control repair at different types of DNA breaks.