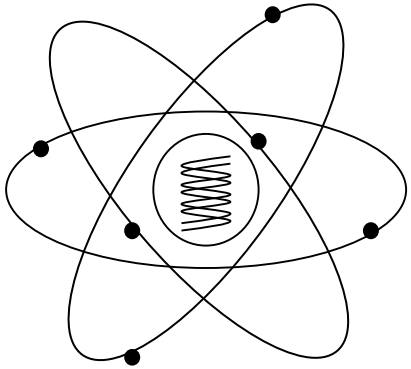
Mechanisms of the Repair of DNA Double-strand Breaks

- *To incorporate DSB repair in the physical modeling of radiation biological effects* -



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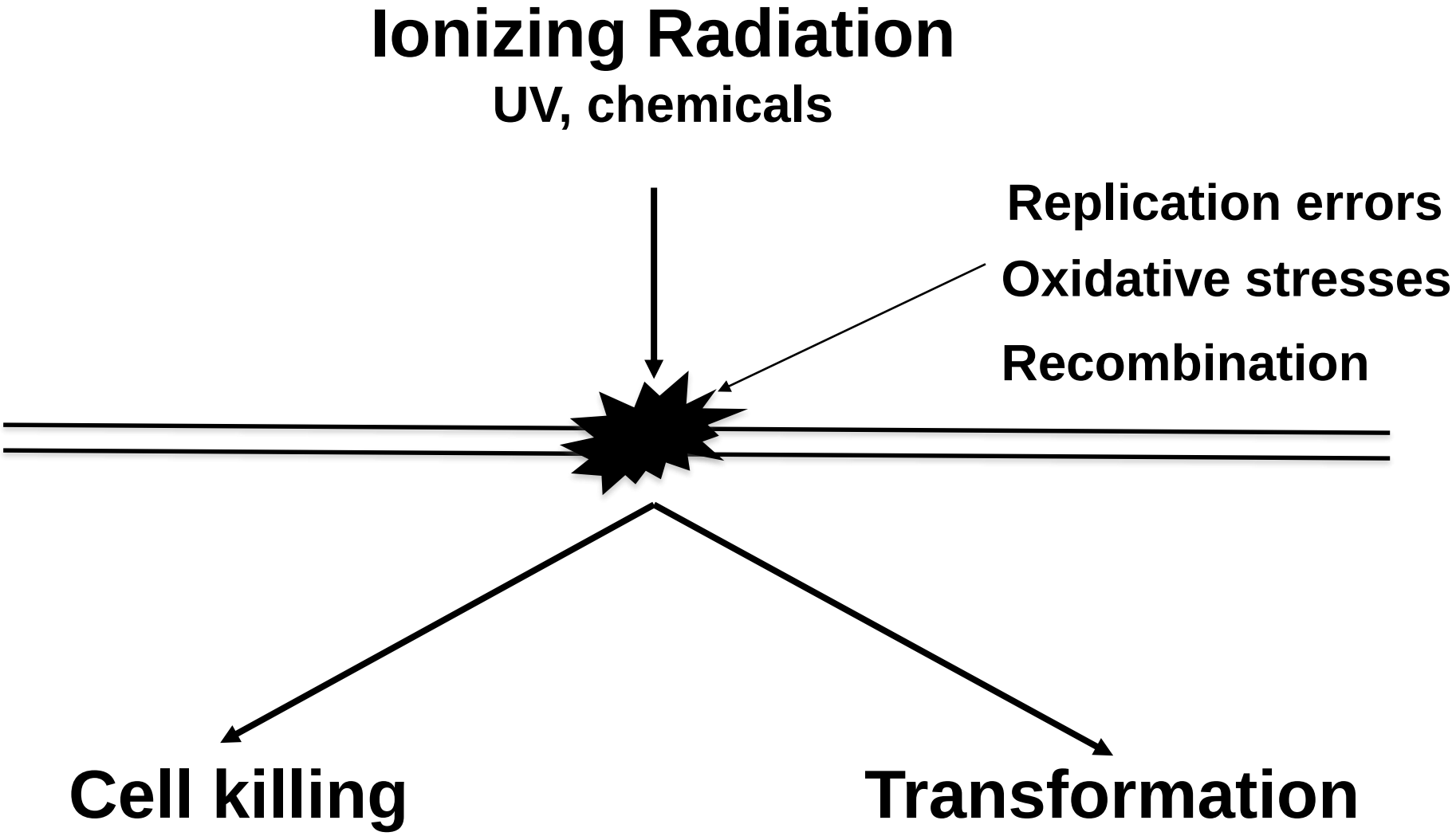
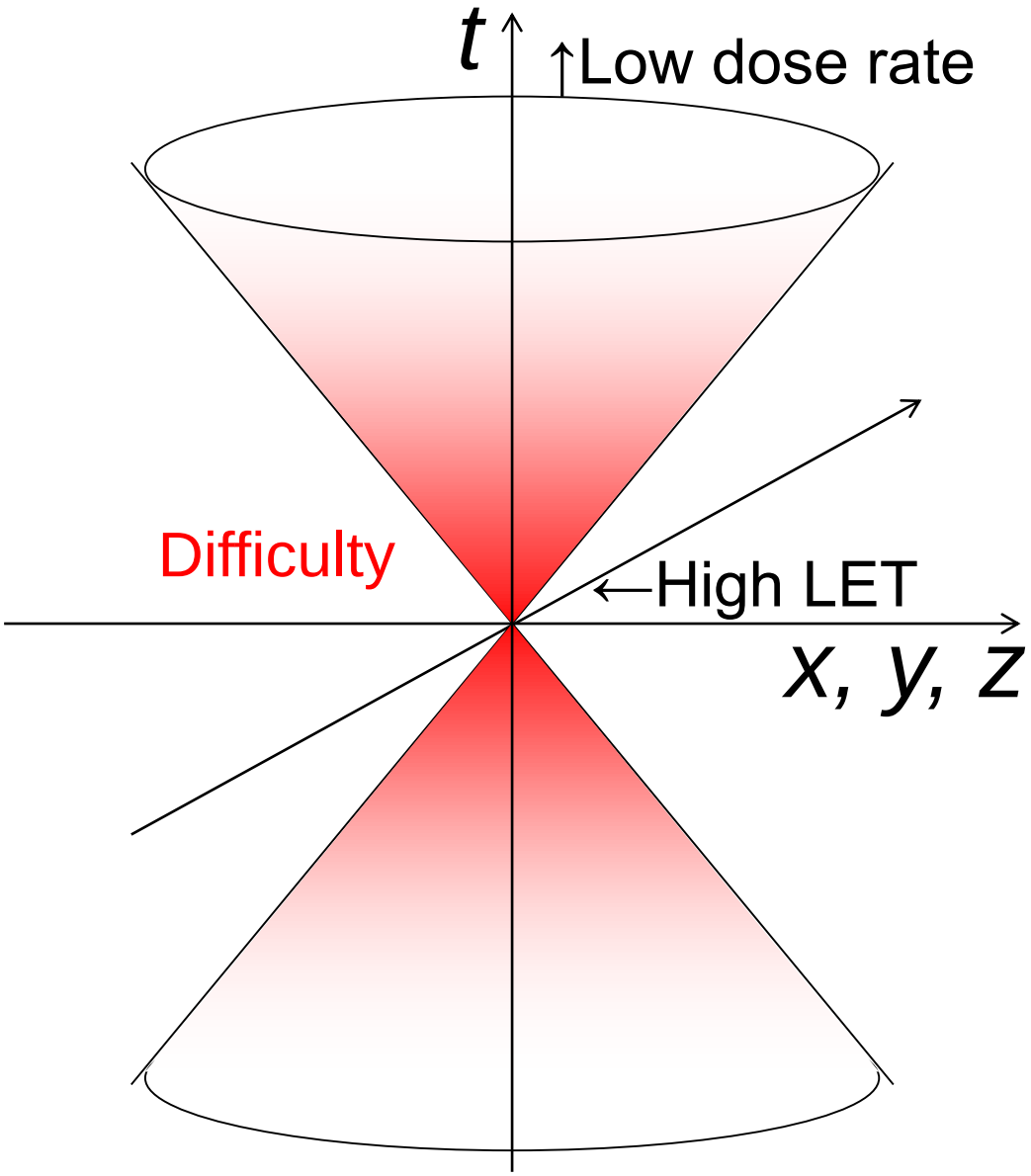
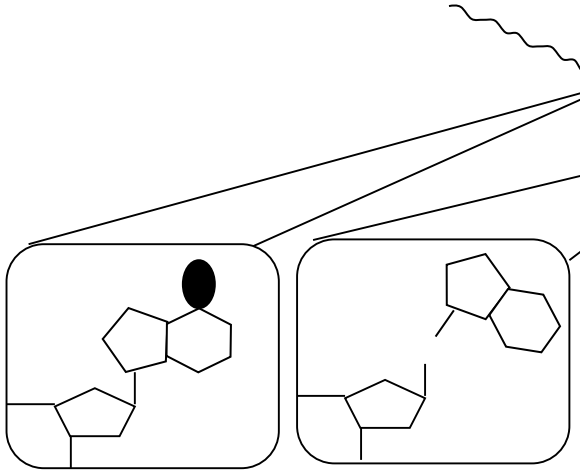
DNA Damage and Consequence



Distance between events (damages) and repairability



Radiation-induced DNA damages



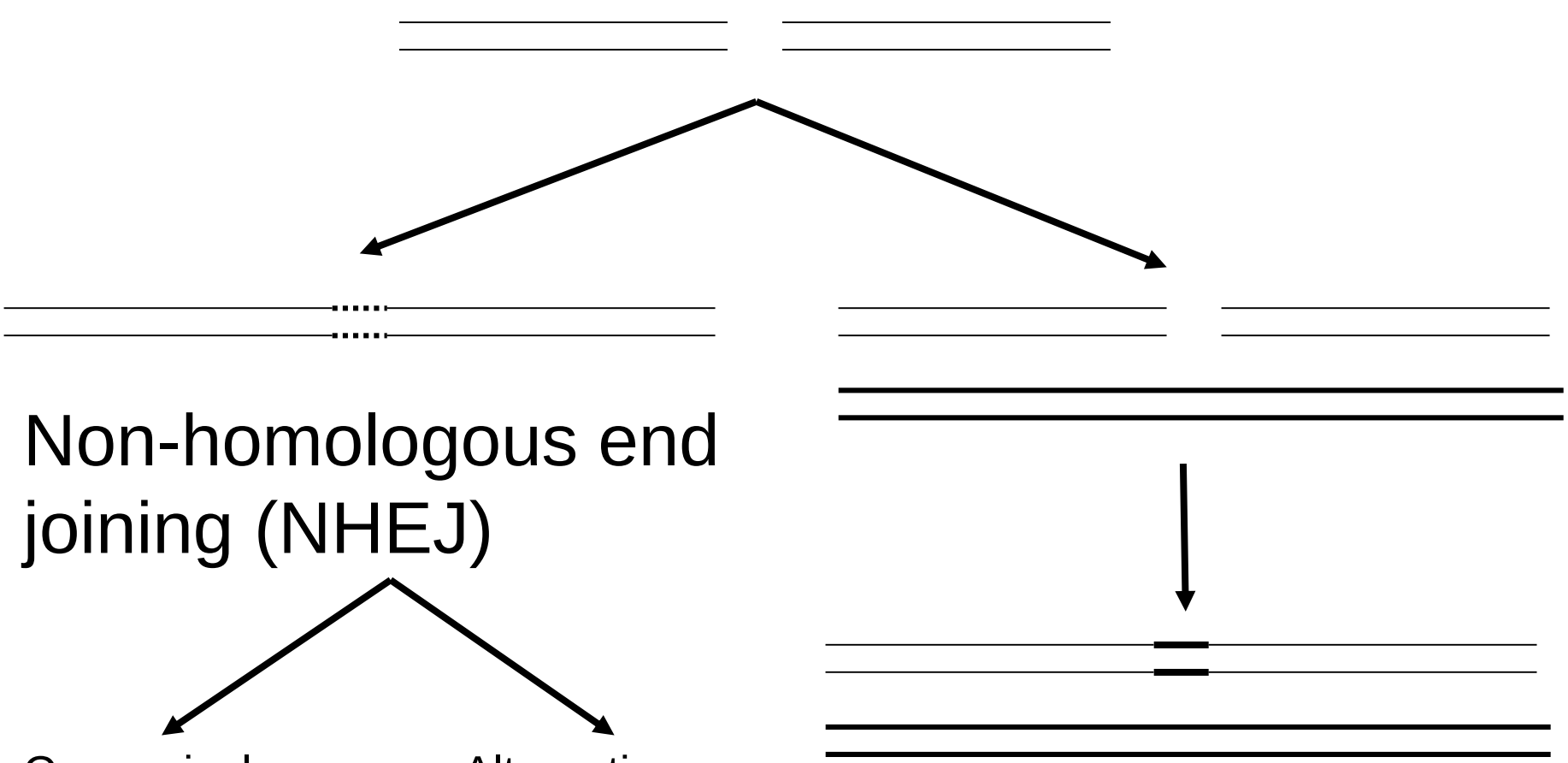
Base damages
1000～3000 / Gy

Single-strand breaks
～1000 / Gy

Crosslinks
-Intrastrand
-Interstrand
-DNA-protein
～150 / Gy

Double-strand breaks
20～50 / Gy

DNA Double-strand Break Repair Pathways

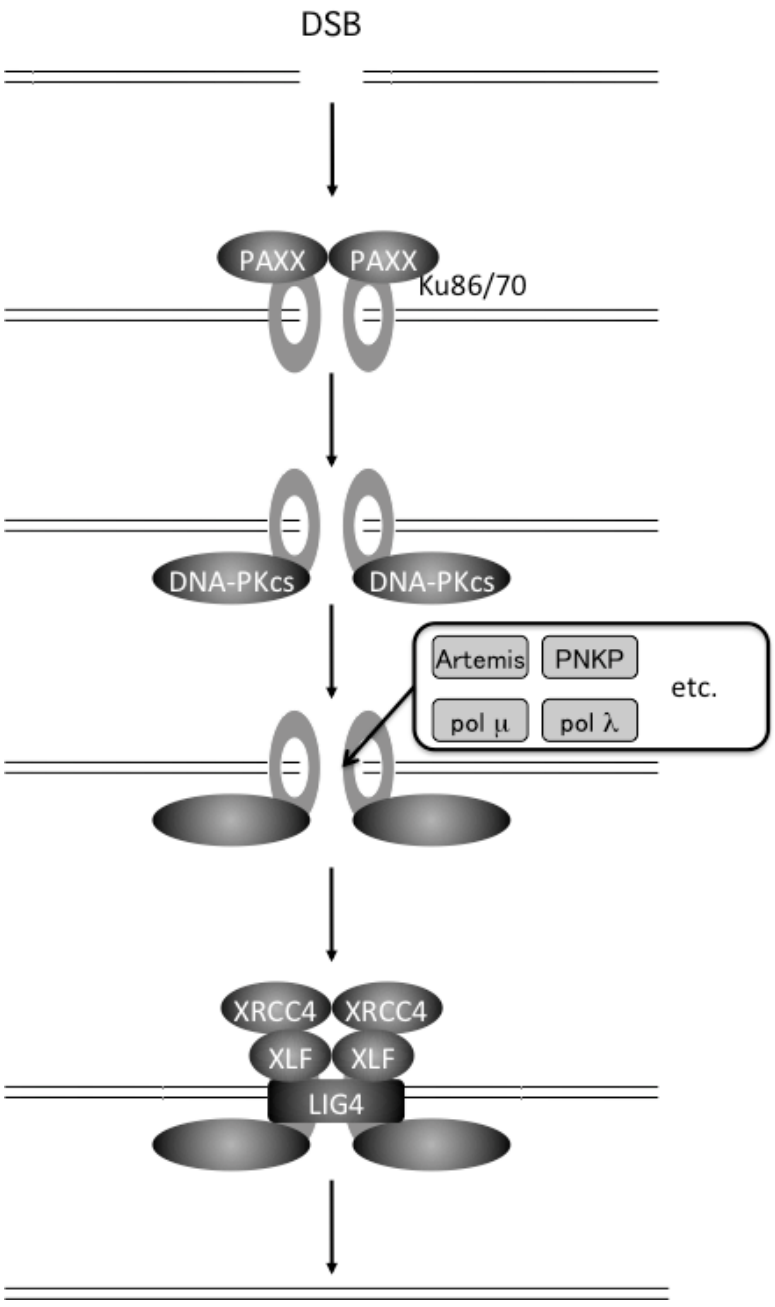


Non-homologous end joining (NHEJ)

Cannonical (Classical) NHEJ (C-NHEJ)

Alternative (Atypical) NHEJ (A-NHEJ)

Homologous recombination (HR)



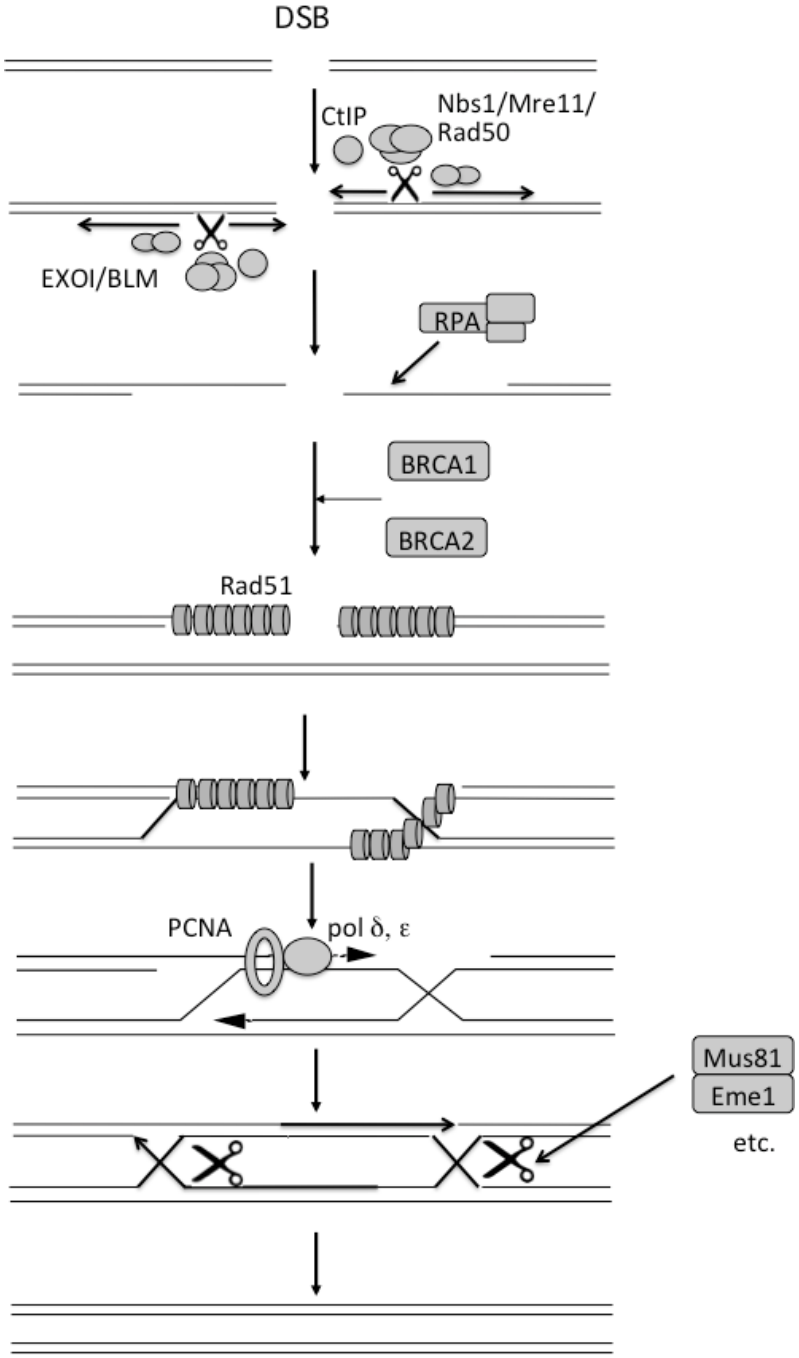
C-NHEJ Reaction

① Ku proteins (Ku86/70) binds to DSB.

② DNA-PKcs is recruited to DSB via Ku.

③ (When necessary) DSB is processed so that it can be ligated.

④ Two ends are ligated by DNA ligase IV (LIG4), where XRCC4 and XLF are thought to regulate LIG4.



HR Reaction

① Complex of Nbs1/Mre11/Rad50 (MNR) and ExoI/BLM degrade one of the strands, generating single strand DNA (Resection).

② RPA binds to single strand DNA.

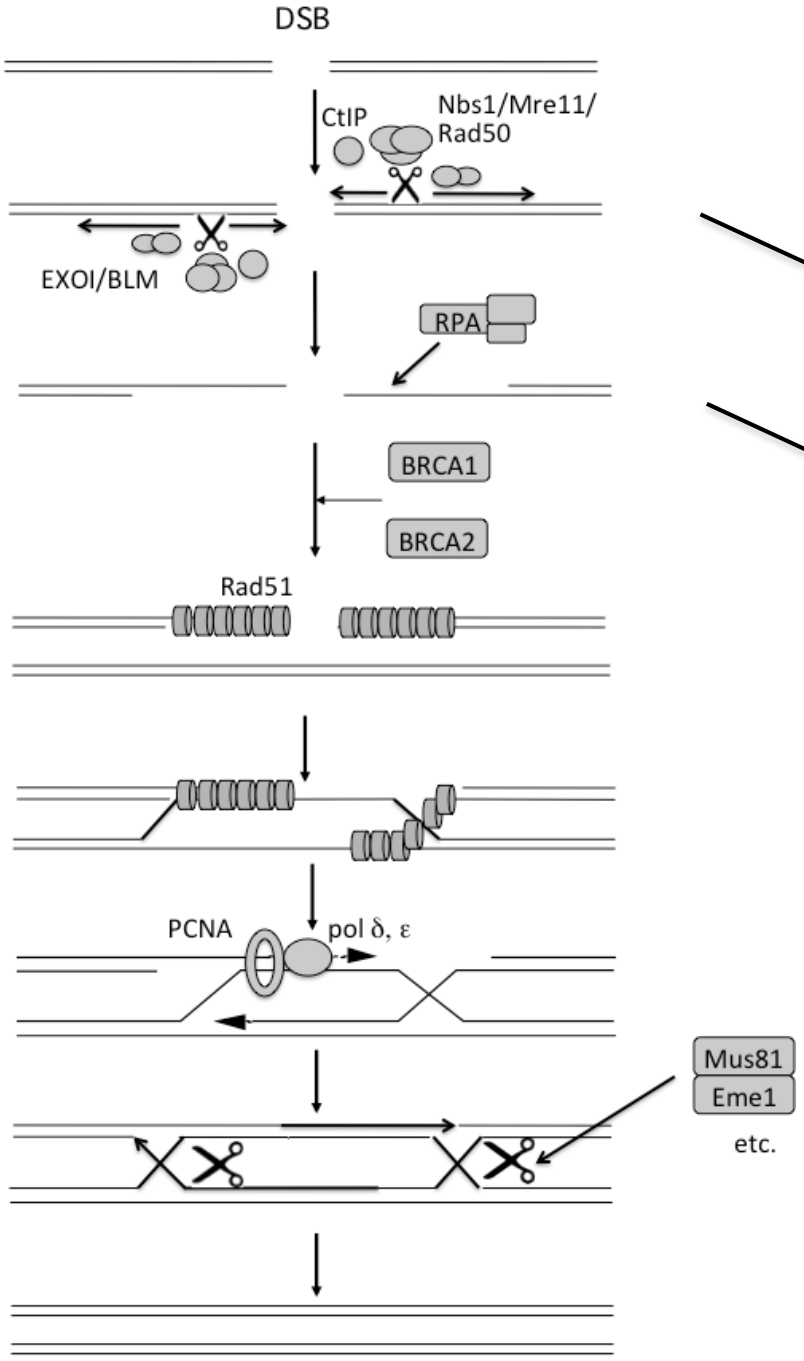
③ RPA is replaced by Rad51, which is promoted by BRCA1, BRCA2, etc.

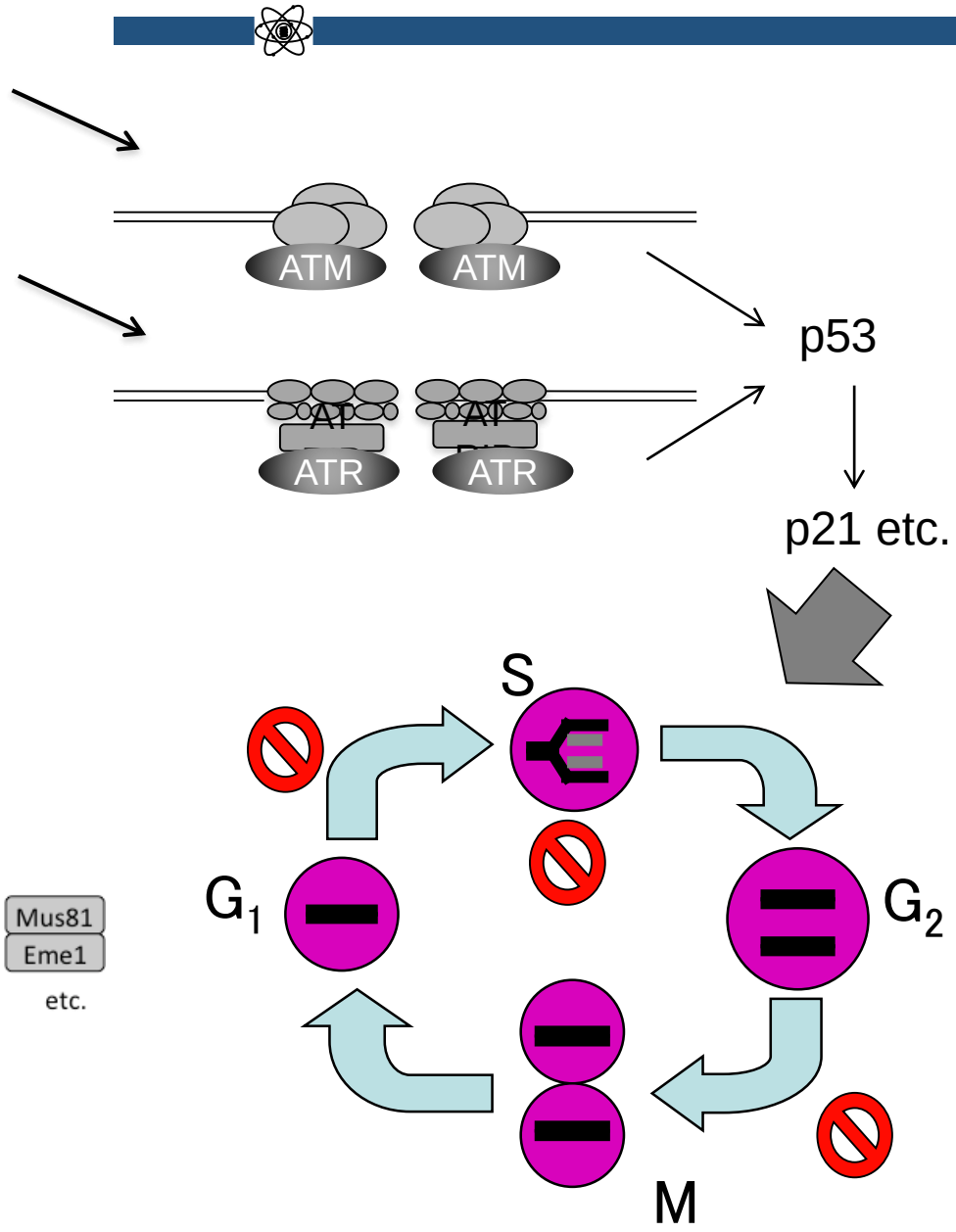
④ Rad51 promotes strand exchange to look for homologous strand.

⑤ Strand synthesized along the template by pol δ, PCNA etc.

⑥ Strands are separated by Mus81/Eme1 etc (Resolution).

Cell Cycle Checkpoints





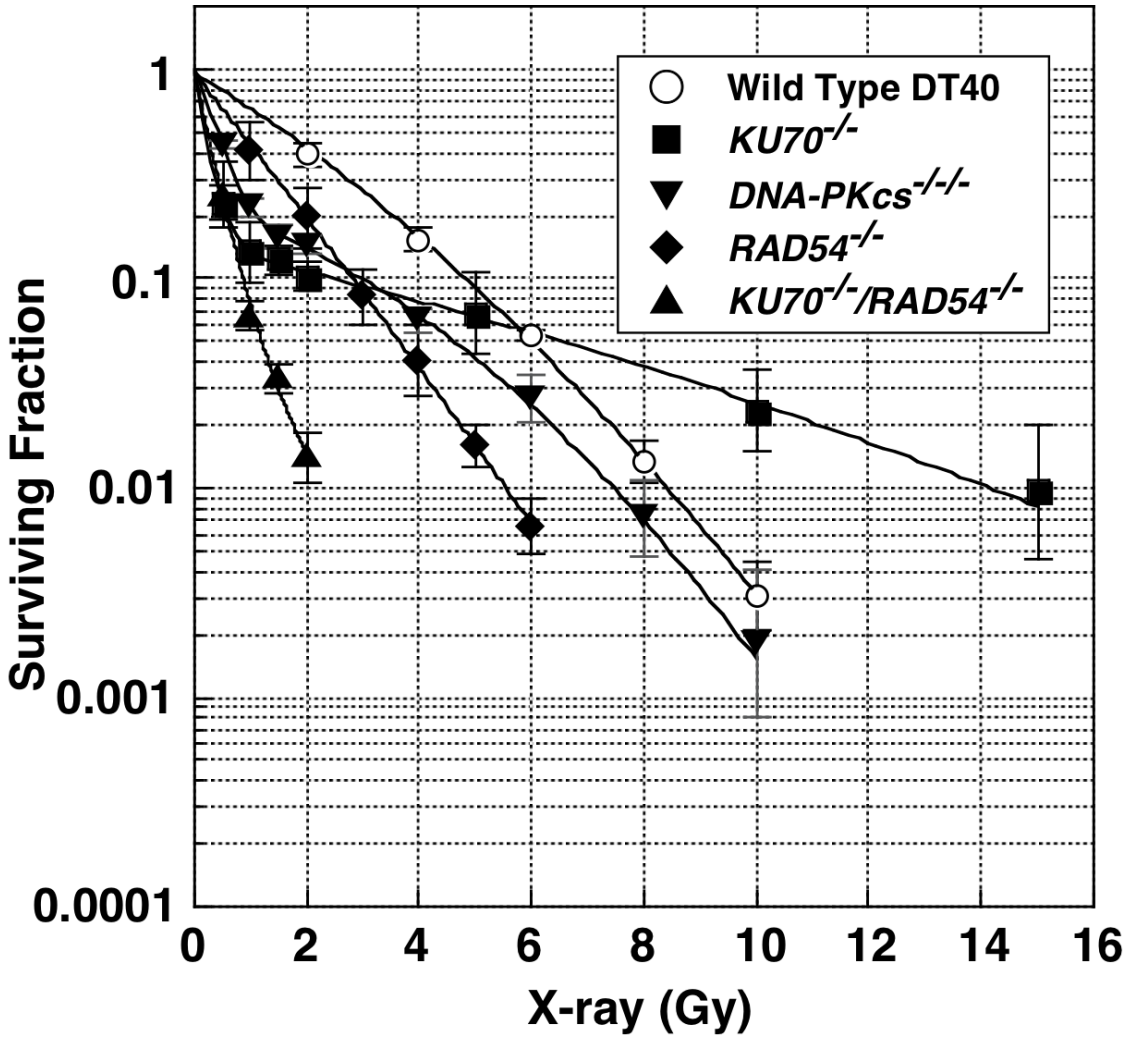
C-NHEJ vs. HR – (1) Accuracy

- C-NHEJ may sometimes incur errors, e.g. loss or addition of nucleotides at the junction or joining wrong pair of DNA ends.
- HR is considered more accurate than C-NHEJ, as it uses undamaged homologous sequence as the template.
- However, most part of the genome of higher eukaryotes is not encoding protein. Therefore, loss or addition of small number of nucleotides does not matter!

C-NHEJ vs. HR – (2) Availability

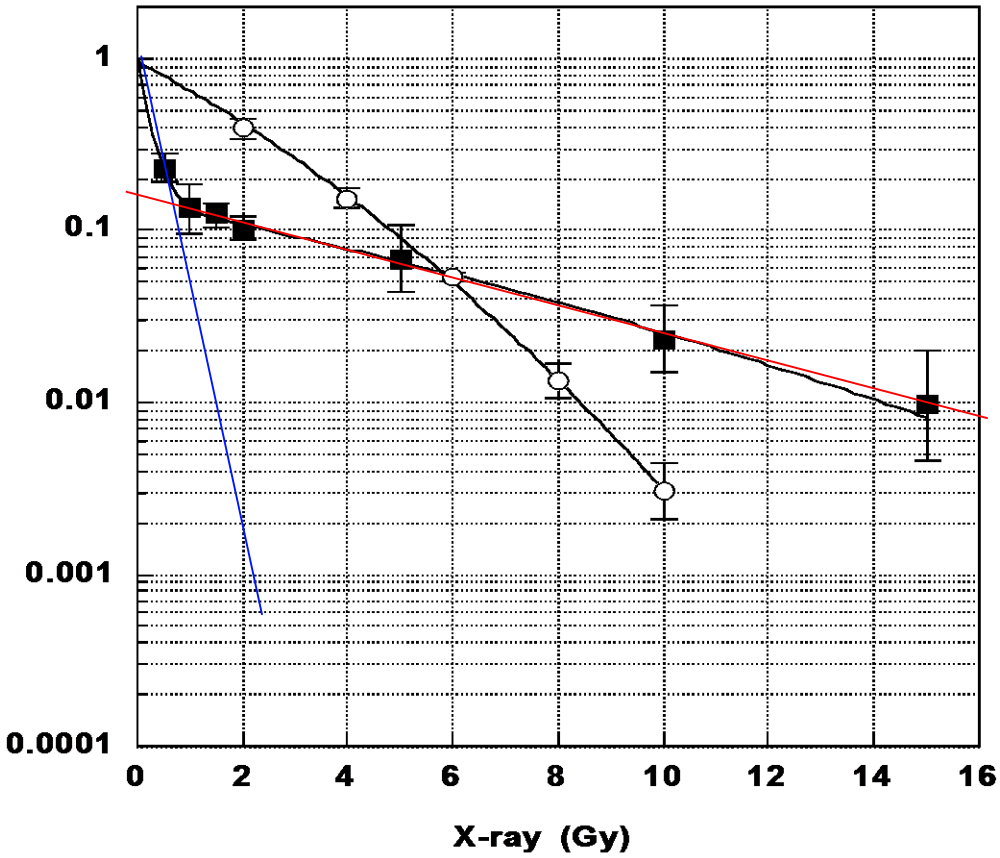
- Lower eukaryotes, such as yeast, can utilize homologous chromosome as well as sister chromatid as the template for HR.
- However, higher eukaryotes, including human, can utilize sister chromatid, but not homologous chromosome as the template for HR. Therefore, HR is limited in late S and G2 phases in cell cycle.
- Most of the cells in the body of higher eukaryotes are in G1 or G0 phases, where HR is not available.

Radiosensitivity of NHEJ and/or HR-deficient cells



(Takata et al. 1998; Fukushima et al. 2001; Yin et al. 2004)

Survival curve of Ku tells us …



○ : Wild-type

■ : Ku70-/-

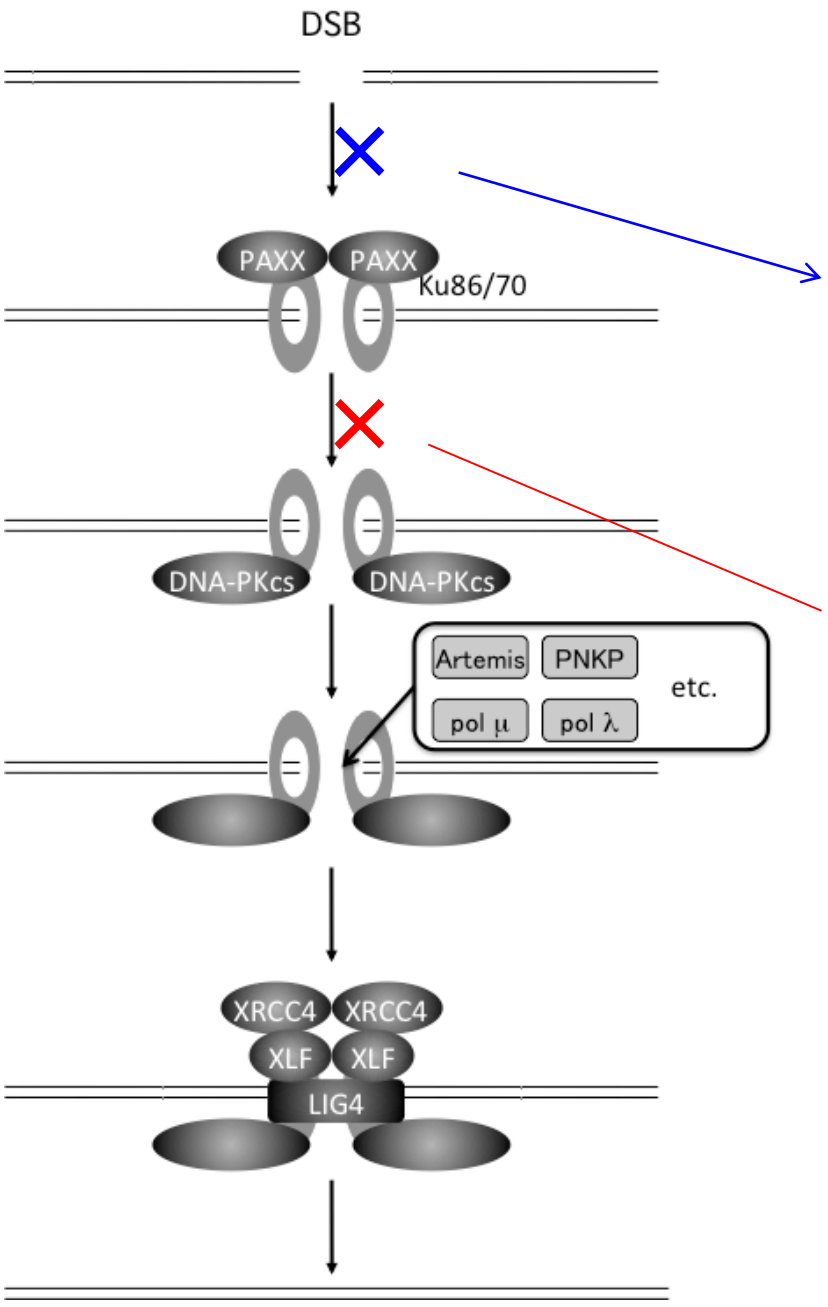
(Takata et al. 1998; Yin et al. 2004)

Sensitive fraction (~85%)

G1/early S cells
- NHEJ-deficient
- HR proficient
  but not allowed to do it!

Resistant fraction (~15%)

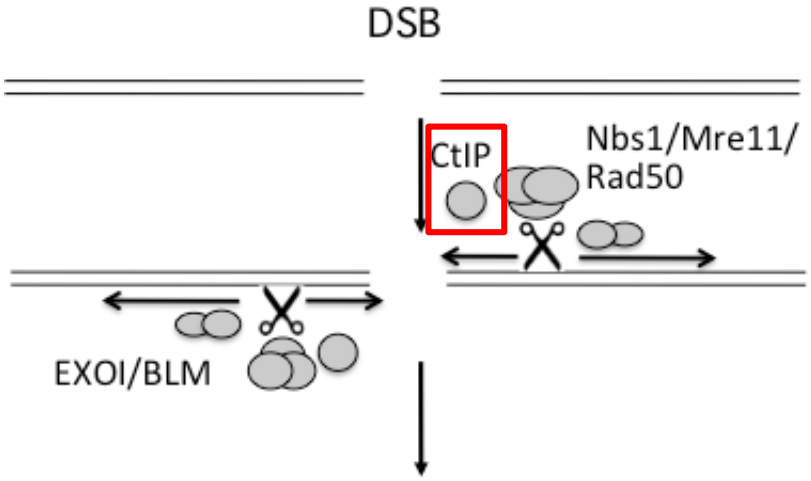
Late S/ G2 cells
- DSB can be repaired through HR
- No NHEJ is good!



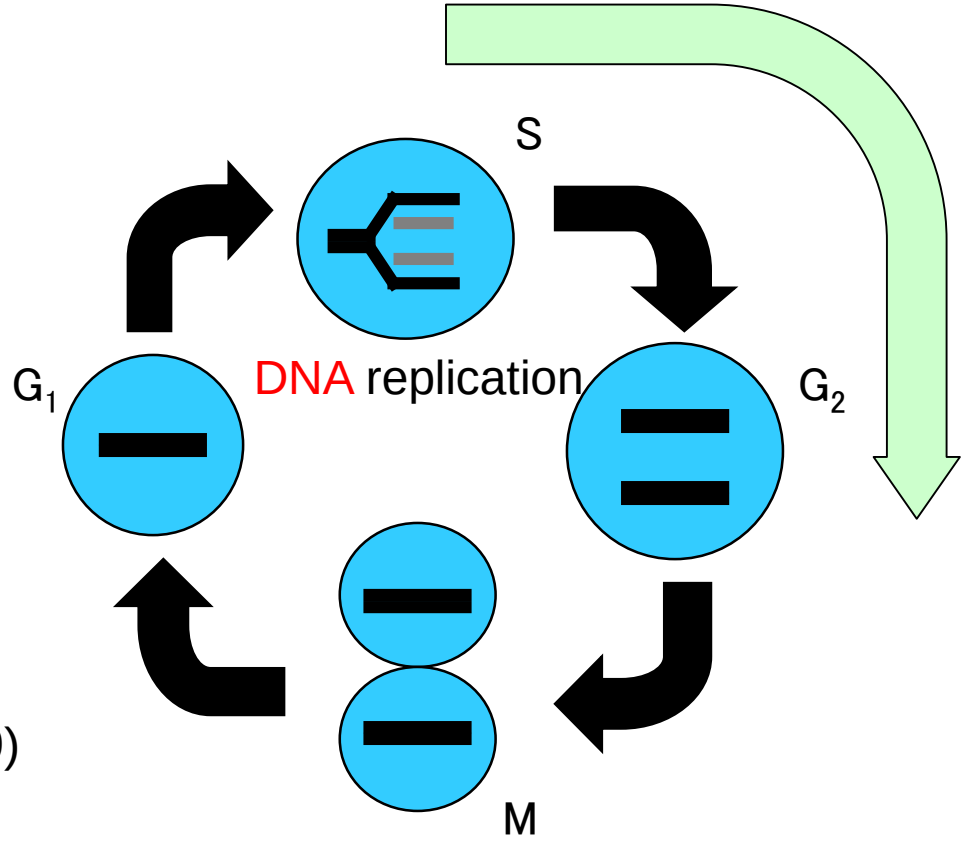
In the absence of Ku,
NHEJ is not started yet.
Just go to HR.

In the absence of DNA-PKcs,
Ku is also bound,
But NHEJ doesn't proceed
Also cannot go to HR!
Dead end!

Cell cycle regulation of DSB repair



CtIP (T847) phosphorylation by S-phase kinase Cdk2 required for resection (license)



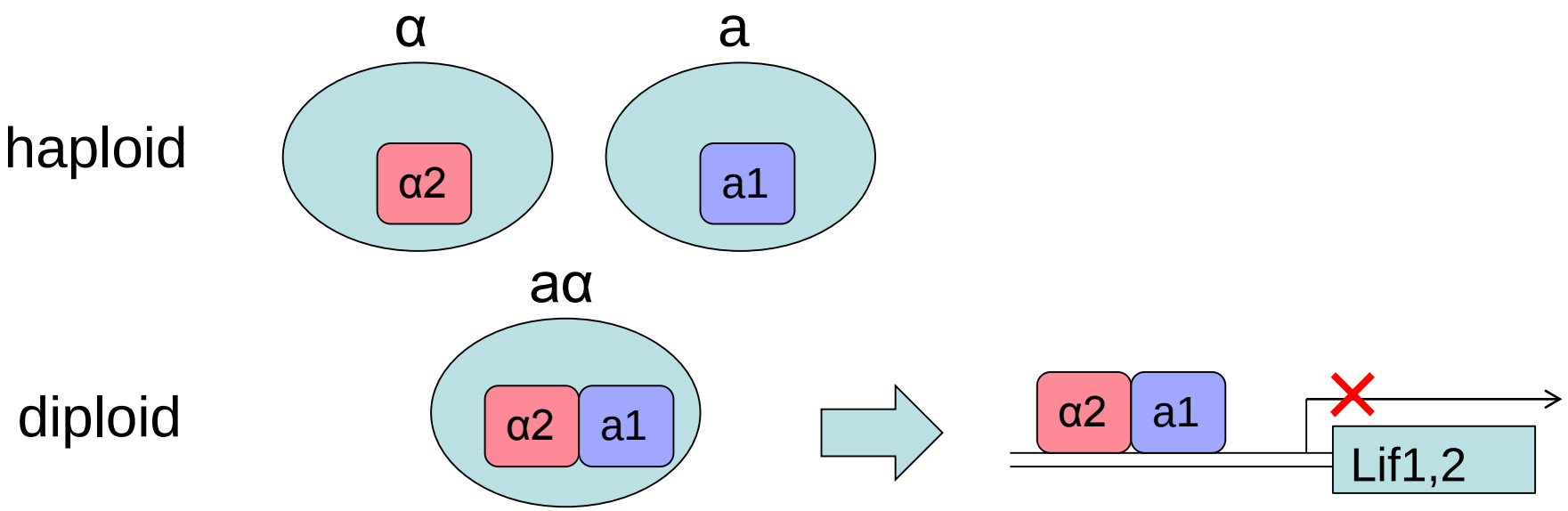
- T847A (phospho-deficient)
  HR-defective, sensitive to G_2 DSB
- T847E (phospho-mimic)
  HR-proficient, sensitive to G_1 DSB
  →HR must be silenced in G_1

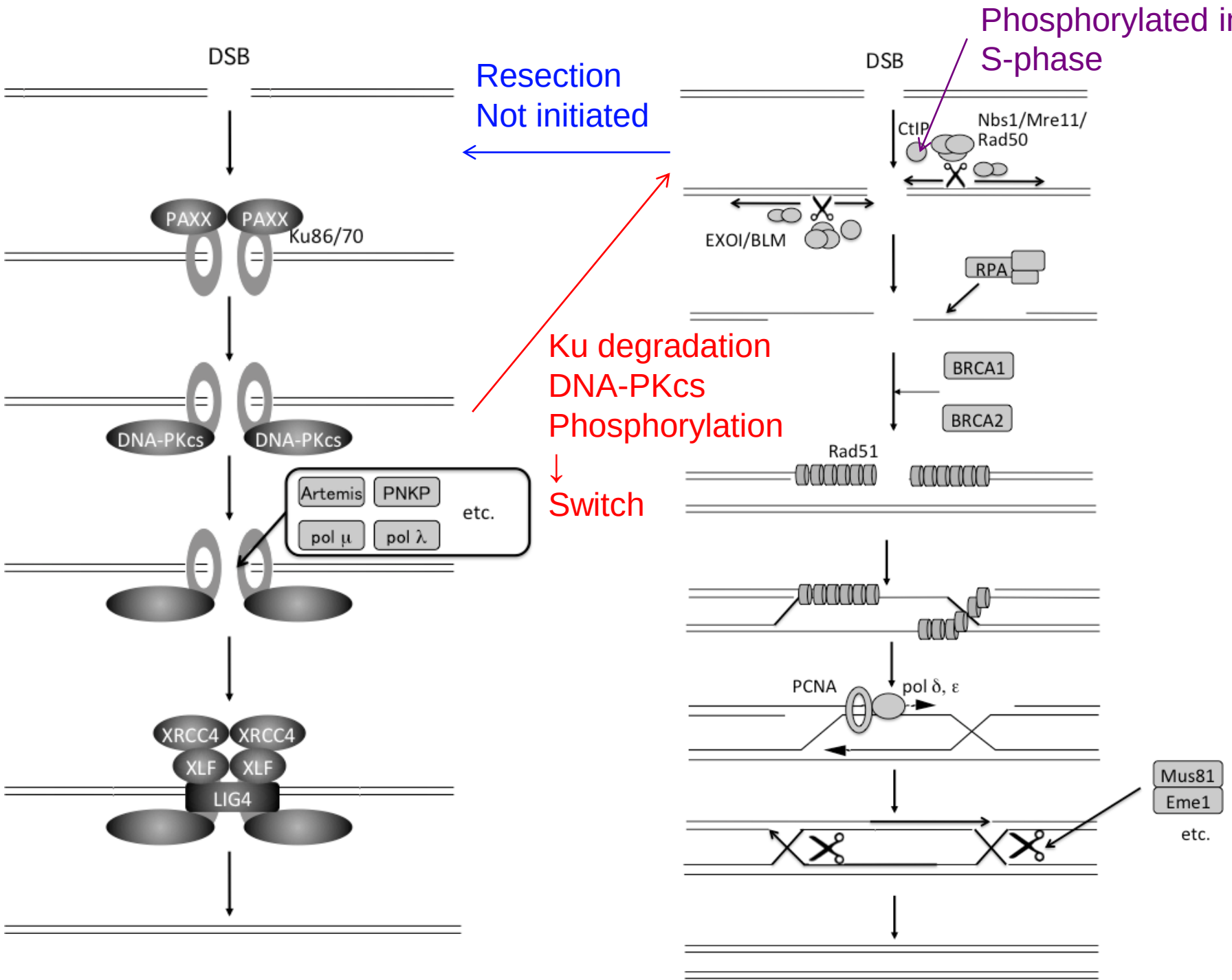
(Huertas and Jackson, JBC 2009)

Ploidy-dependent control of NHEJ

In yeast, NHEJ is unnecessary in diploid regardless of cell cycle phase

Lif1 (XRCC4) and Lif2 (XLF) are silenced in diploid (Frank-Vaillant and Marcand, 2001; Kagel et al, 2001)




DSB
PAXX
PAXX
Ku86/70
DNA-PKcs
DNA-PKcs
Artemis
PNKP
pol μ
pol λ
etc.
XRCC4
XRCC4
XLF
XLF
LIG4
Resection
Not initiated
Ku degradation
DNA-PKcs
Phosphorylation
↓
Switch
DSB
Phosphorylated in
S-phase
CtIP
Nbs1/Mre11/
Rad50
EXOI/BLM
RPA
BRCA1
BRCA2
Rad51
PCNA
pol δ, ε
Mus81
Eme1
etc.

C-NHEJ or HR: which to go?

1) Cell cycle

2) Damage complexity

3) Chromatin status

4) Species – genome organization (?)

The End

Thank you for kind attention