Cancer Biology I :

Topics covered

Week 1:
Lecture 1: **Hallmarks of cancer – an overview; Oncogenes and tumor suppressor genes**
(Chapters 2, 4, 7 (Weinberg book))

Week 2:
DNA repair of DNA double strand breaks; Synthetic lethality
Lecture and paper discussion

Week 3:
Lecture 3/Exercises: **DNA repair and the DNA damage response**

Week 4:
Lecture 4/Exercises: **p53 and apoptosis**
(Chapters 9 (Weinberg))

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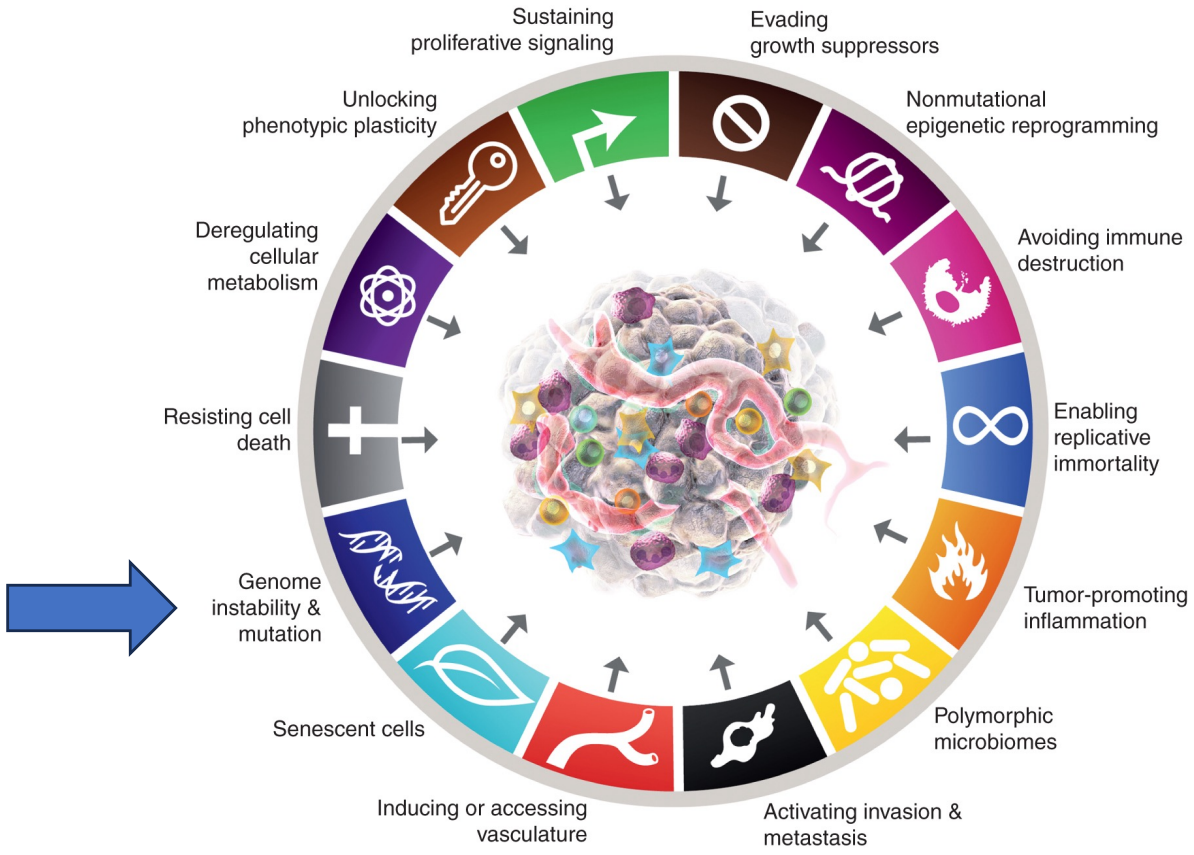
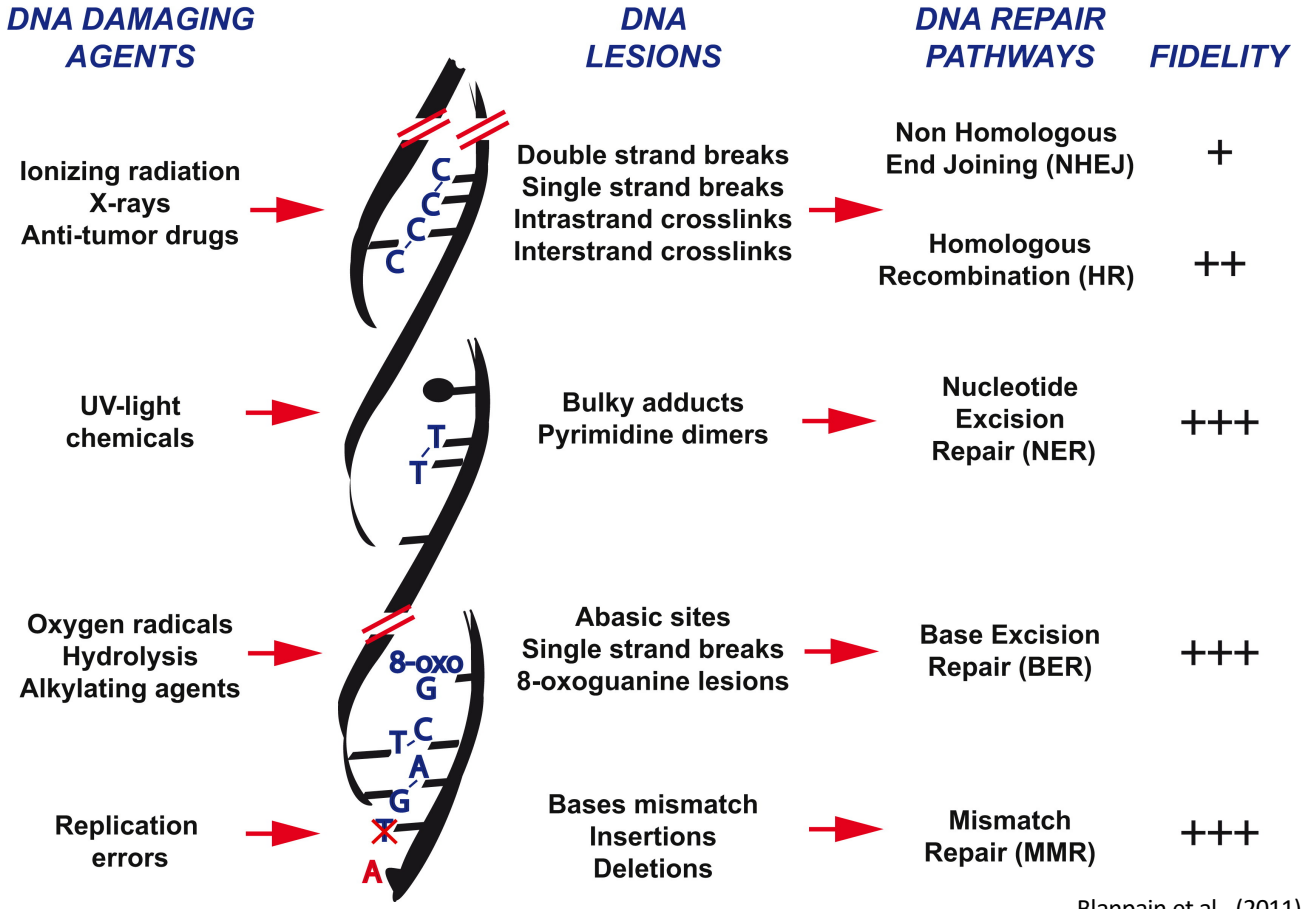
From week 1:

Likelihood of accumulating >6 specific alterations in a single cell is very low



From week 1:

DNA Damage



Blanpain et al., (2011) Cell Stem Cell, Volume 8, Issue 1

From week 1:

DNA Replication Errors are Extremely Rare

- Mutation rate of $1/10^9$ per nucleotide per cell division
  - Copying mistake by DNA polymerases (delta and epsilon): $1/10^5$
  - 3'-5' proofreading overlook: $1/10^2$
  - Mismatch repair enzymes overlook: $1/10^2$

- **10-50 double-strand DNA breaks occur per S phase**
- Human genome: 6.4 billion bp

DNA Repair Mechanisms

Repair by excision

- BER: Base excision repair
- MMR: Mismatch repair
- NER: Nucleotide excision repair
- Ribonucleotide excision repair

Low fidelity DNA polymerases-Translesion polymerases

→ Double strand break repair

- **NHEJ: Non homologous end-joining**
- **MMEJ: Microhomology mediated end-joining (or Alt-NHEJ)**
- **HR: Homologous recombination**

For an exhaustive list of proteins that are implicated in genome stability.
Wood, R. D., Mitchell, M., Sgouros, J., and Lindahl, T. (2001). Human DNA repair genes. Science *291*, 1284-1289.
http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html

DNA double strand breaks and their repair

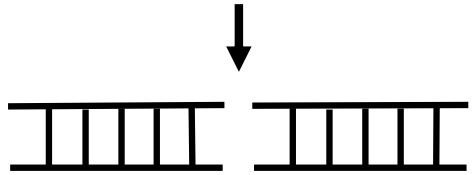
- Non-homologous end joining/Microhomology mediated end-joining
- Homologous recombination, BRCA1, BRCA2

→exploiting synthetic lethality in cancer treatment

Formation of DNA Double-Strand Breaks

CAUSES

- **Exogenous**
  - Ionizing radiations
  - Mutagens
- **Endogenous**
  - Free radicals
  - Replication of damaged DNA
- **Specialized**
  - Meiosis (SPO11)
  - V(D)J Recombination (RAG1/RAG2)



CONSEQUENCES

- **Cell death**
  - Apoptosis
  - Senescence
- **Repair**
  - Non-homologous end-joining
  - Microhomology mediated end joining
  - Homologous Recombination

Incorrect

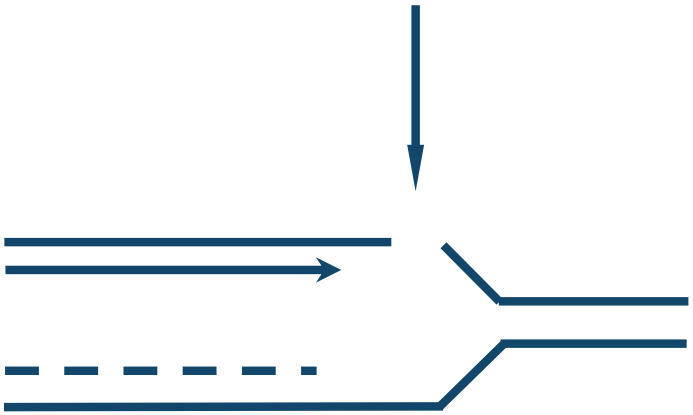
Genome instability

Carcinogenesis

Endogenous Formation of DNA Double-Strand Breaks

Single strand lesions:
$5x10^3$ /cell/cycle

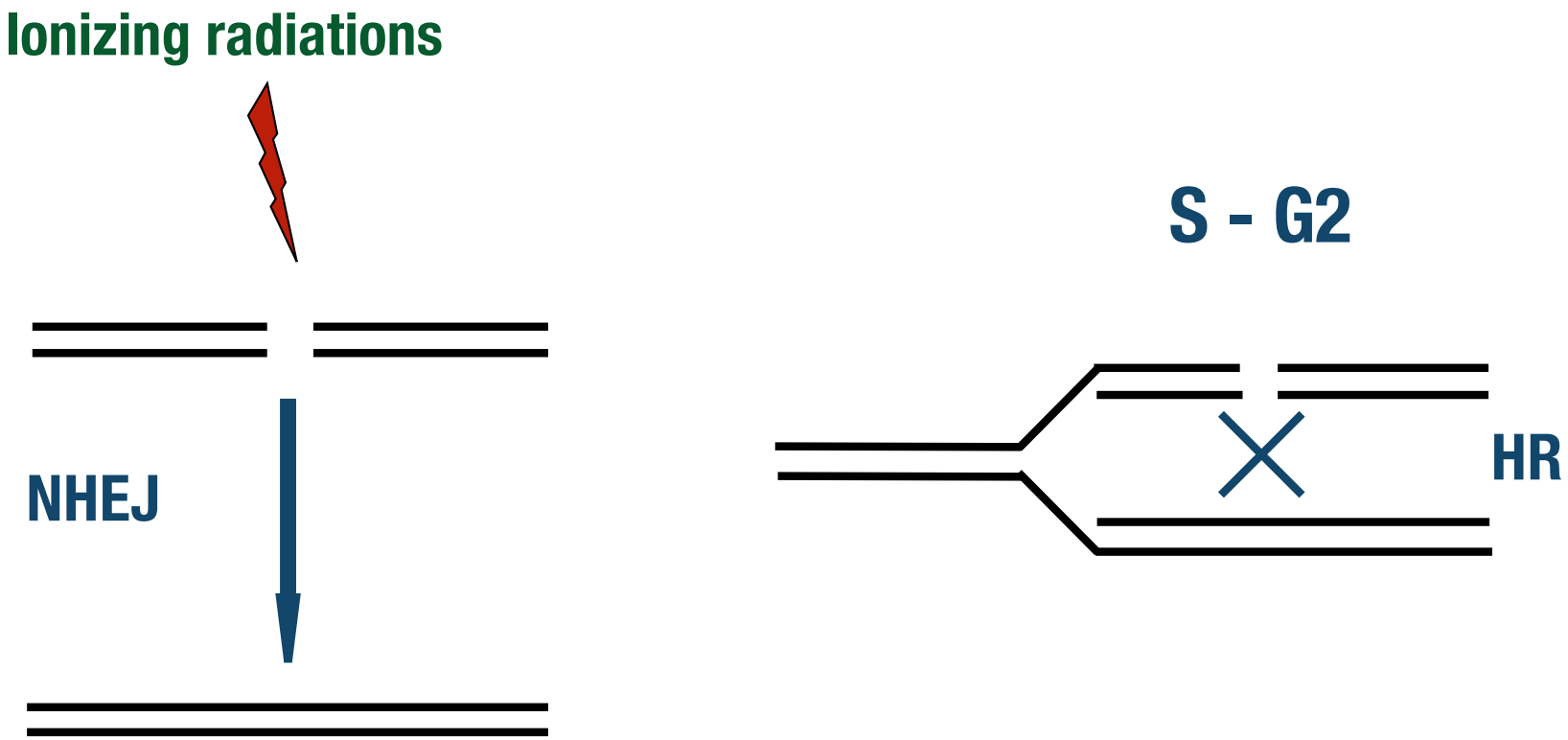
99% repaired: BER, NER



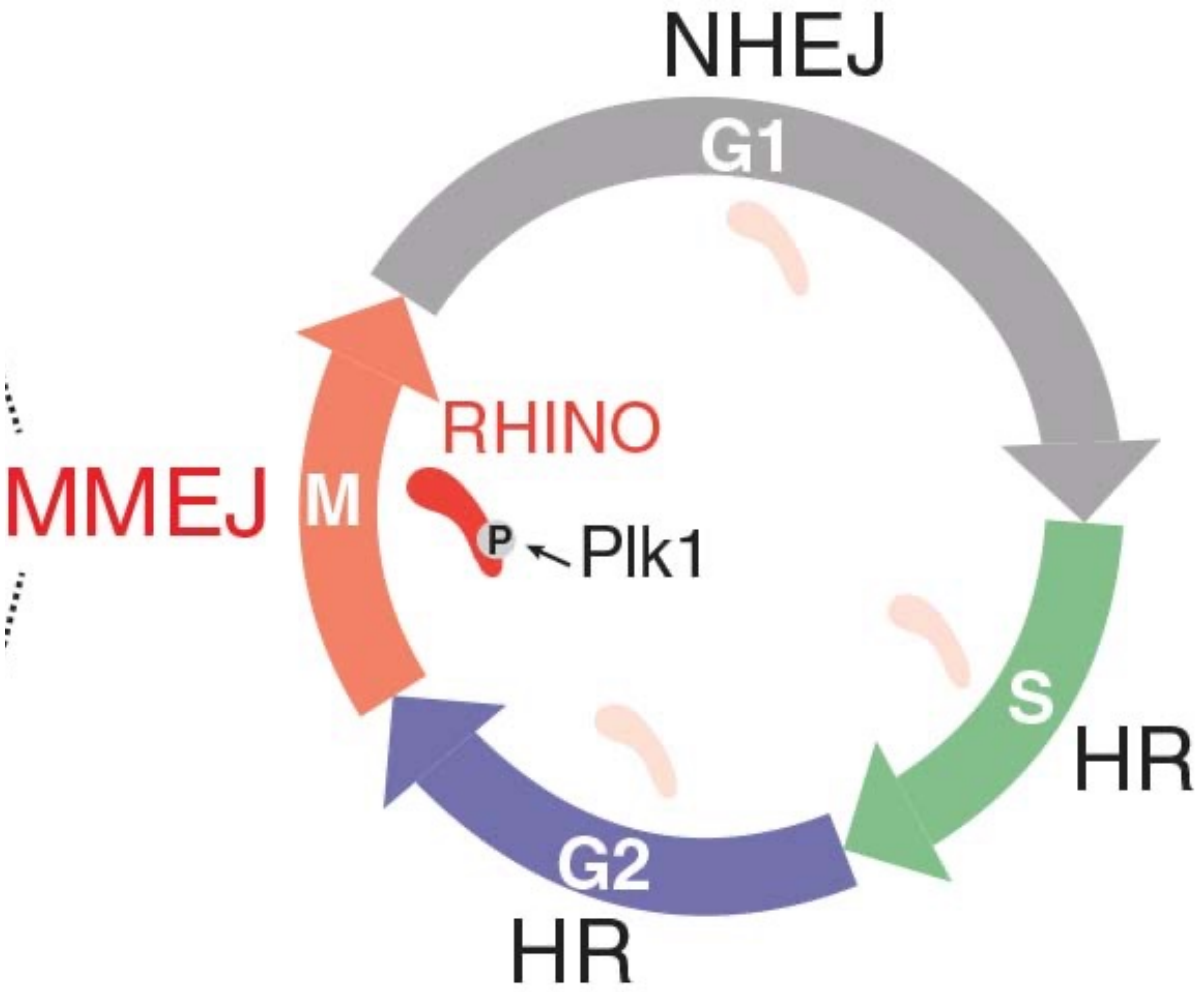
1% is converted into DSB
during replication:
50 DSBs/cell/phase S

Repair

Repair of DNA Double-strand Breaks



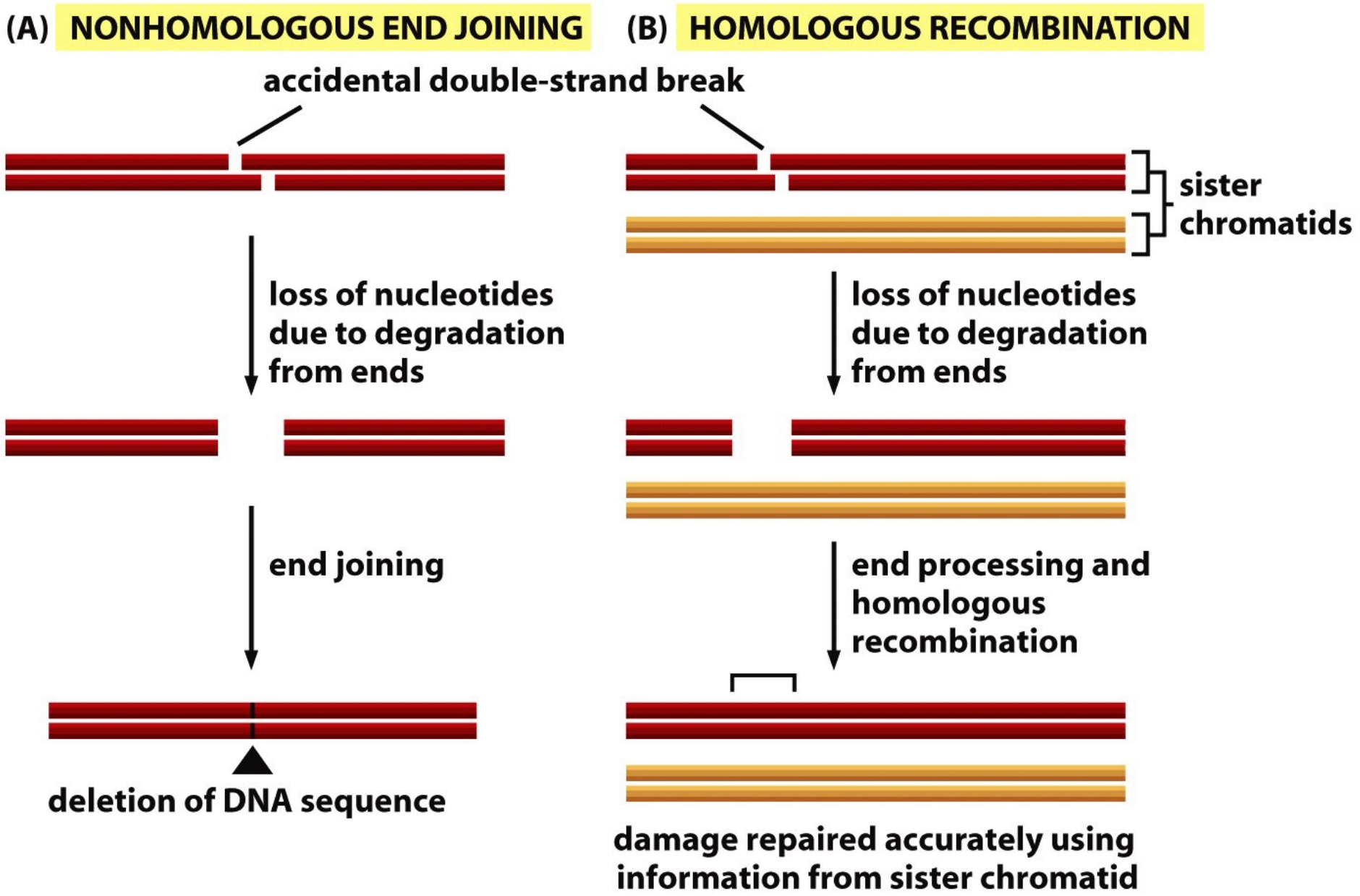
Repair of DNA Double-Strand Breaks During the Cell Cycle



…MMEJ activity in mitosis repairs persistent DSBs that originate in S phase. Of note, NHEJ and HR are not active in mitosis.

Brambati et al., Science **381**, 653–660 (2023)

Two Main Pathways to Repair DNA ds Breaks



Two Main Pathways to Repair DNA ds Breaks

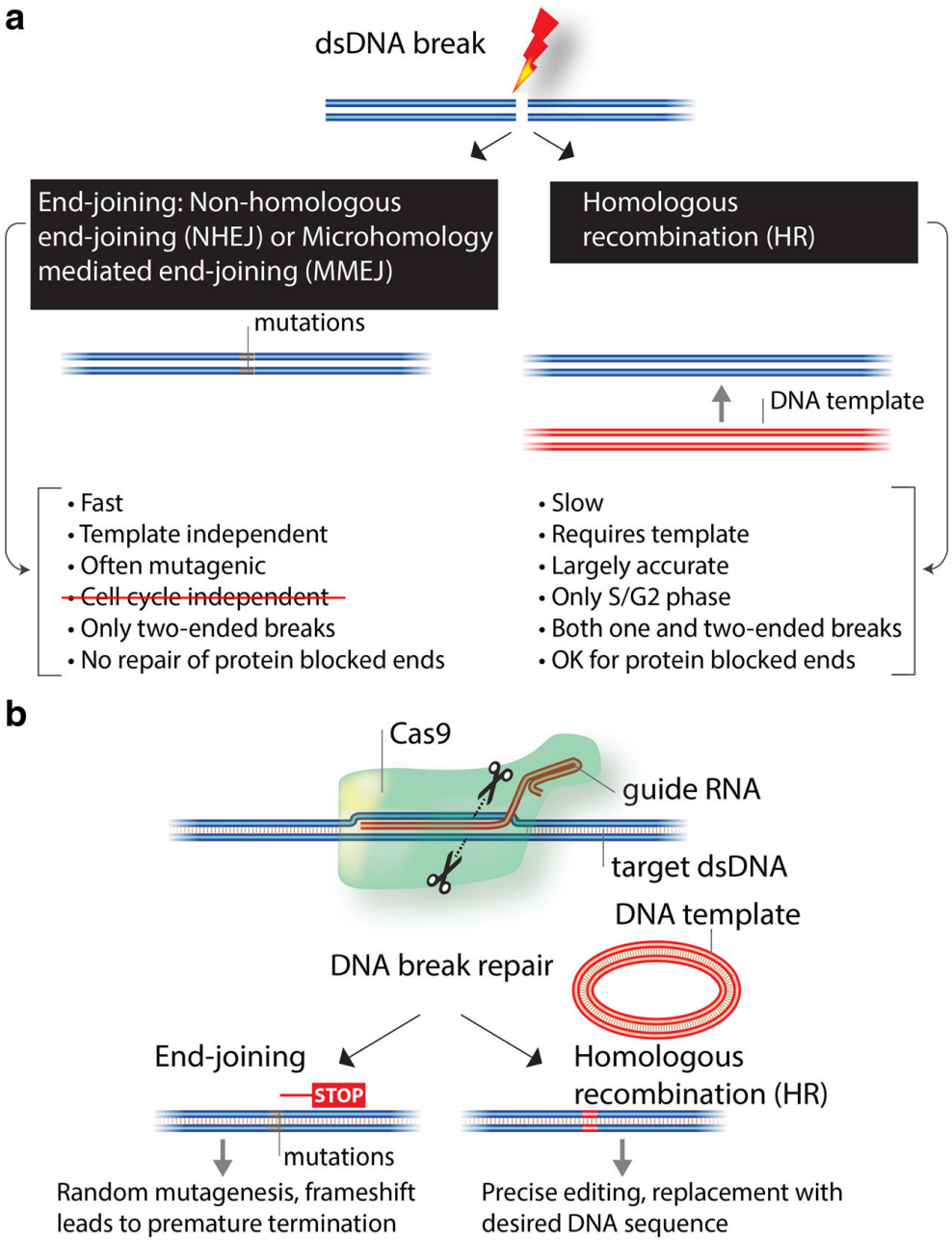


Fig. 2 An overview of the two main pathways for DNA double-strand break repair in human cells. **a** Main differences between end-joining and homologous recombination pathways. **b** DNA double-strand break repair pathway usage gives rise to different outcomes during genome editing with CRISPR-Cas9. Whereas end-joining often results in random mutations in the vicinity of the break site that may disrupt the reading frame of the targeted gene, homologous recombination may mediate the precise replacement of genetic information

DNA End Joining (NHEJ and MMEJ)

Repair DNA ds Breaks by End Joining

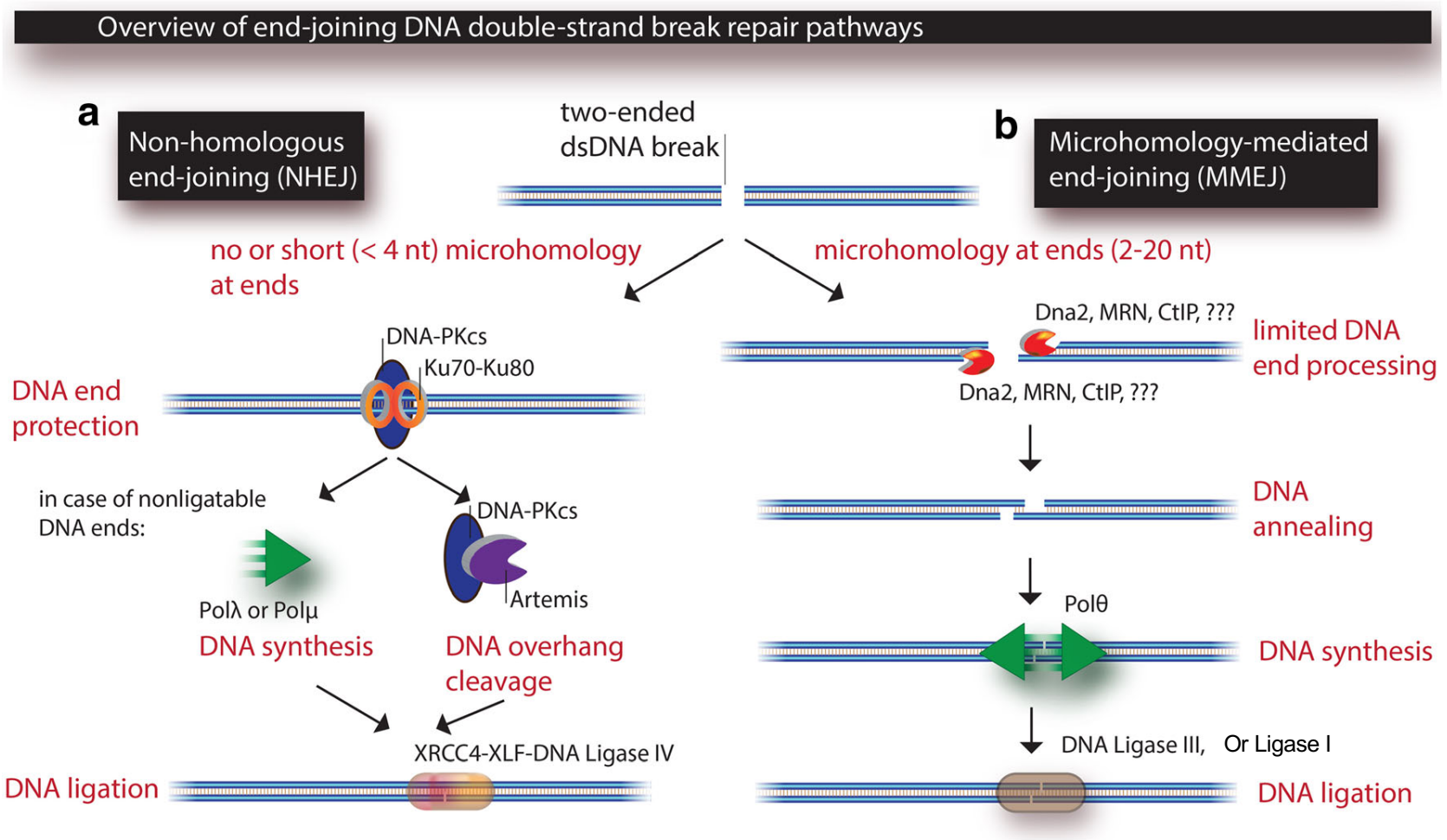
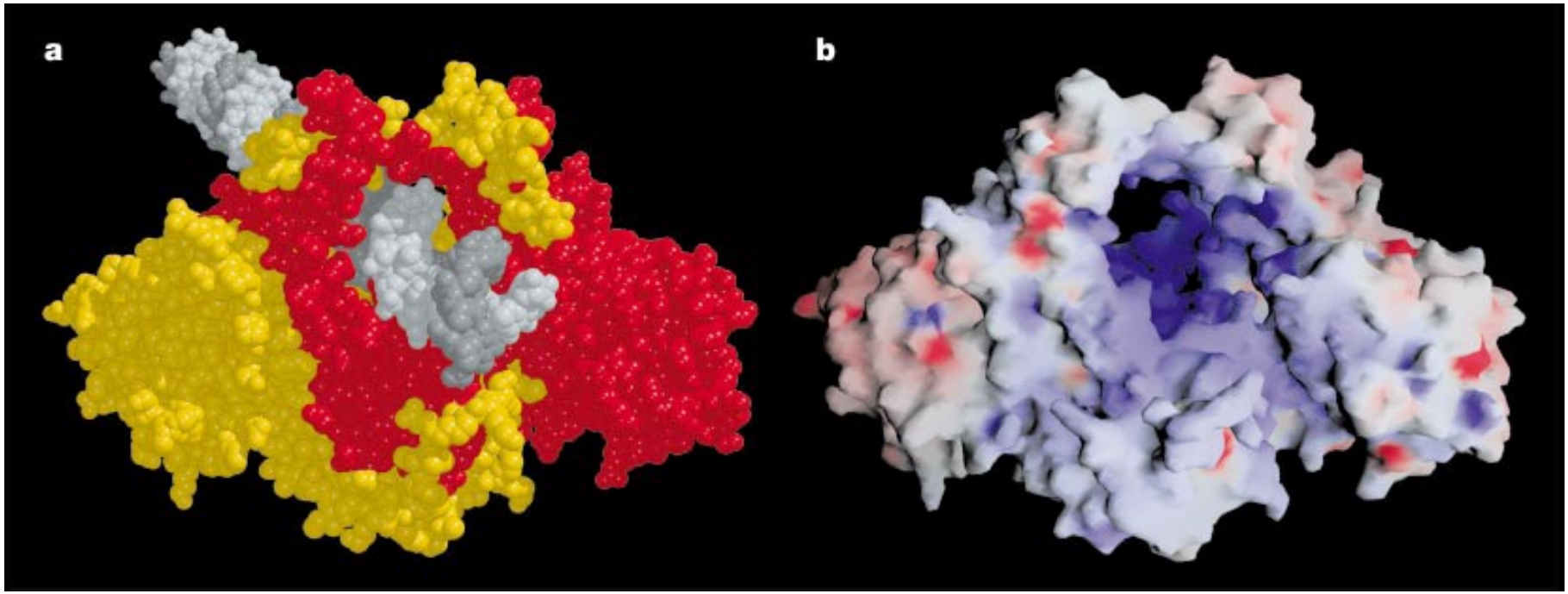


Fig. 3 An overview of DNA end-joining repair mechanisms. **a** Overview and main factors of non-homologous end-joining. **b** Overview and main factors of microhomology-mediated end-joining

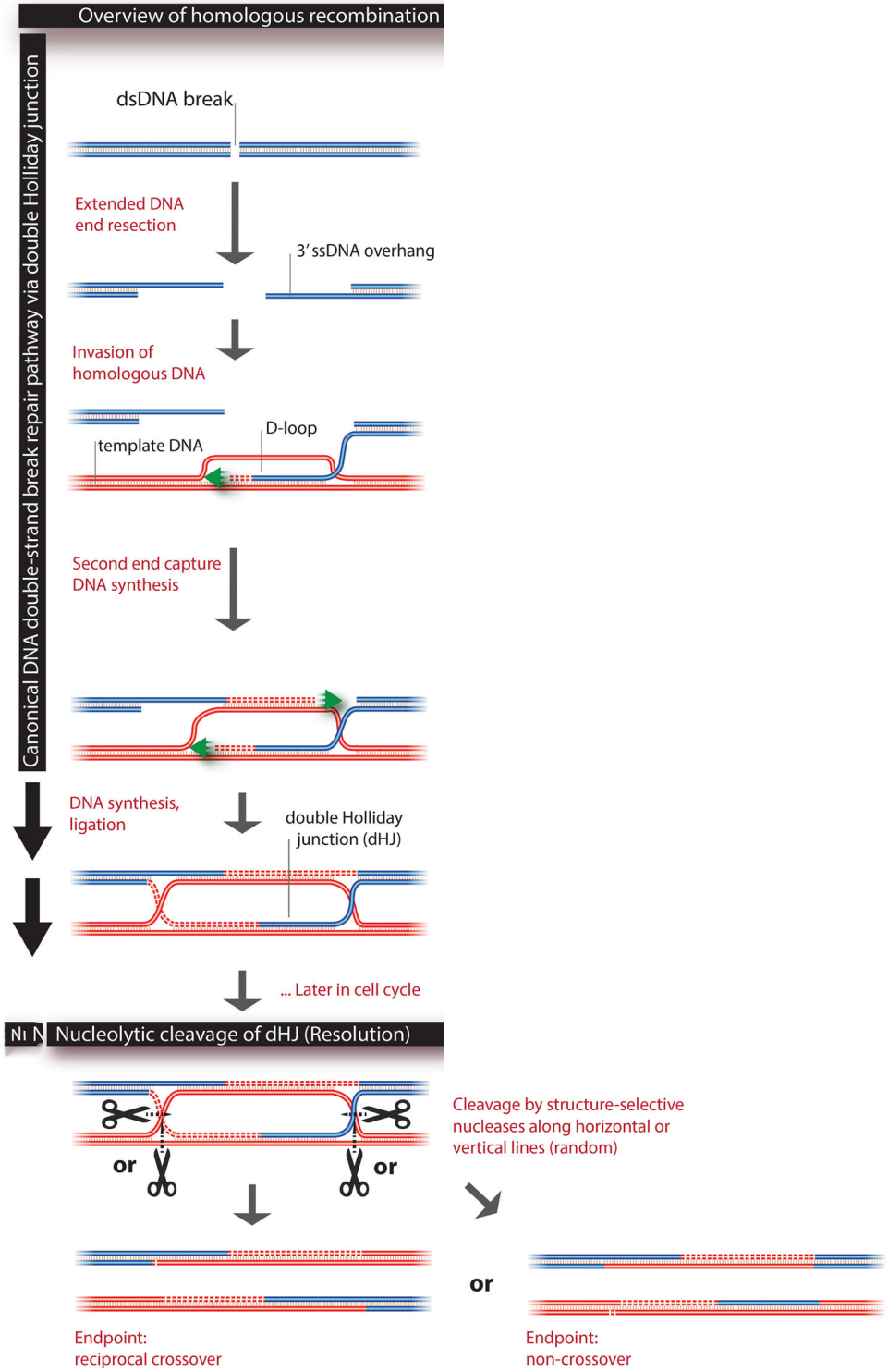
Crystal Structure of the Ku70/80 Heterodimer



Walker JR, Corpina RA, Goldberg J. Nature. 2001 412:607-14.

Homologous Recombination (HR)

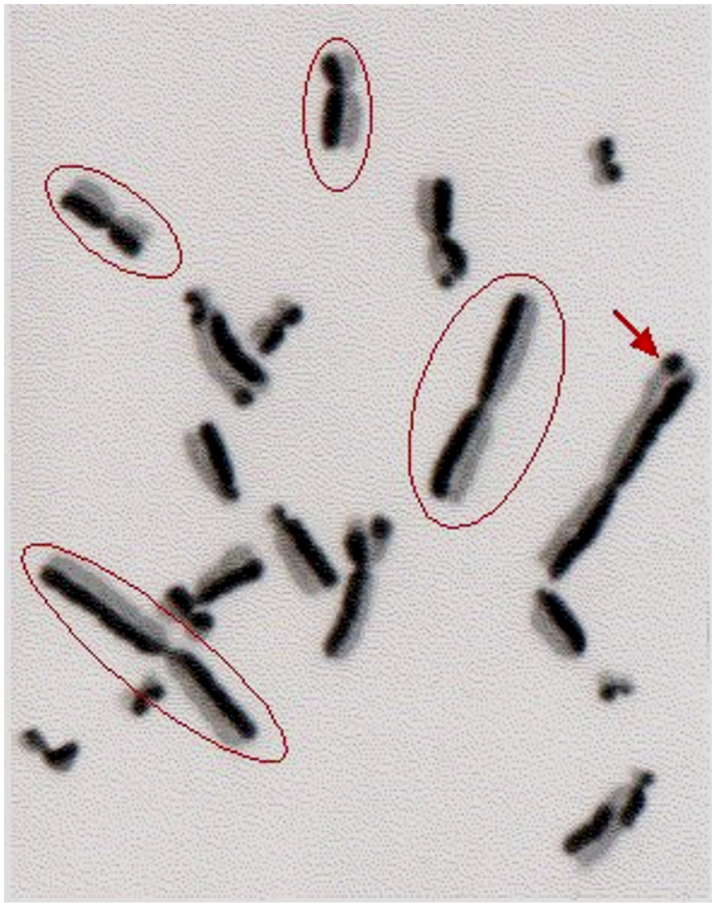
Homologous Recombination (HR)



From: Chromosoma (2018) 127: 187

Sister Chromatid Exchange (SCE)

Spontaneous SCE

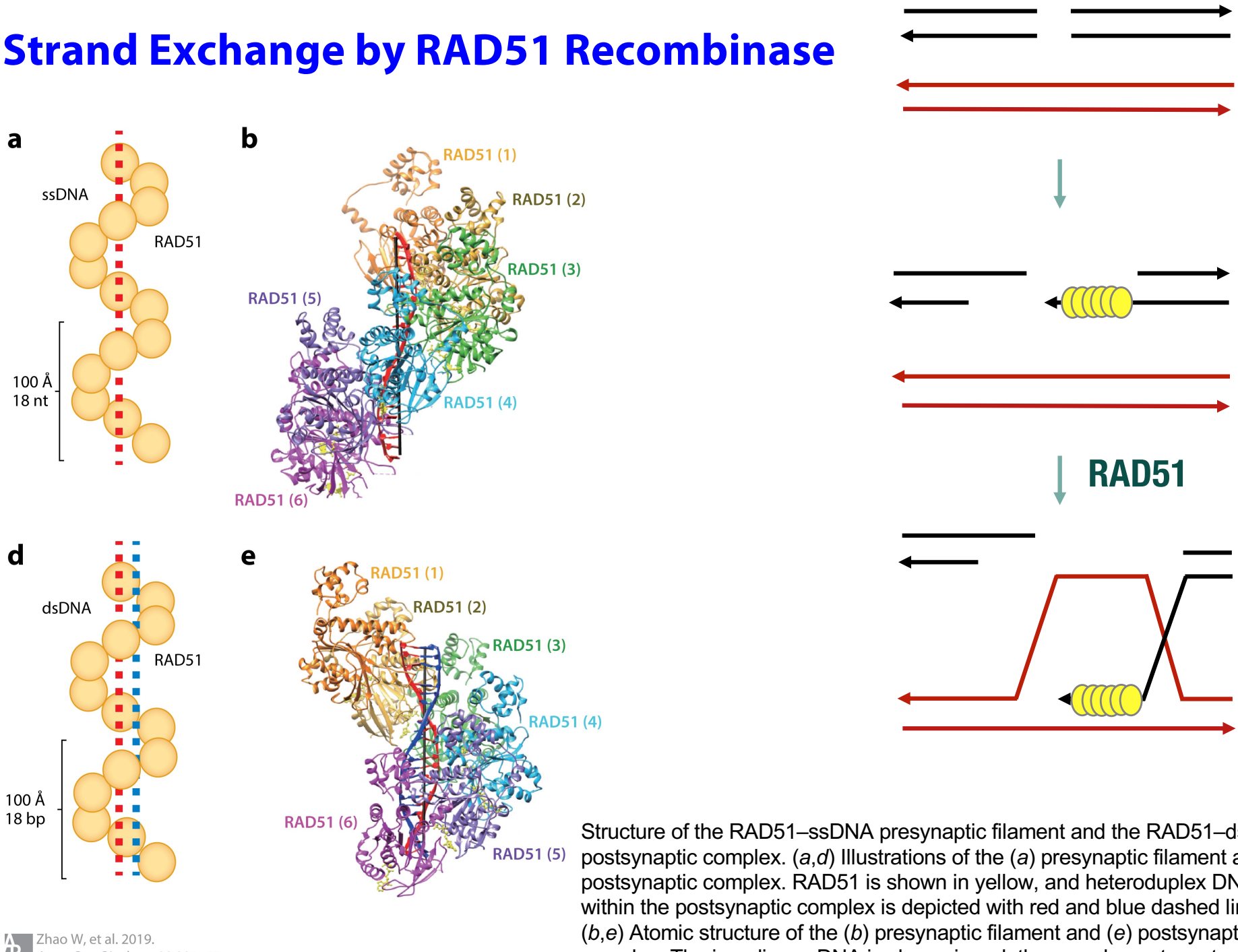


Induced SCE (DNA damage)

"Harlequin chromosomes"

J. Bodycote and S. Wolff

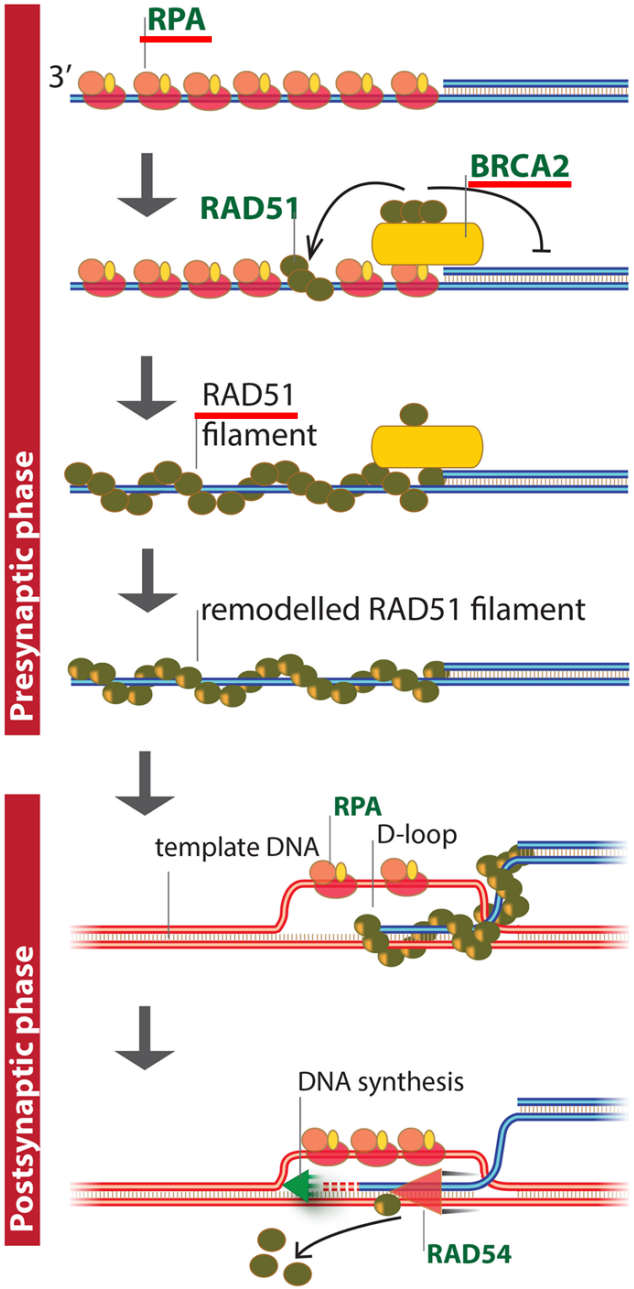
Strand Exchange by RAD51 Recombinase



Zhao W, et al. 2019.
Annu. Rev. Biochem. 88:221–45

Structure of the RAD51–ssDNA presynaptic filament and the RAD51–dsDNA postsynaptic complex. (*a*,*d*) Illustrations of the (*a*) presynaptic filament and (*d*) postsynaptic complex. RAD51 is shown in yellow, and heteroduplex DNA within the postsynaptic complex is depicted with red and blue dashed lines. (*b*,*e*) Atomic structure of the (*b*) presynaptic filament and (*e*) postsynaptic complex. The invading ssDNA is shown in red, the complementary strand is shown in blue.

RAD51 Filament Formation and Strand Invasion



Resected RPA-coated 3′ ssDNA overhang

BRCA2 exchanges RPA for RAD51 on overhanged ssDNA, blocks dsDNA binding of RAD51

RAD51 nucleoprotein filament

Active RAD51 filament

Invasion of template dsDNA, RPA stabilizes D-loop by binding to displaced ssDNA

Removal of RAD51 from dsDNA by RAD54, DNA synthesis by Polδ or Polε

Positive regulation:	Negative regulation:
RAD51 paralogs DSS1 SWS1 ?	
RAD51 paralogs RAD54 RAD52 PALB2	RECQ5 FBH1 PARI BLM
RAD51 paralogs	
BRCA1: stimulation of synaptic complex or homology search	
RAD54	RTEL1 BLM RECQ1 RAD54

From: Chromosoma (2018) 127: 187

Further information on DNA repair by homologous recombination:
Excellent video from Jim Haber online:

https://www.ibiology.org/genetics-and-gene-regulation/homologous-recombination/

Mutations in Homologous Recombination Genes

- BRCA1, BRCA2, PALB2 (cooperates with BRCA2), and RAD51 are mutated in a wide variety of tumors.
- These tumors display severe chromosomal instability, a phenotype referred to as ‘BRCAness’.

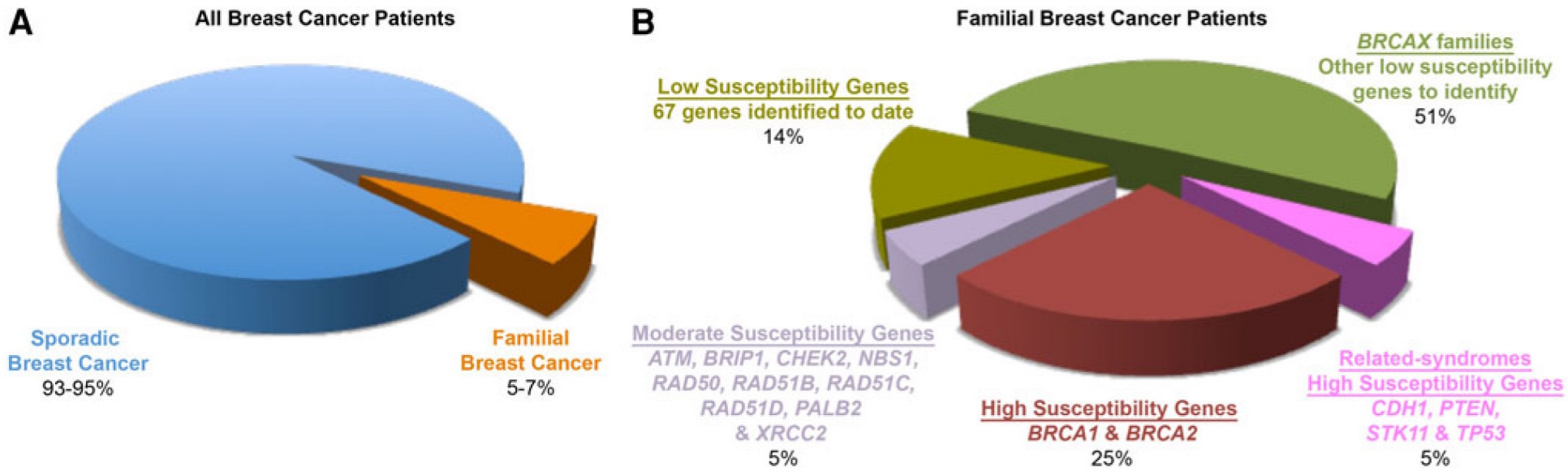


Fig. 1 Distribution of breast cancer patients. **a** Familial breast cancer represents a minor percentage of all breast cancer patients. **b** Proportion of familial breast cancer patients due to germ line mutations in high, moderate, and low penetrance cancer genes. *BRCA1* and *BRCA2* explain the vast majority of familial breast cancer attributed to identified cancer-related genes; however, more families carry no mutations in known susceptibility genes and thus, are suggested to be caused by the inheritance of one or many low penetrance cancer genes (BRCAX families)

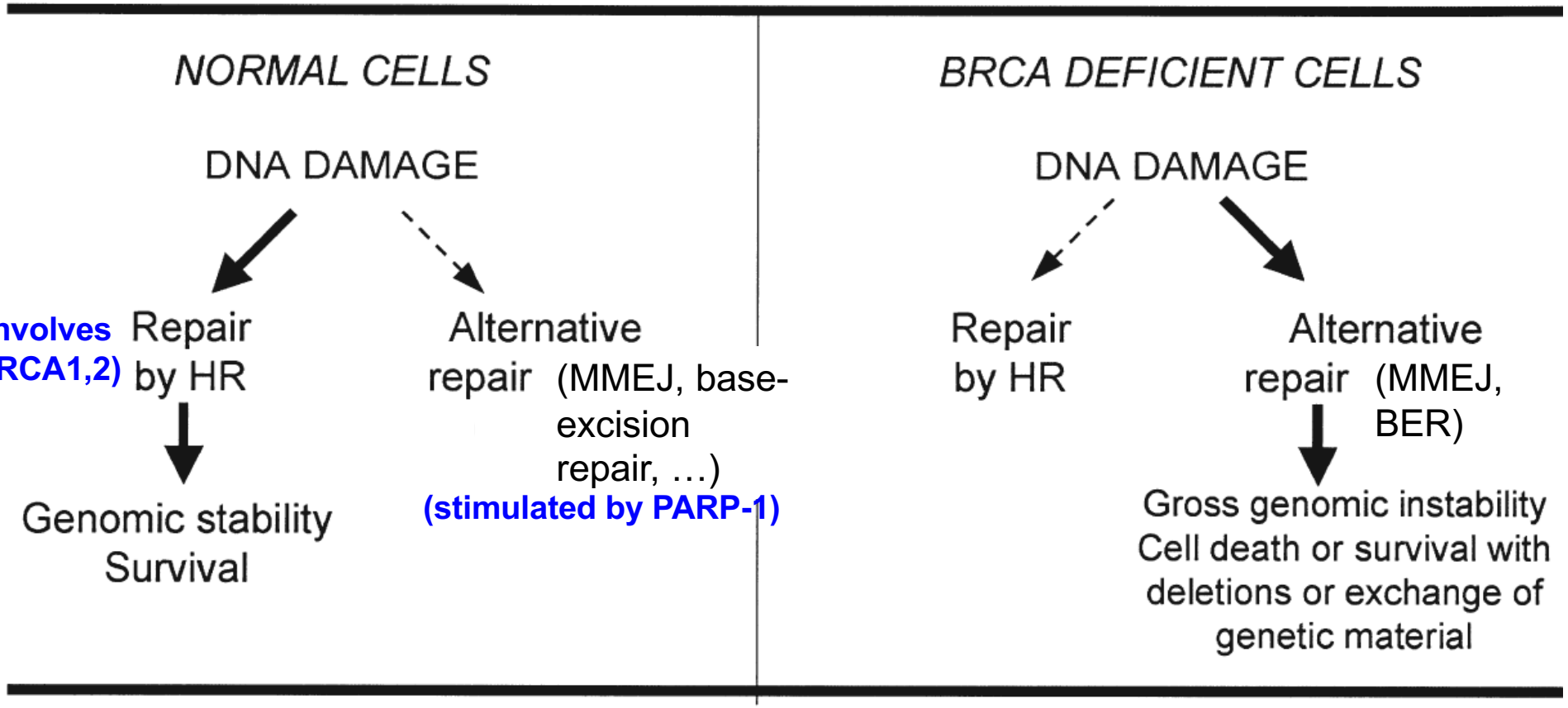
BRCA2: 3,418 amino acids
3 oligonucleotide binding (OB) folds that bind ssDNA
Eight BRC repeats (~40 aa) and C-terminus bind Rad51: 5-6 molecules of Rad51 can be bound/BRCA2

→Rad51 nucleoprotein filament formation and removal of RPA

Germ line mutations (heterozygous) in BRCA1 or BRCA2 confer an average cumulative risk of 65 or 39 % for breast cancer and 39 or 11 % for ovarian cancer by the age of 70 years

from: Hum Genet (2013) 132:845–863

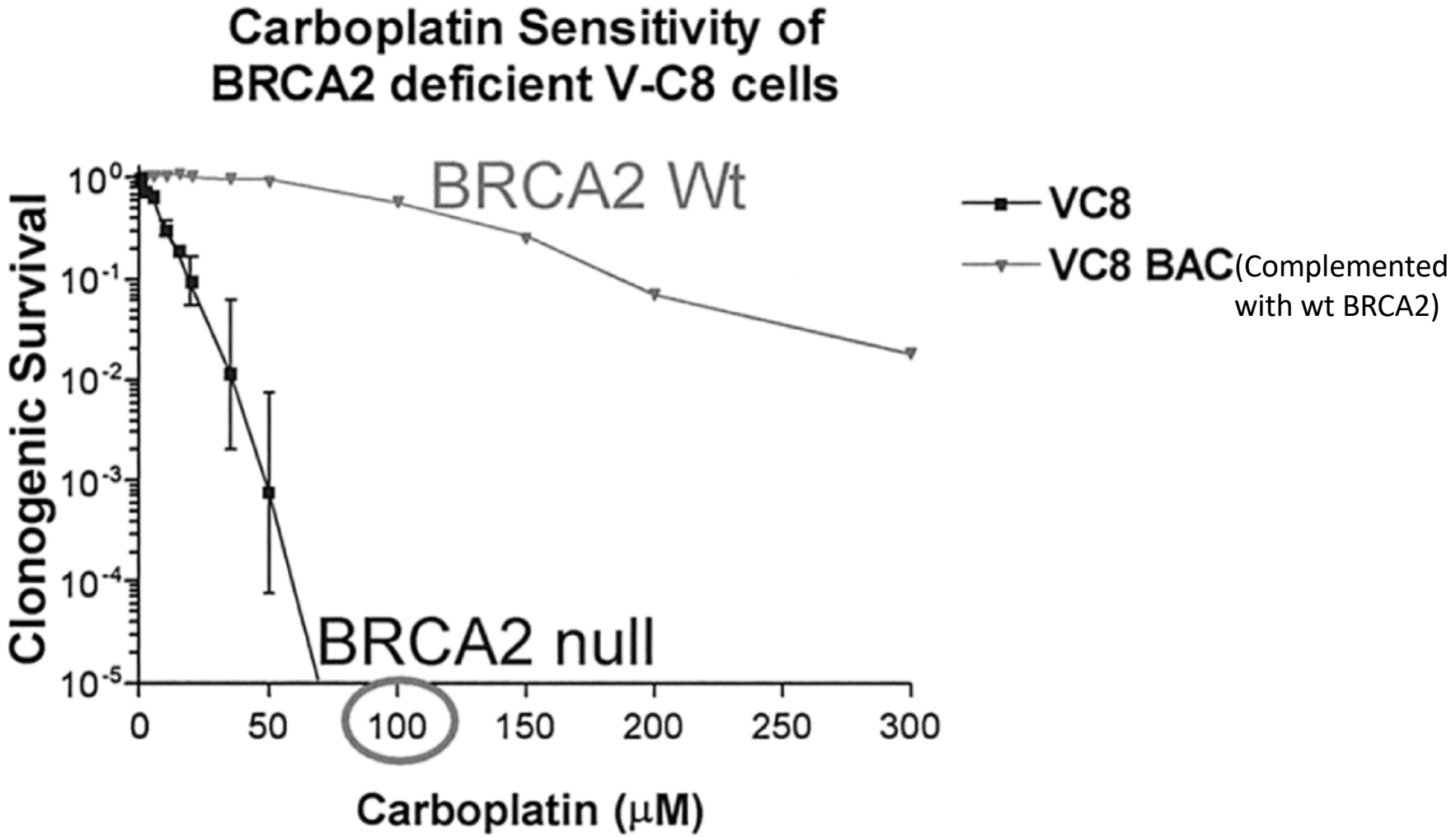
BRCA2 Functions in the Repair of DNA breaks



- **Normal cells:** repair of DNA double strand breaks mostly by Homologous Recombination (HR).
- **BRCA-deficient cell:** HR is defective. Cells become dependent on alternative repair pathways that are error-prone.

Modified from: Cold Spring Harb Symp Quant Biol 70,139 (2005)

BRCA2-deficient Cells are Sensitive to DNA damage

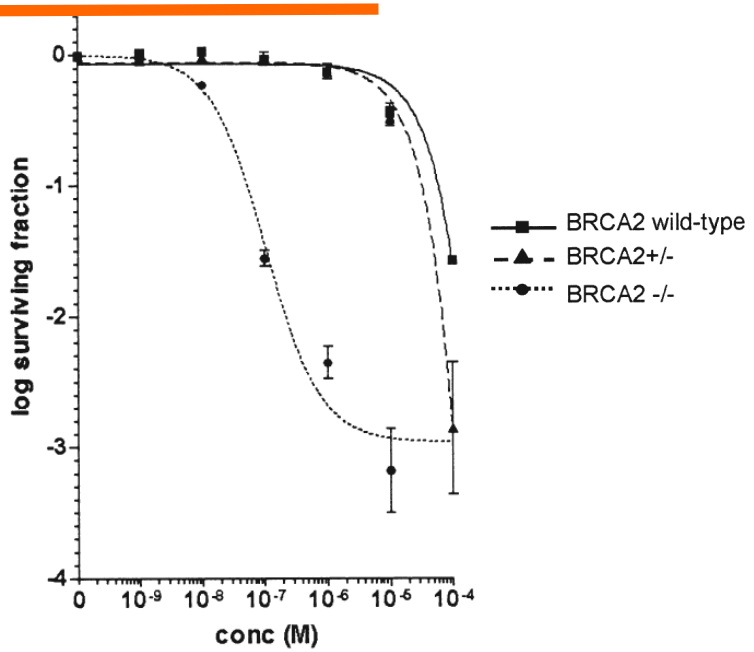


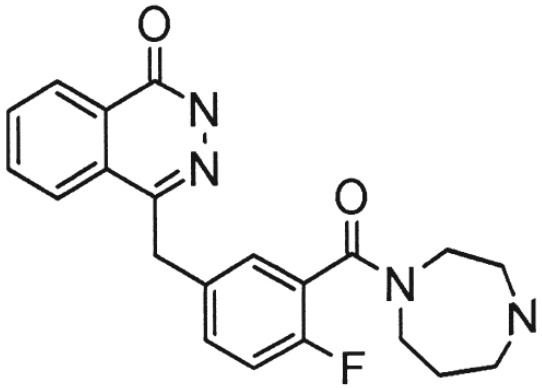
- *BRCA2* mutant cells are hypersensitive to carboplatin (and cisplatin). These chemotherapeutic agents crosslink DNA strands (inter- and intra-strand).

from: Cold Spring Harb Symp Quant Biol 70,139 (2005)

BRCA2-deficient Cells Heavily Rely on PARP1

- KU0058948 inhibits the repair enzyme PARP-1
- Inhibitors of PARP-1 are selectively lethal to cells lacking wild-type BRCA2.
- (*Not shown*: downregulation of PARP-1 by RNA interference has a similar effect as KU0058948)
- PARP1 is stimulating several DNA repair pathways (base-excision repair; microhomology dependent end joining)





KU0058948
IC_{50} = 3.4nM

from: Cold Spring Harb Symp Quant Biol 70,139 (2005)

PARP1 Binds ssDNA Breaks

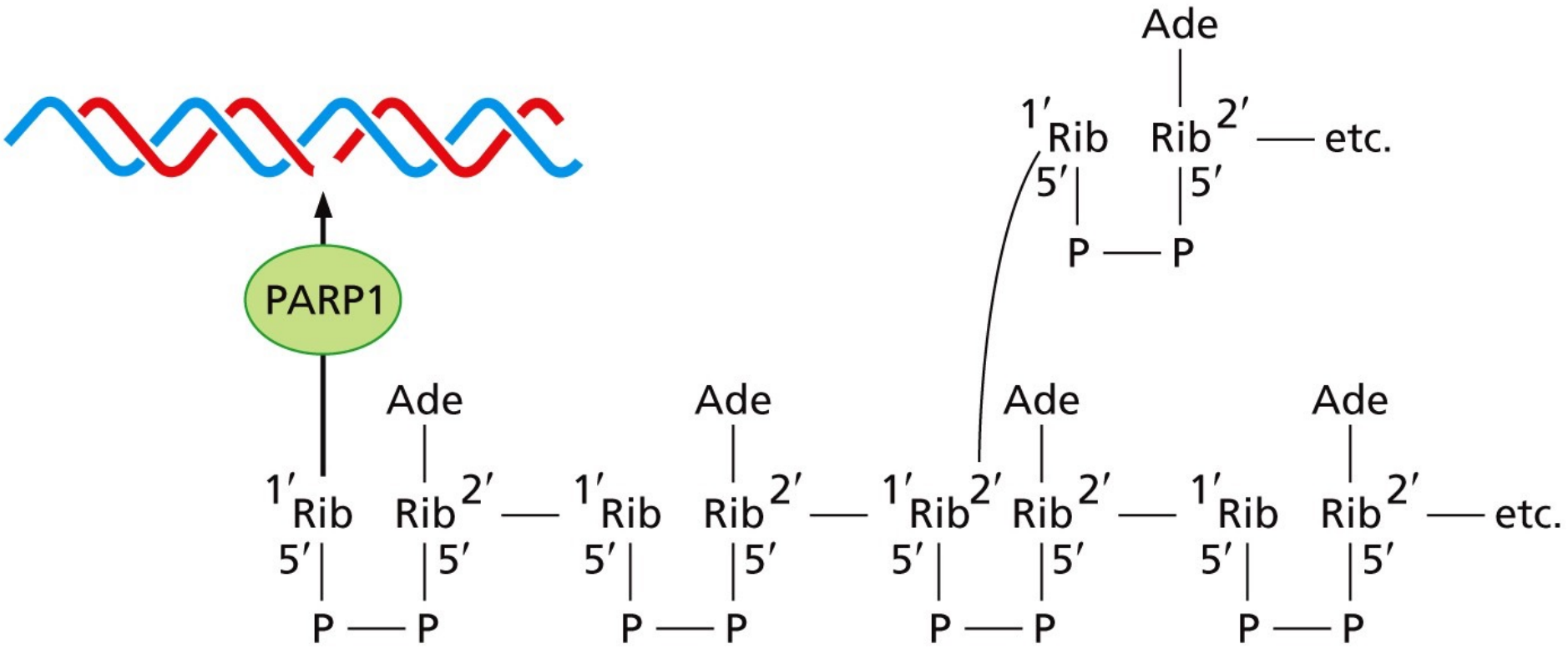


Figure 12.47. Weinberg, The Biology of Cancer

PARP1 (poly(ADP-ribose) polymerase 1)
adds polyADP tails to itself histones and other proteins.
→docking sites for repair enzymes

PolyADP-ribosylation

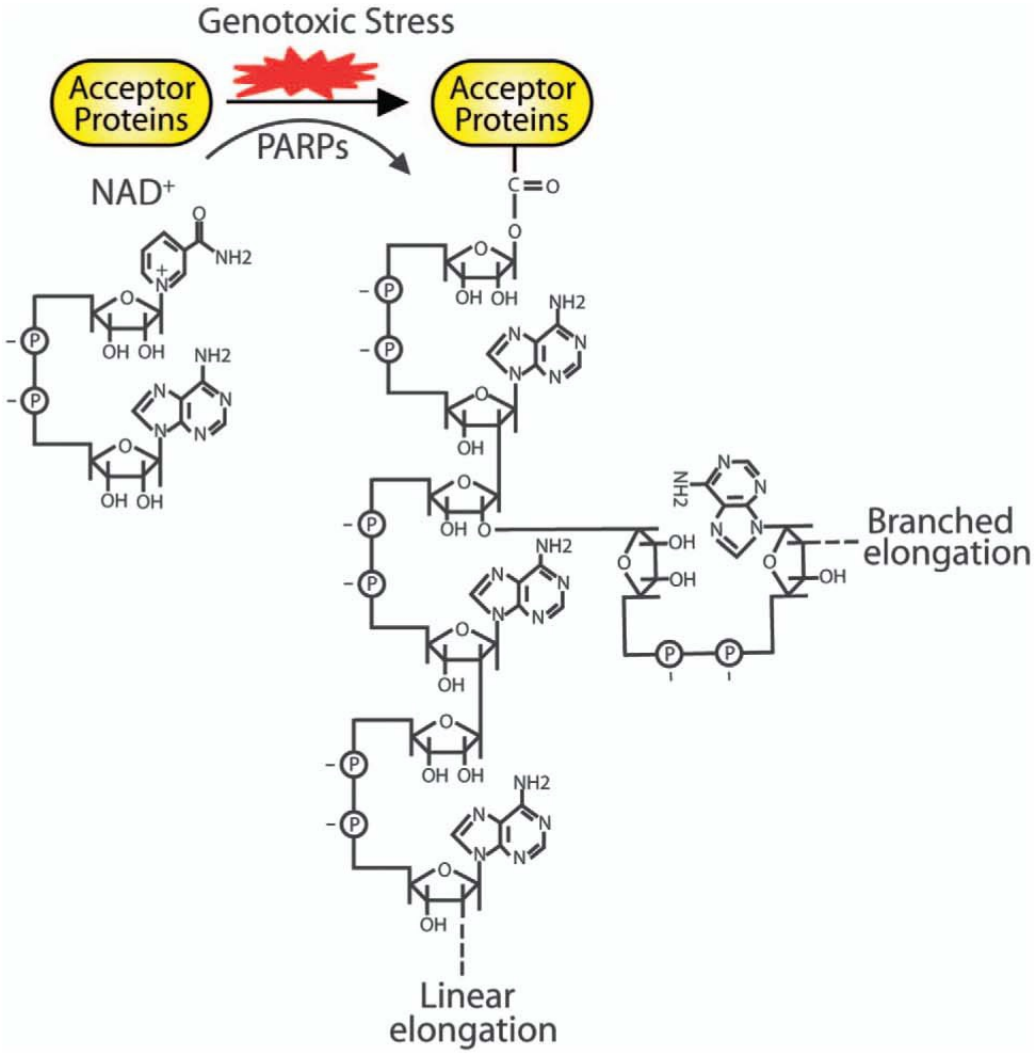


Figure 1. Sketch of poly(ADP-ribosyl)ation. With NAD^+ as the donor, PARPs mediate the genotoxic stress-dependent poly(ADP-ribosyl) ation. ADPr residues are covalently linked to the side chains of arginine, lysine, aspartate or glutamate residues of acceptor proteins. Glycosidic ribose-ribose 1′-2′ bonds between ADPr units generate both linear and branched polymers. The chain length of PAR is heterogeneous, which can reach up to 200 ADPr units, with 20–50 units in each branch.

from: Oncogene (2014), 15 September 2014; doi:10.1038/onc.2014.295

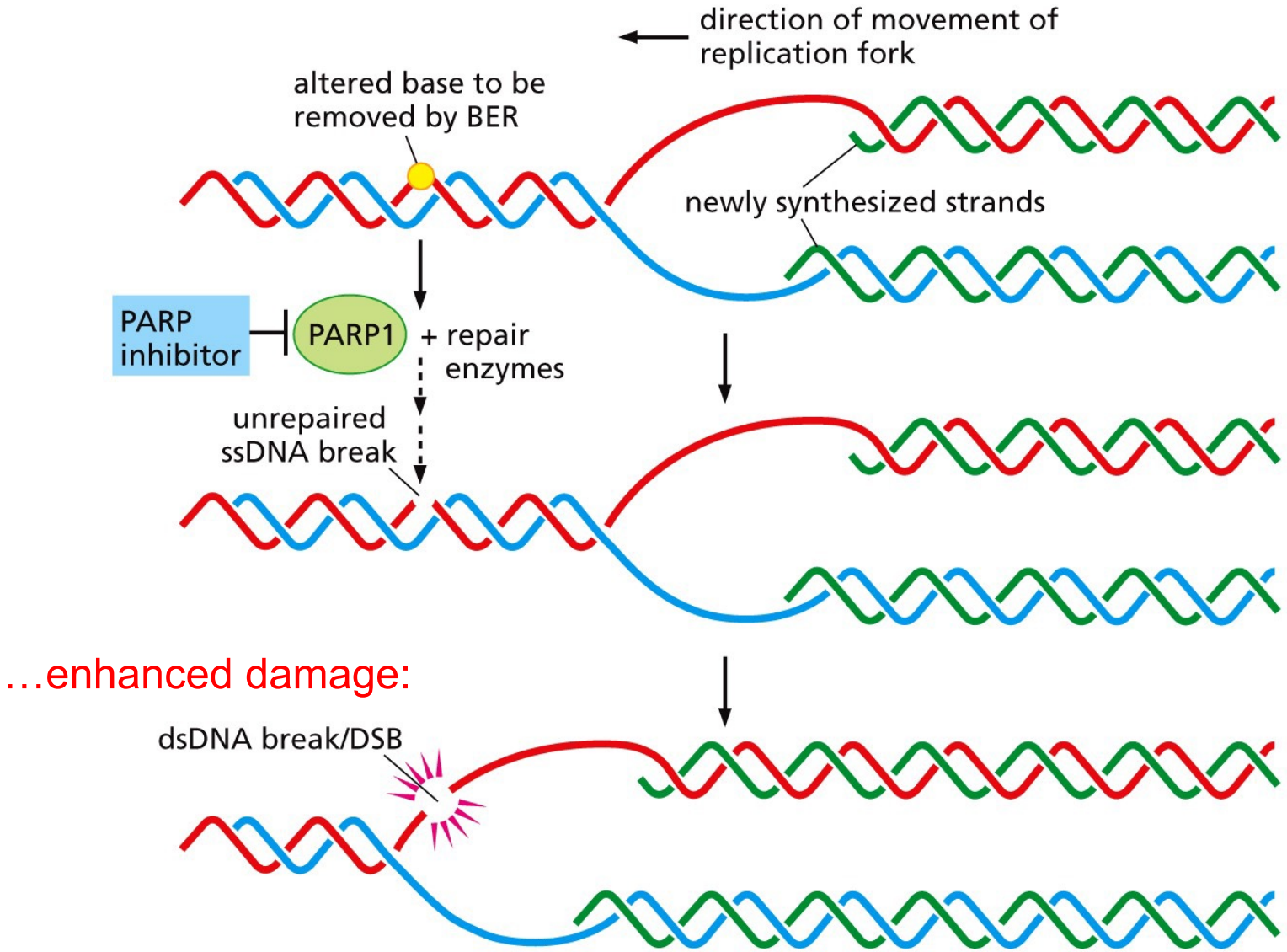
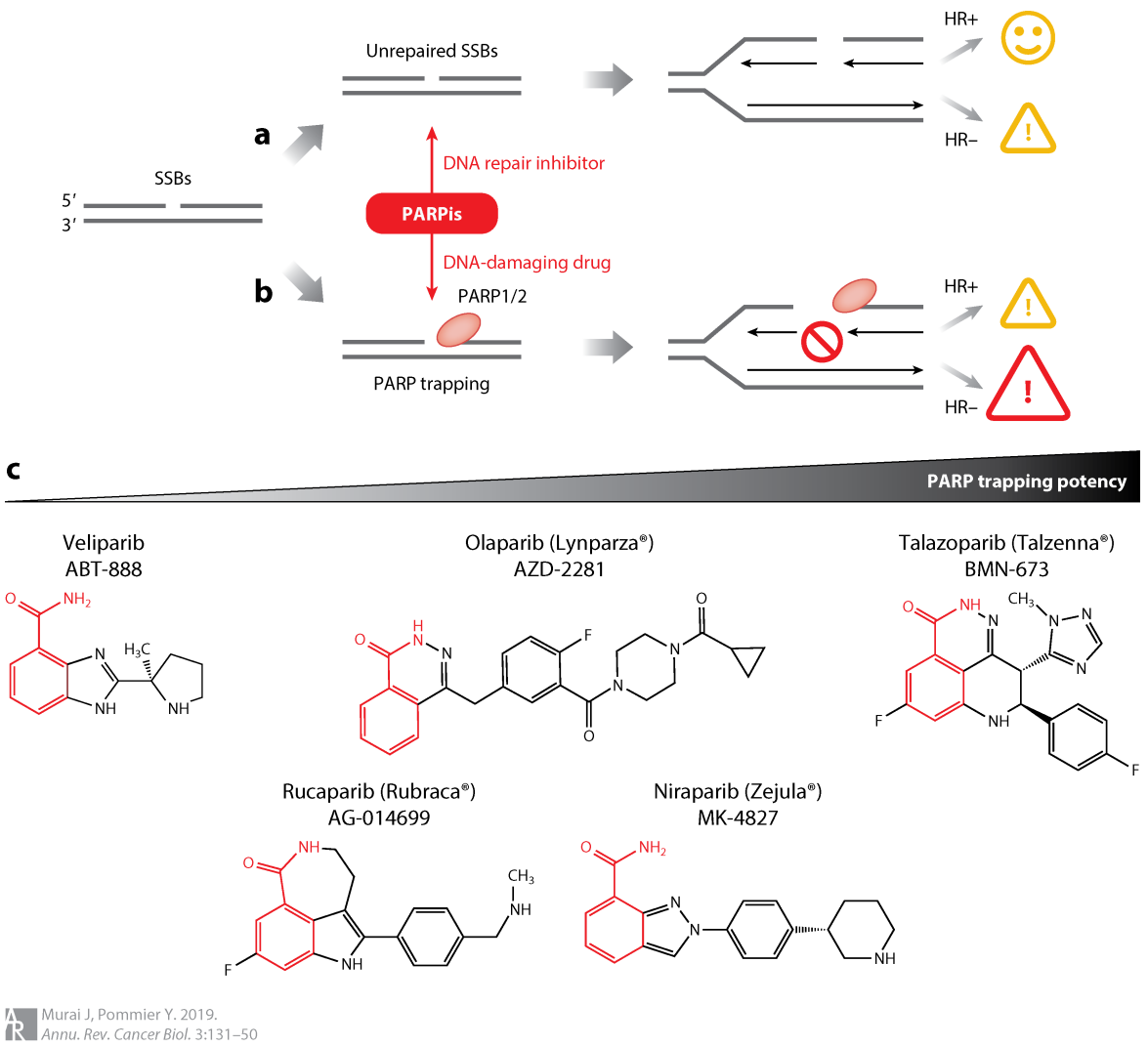


Figure 12.47. Weinberg, The Biology of Cancer

! In addition, more recent data indicate: **Inhibitor-mediated trapping of PARP1 on DNA** may have very potent **toxic effects** in BRCA-deficient tumors. Trapped PARP1 may prevent DNA replication fork movement.

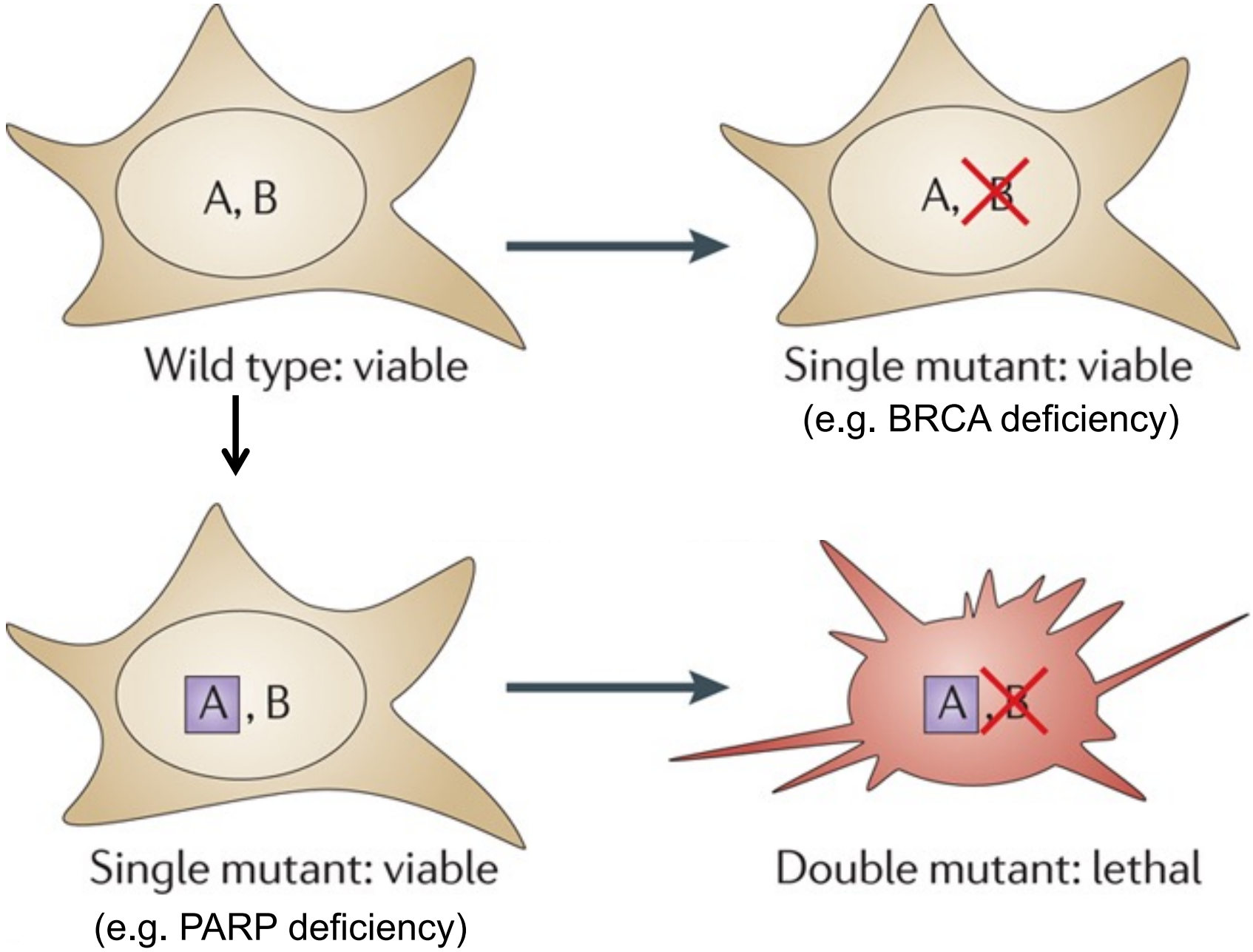


Schematic representation of the dual mechanisms of action of poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) in homologous recombination (HR)-deficient cells. (a) DNA repair inhibition through catalytic inhibition. Double-strand breaks (DSBs) formed upon replication collisions at unrepaired single-strand breaks (SSBs) are not toxic as long as HR is proficient (smiley face). In HR-deficient cells, DSBs cause cytotoxicity (yellow caution sign). (b) DNA damaging by trapping PARP-DNA complexes. DSBs accompanied by PARP trapping strongly blocks replication and activates the S phase checkpoint, which can be cytotoxic even in HR-proficient cells (yellow caution sign). The DSBs induced by PARP trapping are much more cytotoxic in HR-deficient cells (big red caution sign). (c) Clinical PARPis ranked by potency for PARP trapping. The red portions of the molecules correspond to the aminobenzamide group that binds to the NAD+ pocket of PARPs. The commercial names of the FDA-approved PARPis are indicated in parentheses.

Evolution of PARPi resistance in cancer ?

Some examples will be discussed in the exercises: Nature 451, 1111 (2008)

Synthetic Lethality



Key Concepts

- Repair of dsDNA breaks: NHEJ, MMEJ (also called Alt-EJ), HR
- BRCA1 and BRCA2 function in HR
- PARP-1 promotes base-excision repair and micro-homology mediated end joining
- Concept of synthetic lethality