Cancer Biology I :

Topics covered

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

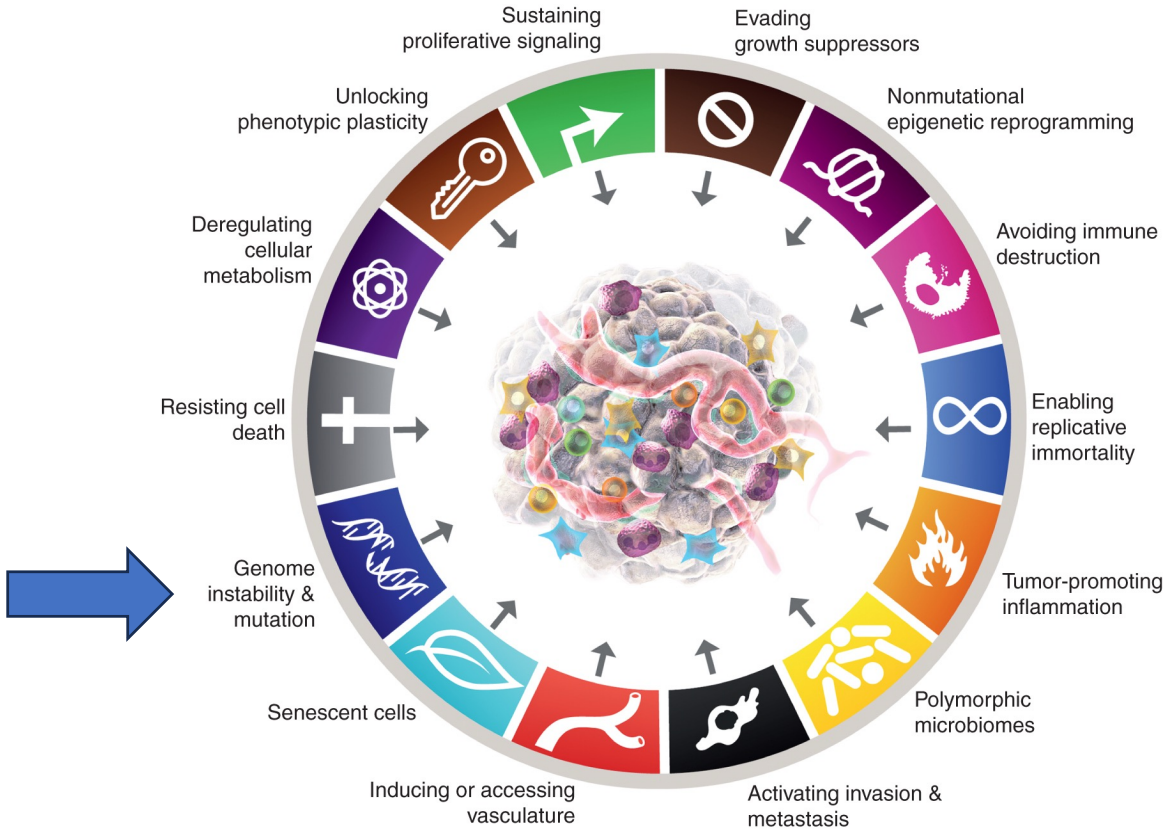
Week 2:
Lecture: **DNA repair of DNA double strand breaks; Synthetic lethality**
Exercises: **Paper discussion on Wednesday**

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

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

From week 1:

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



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 Lesions Generated by Endogenous and Exogenous DNA Damage

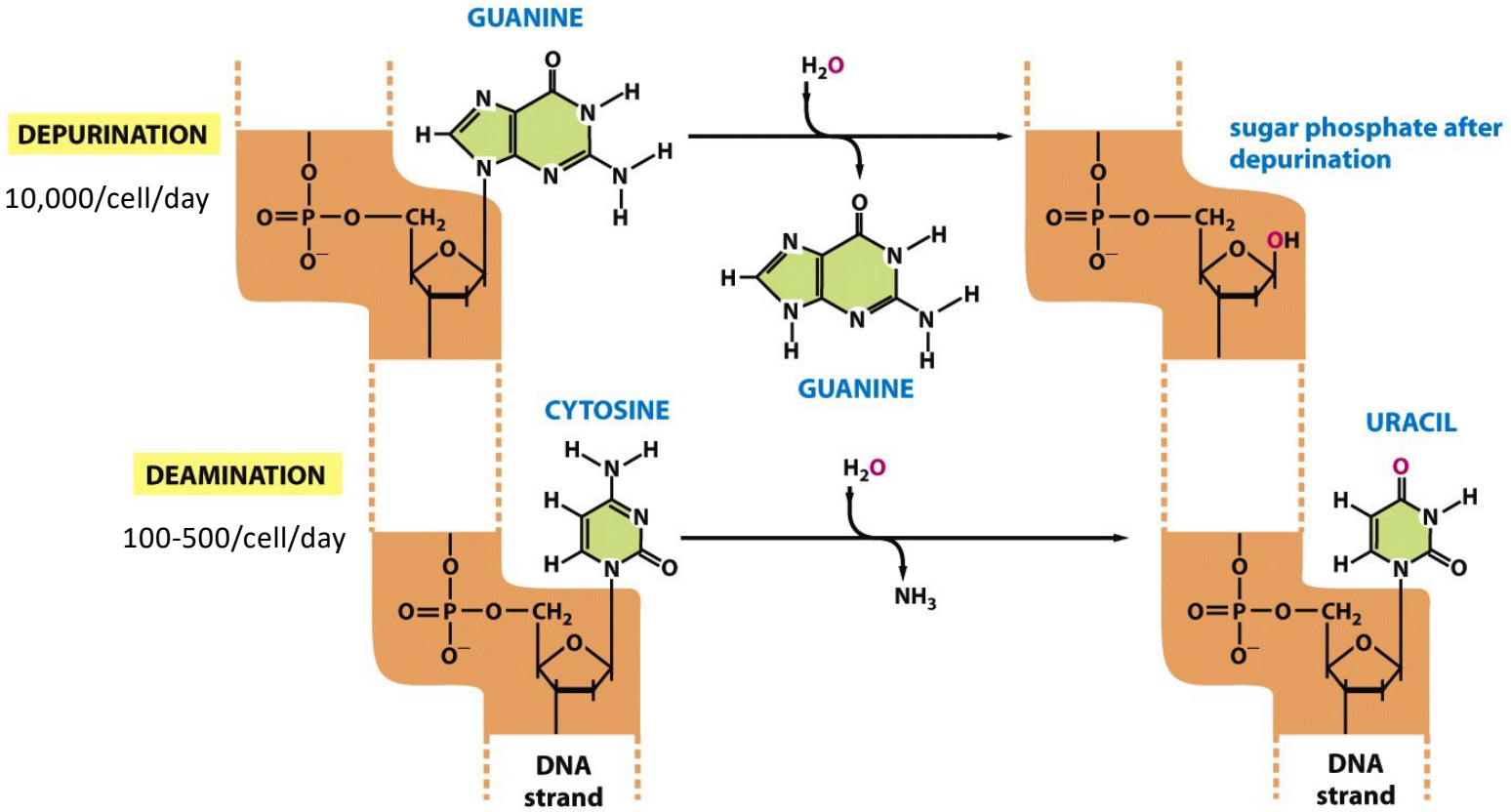
Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

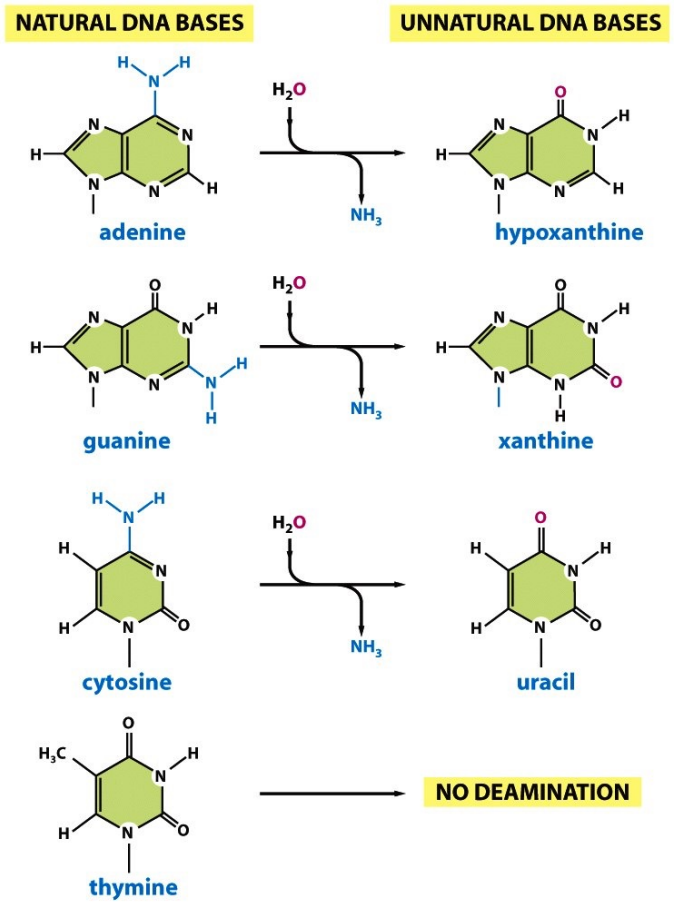
Type and number of DNA lesions are indicated. The number of lesions/cell has been estimated as described.

Ciccia and Elledge, (2010) Mol Cell, Volume 40, Issue 2

Depurination and Deamination

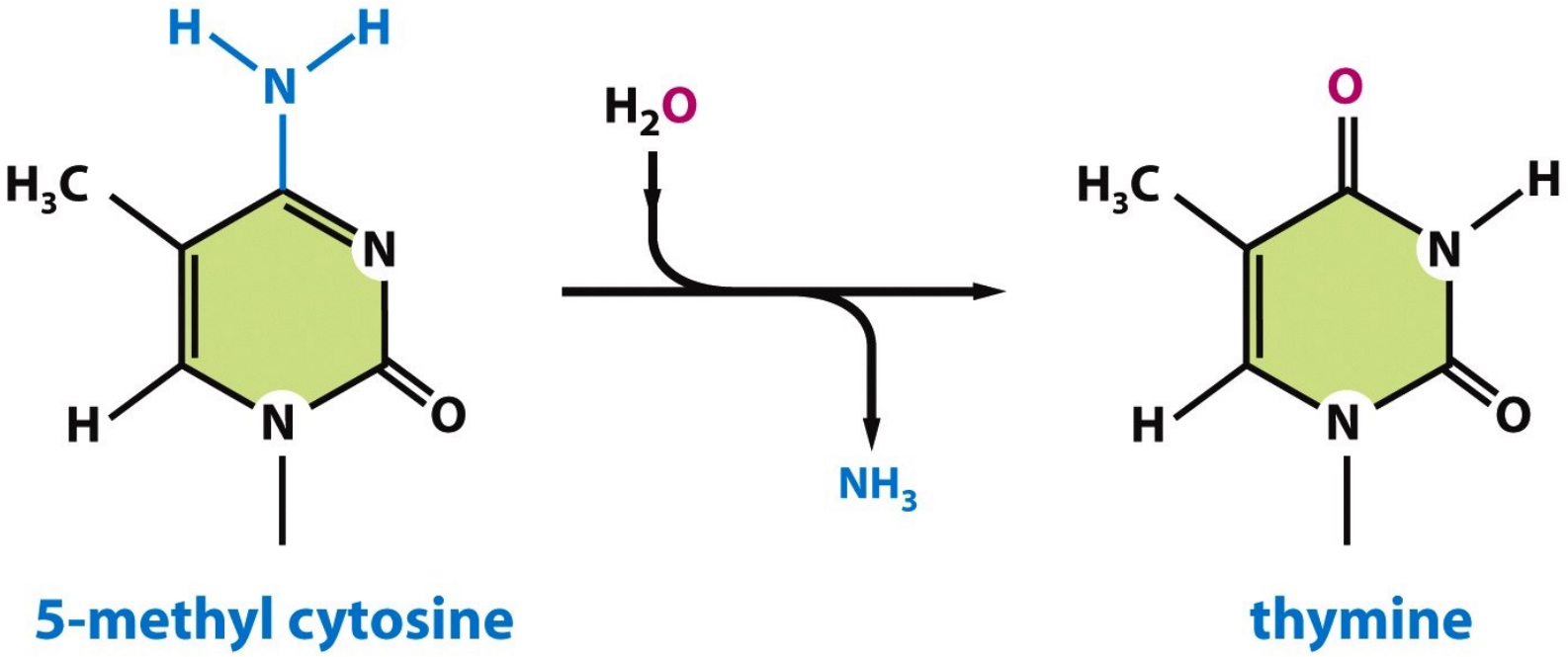


Deamination Yields Unnatural Bases



APOBEC3 proteins catalyze deamination reactions (mostly cytidine deamination) in ssDNA to target viruses and retroelements as part of the innate immune response. However, **APOBEC3-mediated mutagenesis has also been observed in 70% of all cancer types and 50% of all cancer genomes! (***Nature* **607**, pages 799–807 (2022**).**

Deamination of 5-Methyl Cytosine Yields Thymine!



(3% of the C's in human DNA are methylated)

Figure 5-50b *Molecular Biology of the Cell* (© Garland Science 2008)

Thymine-Dimer: Caused by UV-light in the Skin

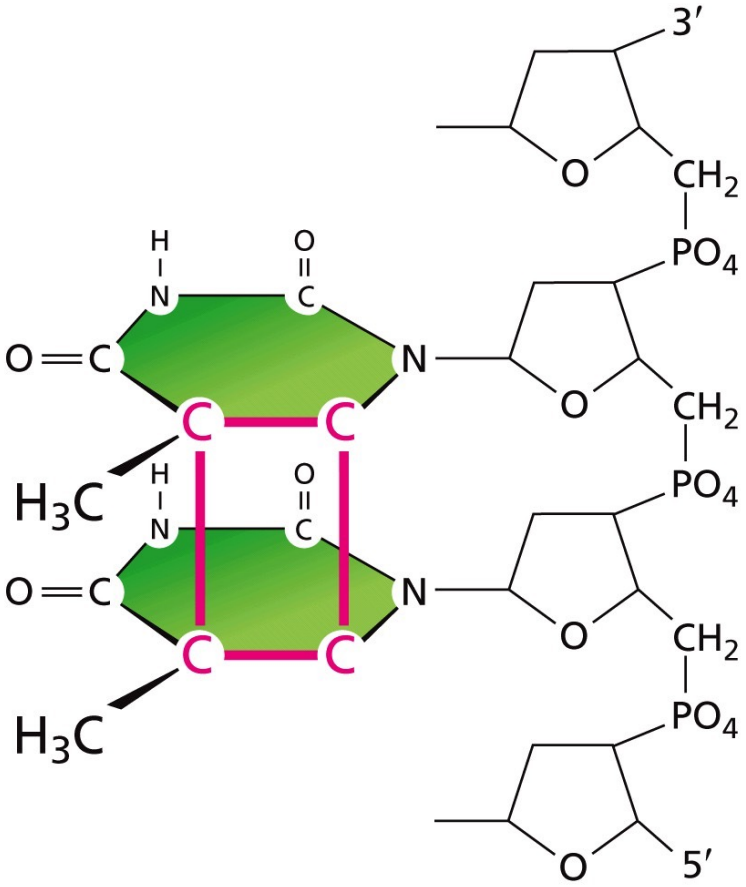


Figure 12.12 the biology of cancer (Weinberg)

6-4 Photoproducts of Pyrimidines

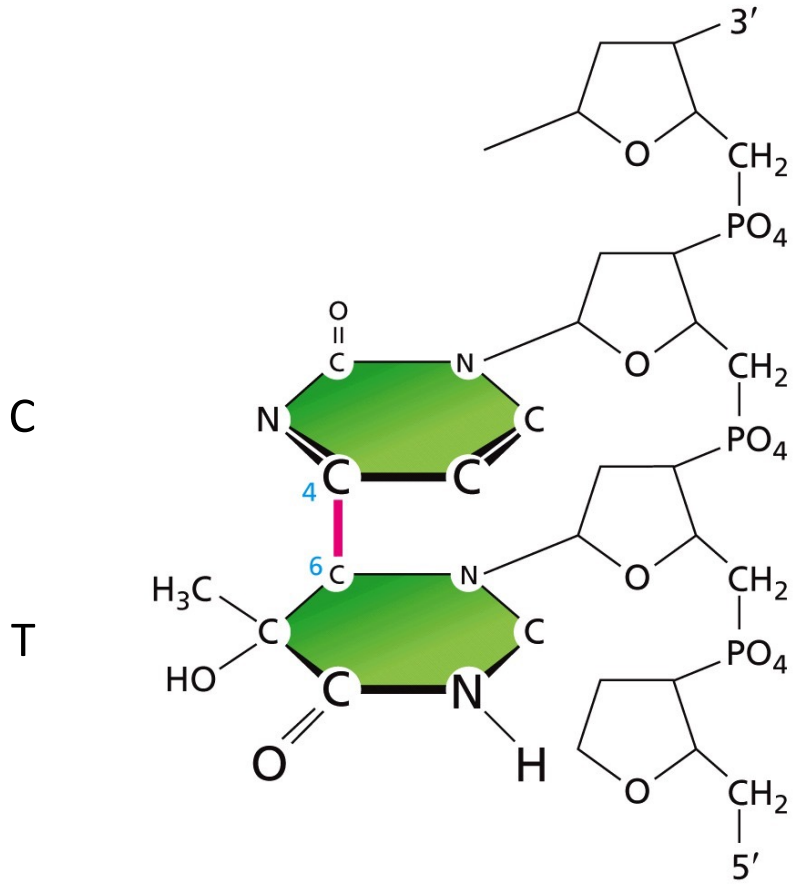


Figure 12.12 the biology of cancer (Weinberg)

Oxidation of DNA Bases

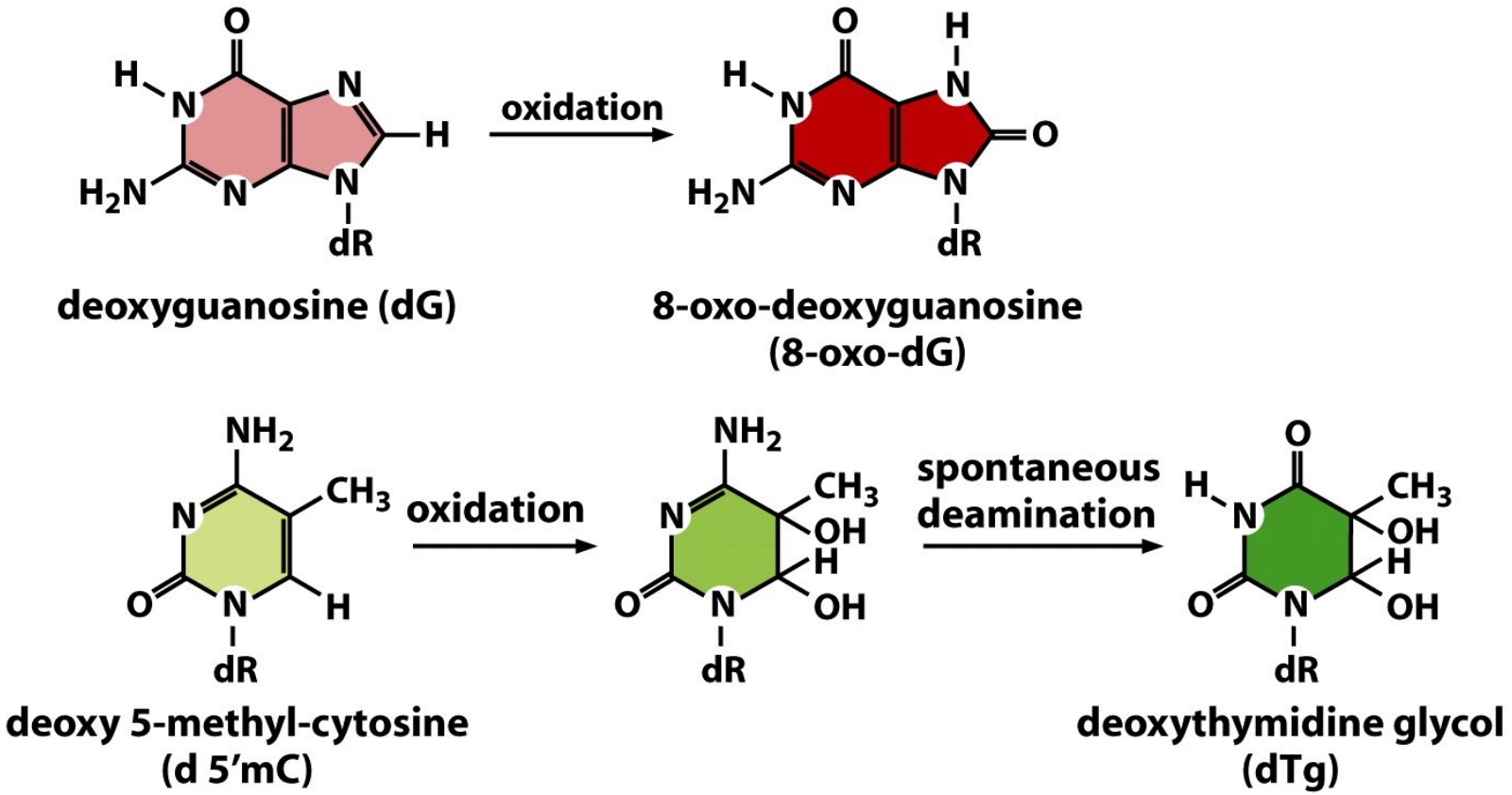
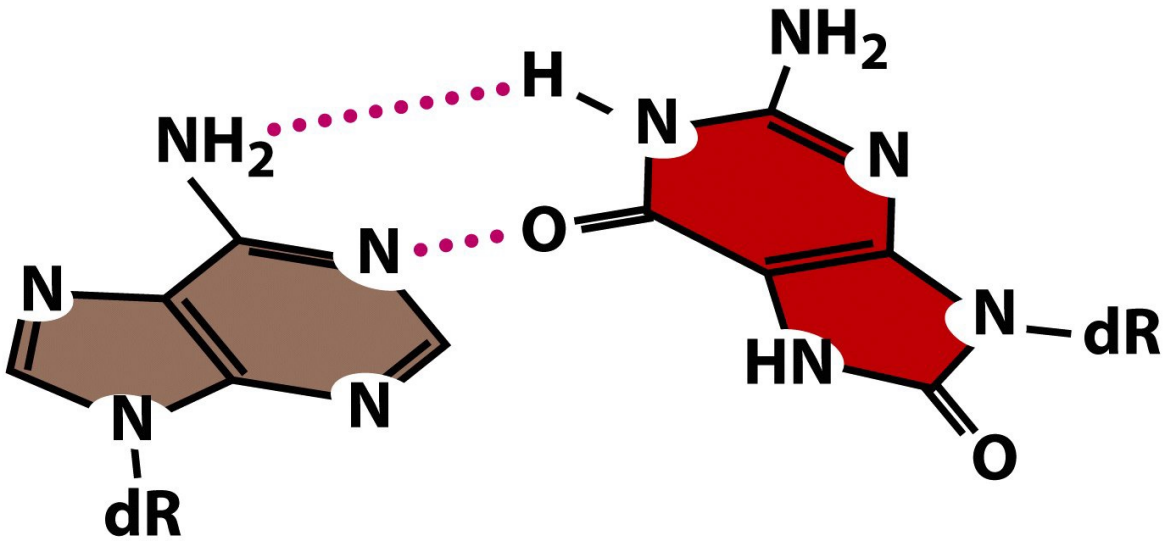


Figure 12.11 the biology of cancer (Weinberg)

8-oxo-dG can mispair with deoxyadenosine during DNA replication



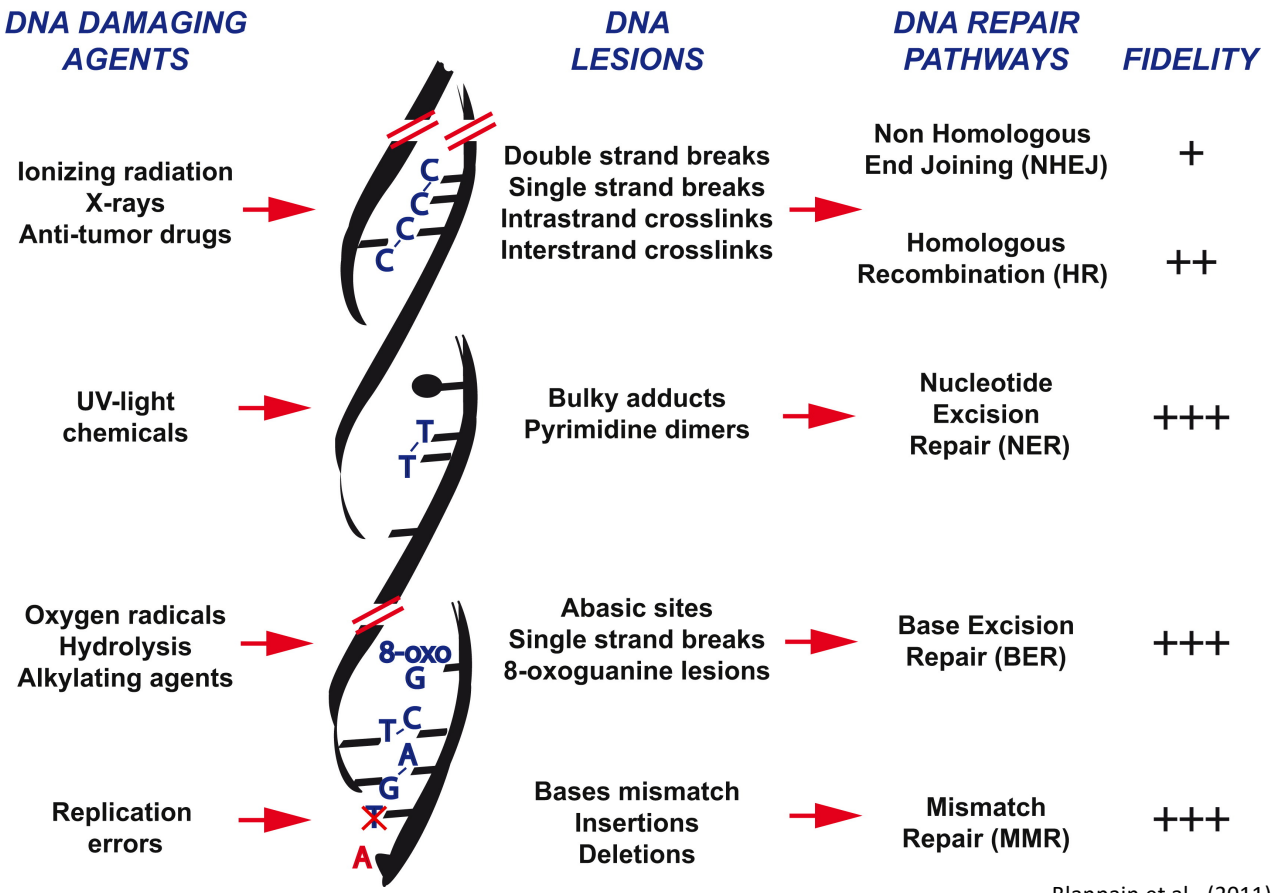
mispairing of 8-oxo-dG with deoxyadenosine (dA)

Figure 12.11 the biology of cancer (Weinberg)

Ionizing Radiation (IR)

- Deposits energy to yield ionized molecules or reactive oxygen species (ROS) that then attack the DNA
- Causes base damage, DNA-protein cross-links, phosphate backbone damage, single-strand breaks, and double-strand breaks (DSBs)
- Cell killing by IR generally reflects the generation of DSBs, as these are difficult to repair
- Does not occur "often" but note that 200,000,000 gamma rays pass through your body every hour (these come from decay of naturally-occurring radioactive isotopes)
- Used in cancer therapy

DNA Damage



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

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 (also called Alt-NHEJ or Theta-mediated end-joining (TMEJ))**
- **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

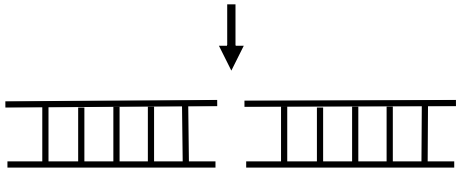
- Non-homologous end joining/Microhomology mediated end-joining
- Homologous recombination, BRCA1, BRCA2

→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

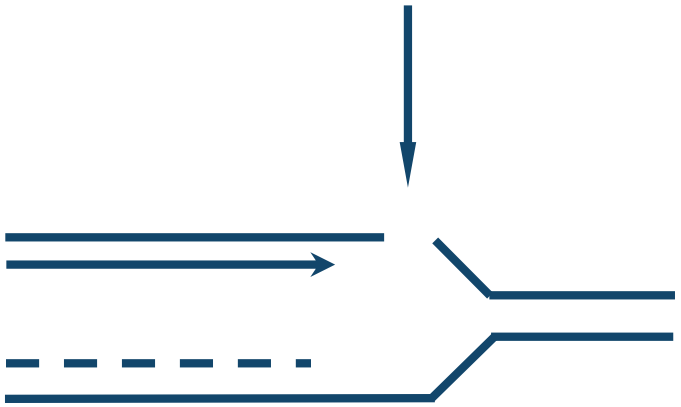
Genome instability

Carcinogenesis

Endogenous Formation of DNA Double-Strand Breaks

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

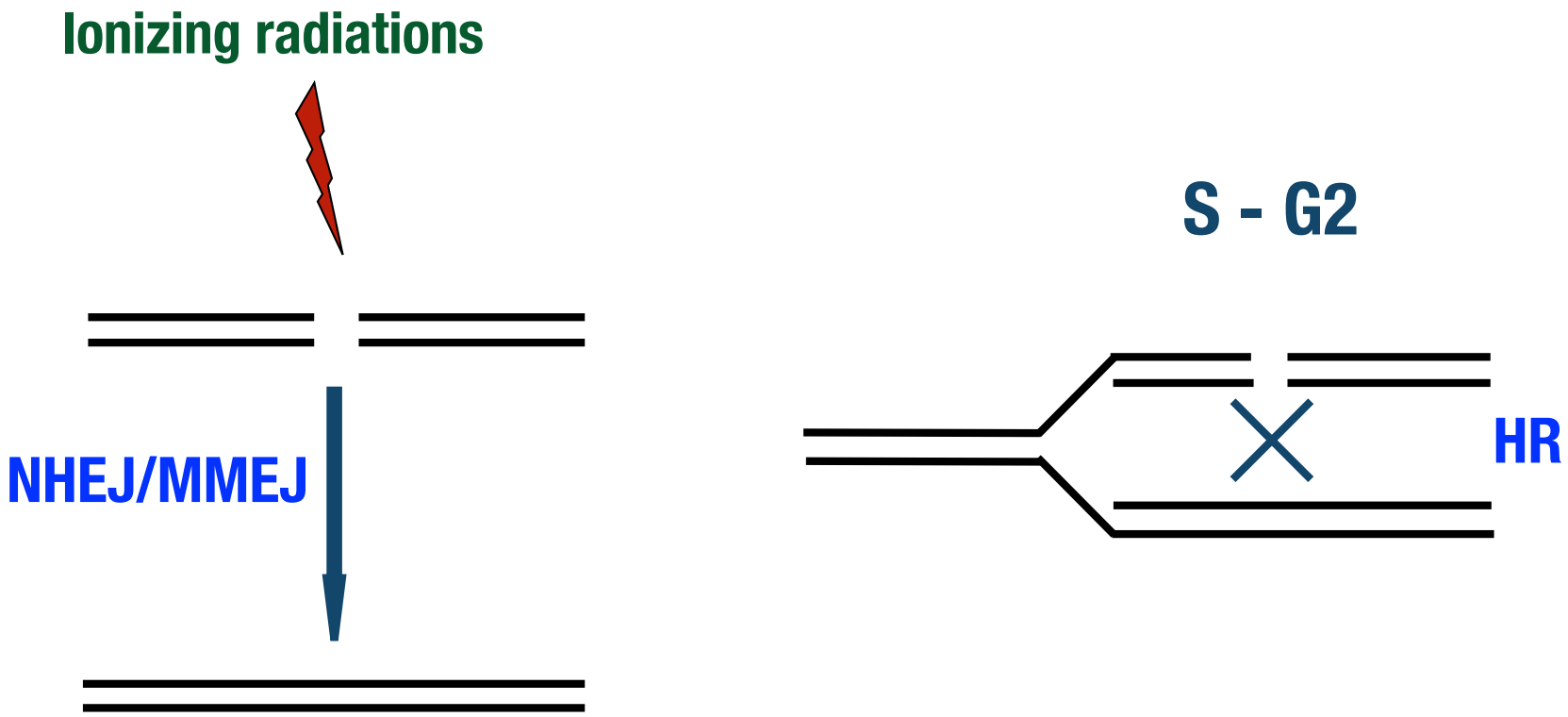
99% repaired: BER, NER



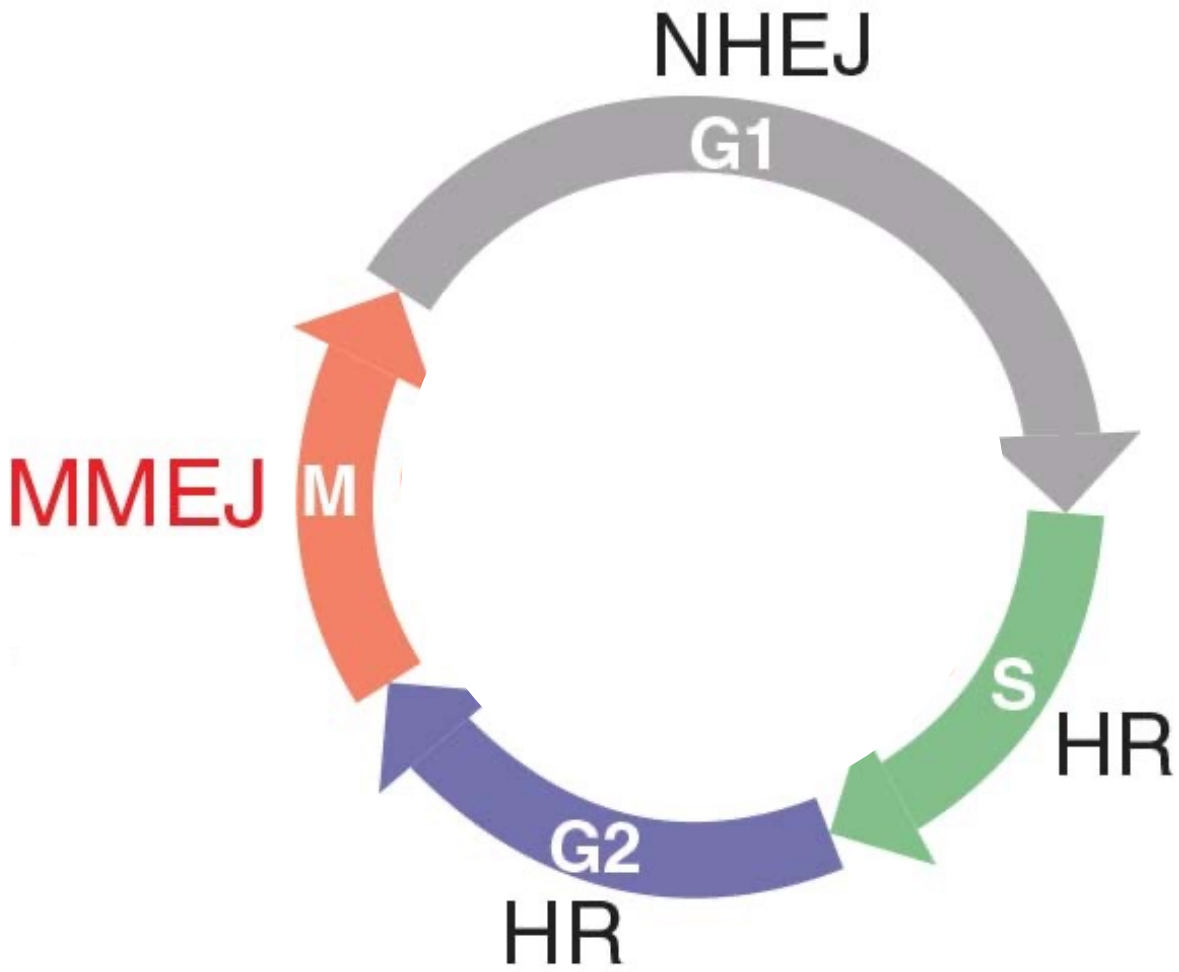
1% is converted into DSB during replication:
50 DSBs/cell/phase S

Repair

Repair of DNA Double-strand Breaks



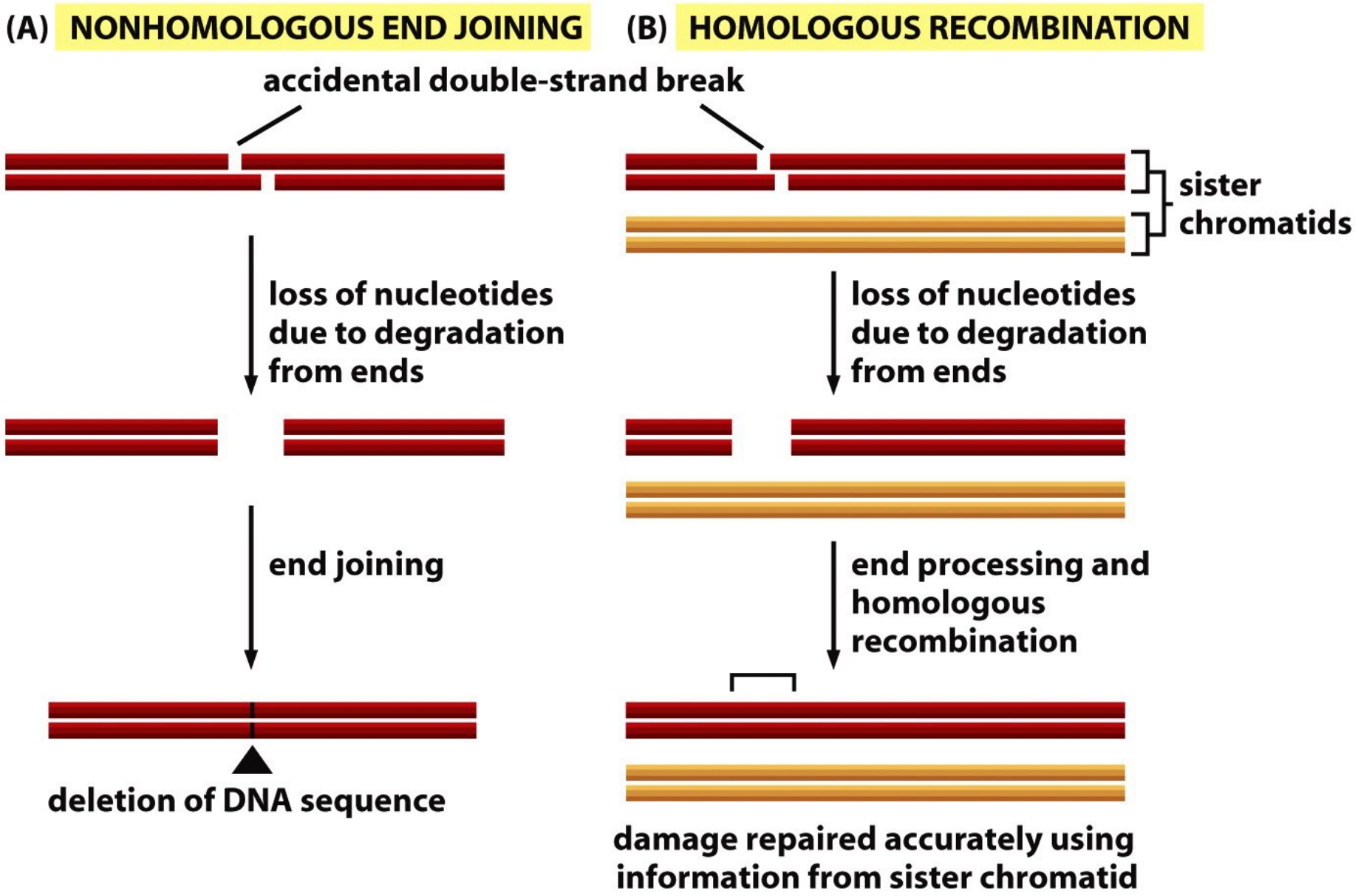
Repair of DNA Double-Strand Breaks During the Cell Cycle



…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



Main Pathways to Repair DNA ds Breaks

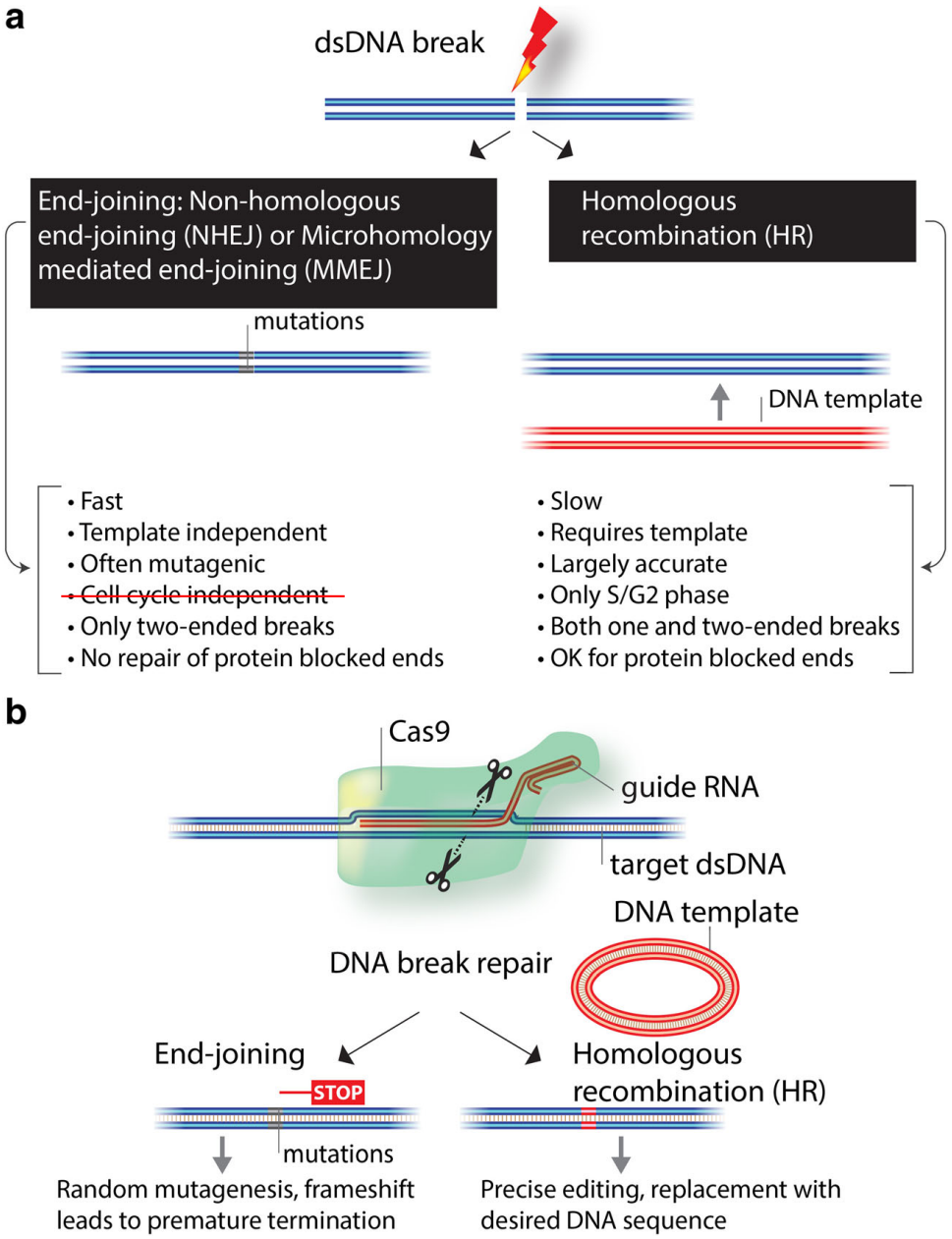


Fig. 2 An overview of the two main pathways for DNA double-strand break repair in human cells. **a** Main differences between end-joining and homologous recombination pathways. **b** DNA double-strand break repair pathway usage gives rise to different outcomes during genome editing with CRISPR-Cas9. 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

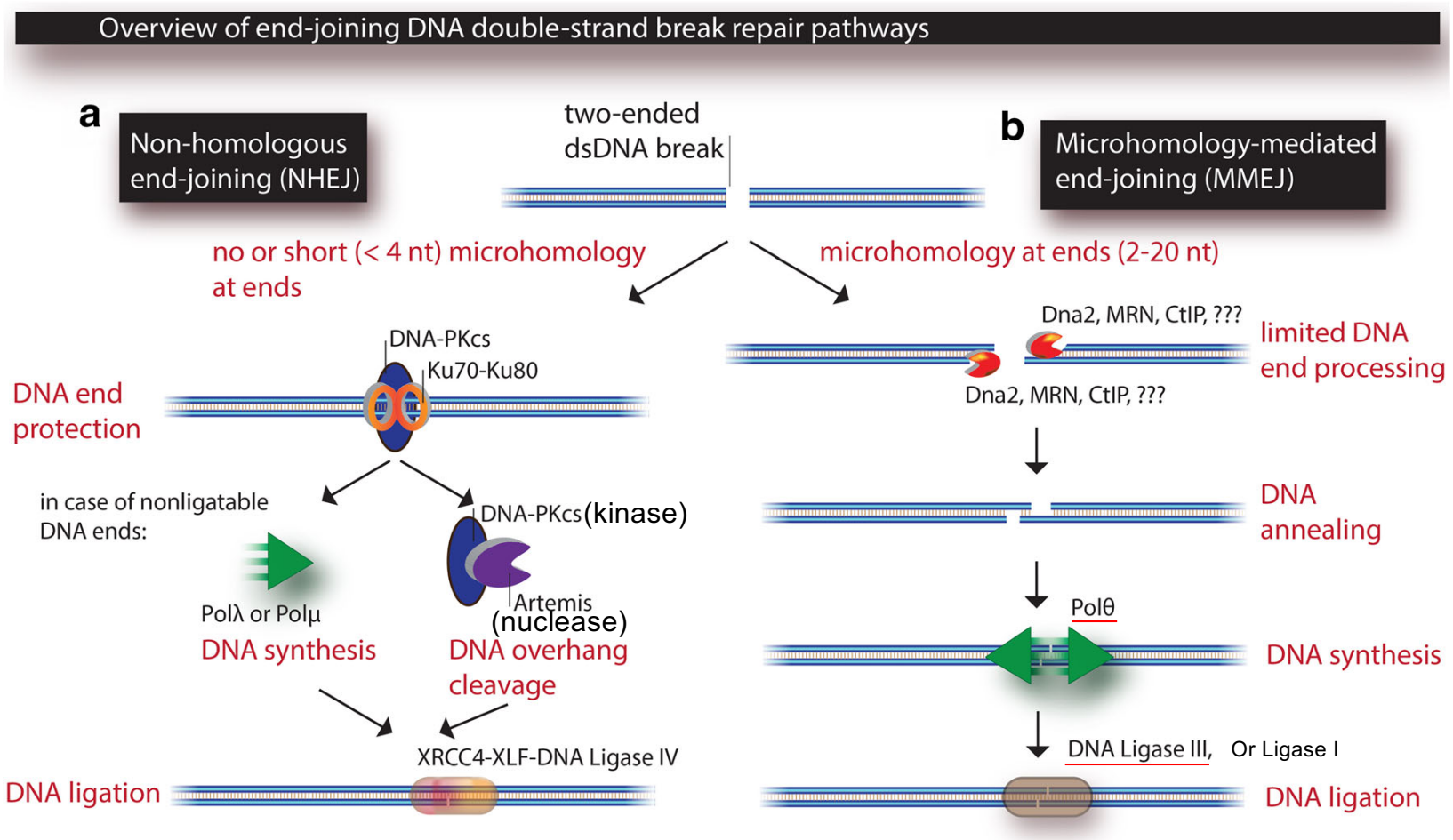
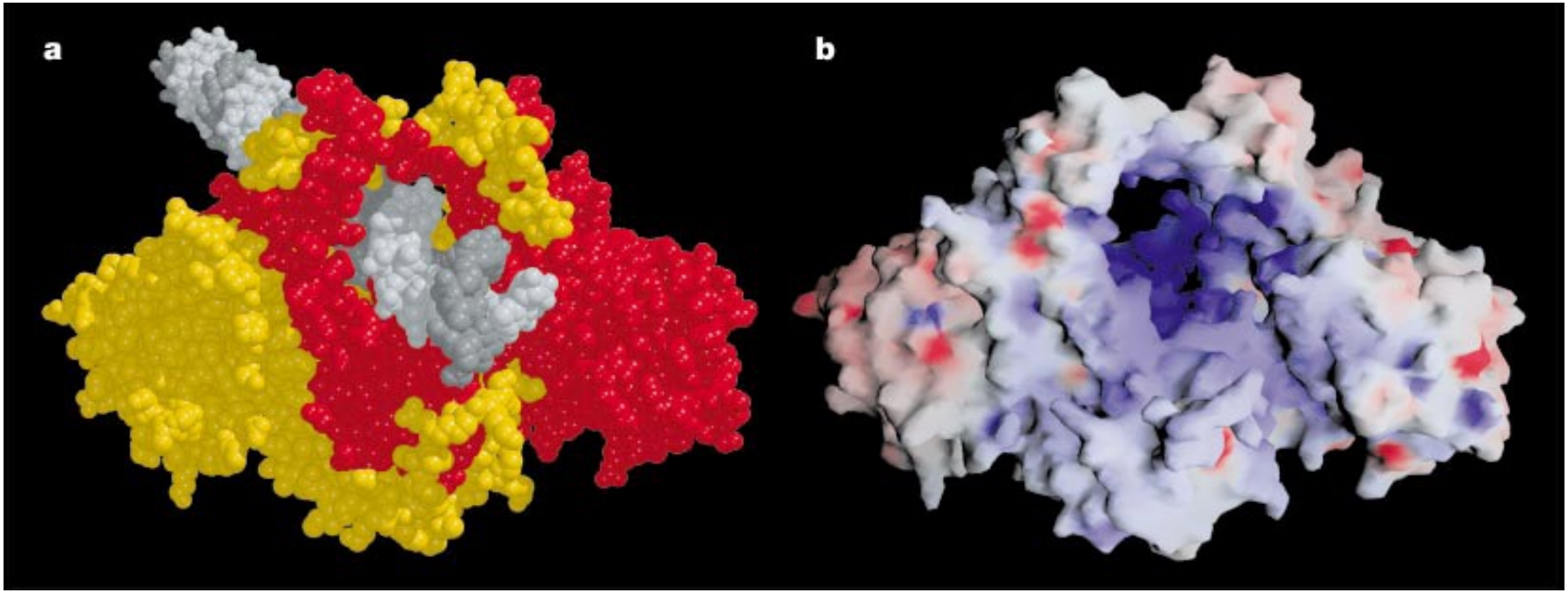


Fig. 3 An overview of DNA end-joining repair mechanisms. **a** Overview and main factors of non-homologous end-joining. **b** Overview and main factors of microhomology-mediated end-joining

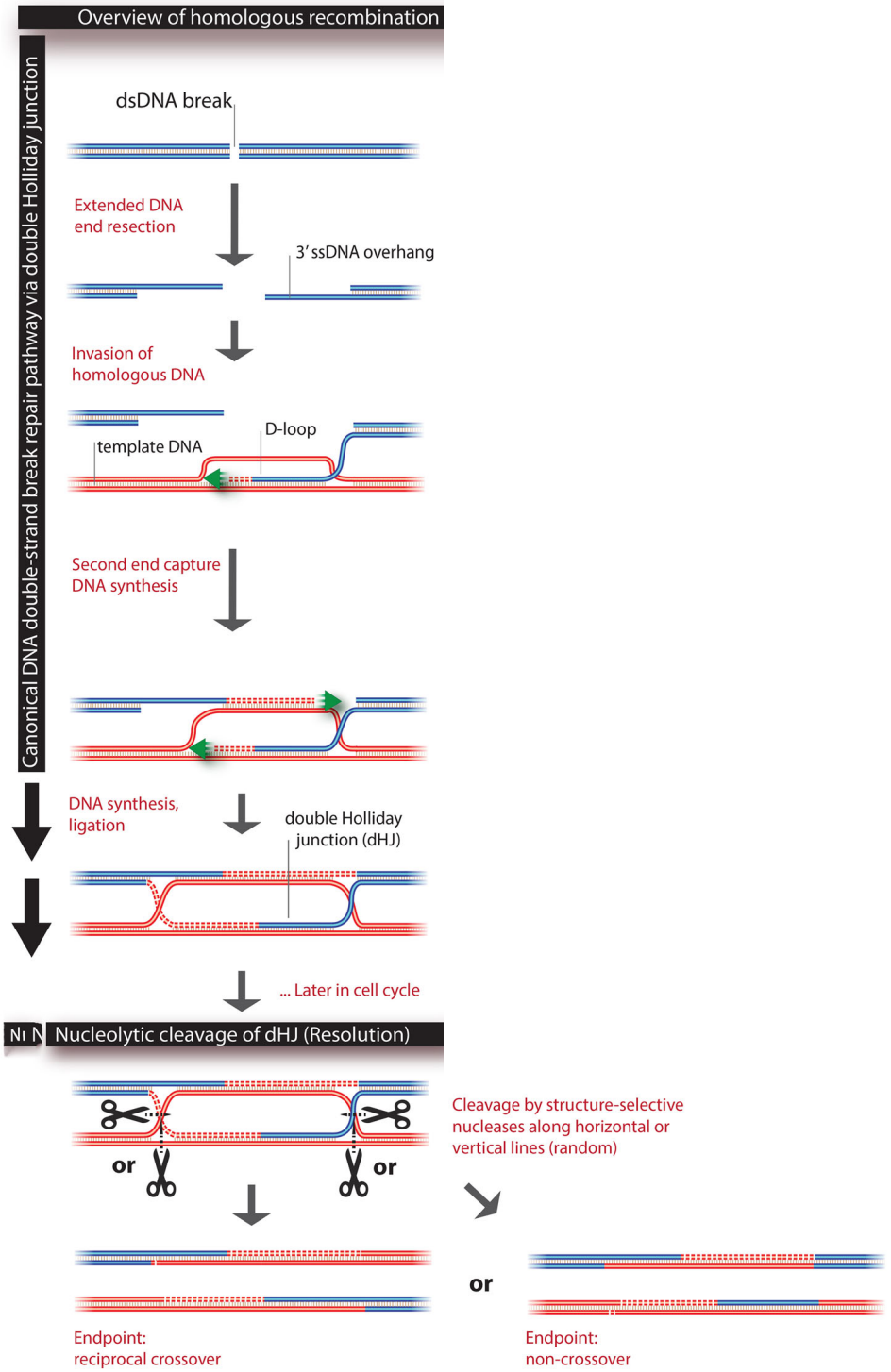
Crystal Structure of the Ku70/80 Heterodimer



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

Homologous Recombination (HR)

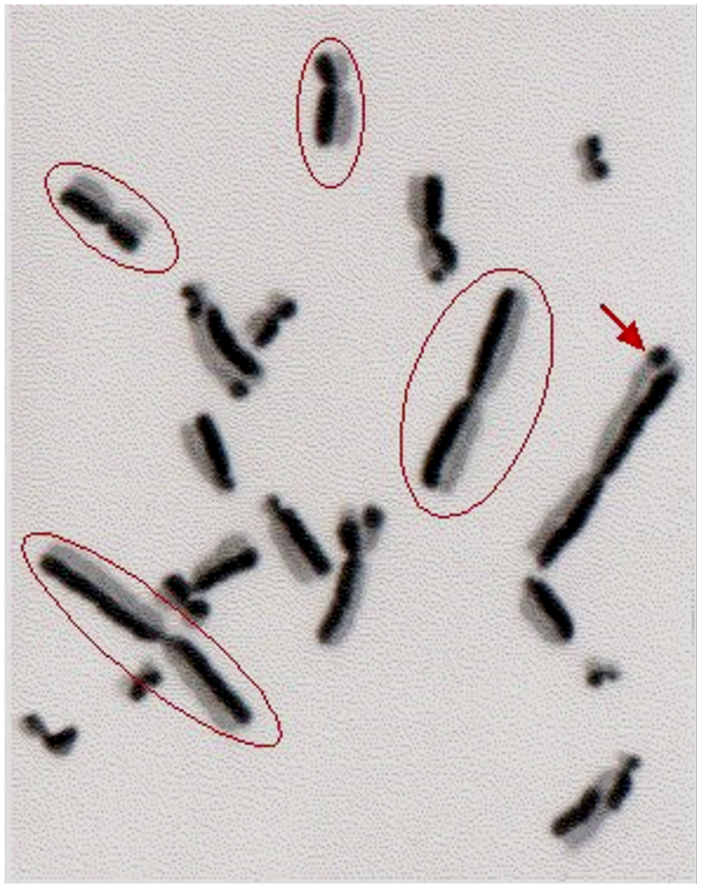
Homologous Recombination (HR)



From: Chromosoma (2018) 127: 187

Sister Chromatid Exchange (SCE)

Spontaneous SCE

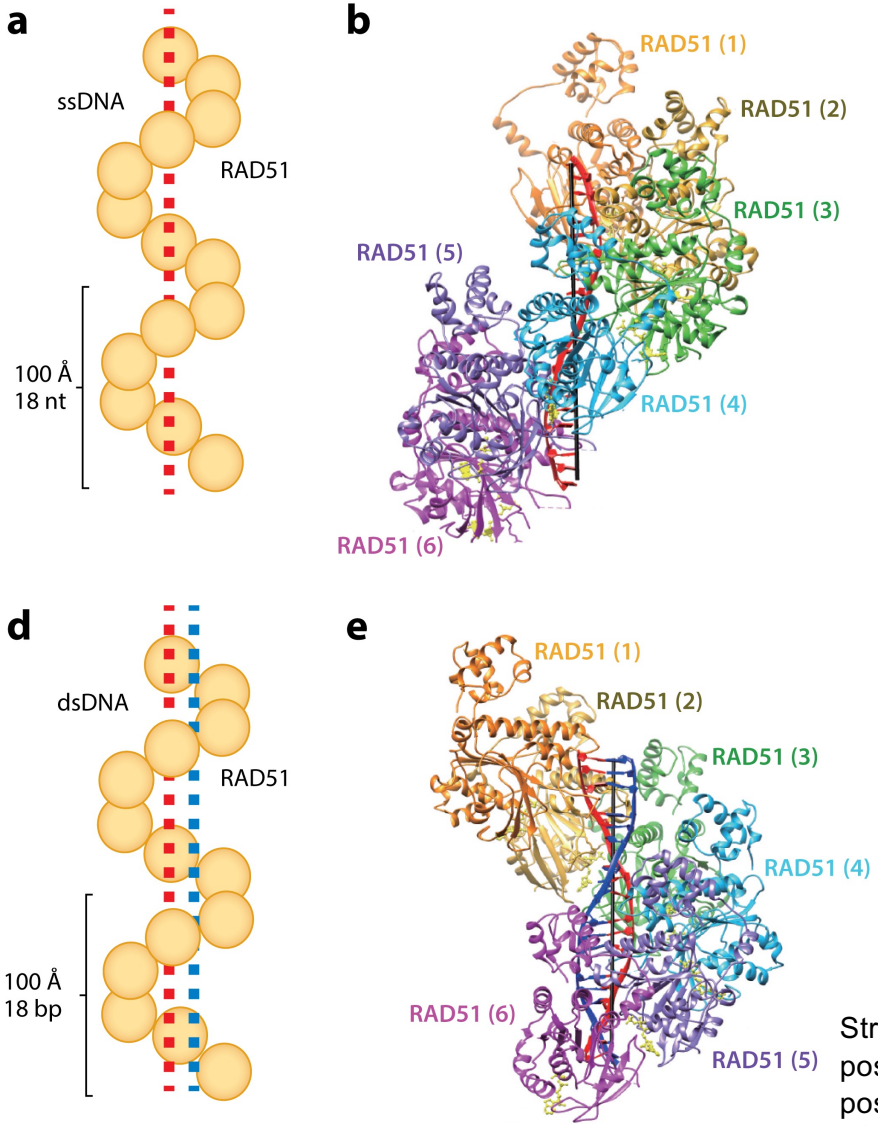


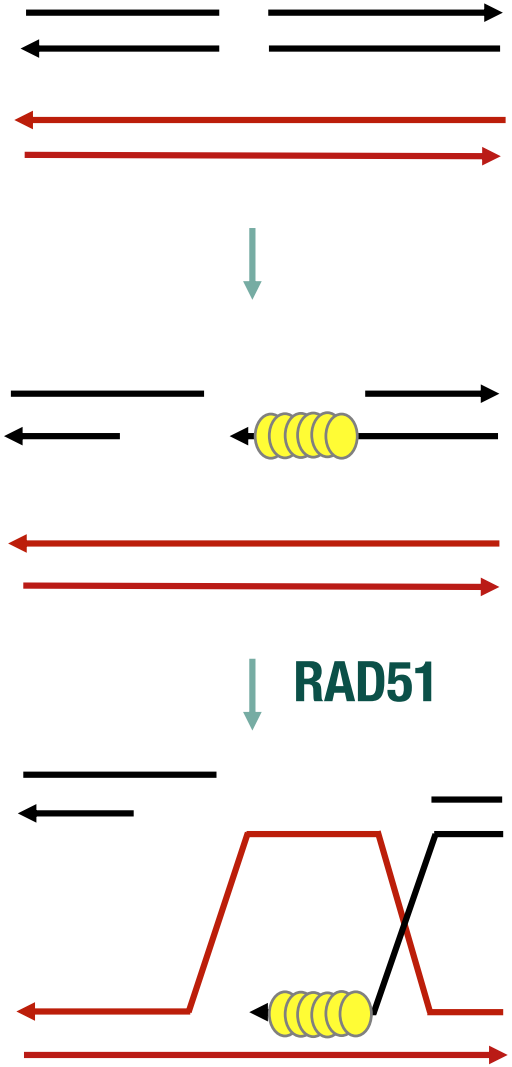
Induced SCE (DNA damage)

"Harlequin chromosomes"

J. Bodycote and S. Wolff

Strand Exchange by RAD51 Recombinase

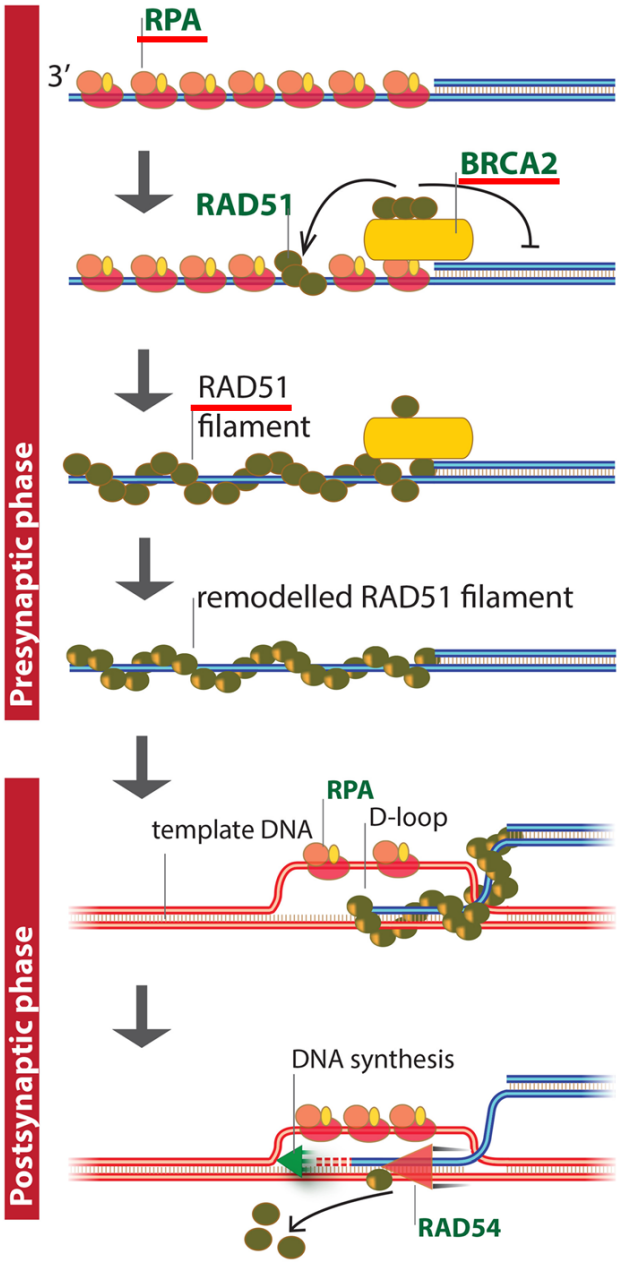




Structure of the RAD51–ssDNA presynaptic filament and the RAD51–dsDNA postsynaptic complex. (*a*,*d*) Illustrations of the (*a*) presynaptic filament and (*d*) postsynaptic complex. RAD51 is shown in yellow, and heteroduplex DNA within the postsynaptic complex is depicted with red and blue dashed lines.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

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

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	
RAD54	RTEL1 BLM RECQ1 RAD54

BRCA1: **Crucial for end resection of ds breaks to create a 3' overhang (top)**; stimulation of synaptic complex or homology search

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’.

Lifetime *BRCA1* and *BRCA2* Cancer Risks

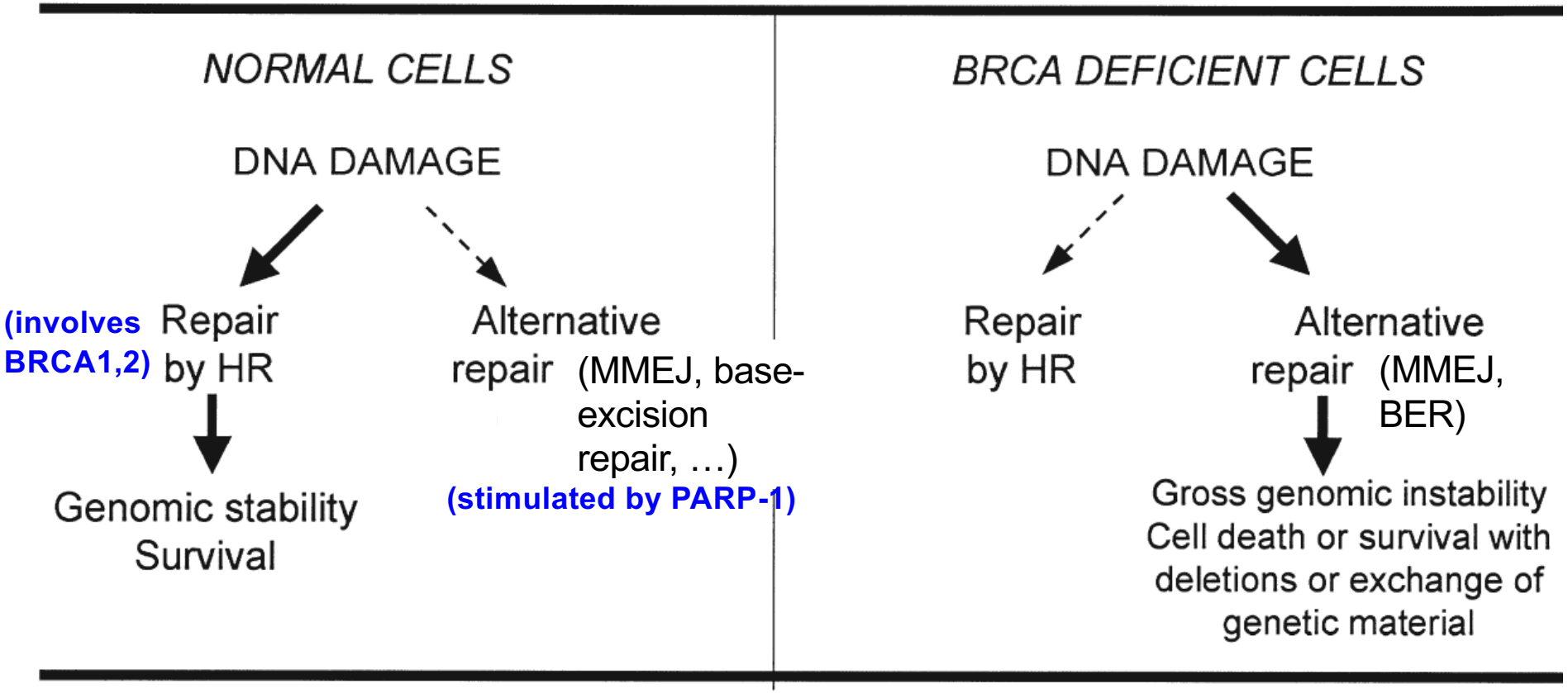
TYPE OF CANCER	WOMEN			MAN		
	Woman with a *BRCA1* mutation	Woman with a *BRCA2* mutation	Average woman in US without mutation	Man with a *BRCA1* mutation	Man with a *BRCA2* mutation	Average man in US without mutation
Breast	60-75%	50-70%	13%	1-5%	5-10%	0.1%
Ovarian	30-50%	10-20%	1-2%	-	-	-
Prostate	-	-	-	[1]	15-25%*	16%
Pancreatic	2-3%	3-5%	1%	2-3%	3-5%	1%
Melanoma	-	3-5%	1-2%	-	3-5%	1-2%

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 increased risk for breast cancer and ovarian cancer.

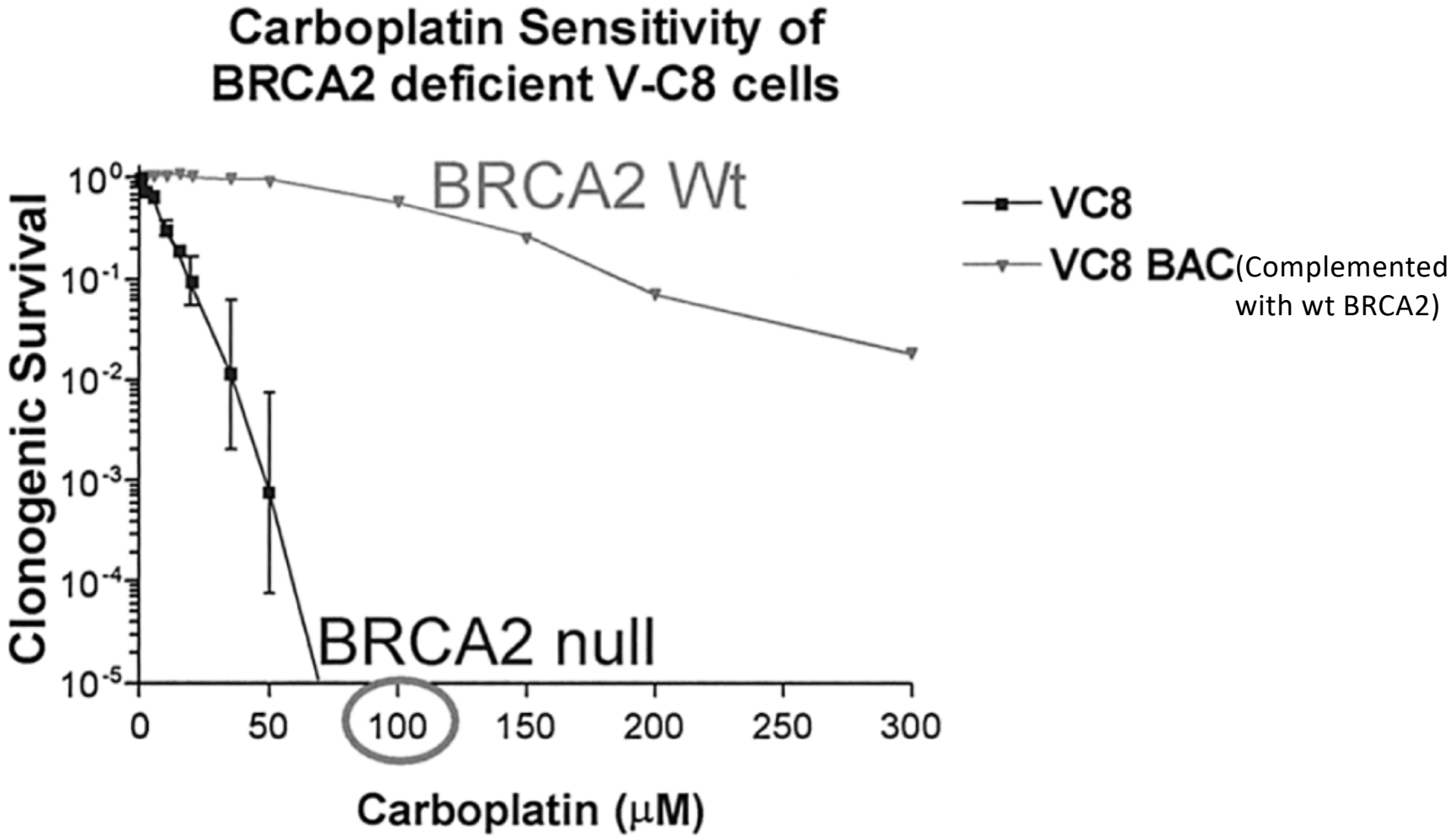
BRCA2 Functions in the Repair of DNA breaks



- **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

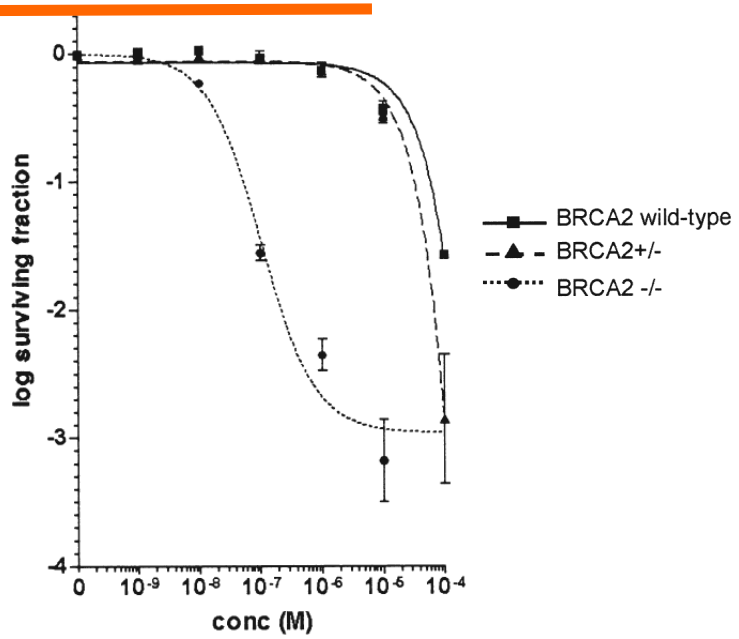


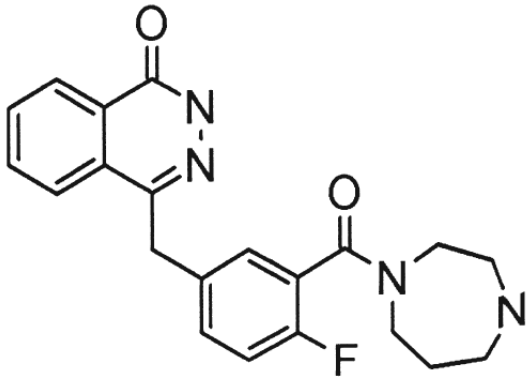
- *BRCA2* mutant cells are hypersensitive to carboplatin (and cisplatin). These chemotherapeutic agents crosslink DNA strands (inter- and intra-strand).

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; stimulates microhomology mediated end joining but is not absolutely required)





KU0058948
IC_{50} = 3.4nM

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

PARP1 Binds ssDNA Breaks (and ds Breaks in competition with Ku)

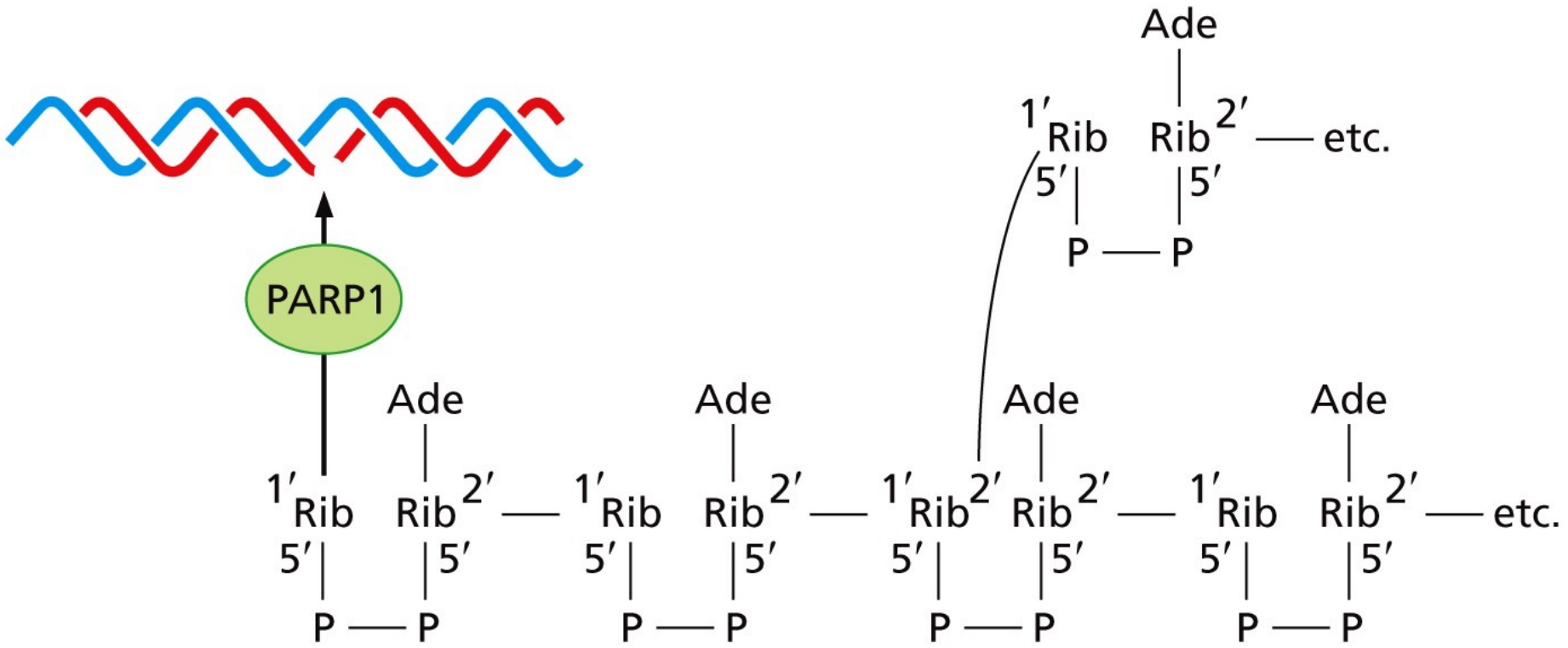


Figure 12.47. Weinberg, The Biology of Cancer

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

PolyADP-ribosylation

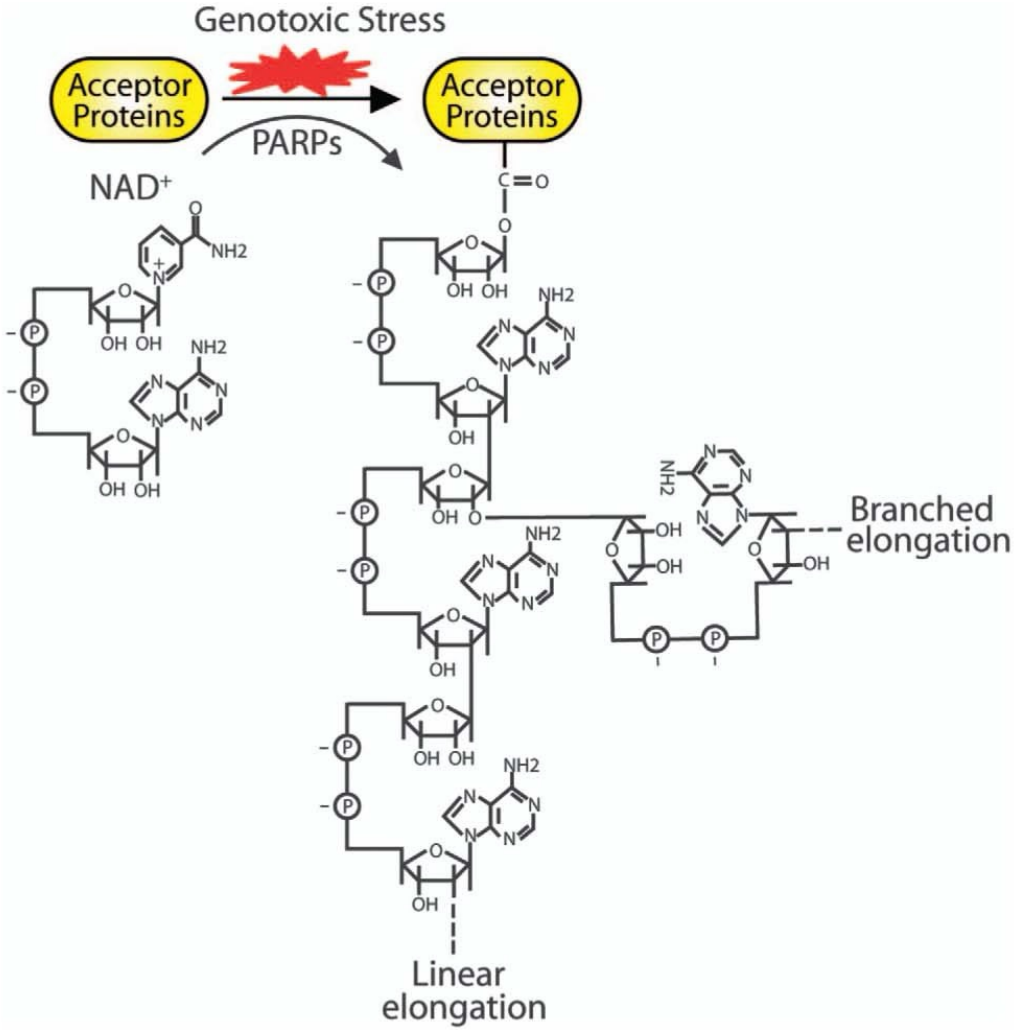


Figure 1. Sketch of poly(ADP-ribosyl)ation. With NAD^+ as the donor, PARPs mediate the genotoxic stress-dependent poly(ADP-ribosyl) ation. ADPr residues are covalently linked to the side chains of arginine, lysine, aspartate or glutamate residues of acceptor proteins. Glycosidic ribose-ribose 1′-2′ bonds 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

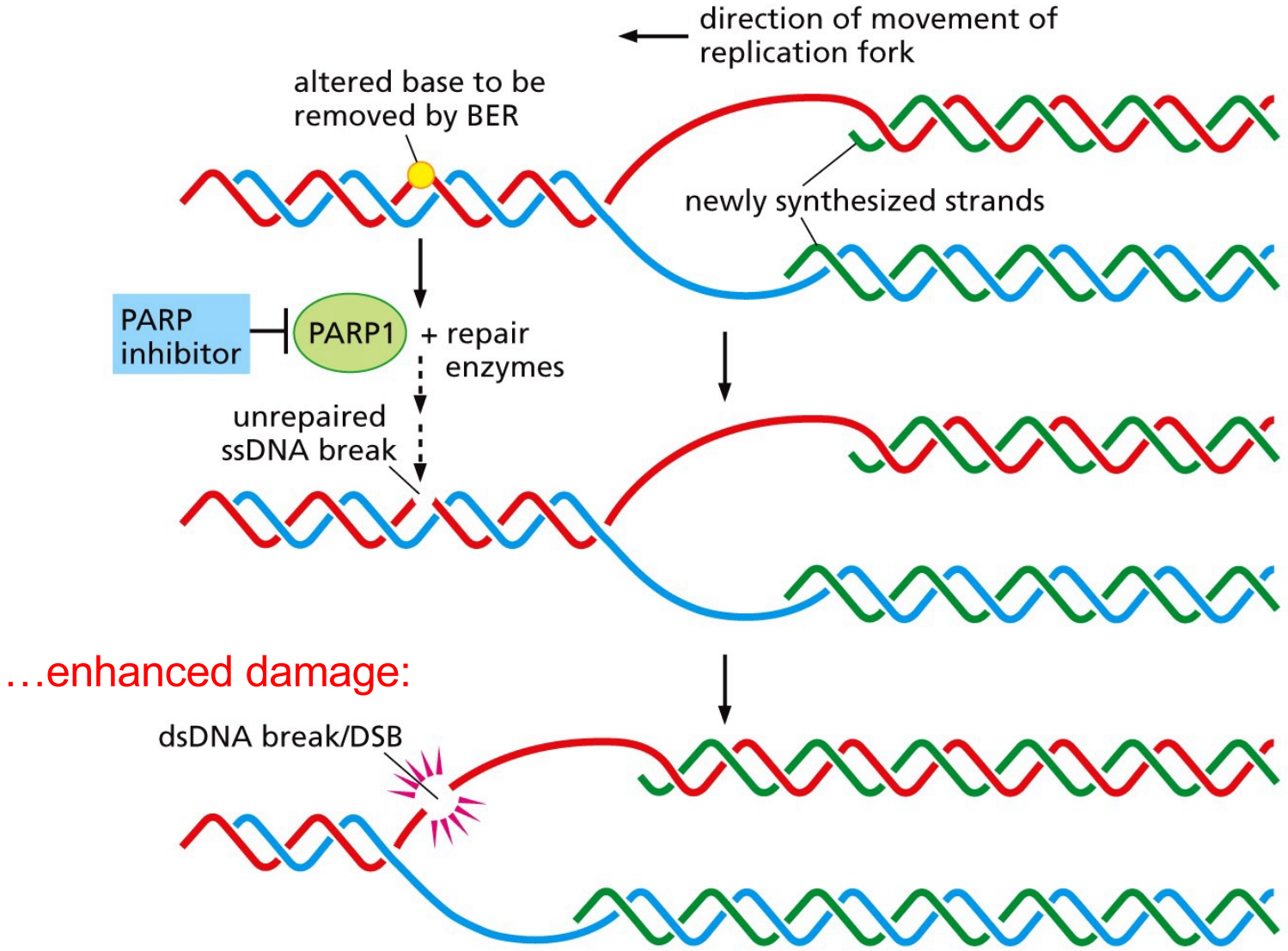
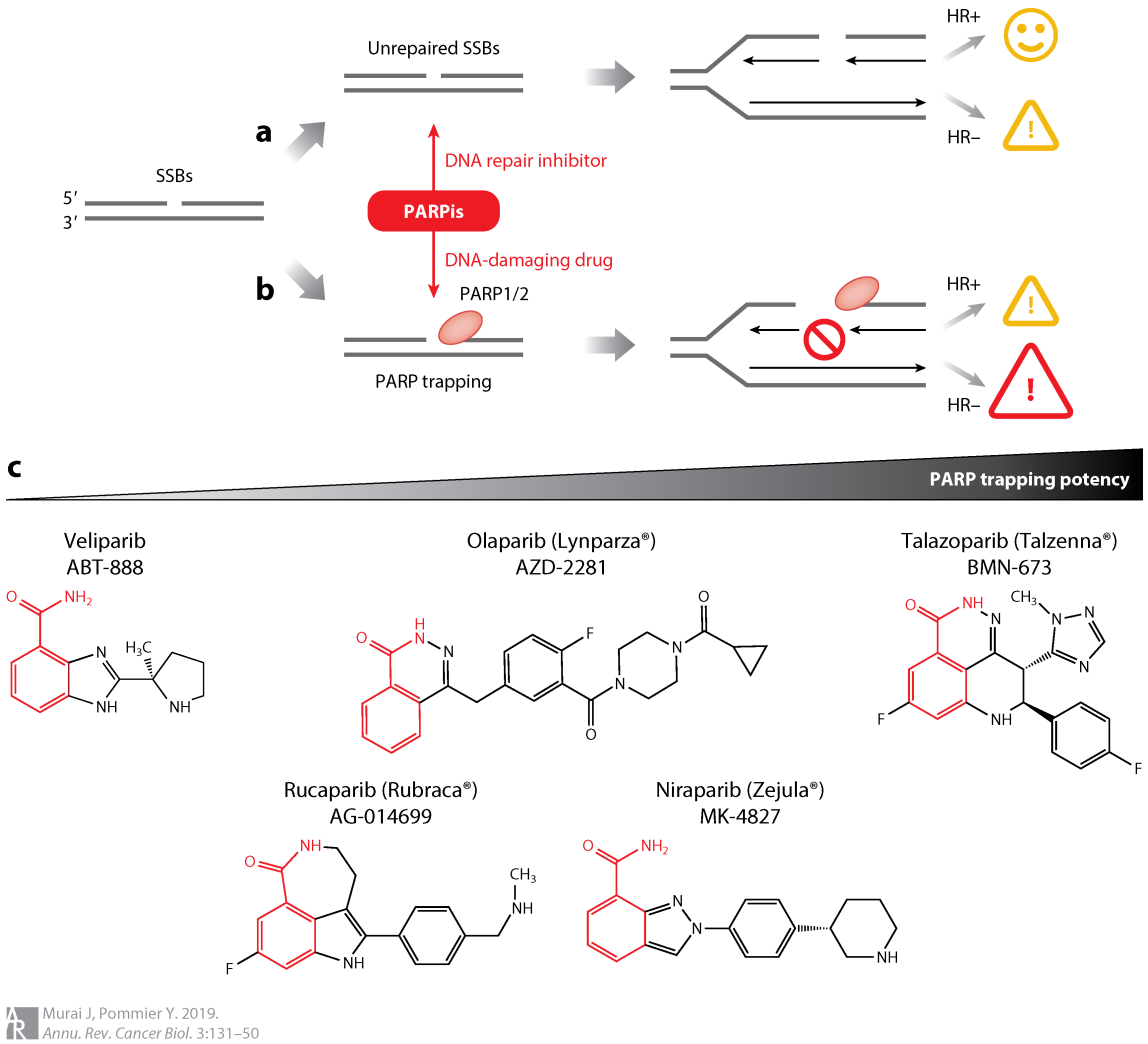


Figure 12.47. Weinberg, The Biology of Cancer

! 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.

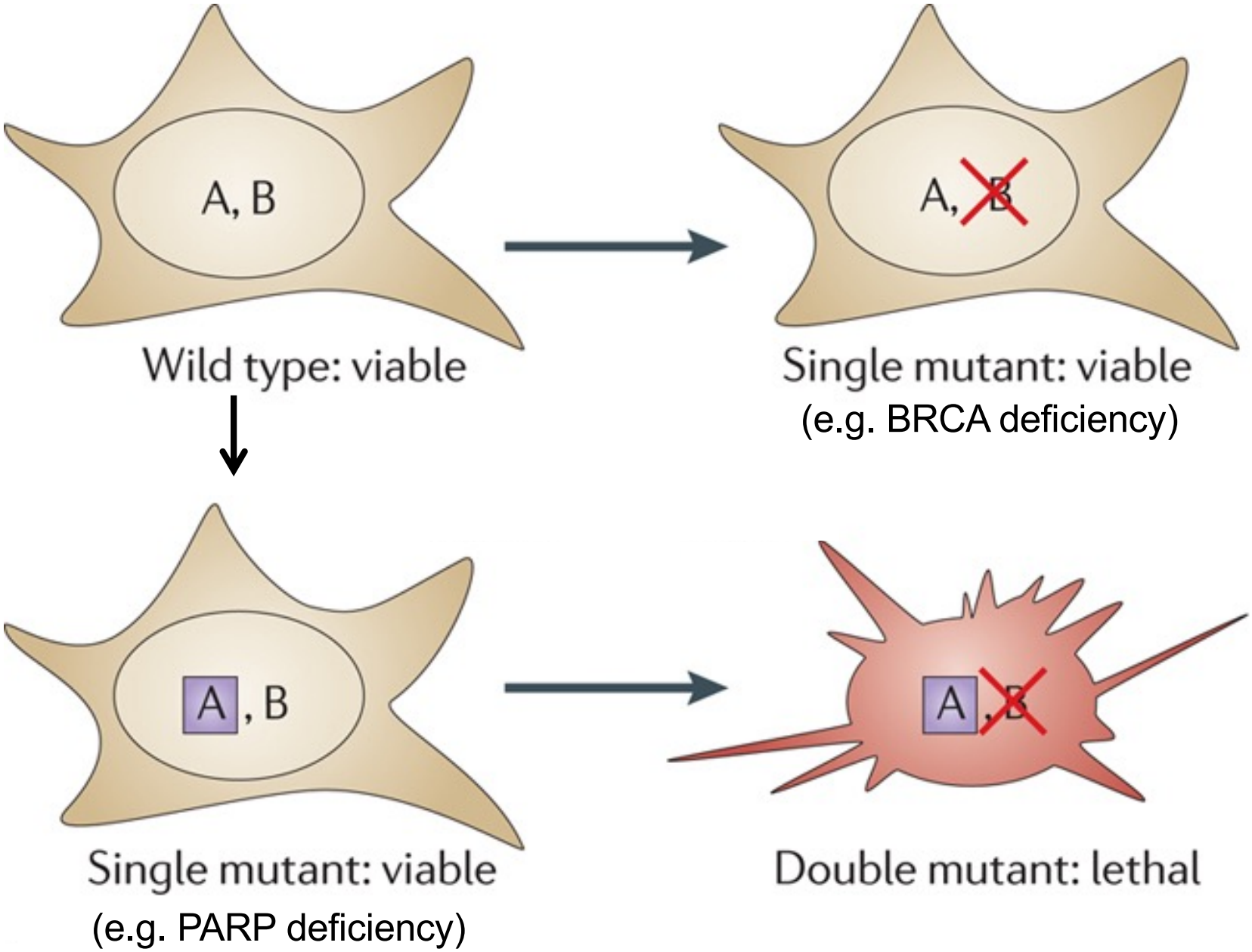


Schematic representation of the dual mechanisms of action of poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) in homologous recombination (HR)-deficient cells. (a) DNA repair inhibition through catalytic inhibition. Double-strand breaks (DSBs) formed upon replication collisions at unrepaired single-strand 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.

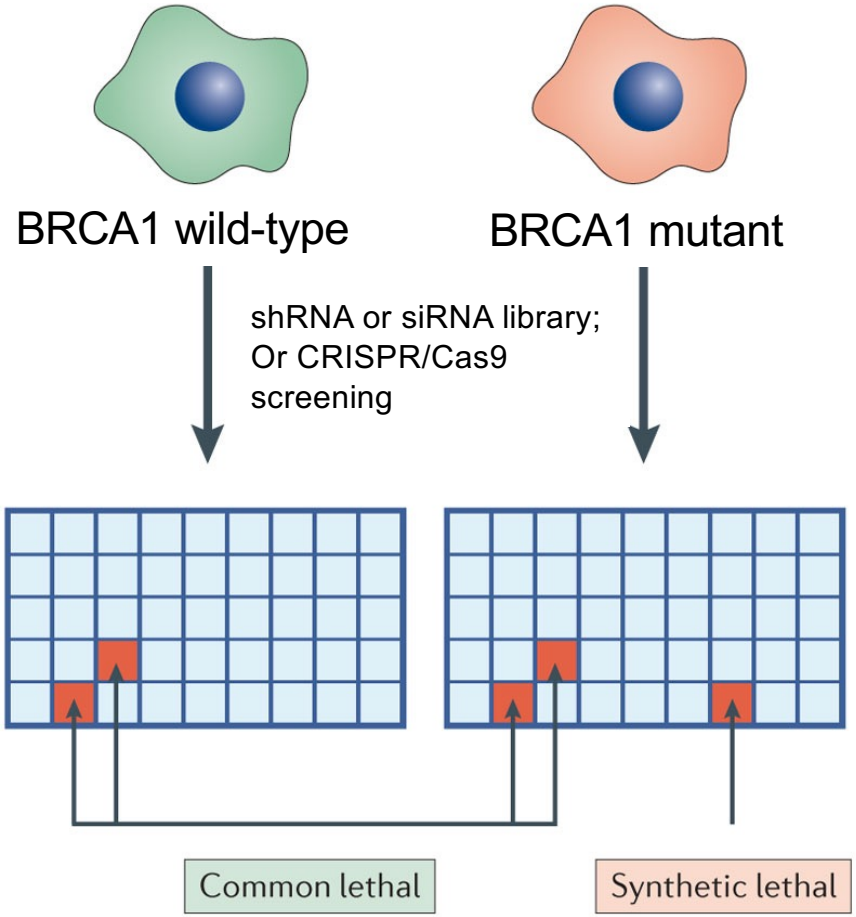
Recapitulation

- Repair of dsDNA breaks: NHEJ, MMEJ (also called Alt-EJ), HR
- BRCA1 and BRCA2 function in HR
- PARP-1 stimulates base-excision repair and micro-homology mediated end joining
- HR-deficient cells can be killed by inhibiting PARP-1. …Concept of **synthetic lethality**

Synthetic Lethality

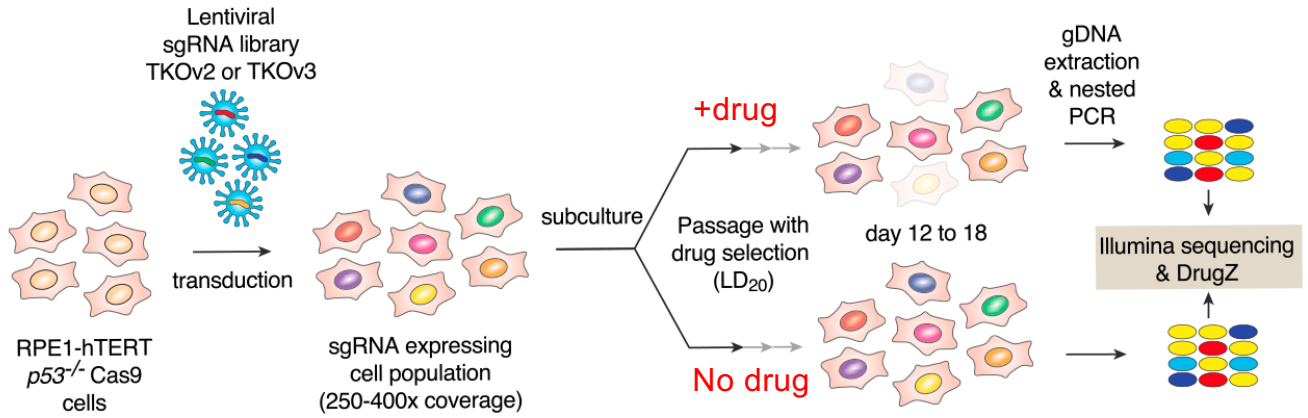


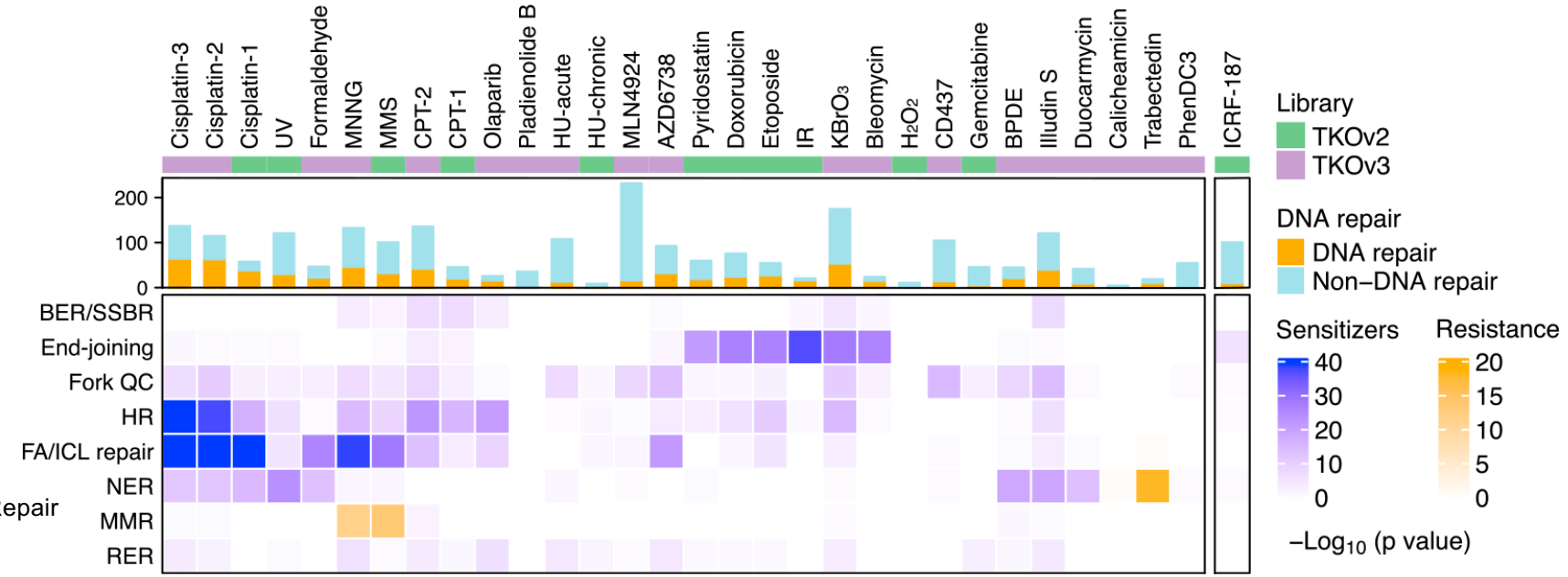
RNAi or CRISPR/Cas9 Screen for Synthetic Lethality



CRISPR-Cas9 Screens: Identification of Genes whose Loss Increases (or Decreases) Sensitivity to a Genotoxic Drug (used in cancer therapy)

RPE: retinal epithelial cells.
hTERT: cells are immortalized.
Cas9: guide RNA-directed DNA endonuclease.
p53 -/- : to avoid cell cycle arrest that may be induced by these drugs.
Essential gene products will not be identified.





BER: Base Excision Repair
HR: Homologous Recombination
NER: Nucleotide Excision Repair
MMR: Mismatch Repair

From Cell 182, 481-496 (2020)

Supplementary Table S1: Screen details including concentrations used, sources and experimenter

Name	Mechanism of Action	Dose used	library	Product supplier / machine ID	Cat#	Author	Set
Cisplatin-2	Inter- intrastrand crosslink/Helix distoring lesion	1.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	NH	1
Bleomycin	DNA strand breaks	25 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/15361?lang=en&region=CA	Cat# 15361	MZ	2
Olaparib	PARP inhibitor	5 µM	TKOv3	https://www.selleckchem.com/products/AZD2281(Olaparib).html	Cat# S1060	MZ	2
CD437	DNA replication stress	200 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/c5865?lang=en&region=CA	Cat# C5865	MO, TC	3
Illudin S	Transcription-interefering	30 nM	TKOv3	https://www.caymanchem.com/product/17451	Cat# 17451	MO, TC	3
KBrO3	Oxidative DNA damage	500 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigald/60085?lang=en&region=CA	Cat# 60085	MO, TC	3
MLN4924 (Pevonedistat)	NAE inhibitor	250 nM	TKOv3	https://www.activebiochem.com/product/231	Cat# A-1139	MO, TC	3
MNNG	Base alkylation	100 nM	TKOv3	https://www.trc-canada.com/product-detail/?CatNum=N493990&CAS=70-25-7&Chemical_Name=N%E2%80%99-Nitro-N-nitroso-N-methylguanidine%20(Stabilized%20with%20Water)&Mol_Formula=C%E2%82%82H%E2%82%85N%E2%82%85O%E2%82%83	Cat# N493990	MO, TC	3
Cisplatin-3	Inter- intrastrand crosslink/Helix distoring lesion	1.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	SA	4
Camptothecin (CPT)-2	DNA strand breaks	6 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/c9911?lang=en&region=CA	Cat# C9911	SA	5
Calicheamicin	DNA strand breaks	0.5 nM - 0.05nM	TKOv3	https://www.medchemexpress.com/Calicheamicin.html	Cat# HY-19609	MO, JY	6
Duocarmycin SA	Base alkylation	1 nM - 0.075 nM	TKOv3	https://www.creative-biolabs.com/adc/duocarmycin-sa-746.htm	Cat# ADC-P-043	MO, JY	6
Formaldehyde	Inter- intrastrand crosslink	63.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sial/252549?lang=en&region=CA	Cat# 252549	MO, JY	6
Phen-DC3	C-quadruplex stabilizer	1 µM	TKOv3	https://www.polysciences.com/default/phen-dc3	Cat# 26000	MO, JY	6
Trabectedin	Transcription-interefering	1 nM - 0.075 nM	TKOv3	https://www.trc-canada.com/product-detail/?CatNum=T703500&CAS=114899-77-3&Chemical_Name=Trabectedin&Mol_Formula=C%E2%82%83%E2%82%89H%E2%82%84%E2%82%83N%E2%82%83O%E2%82%81%E2%82%81S	Cat# T703500	MO, JY	6
AZD6738	ATR inhibitor	0.5 µM	TKOv3	https://www.medchemexpress.com/AZD6738.html?src=google-product&gclid=CjwKCAjwxaXtBRBbEiwAPqPxcL0DcECB0Evzv-IlkhFibqfnHxGvkqQu2_gAQpqgTdDYc7jJTQOcIBoCuQ0QAvD_BwE	Cat# HY-19323	AAQ	7
Camptothecin (CPT)-1	DNA strand breaks	5 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/c9911?lang=en&region=CA	Cat# C9911	MO, TC	8
Cisplatin-1	Inter- intrastrand crosslink/Helix distoring lesion	1 mM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	MO, TC	8
Etoposide (VP-16)	DNA strand breaks	100 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/e1383?lang=en&region=CA	Cat# E1383	MO, TC	8
Hydroxyurea (HU)	DNA replication stress	100 µM (chronic)	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/h8627?lang=en&region=CA	Cat# H8627	MO, TC	8
Ionizing radiation (IR)	DNA strand breaks	3 Gy	TKOv2	Faxitron 43855C	N/A	MO, TC	8
Doxorubicin	DNA strand breaks	5 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/d1515?lang=en&region=CA	Cat# D1515	MO, GSM	9
Hydrogen Peroxide (H2O2)	Oxidative DNA damage	15 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/h1009?lang=en&region=CA	Cat# H1009	MO, GSM	9
Methyl Metanesulfonate (MMS)	Base alkylation	25 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/aldrich/129925?lang=en&region=CA	Cat# 129925	MO, GSM	9
Pyridostatin	C-quadruplex stabilizer	0.75 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/sml0678?lang=en&region=CA	Cat# SML0678	MO, GSM	9
Ultraviolet Light (UV)	Helix distoring lesion	5 J/m2	TKOv2	UVS 254 nm lamp	N/A	MO, GSM	9
ICRF-187	TOP2 inhibitor	25 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/d1446?lang=en&region=CA	Cat# D1446	ASB, IDS	10
benzo(a)pyrene diol epoxide (BPDE)	Helix distoring lesion	200 nM	TKOv3	https://www.scbt.com/p/benzoapyrene-diol-epoxide-58917-67-2?requestFrom=search	Cat# sc-503767A	TC, SF	11
Hydroxyurea (HU)	DNA replication stress	1.5 mM (acute)	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/h8627?lang=en&region=CA	Cat# H8627	TC, SF	11
Pladienolide B (PladB)	splicing-interefering	0.5 nM	TKOv3	https://www.tocris.com/products/pladienolide-b_6070	Cat# 6070	TC, SF	11
Gemcitabine	DNA replication stress	3 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/g6423?lang=en&region=CA	Cat# G6423	TU, MWF	12

From Cell 182, 481-496 (2020)

Example: PARP-1 inhibitors

- BRCA-silenced or mutated cells are sensitive to PARP-1 inhibitors (as discussed previously)
- Recent work (published in 2015): Pol θ (teta) which is involved in microhomology-mediated end joining is also a lethal target for the treatment of BRCA1-mutated cancers.

→ Development of PARP-inhibitors

- Olaparib: cellular model (*BRCA2*-deficient Capan-1 cells); concentration for 50% reduction in survival: IC_{50} = 259 nM ; approved by the FDA (US food and drug administration) and EMA (European medicines agency) for the treatment of BRCA-mutated advanced ovarian cancer.
- Talazoparib has also been approved. Talazoparib traps PARP on DNA and is effective at low concentrations in a cellular model (Capan-1 cells) (IC_{50} =5 nM).

Table 1 Comparison of clinical PARP inhibitors based on the individual parameters[a]

	Catalytic inhibition (IC_{50} in wild-type DT40 cells) (μM)	Cytotoxicity (IC_{90} in wild-type DT40 cells) (μM)	Cytotoxicity (IC_{90} in *Brca2*-deficient DT40 cells) (μM)	PARP trapping potency (relative to olaparib)	Anticancer clinical application (dose)
Olaparib	0.006	4.6	0.20	1	Approved as single agent for ovarian and breast cancers for maintenance and for ovarian cancers with *BRCA* mutations (300 mg × 2/day)
Niraparib	0.060	2.3	NA	2	Approved as single agent for ovarian cancer for maintenance (300 mg × 1/day)
Rucaparib	0.021	3.1	0.15	1	Approved as a single agent for ovarian cancer for maintenance and for ovarian cancers with *BRCA* mutations (600 mg × 2/day)
Talazoparib	0.004	0.5	0.006	100	Approved as a single agent for advanced metastatic HER2-negative breast cancers with deleterious or suspected deleterious germline *BRCA* mutations (1 mg × 1/day)
Veliparib	0.030	>50	15	<0.2	Combination clinical trials (400 mg × 2/day)

Abbreviations: IC_n, *n*% of maximal inhibitory concentration; NA, not available; PARP, poly(ADP-ribose) polymerase.

[a]Data from Murai et al. (2012, 2014a) and Murai & Pommier (2015).

Evolution of PARPi resistance in cancer ?

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

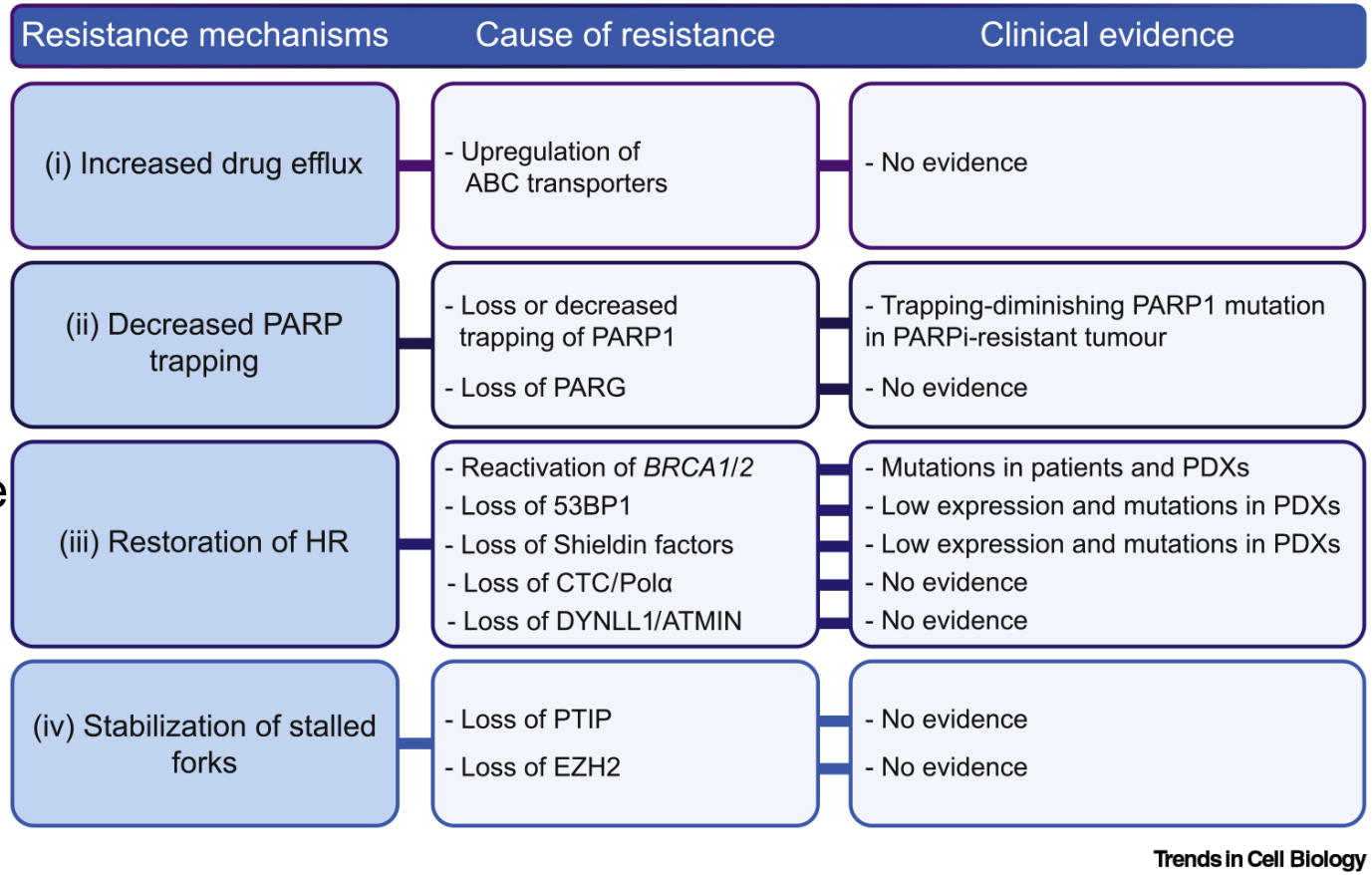
Resistance to therapy

- Resistance to platinum-based chemotherapies is a strong predictor for PARPi resistance, indicating sharing of common mechanisms.

PARP Inhibitor Resistance

Strong evidence

Not discussed in this course:



PDX: patient derived xenografts

Figure 2. Modes of Resistance to PARP Inhibitors (PARPi). An overview of the four distinct categories of PARPi resistance mechanisms. In each category (left column), all molecular mechanisms that have been identified in preclinical studies are mentioned (middle column). In addition, whether direct clinical evidence for PARPi resistance has been observed in primary tumor material or PDX-models until this date is indicated (right column). Abbreviations: HR, homologous recombination; PARP, poly(ADP-ribose) polymerase; PDX, patient-derived xenograft.

- PARG: Poly (ADP-ribose) glycohydrolase; removes poly ADP-ribose moieties
- Loss of 53BP1, shieldin, CTC1 →increased 3' overhangs at DSBs

→ reactivates resection and HR in BRCA1 cells (in BRCA1 deficiency resection is inhibited). **BRCA2 deficient cells are not acquiring PARP inhibitor resistance by loss of 53BP1!**

From: Trends Cell Biol 29, 820 (2019)

Loss of 53BP1 in *BRCA1*-mt Cells Leads to PARPi-resistance

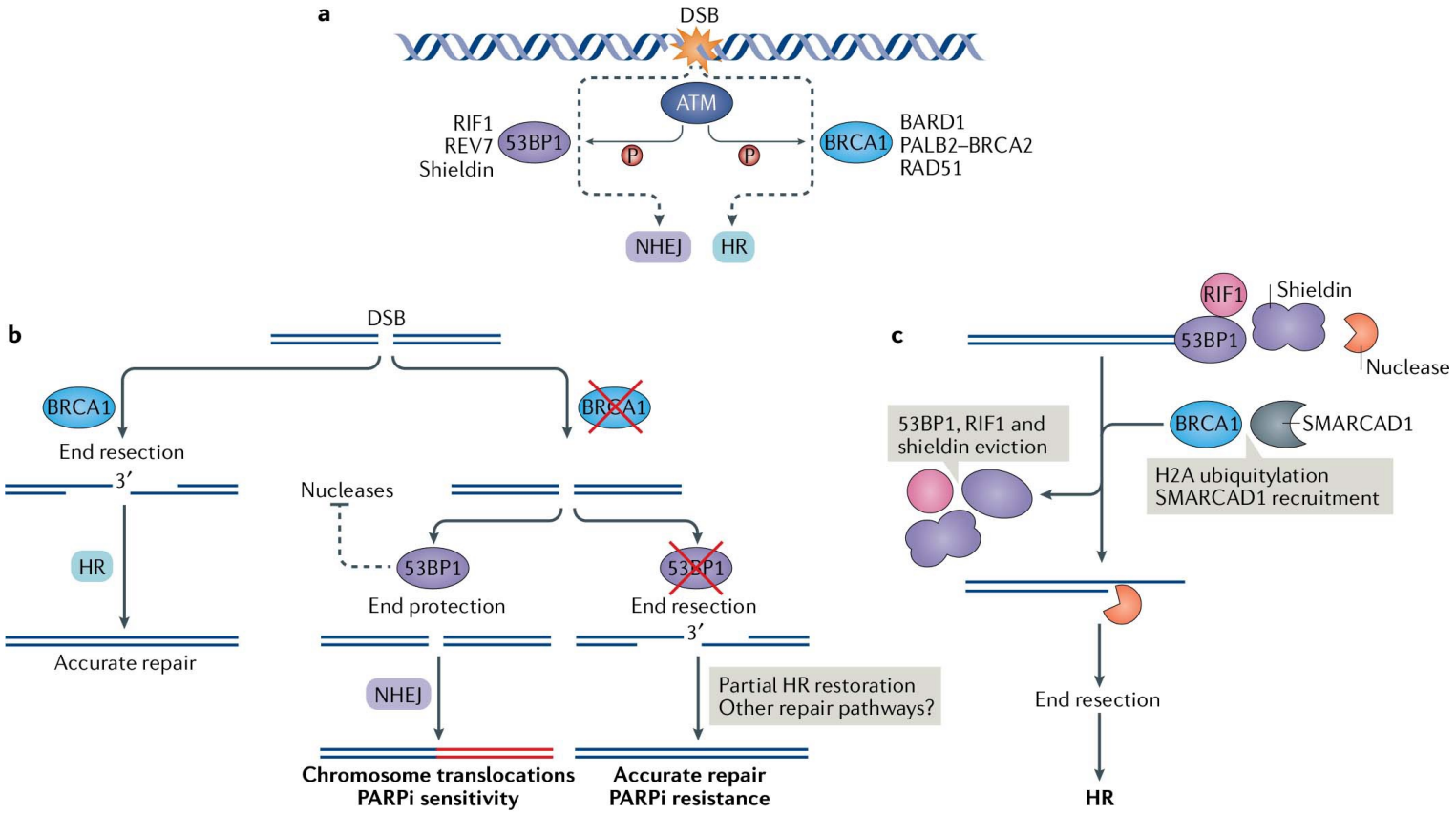


Fig. 3 | **Antagonistic roles of BRCA1–BARD1 and the 53BP1 complex. a** | A DNA double-strand break (DSB) elicits ataxia telangiectasia mutated (ATM)-dependent checkpoint responses, which activate and recruit breast cancer type 1 susceptibility protein (BRCA1)-containing and/or p53-binding protein 1 (53BP1)-containing complexes to the DNA lesion. BRCA1 and 53BP1 compete and perform opposing functions at broken DNA ends by engaging either homologous recombination (HR) or non-homologous end joining (NHEJ) for DSB repair, respectively. **b** | BRCA1 promotes DNA end resection and HR repair (left). If BRCA1 is abrogated, DSB ends are protected from resection by 53BP1-containing complexes and are channelled into NHEJ for repair (right). This process can lead to deleterious chromosome translocations, which underline the sensitivity of BRCA1-deficient cells to poly(ADP-ribose) polymerase inhibitors (PARPi). If, in addition to BRCA1, 53BP1 is concomitantly abrogated, the DNA ends are accessible to resection nucleases, leading to partial HR restoration and PARPi resistance. **c** | 53BP1-containing complexes (53BP1–RIF1–shieldin) protect DNA ends from nucleolytic digestion by nucleases. This end protection is overcome by BRCA1-mediated histone H2A ubiquitylation and recruitment of the helicase SMARCAD1. The eviction of the 53BP1-containing complexes leads to DNA end resection and HR.

From: NatRevMolCellBiol 2020: https://doi.org/10.1038/s41580-020-0218-z

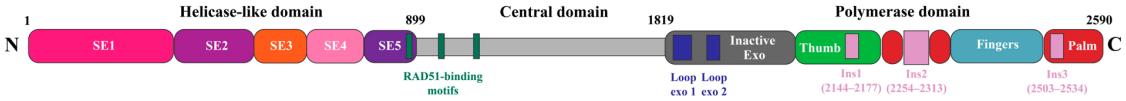
Targeting MMEJ

- BRCA-silenced or mutated cells are sensitive to PARP-1 inhibitors (as discussed previously)
- Recent work (published in 2015): Pol θ (teta) which is involved in microhomology-mediated end joining is also a lethal target for the treatment of BRCA1-mutated cancers.

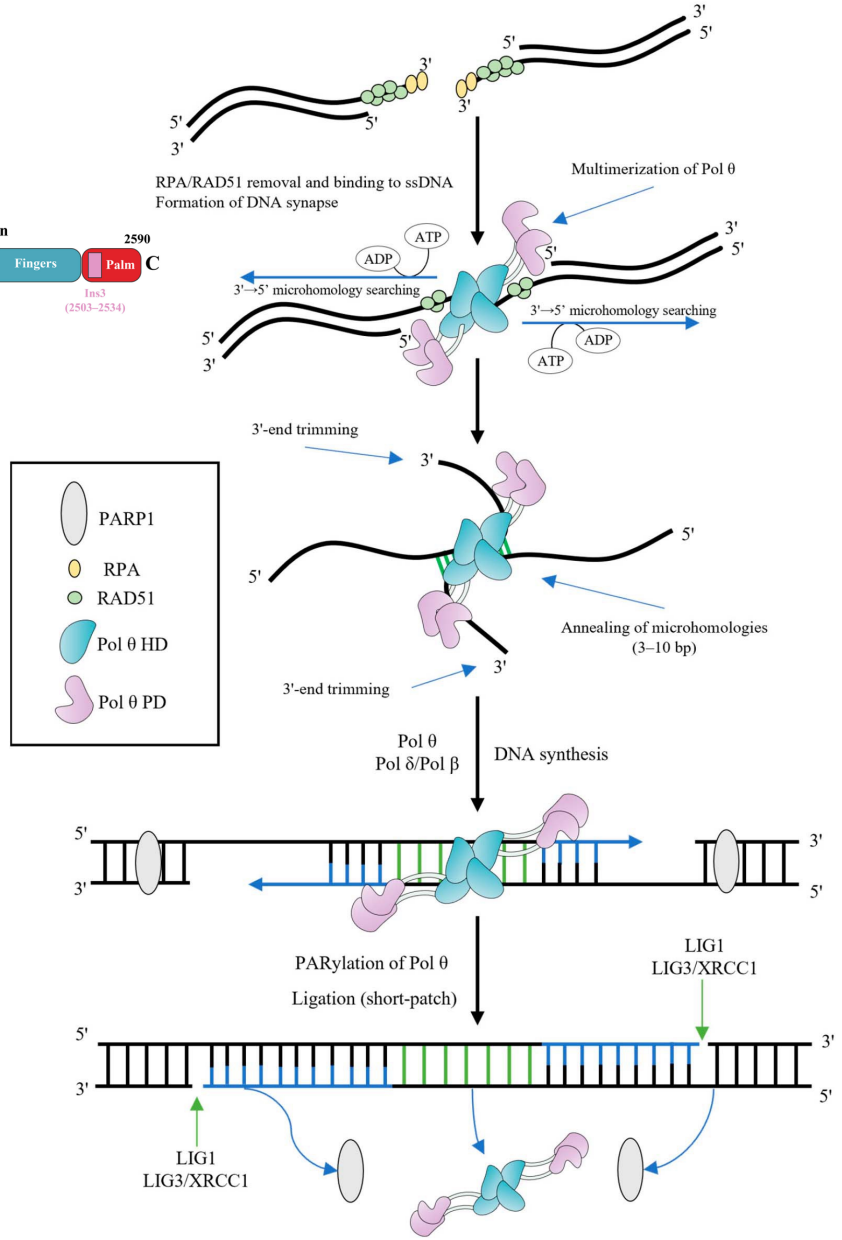
→ Development of PARP-inhibitors

- Olaparib: cellular model (*BRCA2*-deficient Capan-1 cells); concentration for 50% reduction in survival: IC_{50} = 259 nM ; approved by the FDA (US food and drug administration) and EMA (European medicines agency) for the treatment of BRCA-mutated advanced ovarian cancer.
- Talazoparib has also been approved. Talazoparib traps PARP on DNA and is effective at low concentrations in a cellular model (Capan-1 cells) (IC_{50} =5 nM).

Pol θ (teta) and MMEJ



Domain organization of Pol θ. The N-terminal helicase-like domain (HD), the C-terminal polymerase domain (PD), the unstructured central domain (CD), and the exonuclease-like subdomain are shown.



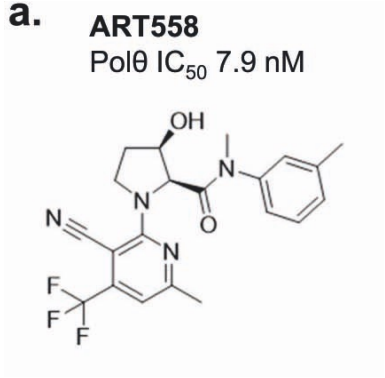
Model of MMEJ. . The resected 3′-overhangs are coated by RPA and RAD51 filaments. The HD of Pol θ removes both of them in ATP-dependent manner, contributing to TMEJ. Multimerization of Pol θ promotes the DNA synapse formation and initiates the 3′→5′ bidirectional searching for microhomologies, using the energy of ATP hydrolysis. Aligned 3′-ends are annealed in microhomology-rich regions. Unannealed 3′-overhangs are trimmed before DNA extension. Pol θ (or Pol β) fills gaps from annealed microhomologies, using them as primers. PARP1 PARylates Pol θ in order to remove the polymerase from DNA and complete the synthesis step. LIG1 or LIG3/XRCC1 complex finish the end joining (the short-patch resolution is shown).

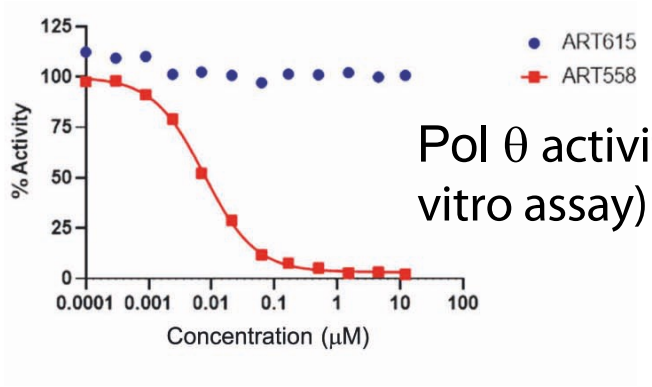
(MMEJ is referred to as TMEJ in this review)

From https://doi.org/10.3390/ijms24043619

Development of Pol θ (teta) Inhibitors

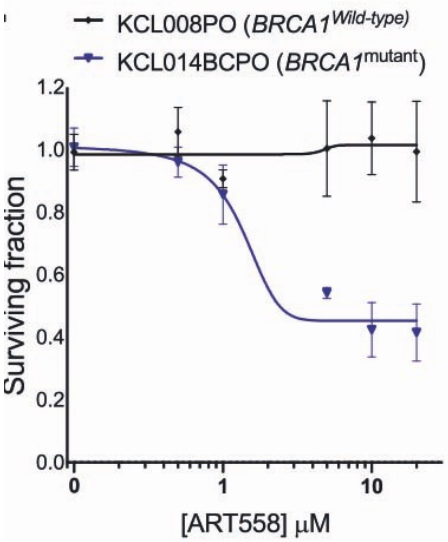
(e.g. NatComm 12: 3636 (2021); NatCancer 2, 598 (2021))

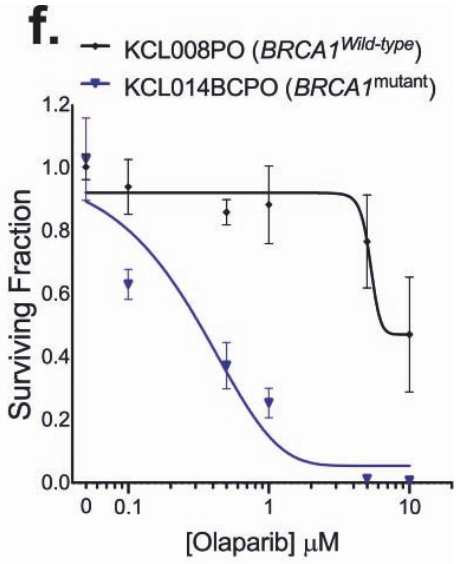




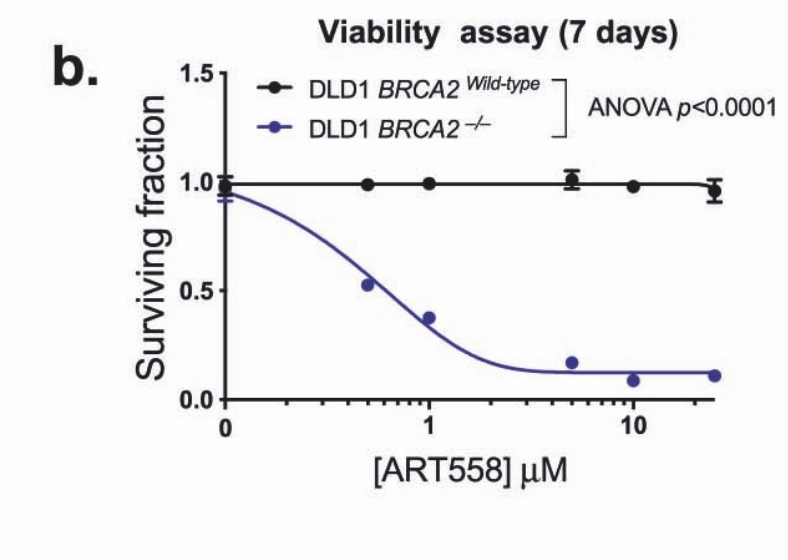
Pol θ activity (in vitro assay)

Breast cancer cells:





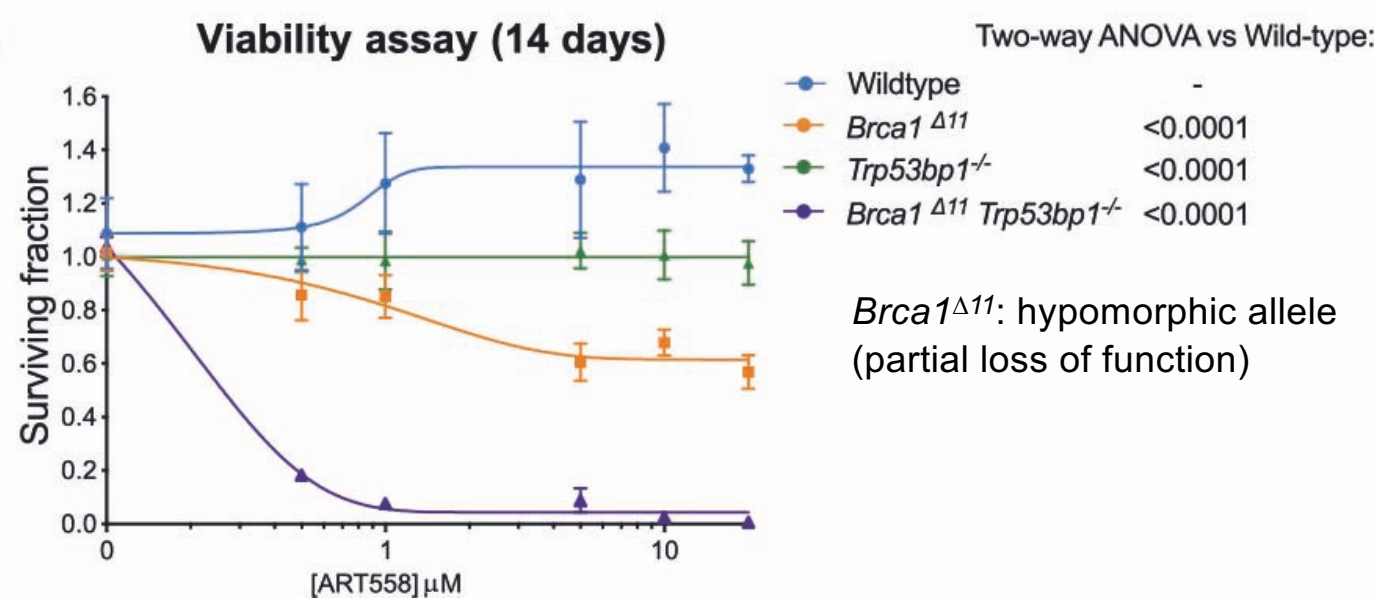
Colorectal adenocarcinoma cells:



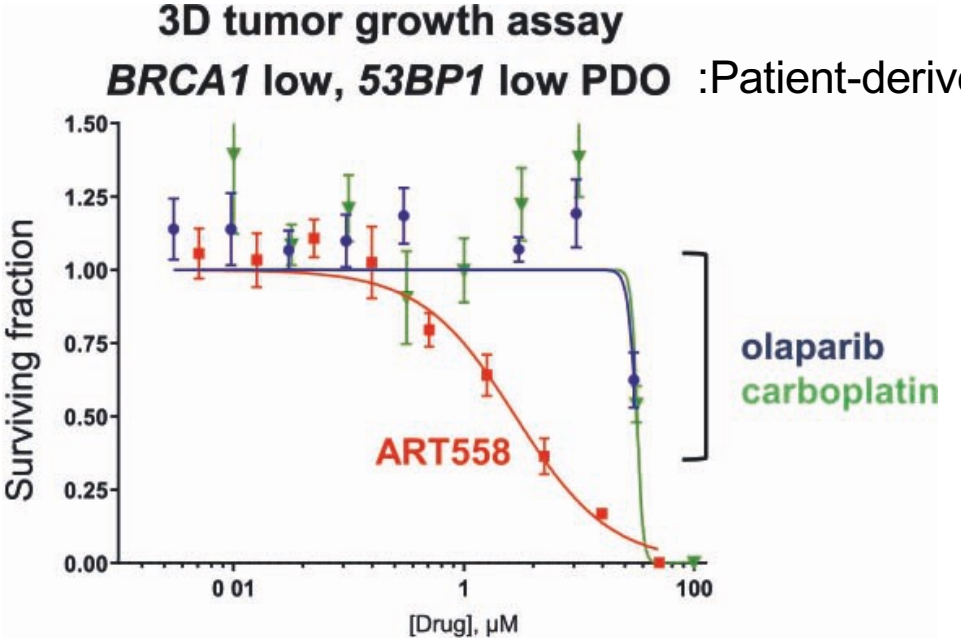
…the Pol θ inhibitor targets BRCA1 and BRCA2-deficient cells

Pol θ (teta) Inhibitor Targets even a PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))



Brca1$^{\Delta 11}$: hypomorphic allele (partial loss of function)

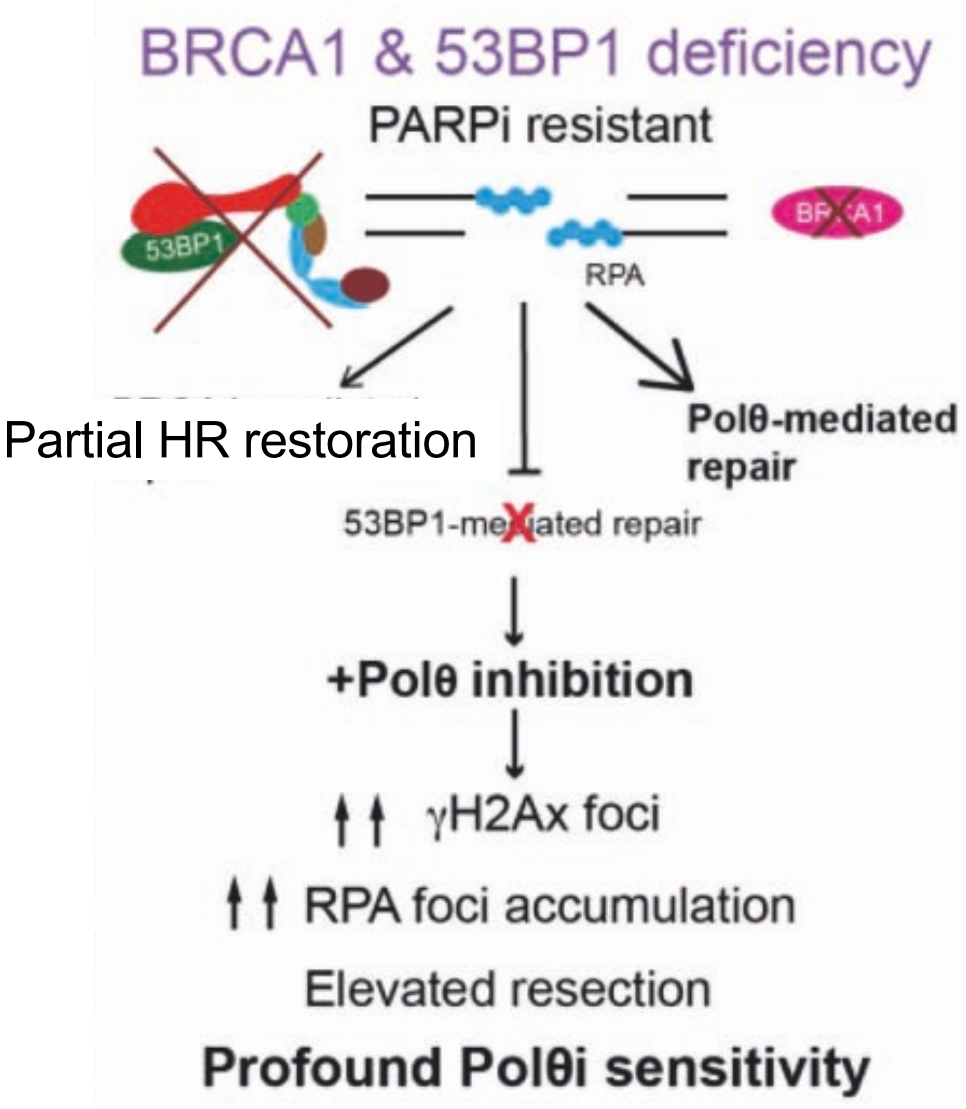


:Patient-derived Organoid

Pol θ (teta) Inhibitor Targets PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))

Model: the authors observed elevated DNA end resection upon Pol θ inhibition.
Therefore, they speculate that when both BRCA1 and 53BP1 function become impaired, Pol θ becomes essential for repairing resected ssDNA caused by the exposure of DSB ends due to 53BP1-loss.
I.e.This would correspond to a MMEJ-independent function of pol θ.
…to be investigated further.



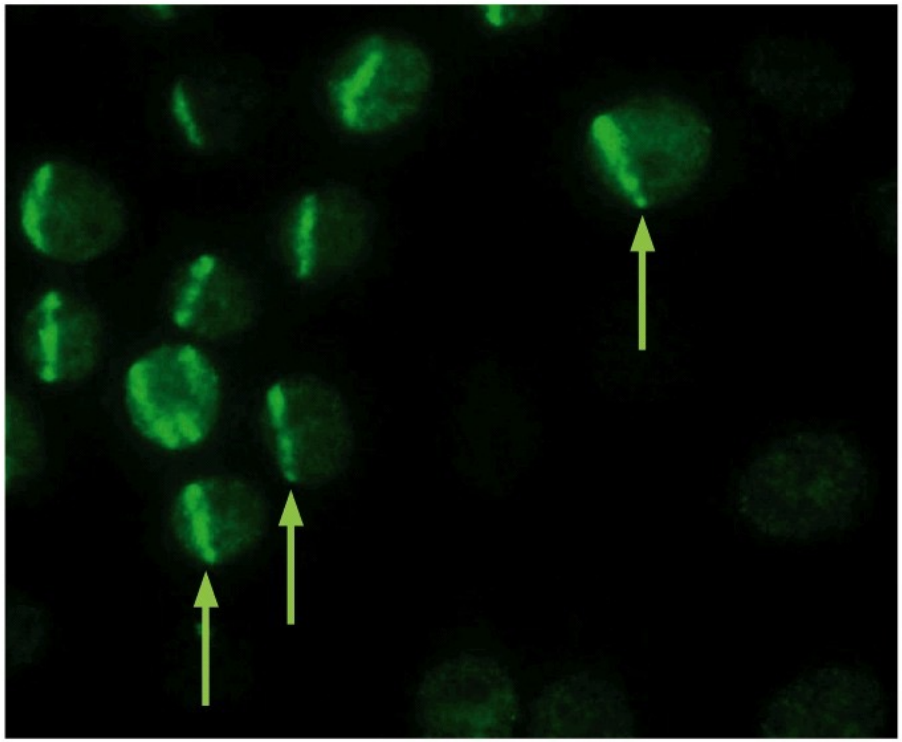
Chromatin and DNA Repair

Repair of DSB Involves Posttranslational Modifications of Nucleosomes and other Proteins

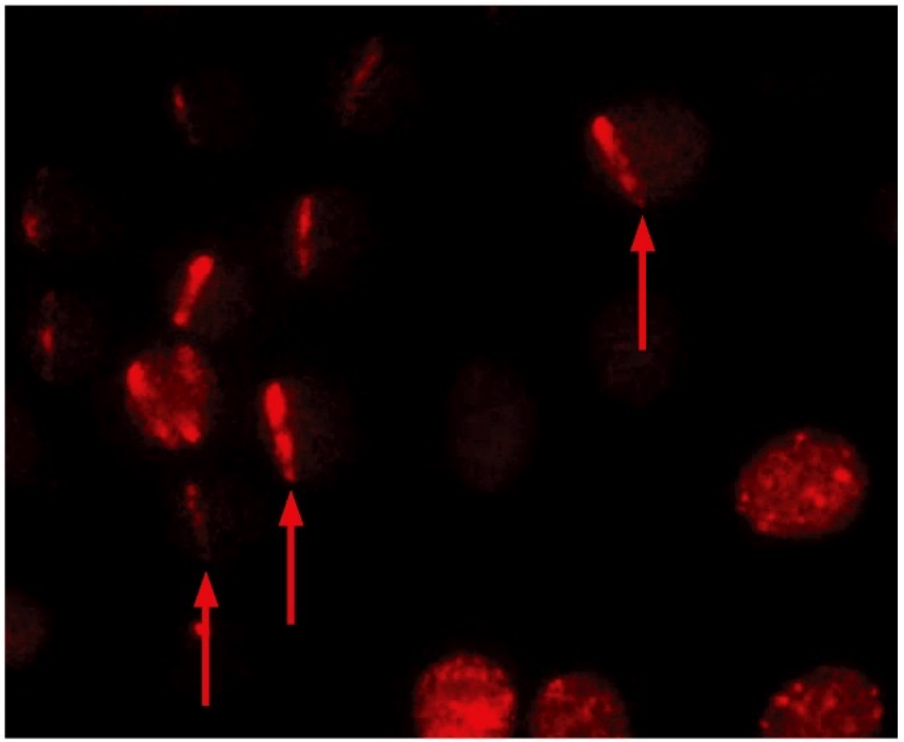
- Detect DNA damage
- Remodel local chromatin to provide access
- Reorganize nucleosome-DNA template for processing and repair
- Restore local chromatin organization after repair

See *Cell* 152, 1344 (2013) for a Review

Colocalization of BRCA1 with γ-H2AX at ds DNA Breaks



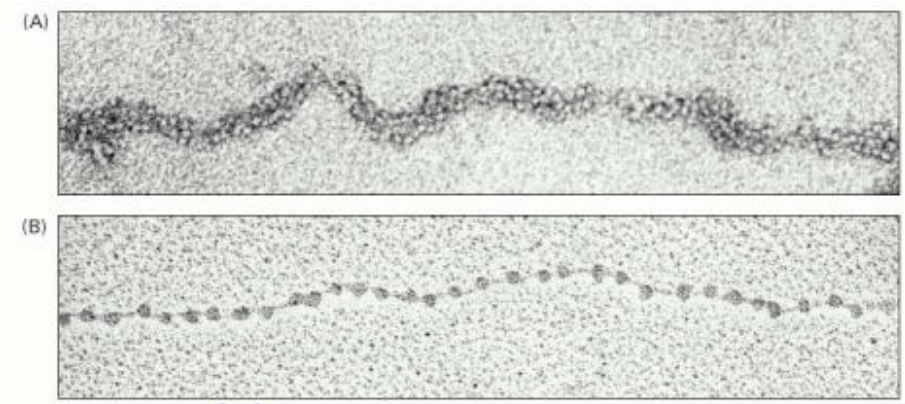
γ-H2AX



BRCA1

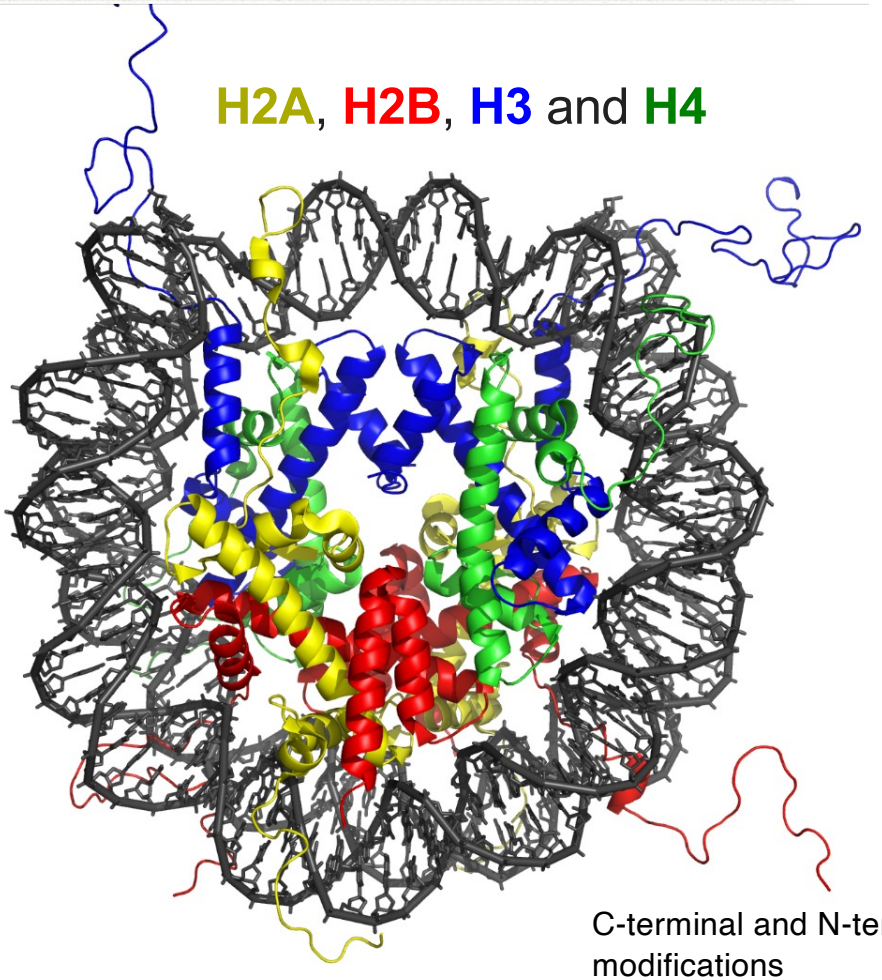
Figure 12.28. Weinberg, The Biology of Cancer

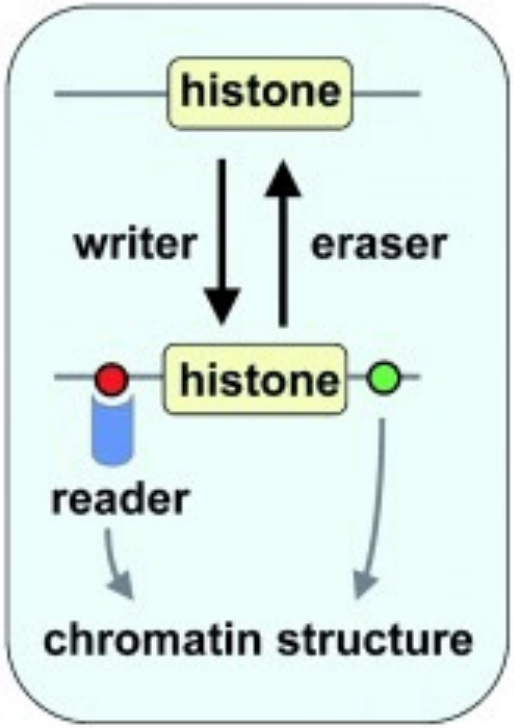
A 355-nm UV laser was used to paint stripes across nuclei



30 nm fiber (interphase chromatin analyzed by EM)

Linker histone H1 removed: “beads on a string”
147 bp are rolling 1.7 times around a histone octamer (two of histones H2A, H2B, H3 and H4).





C-terminal and N-terminal tails are subject to post-translational modifications

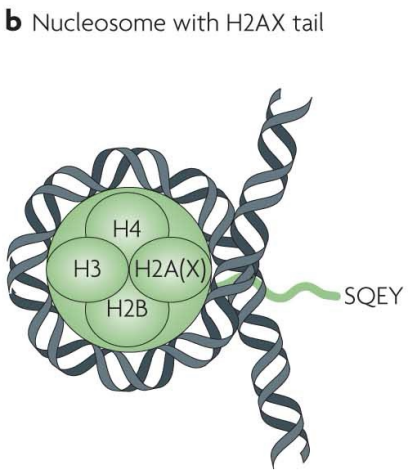
H2AX

10 % of the H2A pool of mammals
H2AX is the ‘normal’ histone H2A in budding yeast

Double strand breaks ⟶ Phosphorylation of Ser 139 (Ser c-4)
→γH2AX

a

Species	Core (C-terminal portion)	Linker	Tail
Human	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Chimpanzee	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Mouse	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSATVGPKAPAVGKKASQA	SQEY
Chicken	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGGGAASPAKAGKKGSGQQ	SQEY
Zebrafish 1	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGQAAASSGKSGKKGSSQ	SQEY
Xenopus laevis	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSGGVSTSGKKSSQQ	SQEY
Anopheles gambiae	NDEELSKLLQGTTISQGGVMPNIHSVLLPRK	TGAKAAGNSSSQEKQ	SQEY
Zebrafish 2	NDEELNTLLGGVTISEGGVLPNIQAVLLPKK	TKAAREPNAGTEAQ	SQDF
Chickpea	NDEELSKLLGSVTIANGGVLPNIHQTLLPKK	VGKGKGEIGSA	SQEF
Tetrahymena pyriformis	NDEELNKLMANTTIADGGVLPNINPMLLPSK	SKKTESRGQA	SQDI
Budding yeast	NDDELNKLLGNVTIAQGGVLPNIHQNLLPKK	SAKATKA	SQEL
Fission yeast	NDEELNKLLGHVTIAQGGVVPNINAHLLPKT	SGRTGKP	SQEL
Giardia intestinalis	KDKELATIFANVTIREGGVARSAKEGREGKG	SHR	SQDL



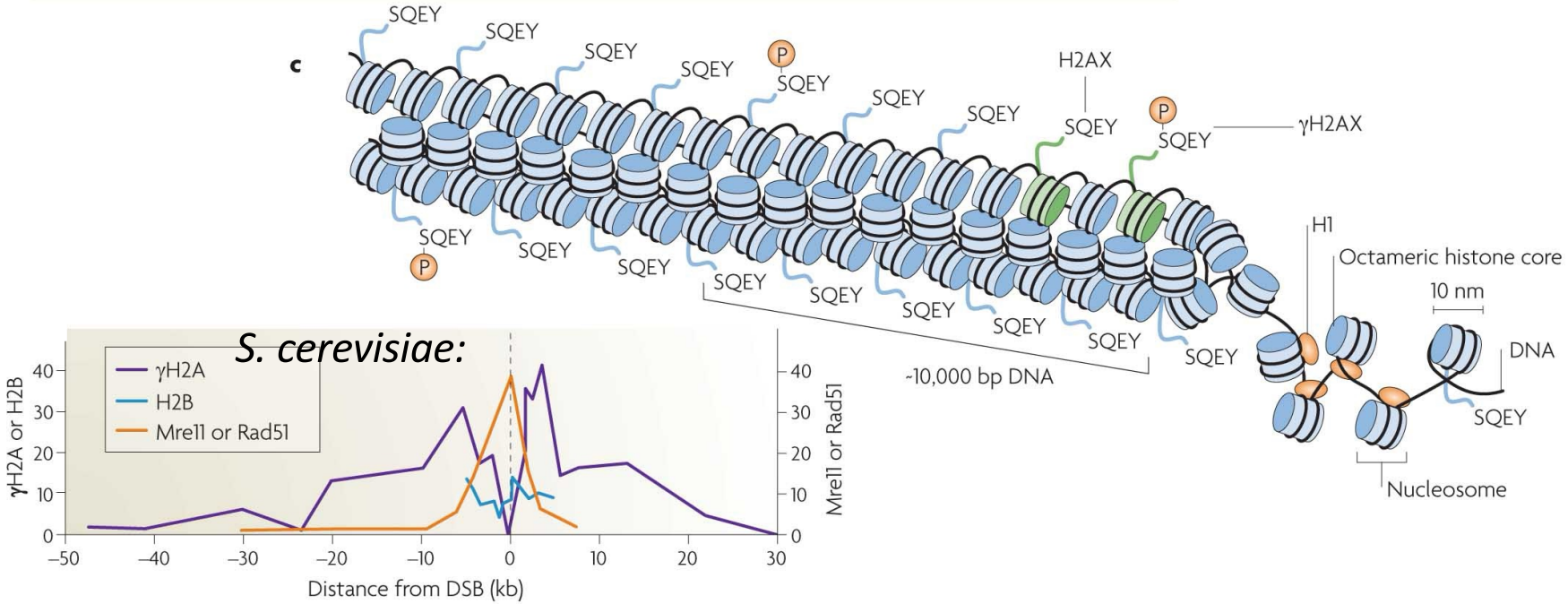
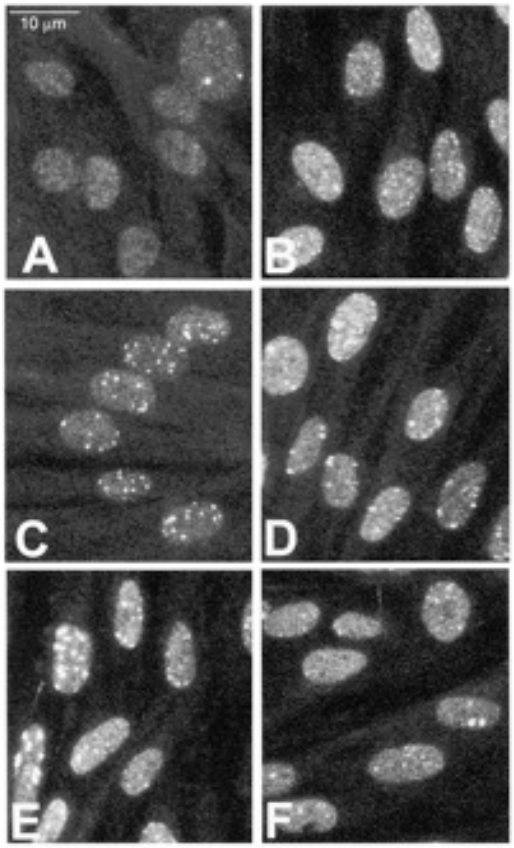


Figure 3 | **H2AX and γH2AX foci. a** | H2AX is an H2A histone with a core sequence conserved with other H2A species and a tail conserved through evolution connected by a linker of variable length. **b** | The SQEY tail extends from the core nucleosome near the entry and exit point of the DNA. **c** | The nucleosomes form a 30 nm fibre with H2AX molecules in every fifth nucleosome on average in mammals and every nucleosome in yeast. Approximately 10% of the H2AX molecules are phosphorylated at any one time in a focus.

From NatureRevCancer 8, 957 (2008)

Nuclear γ-H2AX foci are a Direct Consequence of DNA Double Strand Breaks

Irradiation: 0.6 Gy



Control

3 min.

15 min.

30 min.

60 min.

180 min.

Rogakou et al. (1999). JCB (146) pp. 905-916

Ionizing radiation
Replicative stress

↓

ATM/ATR

↓

γH2AX

Spreading of γ-H2AX at DSB

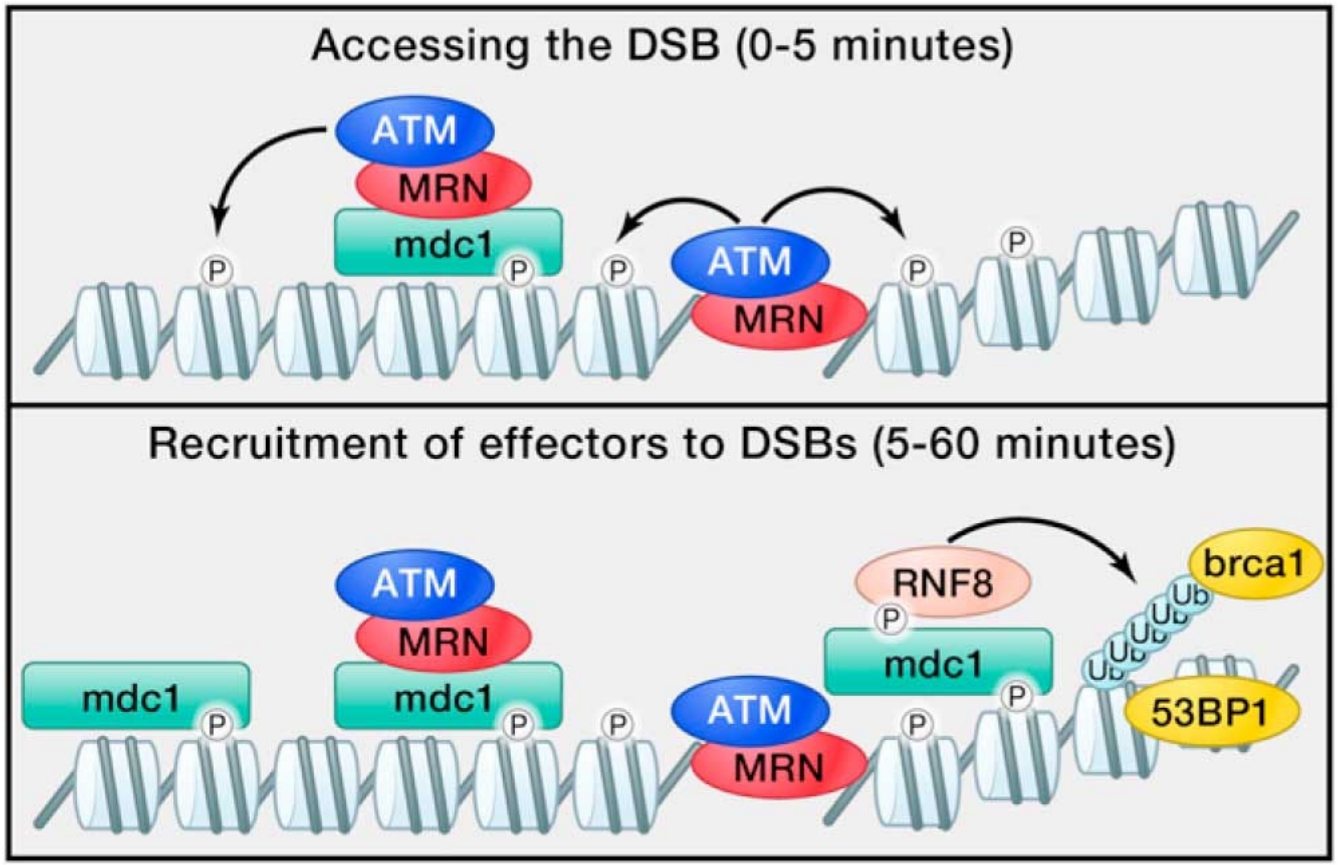


Figure 1. The Mechanism of DSB Repair

Top: ATM phosphorylates H2AX at DSBs, creating a binding site for the mdc1 protein. ATM-MRN complexes then associate with mdc1, promoting the spreading of γH2AX along the chromatin for hundreds of kilobases.

Bottom: mdc1 recruits multiple DSB-repair proteins, including the RNF8/RNF168 ubiquitin ligases, to sites of damage. Chromatin ubiquitination then facilitates loading of the brca1 complex and 53BP1 DSB-repair proteins.

P = phosphorylation, Ub = ubiquitination, MRN = mre11-rad50-nbs1 complex.

Role of H2AX

- **Repair of double-strand breaks: NHEJ & HR**

(impaired recruitment of 53BP1 and BRCA1 in absence of H2AX)

- **Mouse H2AX -/- :**

Chromosomal breaks and translocations
Small size
Lymphomas & solid tumors

Key Concepts

- Overview of DNA repair machineries.
- DNA double strand breaks: cell cycle regulation and mechanisms involving HR, NHEJ, MMEJ
- Concept of synthetic lethality: BRCA1 and BRCA2 mutant cells are addicted to PARP-1 and MMEJ.
- Resistance: one important mechanism to be discussed (paper on Wednesday: please start reading); BRCA1-mutant cells become resistant to PARPi by loss of 53BP1 which counteracts end resection.
- MMEJ inhibitors are an alternative target to kill HR-deficient cancer cells. They can also overcome PARP-1i resistance.
- H2AX and gamma-H2AX at damaged DNA.