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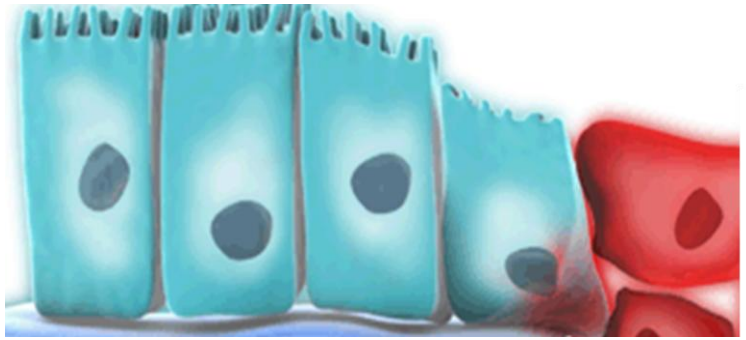
Instituto_Nacional de Saúde Doutor Ricardo Jorge

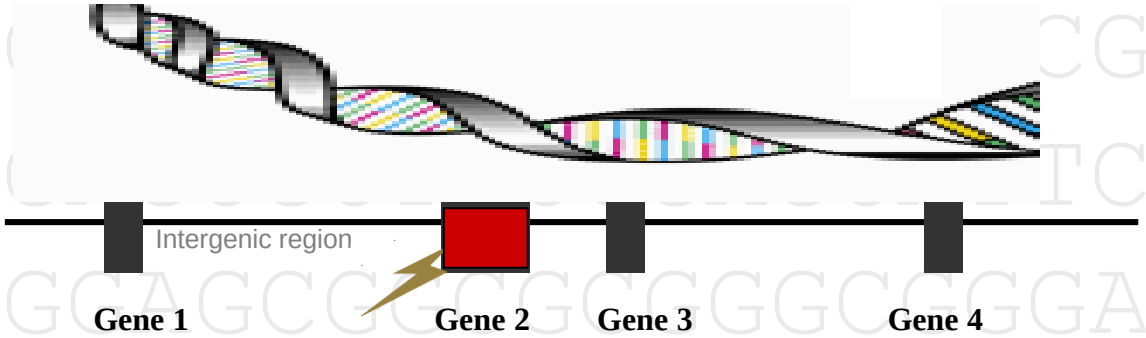


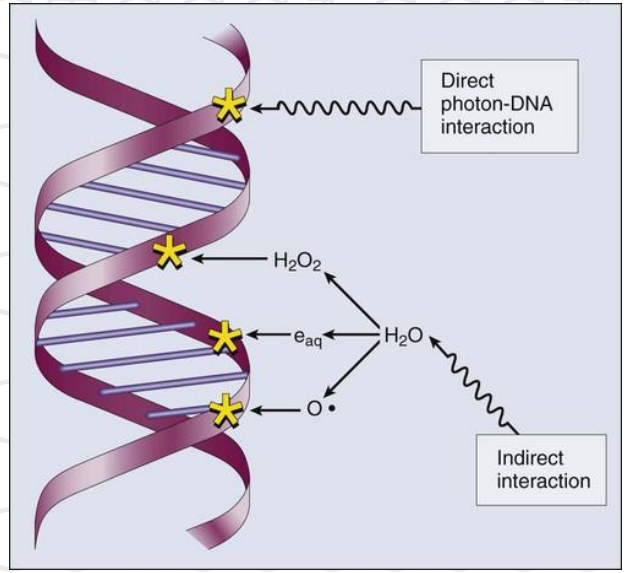
Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

DNA damage repair

DNA damage

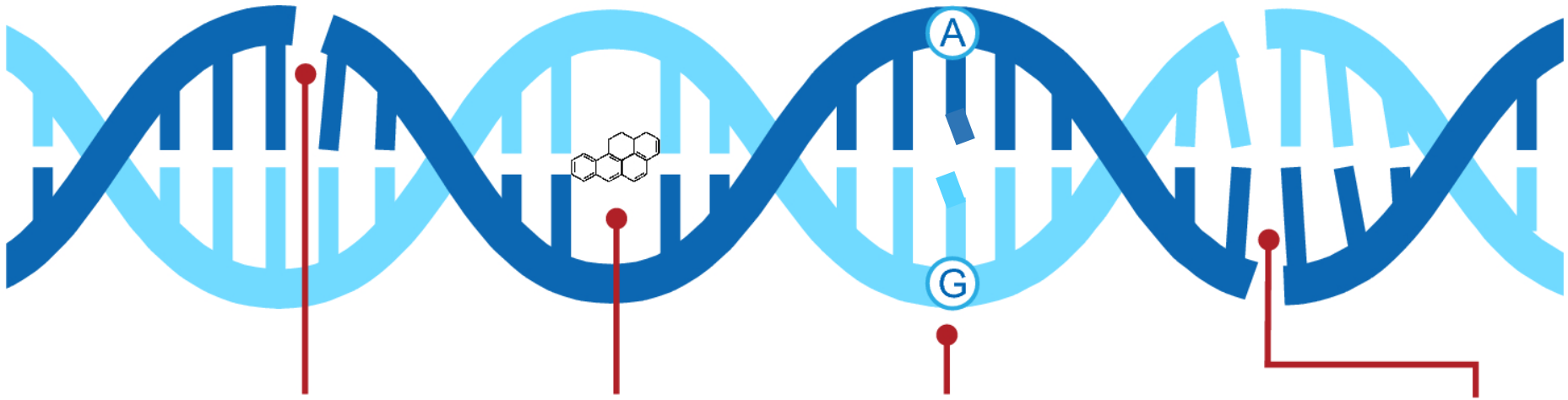




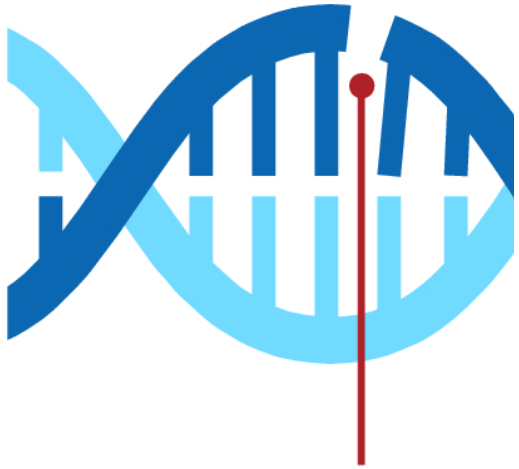
..any alteration in the structure or chemical composition of DNA

- **radiation (UV light, X rays, radioactivity; radiotherapy)**
- **environmental chemicals (benzopyrenes, nitrosamines,..)**
- **reactive oxygen species**
- **errors during DNA replication and cell division/spontaneous deamination**

Photons are electrically neutral ‘wave packets’ with electromagnetic force of sufficient energy to dislodge electrons when hitting a chemical bond



Type of damage	DNA single-strand breaks	Bulky adducts	Base mismatches, insertions and deletions	DNA double-strand breaks	
DNA damage response pathway	Base excision repair	Nucleotide excision repair	Mismatch repair	Homologous recombination repair	Non-homologous end joining
	BER	NER	MMR	HRR	NHEJ



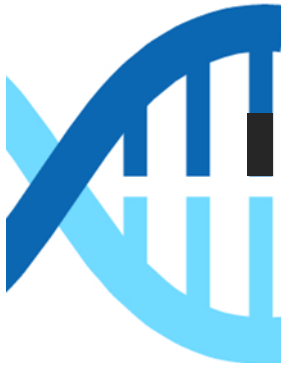
Type of damage

DNA single-strand breaks

DNA damage response pathway

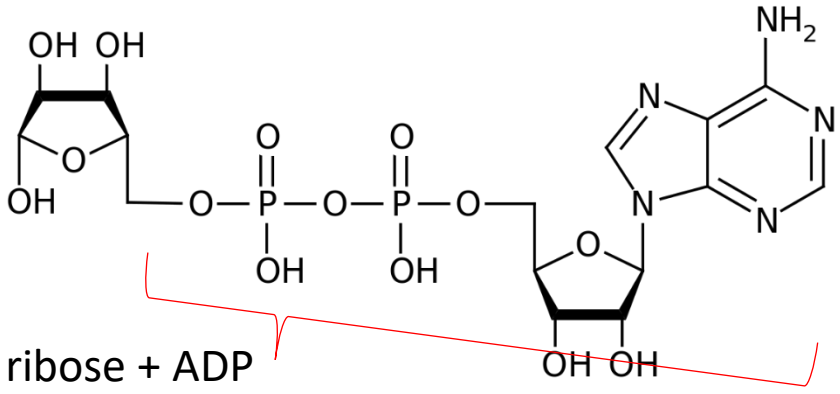
Base excision repair

BER



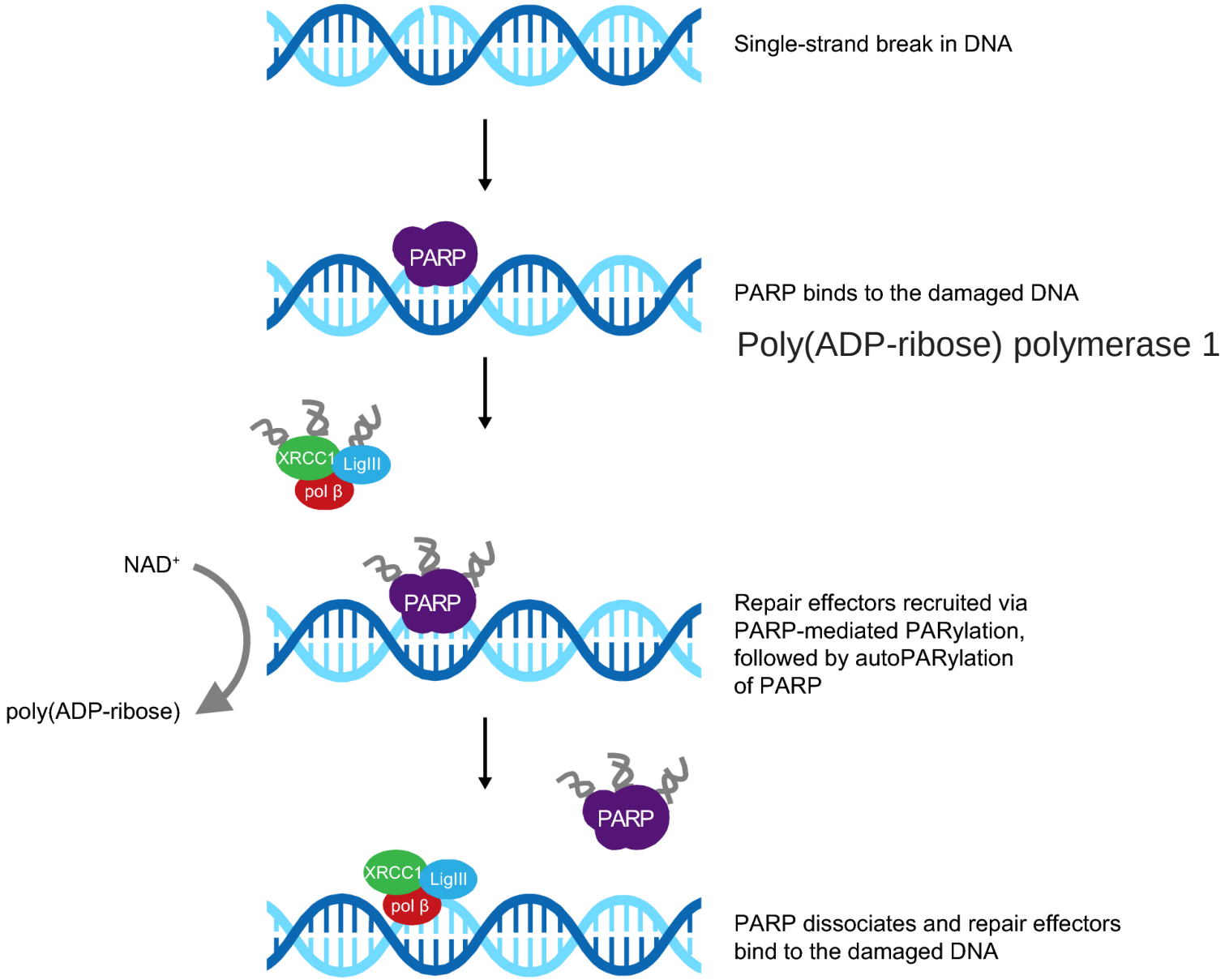
also repairs
- oxidized bases
- apurinic/apyrimdinic (AP) sites

BER of a DNA single-strand break



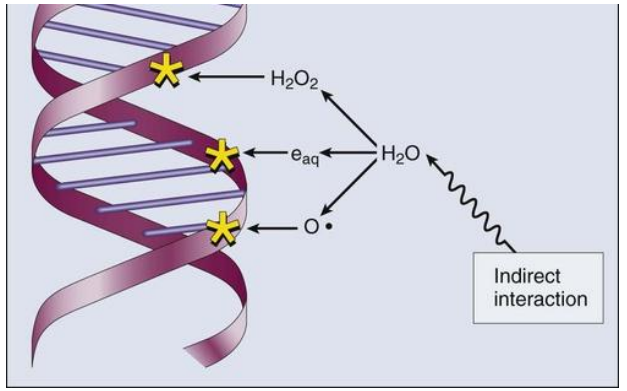
ribose + ADP

PAPylation= reversible post-translational modification on hydrophilic amino acids of target proteins



BER of
- oxidized bases
- AP sites
(apurinic/apyrimidinic site)

ROS = Reactive oxygen species



Cellular anti-oxidant defense mechanisms

Oxygen (O_2)

+e

Superoxide (O_2^-)

+ O_2^- (superoxide dismutase)

Hydrogen peroxide (H_2O_2)

+ NO

Peroxynitrite anion ($ONOO^-$)

Fe^{II} (Fenton reaction)

Hydroxyl radical ($\bullet OH$) + Fe^{III}

Hypochlorite ion (OCl^-)

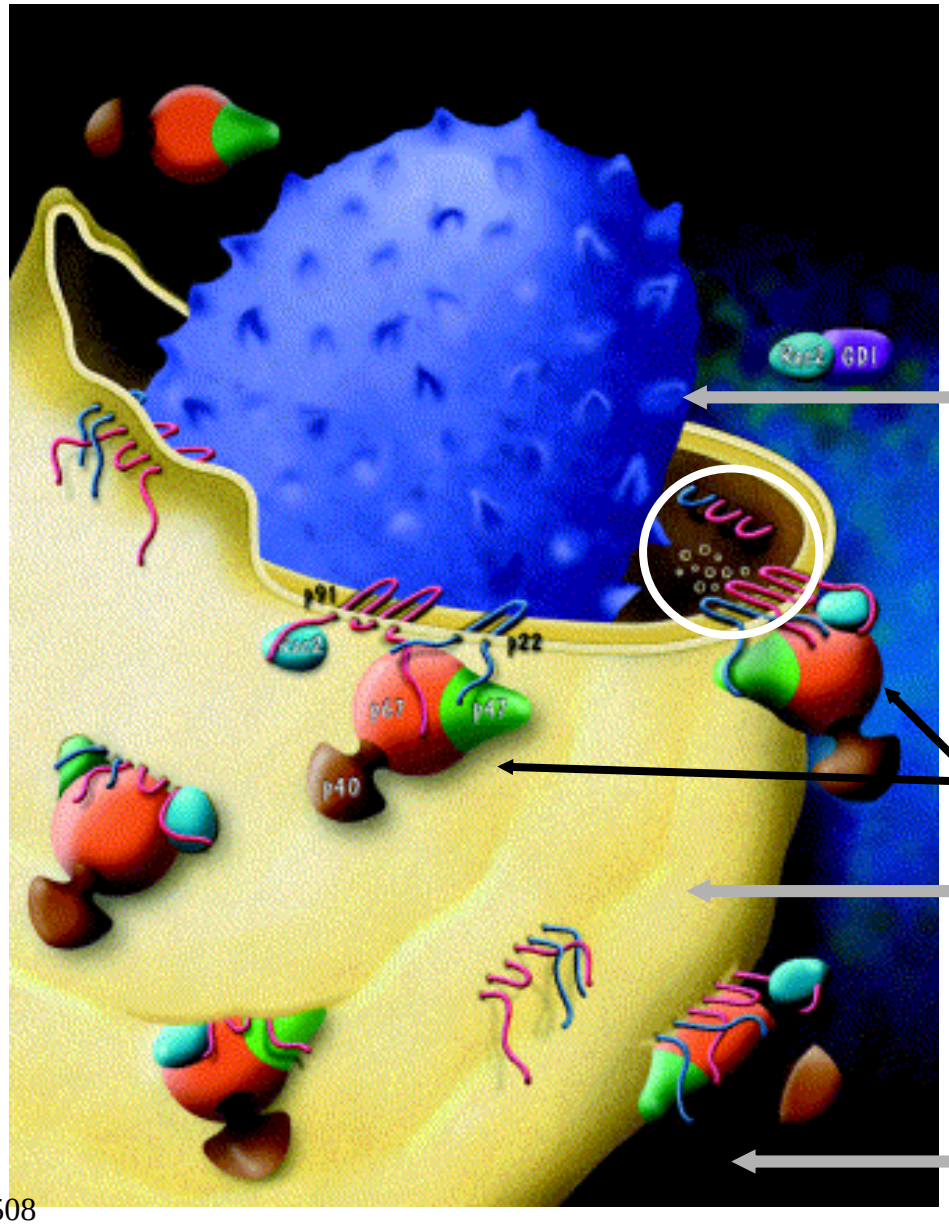
+ Cl^-

Mutagenic !!
Causes oxidized bases (e.g. 8-oxoguanine) or strand breaks

Hool, LC (2006).C*linExperim Pharmacol Physiol* **33** (1-2), 146-151.

Besides radiation, our cells can generate ROS..

Neutrophils generate ROS for cellular immunity



Phagocytosed bacteria

NADPH oxidase (Nox2)

Phagosome

Cytoplasma

Bokoch and Knaus (2003) TIBS 28, 502-508

Besides radiation, our cells can generate ROS..

Source 1 : Cellular NADPH oxidases

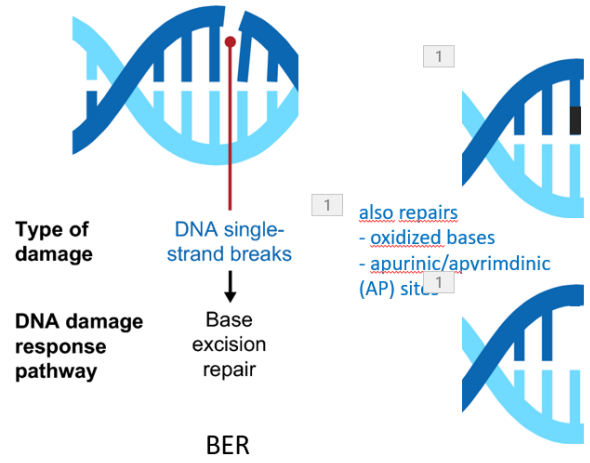
Source 2 : Nitric oxide synthases

Controlled generation of small and local amounts of ROS as cell signalling response

Source 3: mitochondrial metabolism

- by-product of mitochondrial electron transport chain;

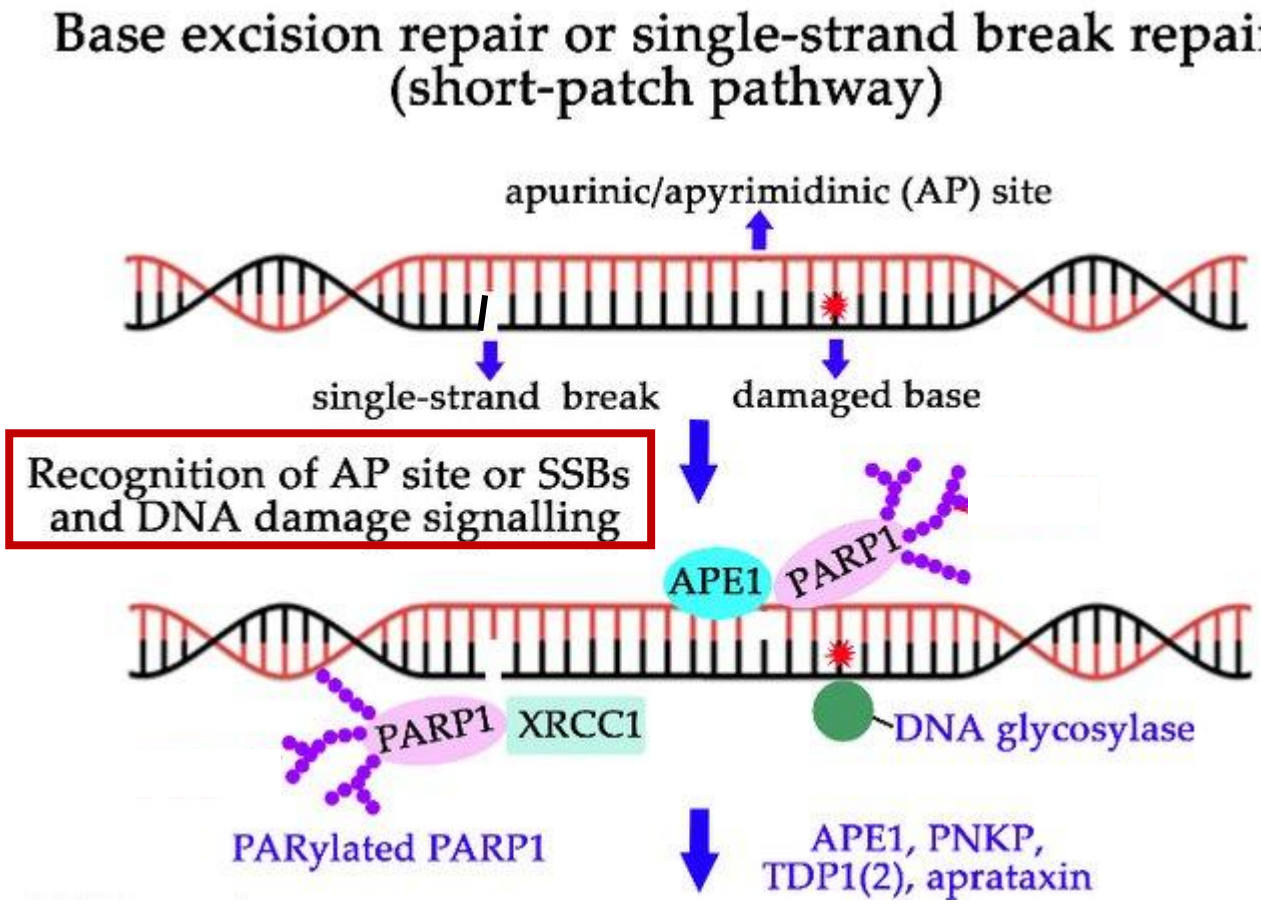
- metabolic imbalances can lead to **oxidative stress** (reduced antioxidant defense and increased ROS in tissues (oxidation of DNA, lipid and proteins)

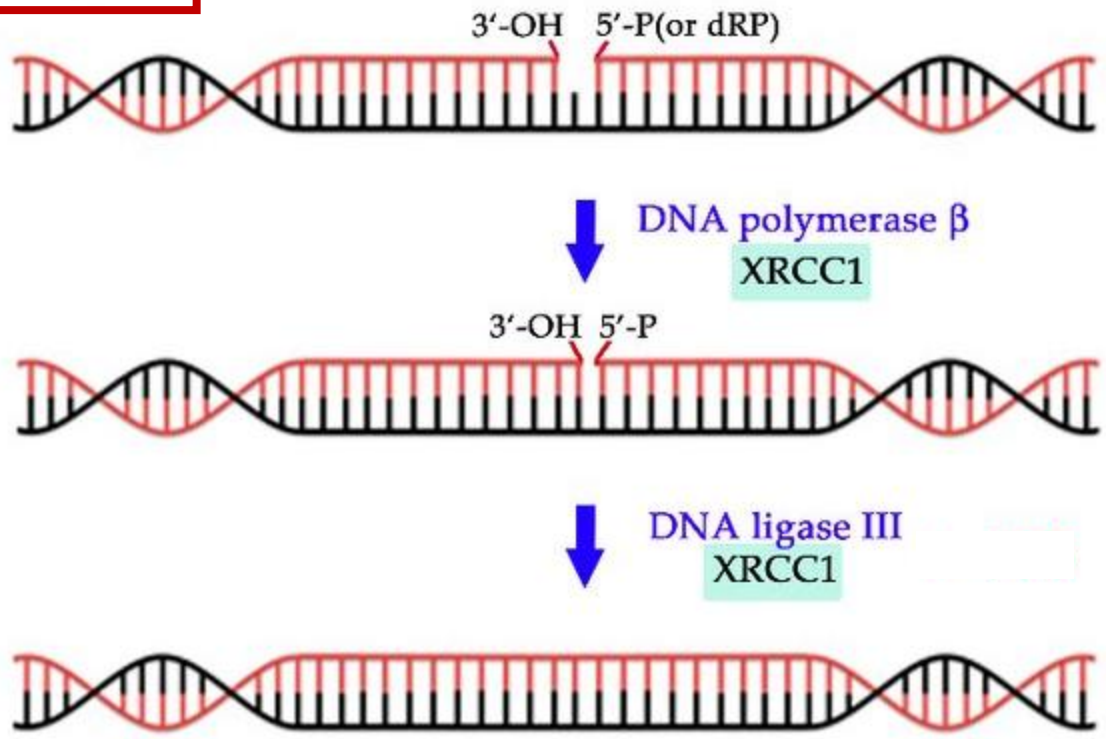


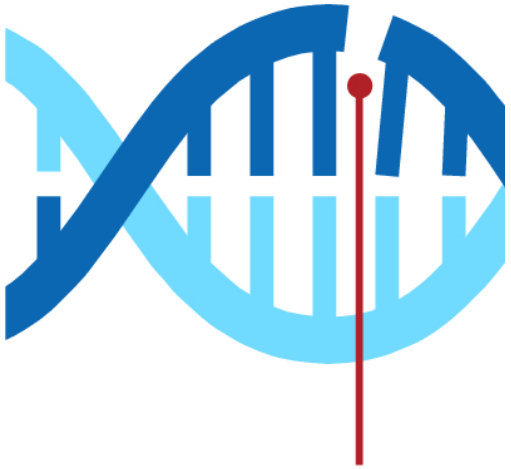
BER

Repair:
- Glycosylase creates a 1-bp gap;
- DNA pol **β** closes gap

DNA repair







Type of damage

DNA single-strand breaks

DNA damage response pathway

Base excision repair

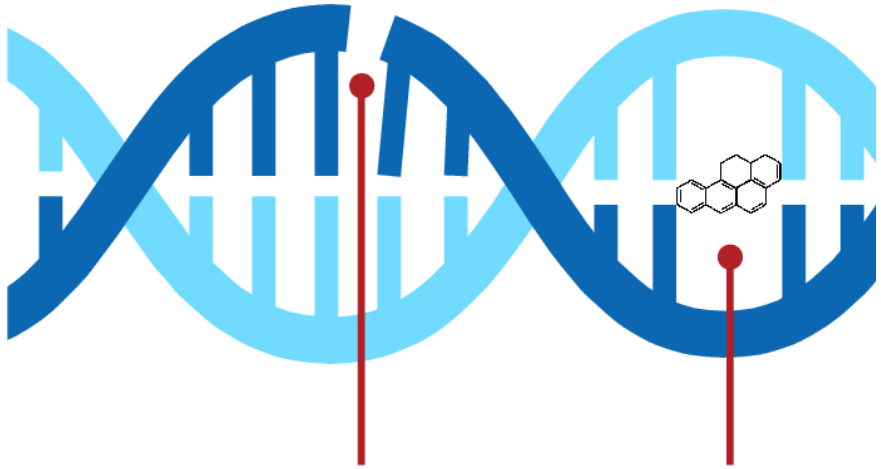
BER in cancer

- somatic missense mutations in the DNA pol β gene (*POLB*) found in ~30% of epithelial cancers
  → increased mutation rate and tumour mutation burden;

- genetic predisposition to cancer: SNPs in the POLB gene (promoter or coding sequence) as a potential risk factor?

- homozygous mutations in the DNA glycosylase MYH in a rare form of hereditary colon cancer with polyposis
  → increased mutation rate and mutation burden

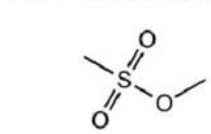
Alkylating chemicals
Platinum drug cross-links



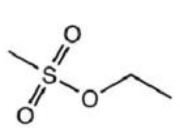
Type of damage	DNA single-strand breaks	Bulky adducts	also for UV light-induced damage
	↓	↓	
DNA damage response pathway	Base excision repair	Nucleotide excision repair	

Structure of representative DNA damaging chemicals

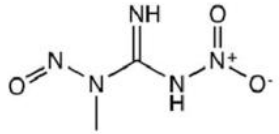
(A) **Alkylating agents**



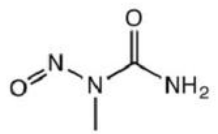
Methyl methanesulfonate



Ethyl methanesulfonate



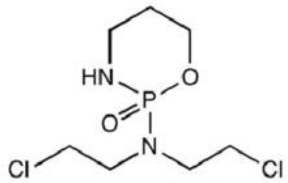
N-methyl-N'-nitro-N-nitrosoguanidine



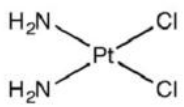
Methylnitrosourea

experimental mutagens

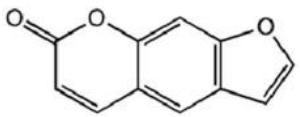
(B) **Crosslinking agents**



Cyclophosphamide



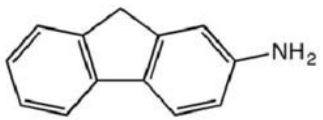
Cisplatin



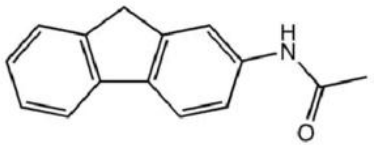
Psoralen

chemotherapeutic

(C) **Aromatic amines**

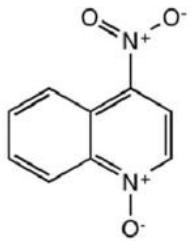


2-Aminofluorene



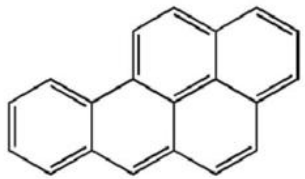
N-acetyl-2-Aminofluorene

(E) **Reactive electrophiles**

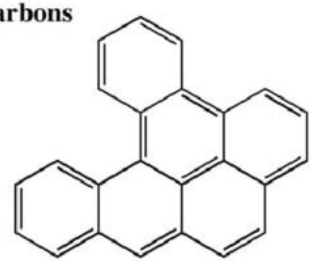


4-Nitroquinoline 1-oxide

(D) **Polycyclic aromatic hydrocarbons**

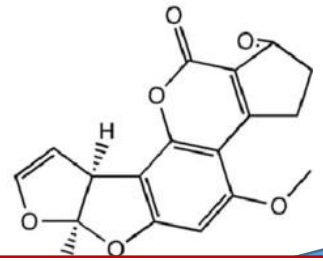


Benzo[α]pyrene



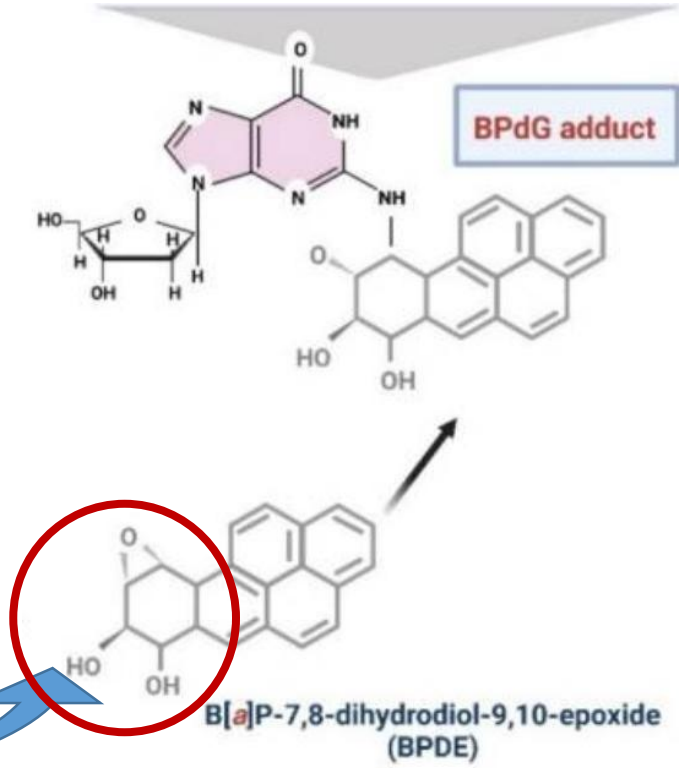
Benzo[α,l]pyrene

(F) **Toxins**



Tobacco smoke

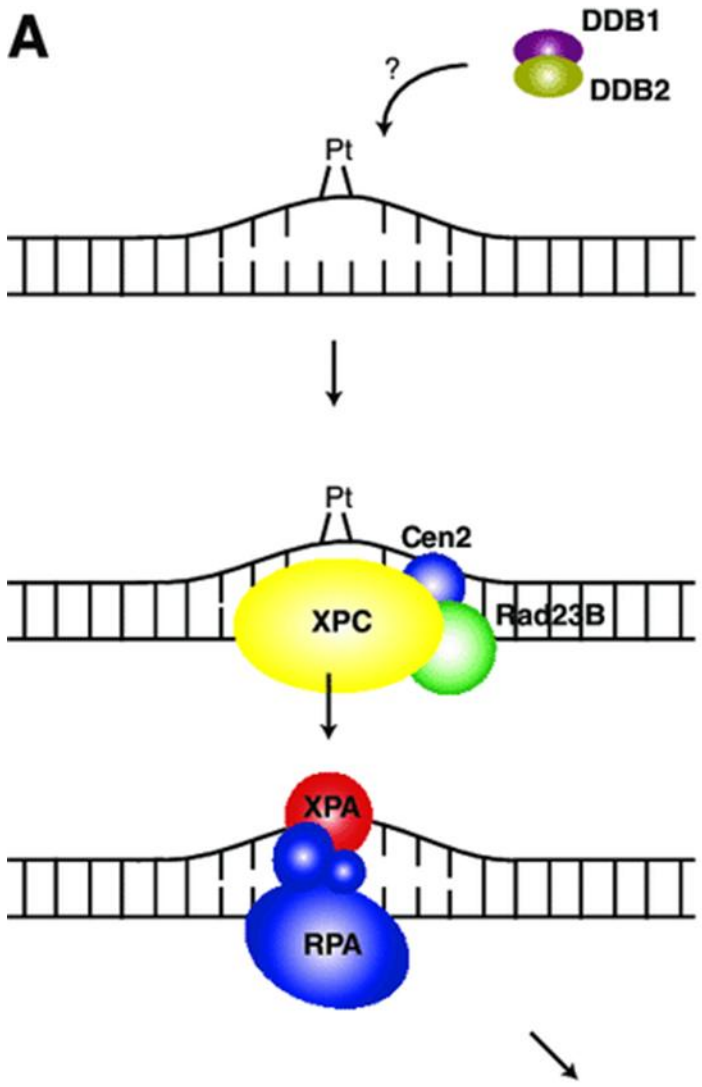
Biotransformation by CytP450



Tobacco =

has a systemic mutagenic effect due to bio-transformation of BP in the liver into highly reactive alkylating compounds

NER
to repair
DNA
adducts



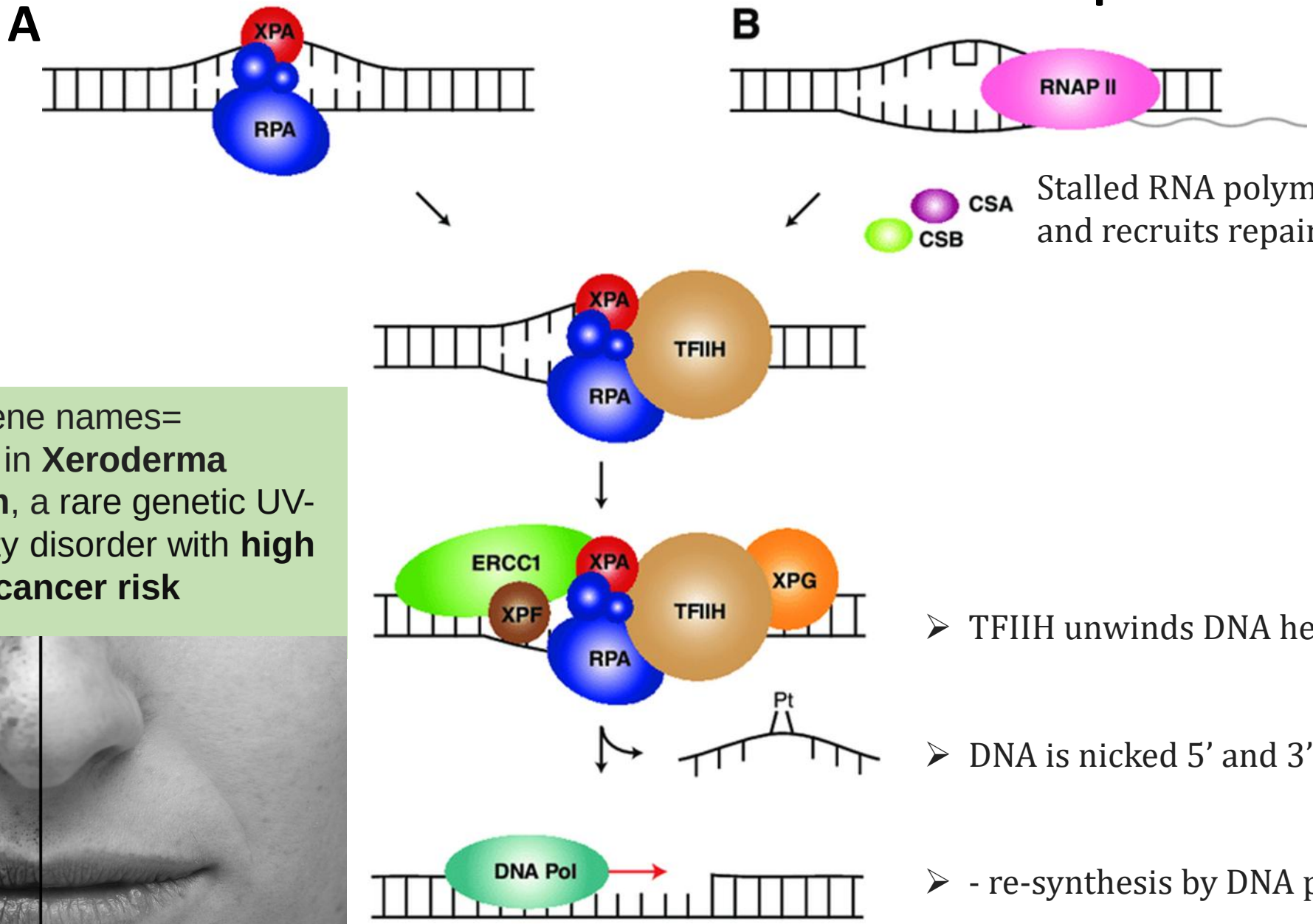
A= global genome NER

Recognition of DNA helix-destabilizing adducts

Recruitment of repair-promoting factors

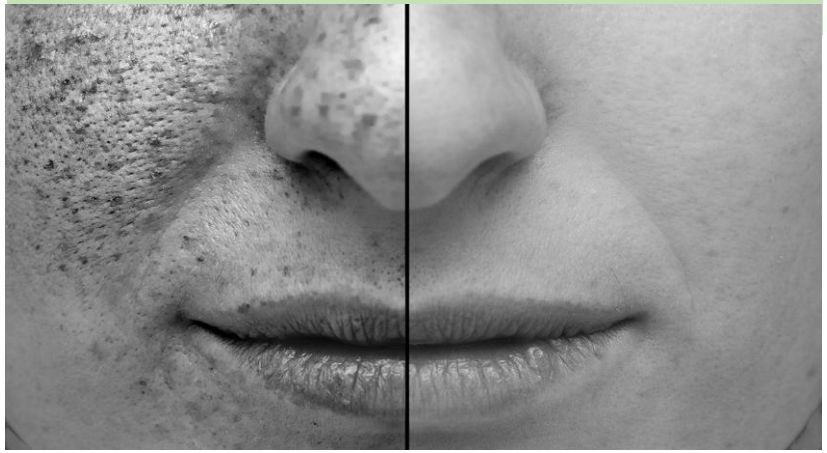
A= global genome NER

B= Transcription-coupled repair



Stalled RNA polymerase is recognized and recruits repair factors

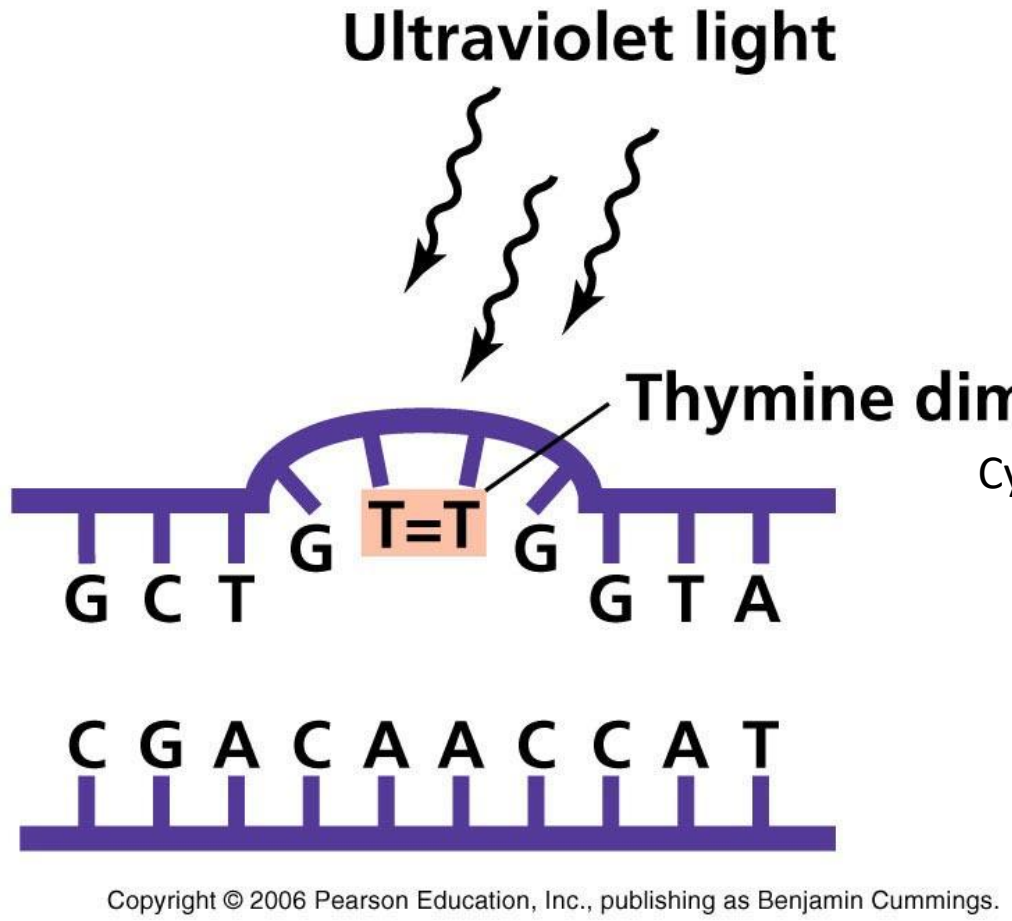
XP gene names= mutated in **Xeroderma pigmentosum**, a rare genetic UV-hypersensibility disorder with **high skin cancer risk**

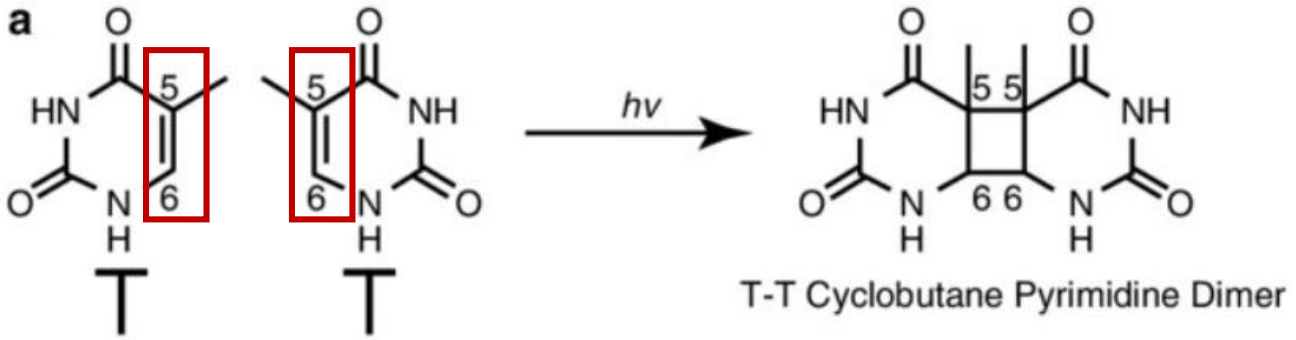


- TFIIH unwinds DNA helix;
- DNA is nicked 5' and 3' of detected site;
- - re-synthesis by DNA pol

NER
to repair
UV lesions

UV lesions removed by the **nucleotide excision repair pathway**





Thymine dimer or Cytosine dimer or C/T heterodimer

Cyclobutane Pyrimidine dimer (CPD)

- around 100 daily lesions per skin cell → **melanoma!!;**

- typical mutation signature =

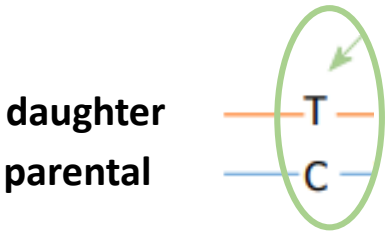
predominant CC→TT substitutions at dipyrimidine sites

(C deamination to U at unrepaired sites, then U pairs with A)

Alkylating chemicals
Platinum drug cross-links

Type of damage	DNA single-strand breaks	Bulky adducts	Base mismatches, insertions and deletion
DNA damage response pathway	Base excision repair	Nucleotide excision repair	Mismatch repair

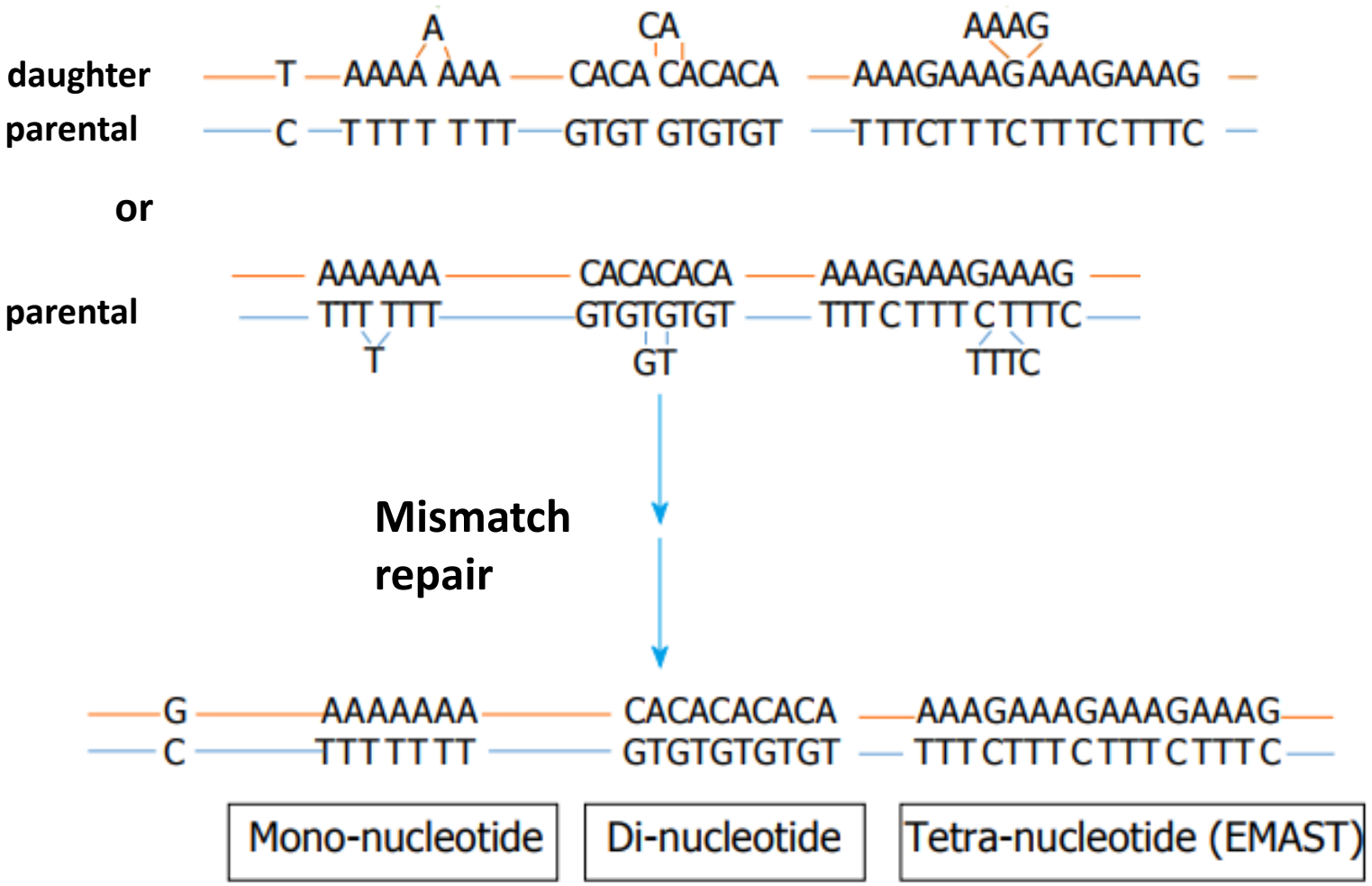
Mismatch errors happen during DNA replication



The spontaneous DNA polymerase error rate during DNA replication is low, being 1 in 10^9 to 10^{10} nucleotides…

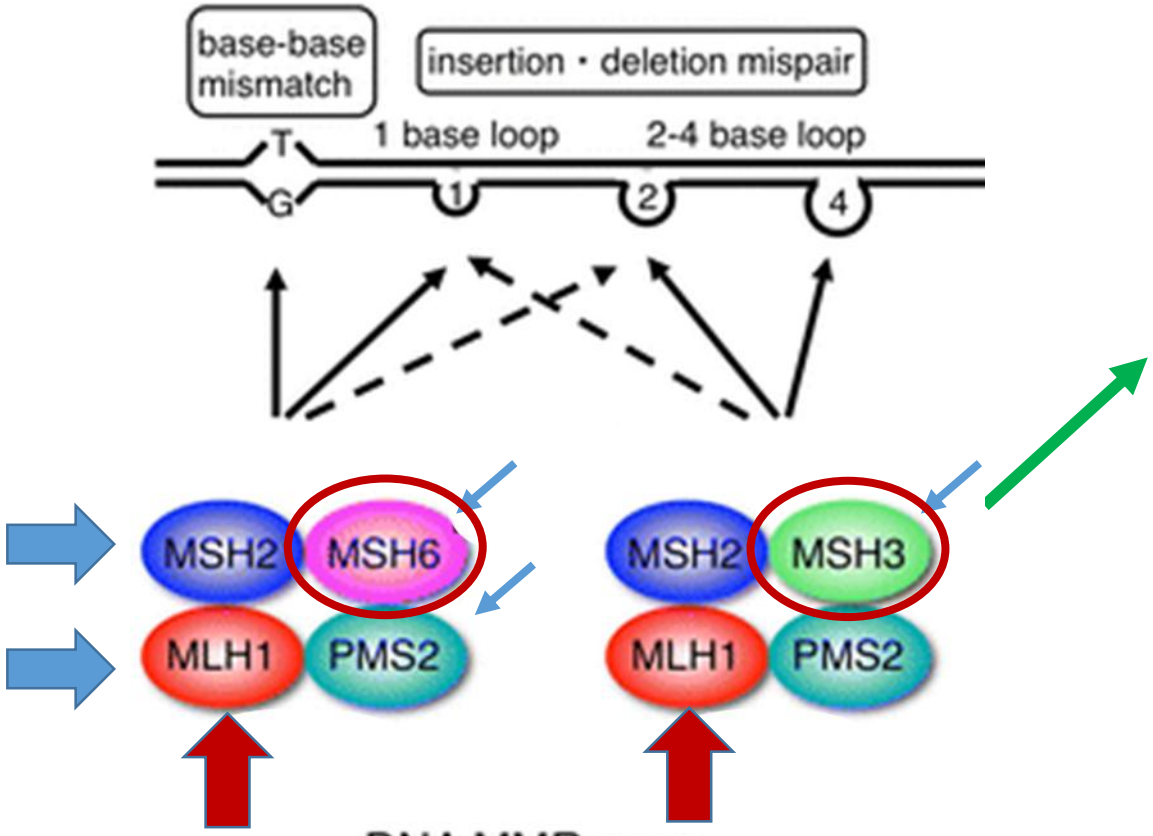
…… except at regions of repetitive sequence

Mismatch errors happen during DNA replication in repetitive sequences



Koi et al. (2018) World J Gastrointest Oncol 10, 1-14, DOI: 10.4251/wjgo.v10.i1.1

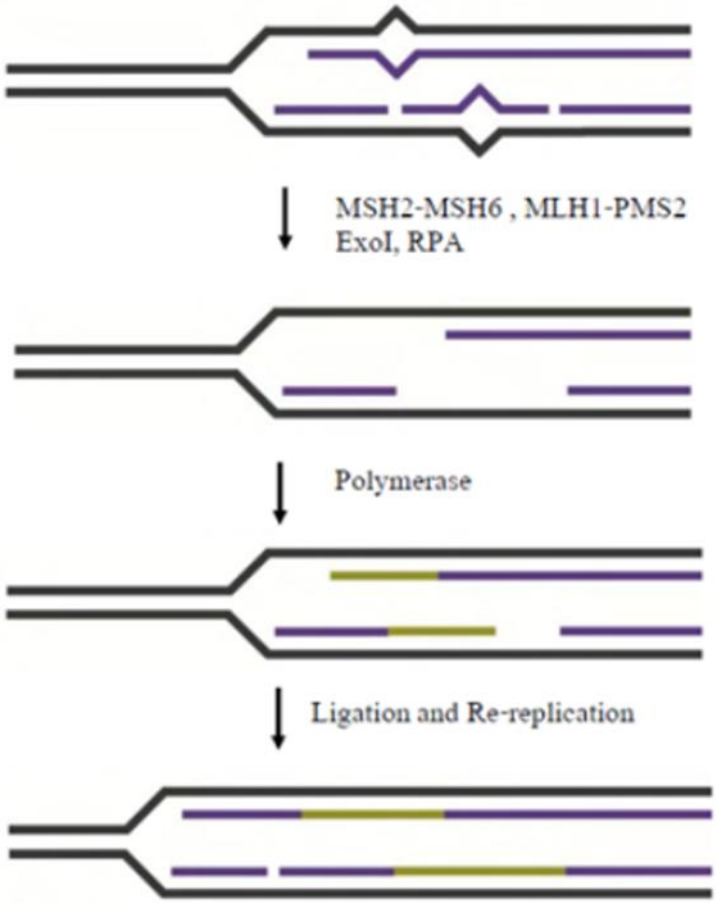
Mismatch error recognition



Mutated in hereditary colon cancer (HNPCC)

Gene silenced in sporadic tumours

Error repair during replication



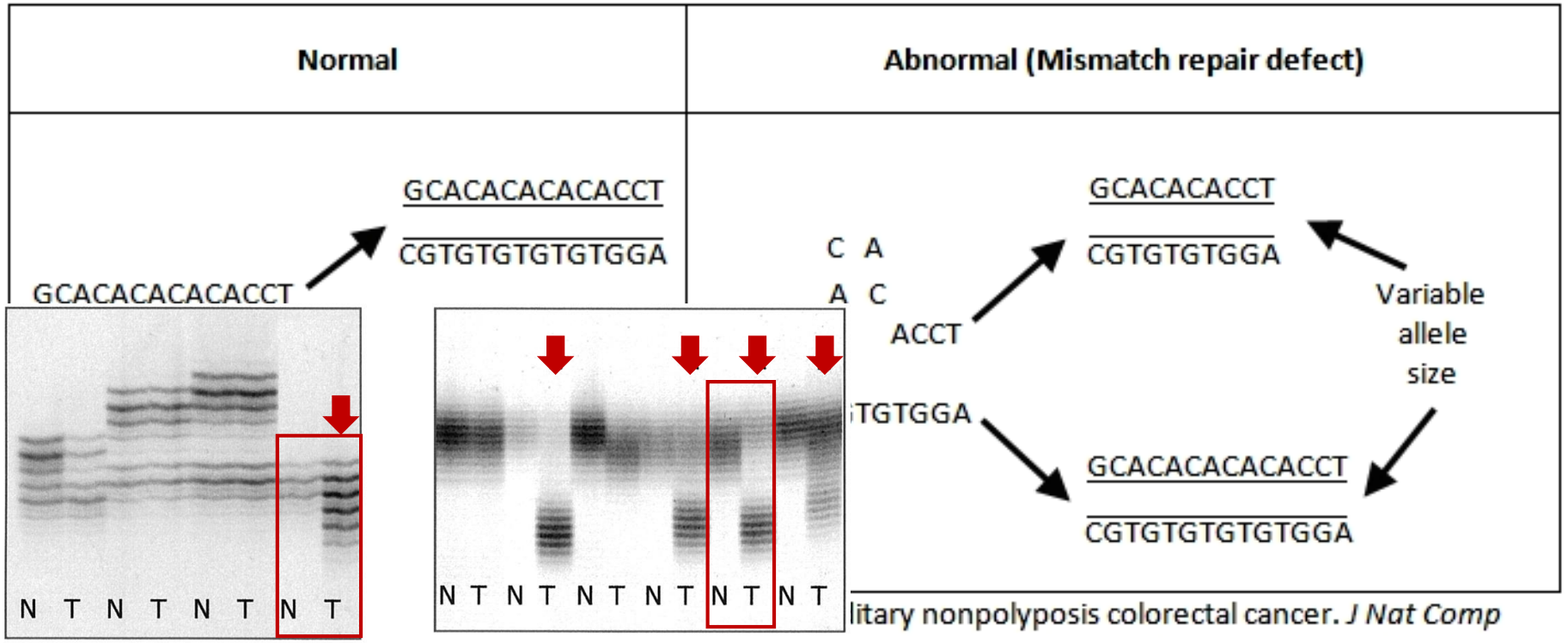
recognition

excision

gap filling

ligation

Mismatch repair deficiency causes Microsatellite instability

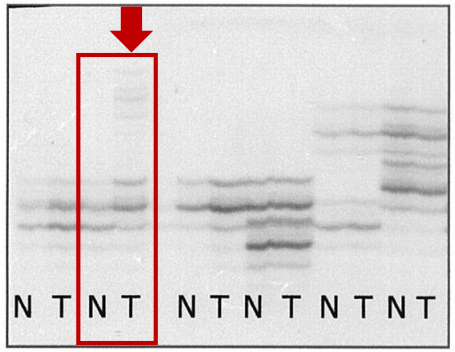


D17S250

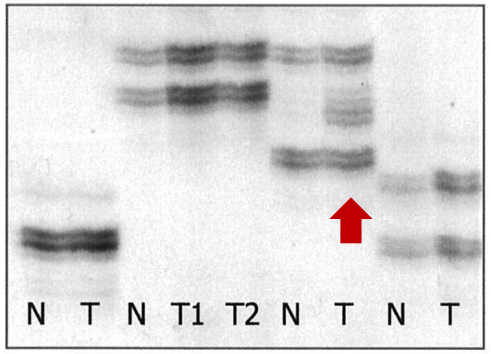
Bat 26

N= normal
T= tumour

(Single-stranded DNA electrophoresis followed by silver staining of PCR-amplified MSI markers)



D5S346



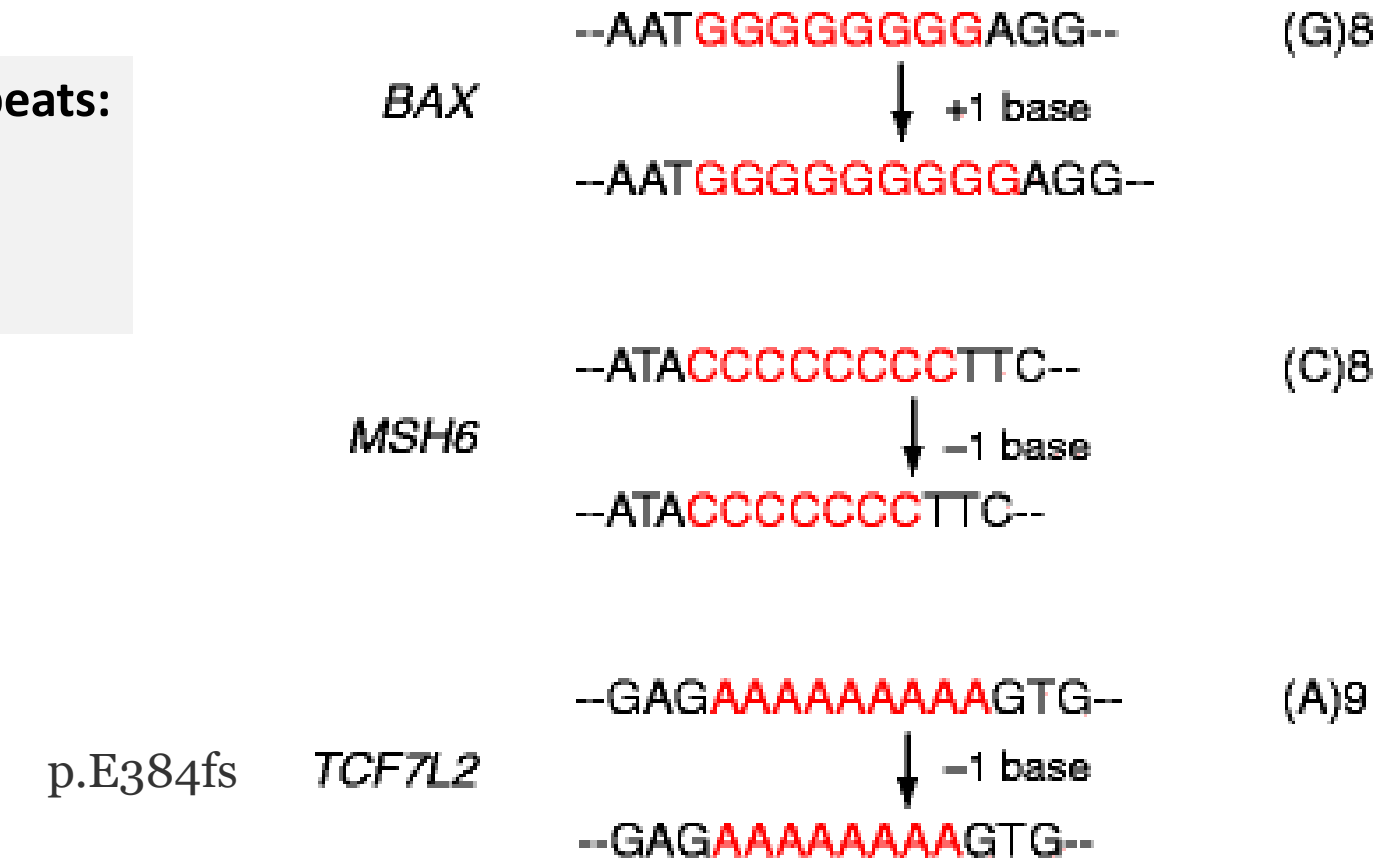
MYCL

MSI=
expansion or contraction of the size of a microsatellite repeat sequence

why care about non-coding microsatellites?

…what happens in microsatellites also happens genome-wide,
and thus also in coding sequences of some tumor supressor genes

Nucleotide repeats:
GGG- Gly
CCC- Pro
AAA- Lys

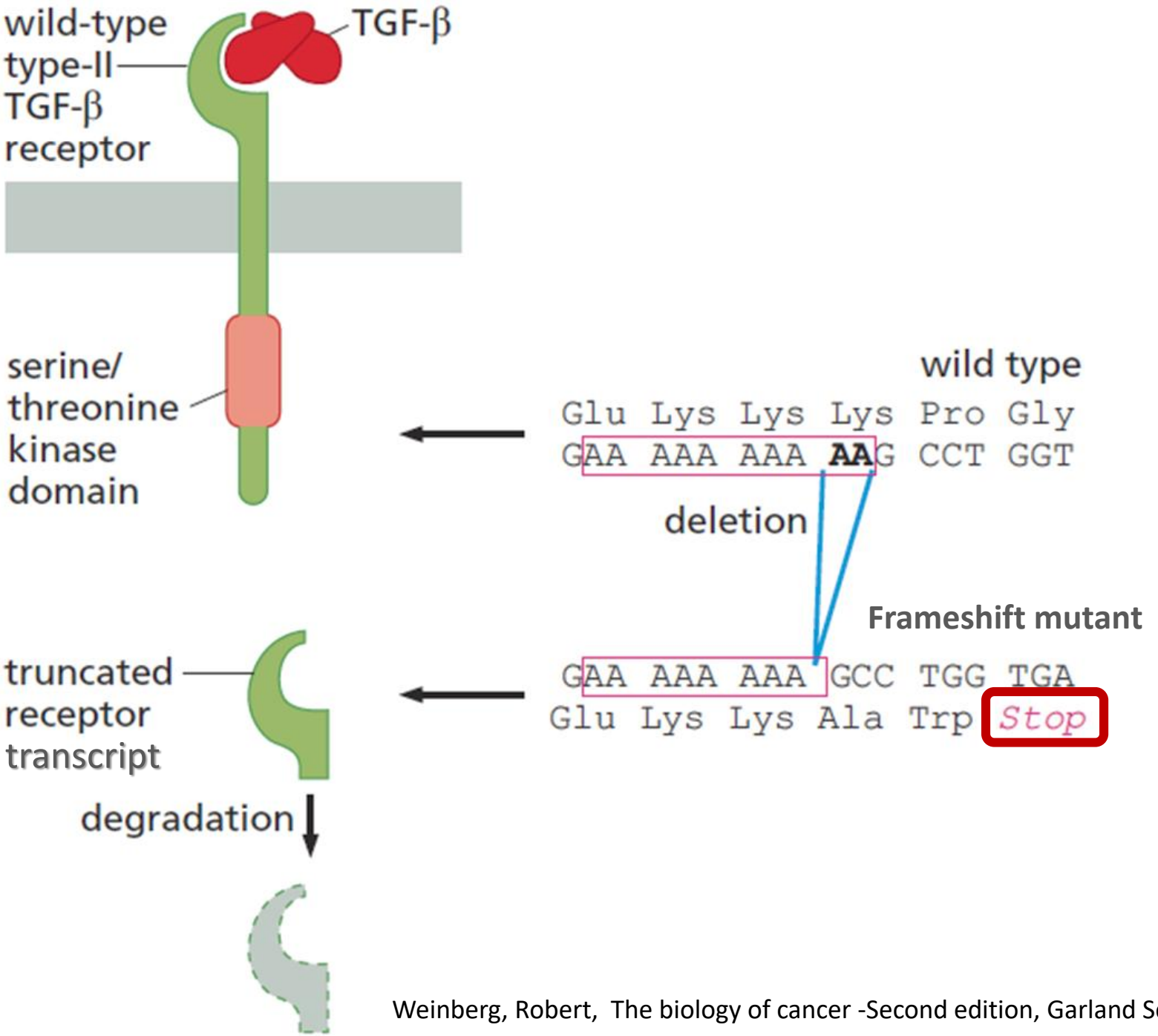


Cancer-related genes with repetitive stretches in their coding sequences:

<u>cell cycle regulation</u>
TGFBR2, IGFR2, TCF7L2, AXIN2, PTEN;

<u>apoptosis</u>
BAX, CASP5, BLC10, APAF1, FAZ;

<u>DNA repair and integrity</u>
ATR, DNA-PK, RAD50, MSH3, MSH6, CHK1



Frameshift mutation in consequence of mismatch repair deficiency

↓

loss of receptor expression

↓

progenitors of this colon cell lose the growth-inhibitory effect of TGF-β

Weinberg, Robert, The biology of cancer -Second edition, Garland Science 2014

Designations

	Normal counterpart:
Microsatellite instability (or MSI),	**Microsatellite stability (or MSS),**
Mismatch repair deficiency (dMMR),	**Mismatch repair proficiency (pMMR),**
DNA sequence instability,	**DNA sequence stable**
Mutator phenotype,	
High tumour mutation burden* ≥ 10 mut/Mb	**Low tumour mutation burden** ~1 mut/Gb

* in rare cases also caused by missense mutations in **POLE/ POLD** genes
→ loss of proofreading during replication;
* also observed in skin and lung tumours due to high exposure to mutagenic agents

MSI= the mutator phenotype

MSI is found in endometrial> colon, stomach> cervix, kidney tumours

a large number of mutations are produced randomly throughout the genome of cancer cells

Whole-genome sequencing of tumour samples

detected 1000 – 100 000 genetic alterations

Distinguish the mutations that cause or accelerate cancer

— the **driver mutations**

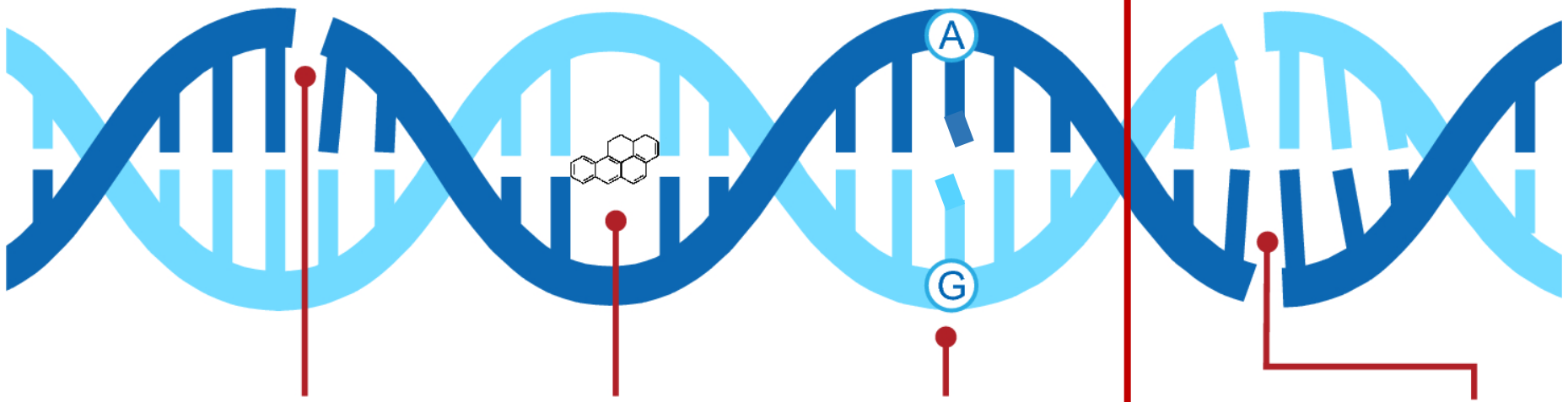
from

by-products of high mutation rate that do not provide growth advantage

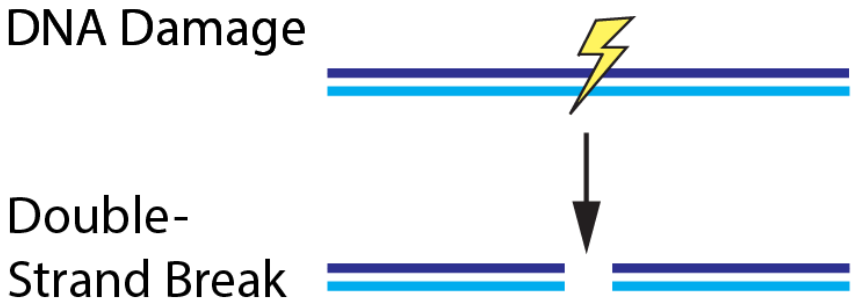
— the **passenger** mutations

passenger mutations can be:

neutral, or deleterious or, source for potential adaptability to future environmental changes



Type of damage	DNA single-strand breaks	Bulky adducts	Base mismatches, insertions and deletions	DNA double-strand breaks
DNA damage response pathway	Base excision repair	Nucleotide excision repair	Mismatch repair	Homologous recombination repair / Non-homologous end joining



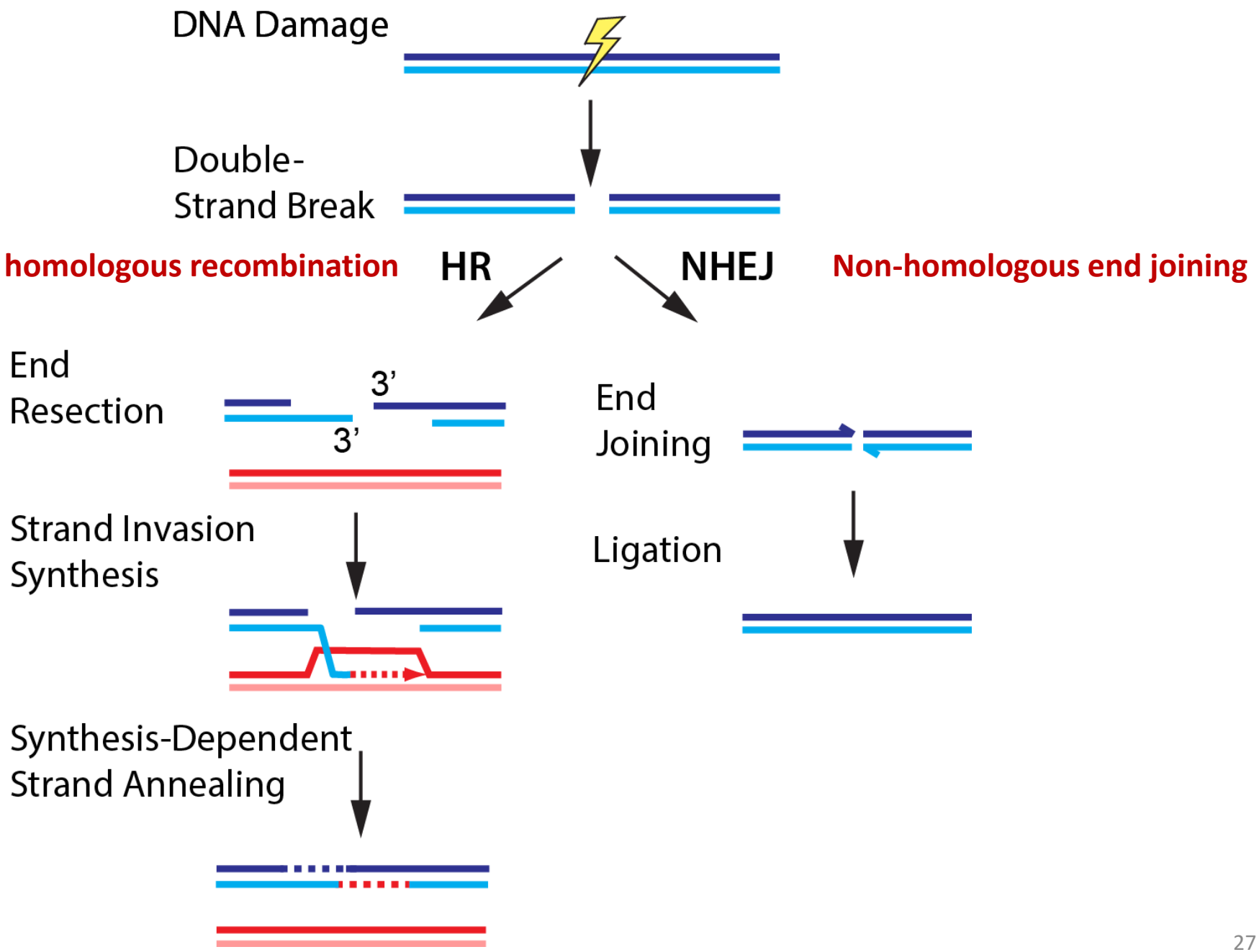
especially by ionizing radiation, including radiotherapy

DSBs also occur:

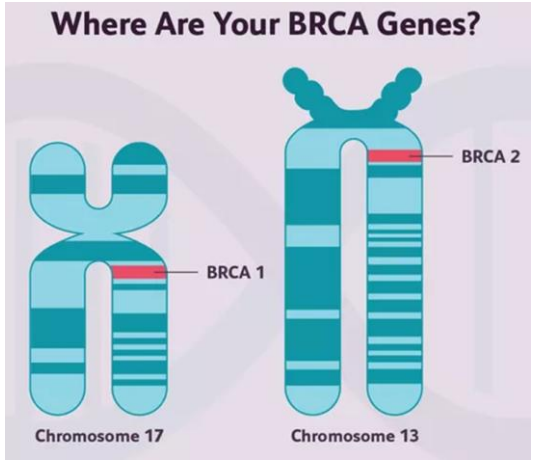
- at stalled replication forks,
- after extensive single-strand DNA damage,
- during IgG class switching in B cells

What biological consequences?

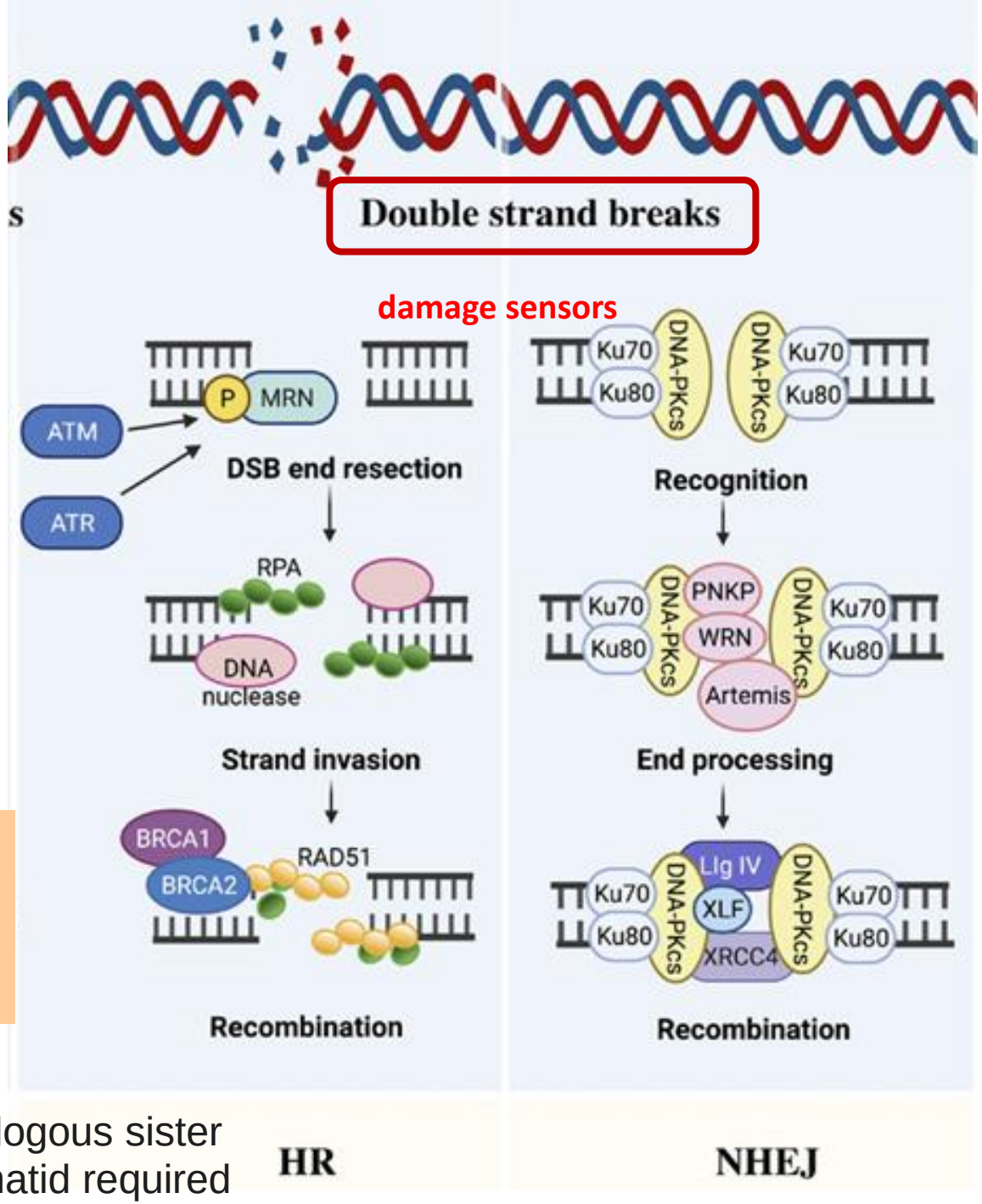
- Loss of chromosome parts in next metaphase plate
- Chromosomal translocations
- Deletions


DNA Damage
Double-
Strand Break
homologous recombination
HR
NHEJ
Non-homologous end joining
End
Resection
3'
3'
Strand Invasion
Synthesis
Synthesis-Dependent
Strand Annealing
End
Joining
Ligation

BRCA1
BRCA2



BRCA1 and BRCA2 proteins act mostly as **scaffolds** to assemble other proteins into a DNA repair complex required for HRR



homologous sister chromatid required

more error-prone repair mechanism

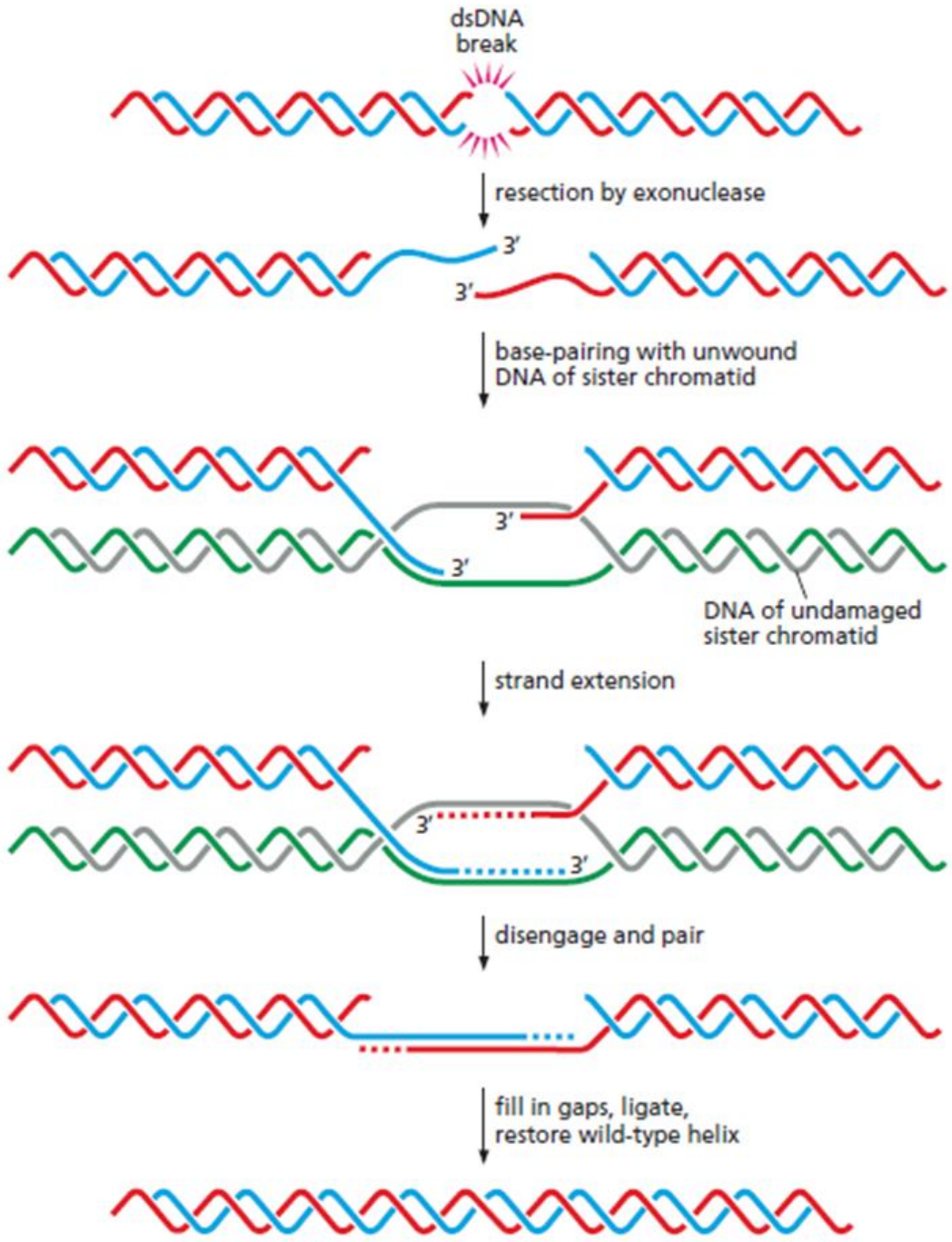
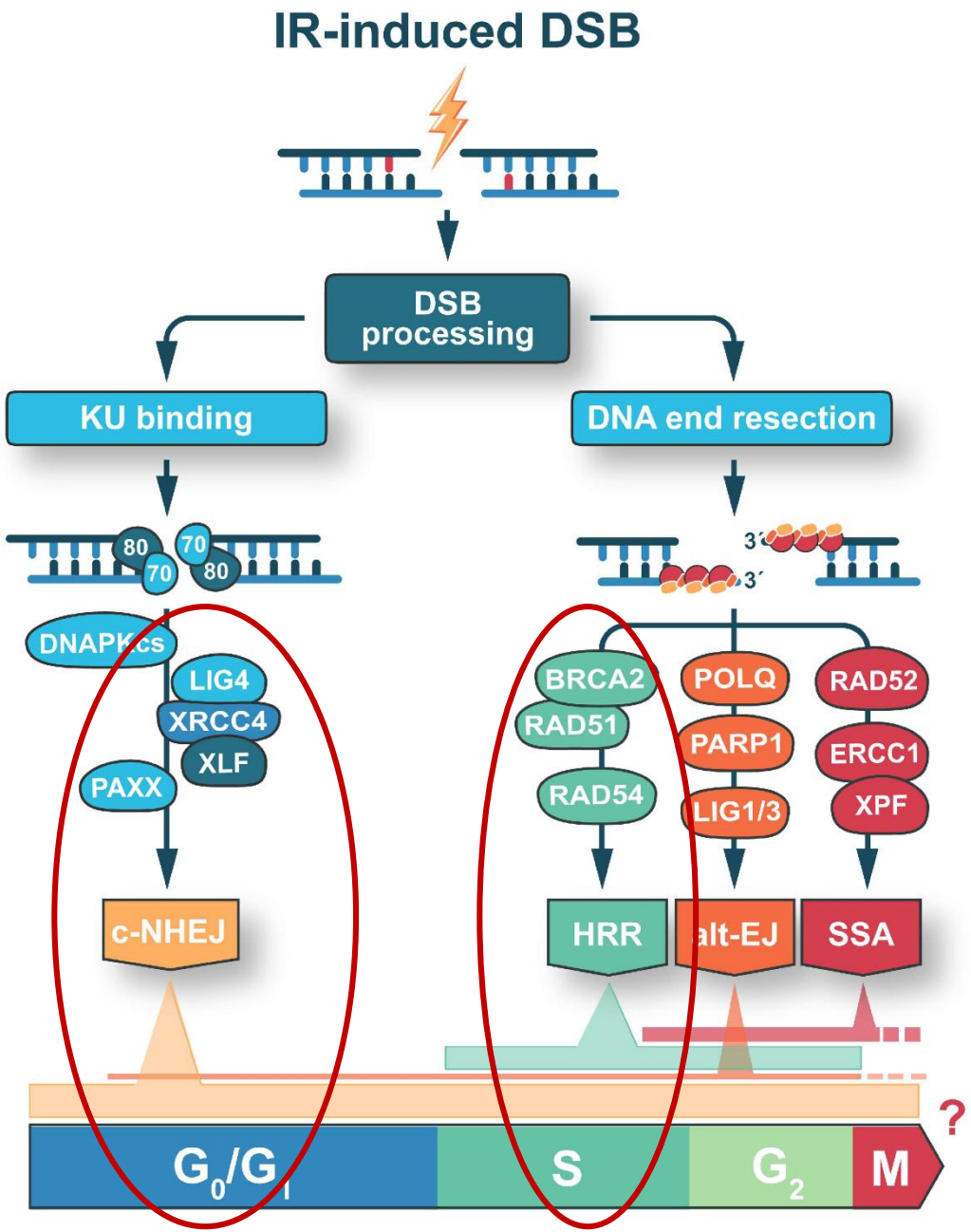


Figure 12.31
Homology-directed repair

The repair of dsDNA breaks that involves the BRCA proteins can occur during the late S phase of the cell cycle, and depends on the ability of the repair apparatus to “consult” the sequences in the undamaged sister chromatid that was formed during the most recent S phase.

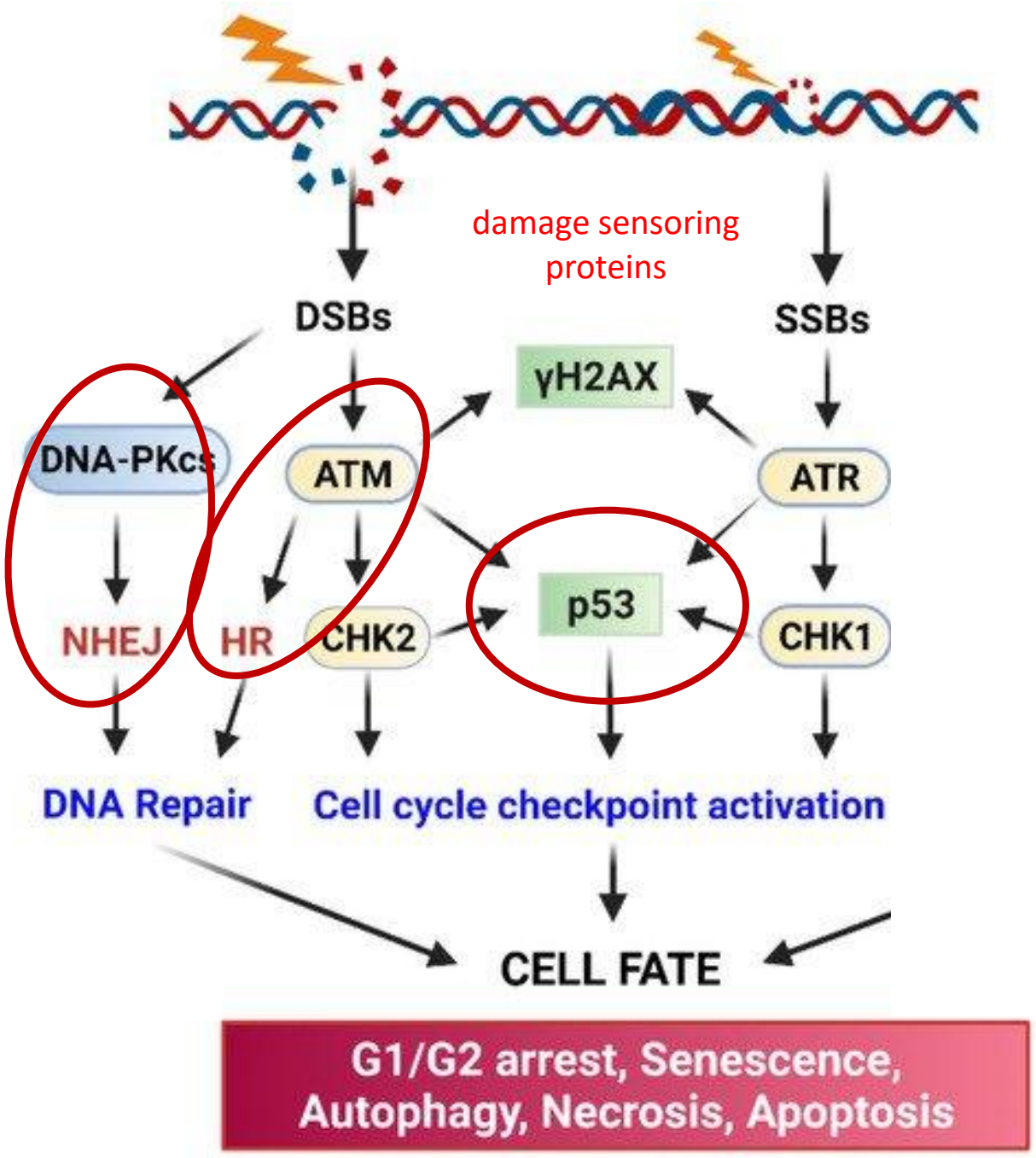
Weinberg, Robert, The biology of cancer -Second edition, Garland Science 2014

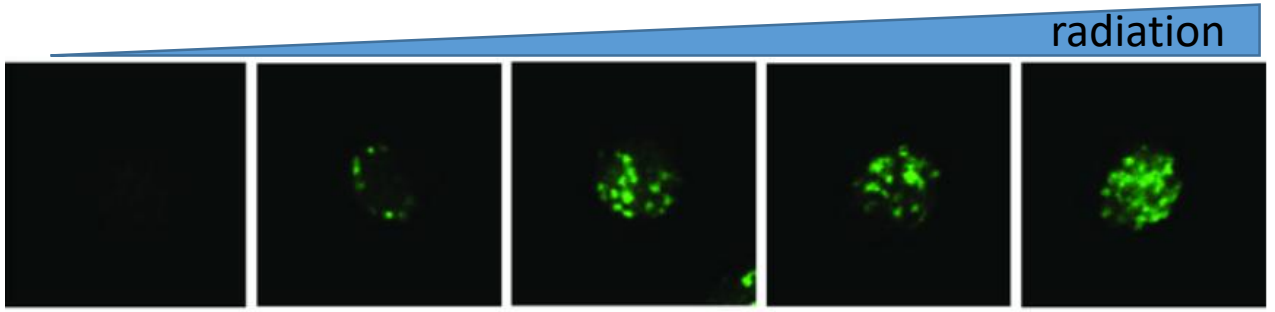


Double strand repair pathways

Pathway and fidelity depends on the phase of the cell cycle.

alt-EJ= alternative end joining
SSA= single-strand annealing

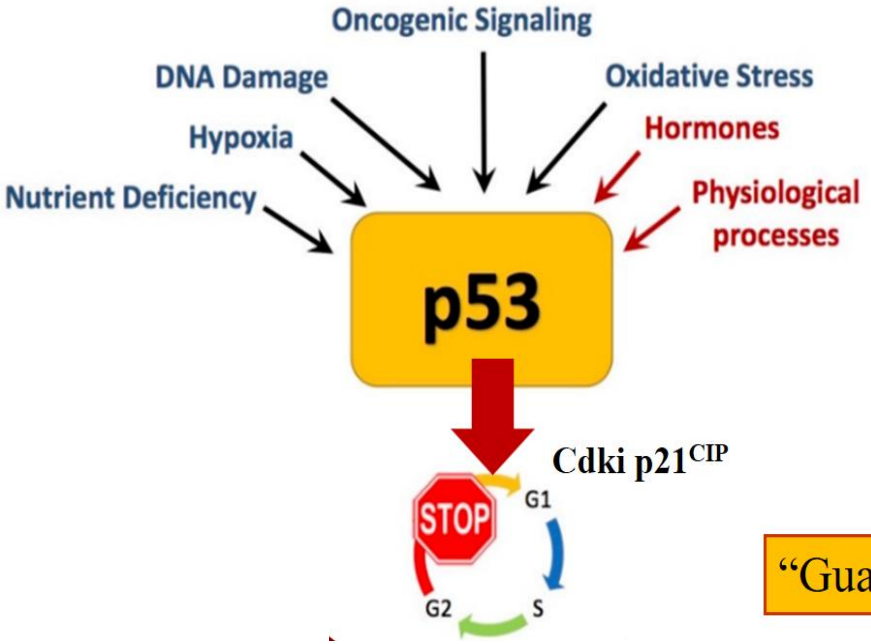




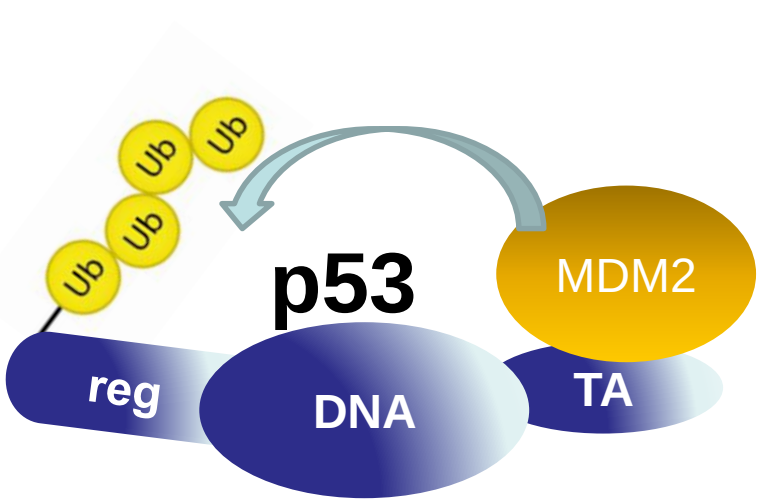
yH2AX foci

DNA strand breaks trigger signalling responses:

ATM- ataxia telangiectasia mutated protein kinase
ATR- ataxia telangiectasia and Rad3-related

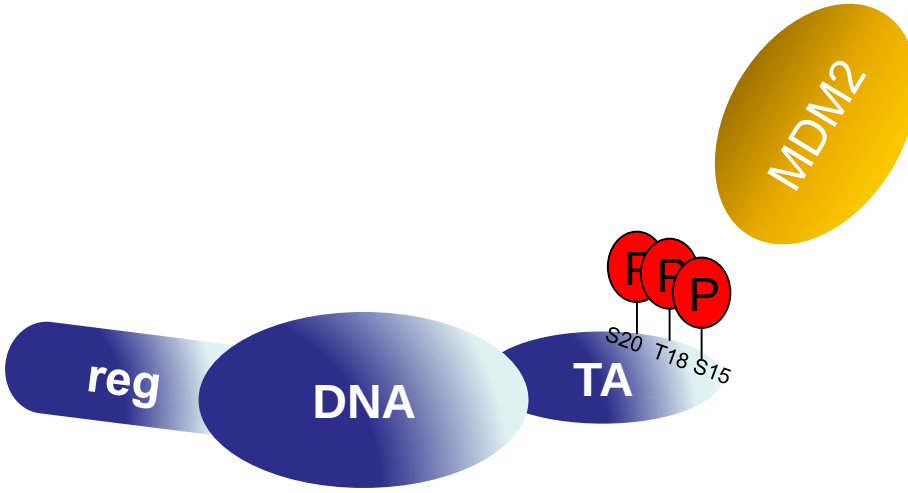


"Guardian of the genome"



DNA damage-activated kinases

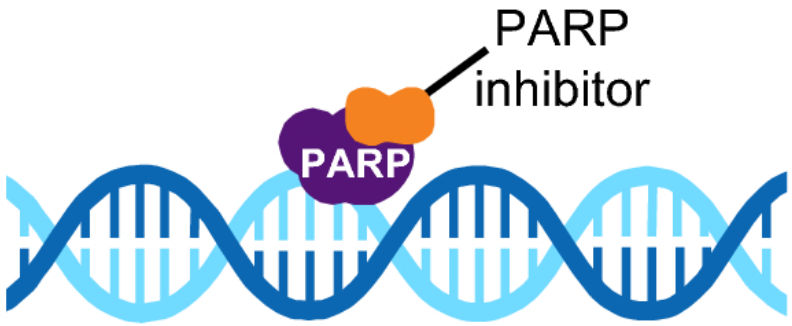
(ATM/ATR, Chk1/Chk2, DNA-PK)



MDM2 binds p53 to promote degradation and inhibit binding to target gene promoters

p53 stabilization and activation of target genes

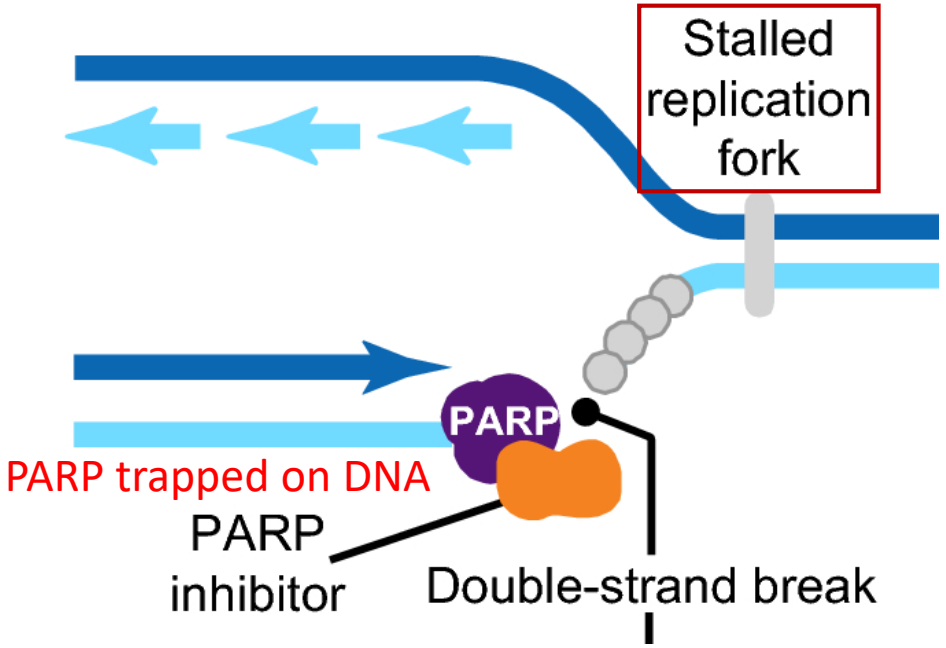
Targeted therapy opportunity in BRCA-mutant tumours



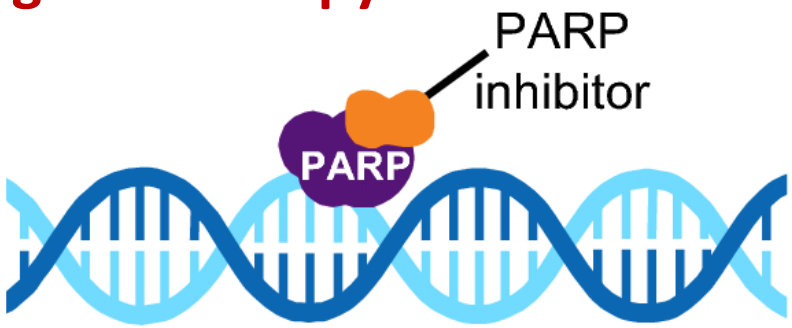
PARylation inhibited and PARP trapped on single-strand breaks

Increase in double-strand breaks in replicating cells

S-phase



Targeted therapy



PARylation inhibited and PARP trapped on single-strand breaks

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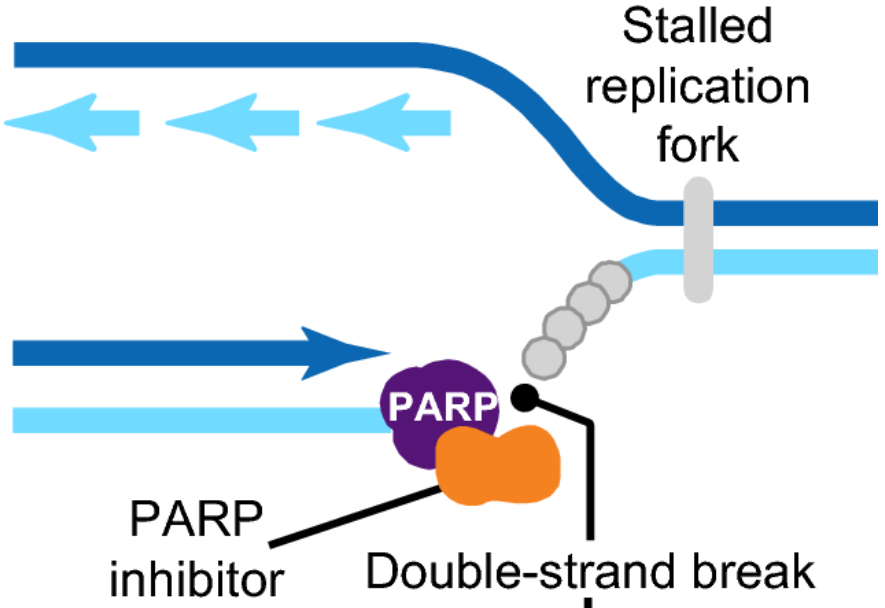
S-phase



Olaparib (Lynparza) and talazoparib (Talzenna) for the treatment of BRCA1 or BRCA2 mutant breast cancer

ovarian, breast, prostate, pancreatic cancer

HRR-deficient cancer cell

Reliance on error-prone NHEJ pathways leads to accumulation of genomic instability and cell death

Normal cell

Repair of double-strand breaks via the HRR pathway and cell survival

DOI: 10.1007/s11523-021-00796-4

Targeted therapy in BRCA-mutant tumours

Concept of *Synthetic Lethality*

loss-of-function of one component in a cell does not have a significant impact on viability, but the combined loss of two components results in cell death (due to the interdependent and/or compensatory nature of the two pathways)

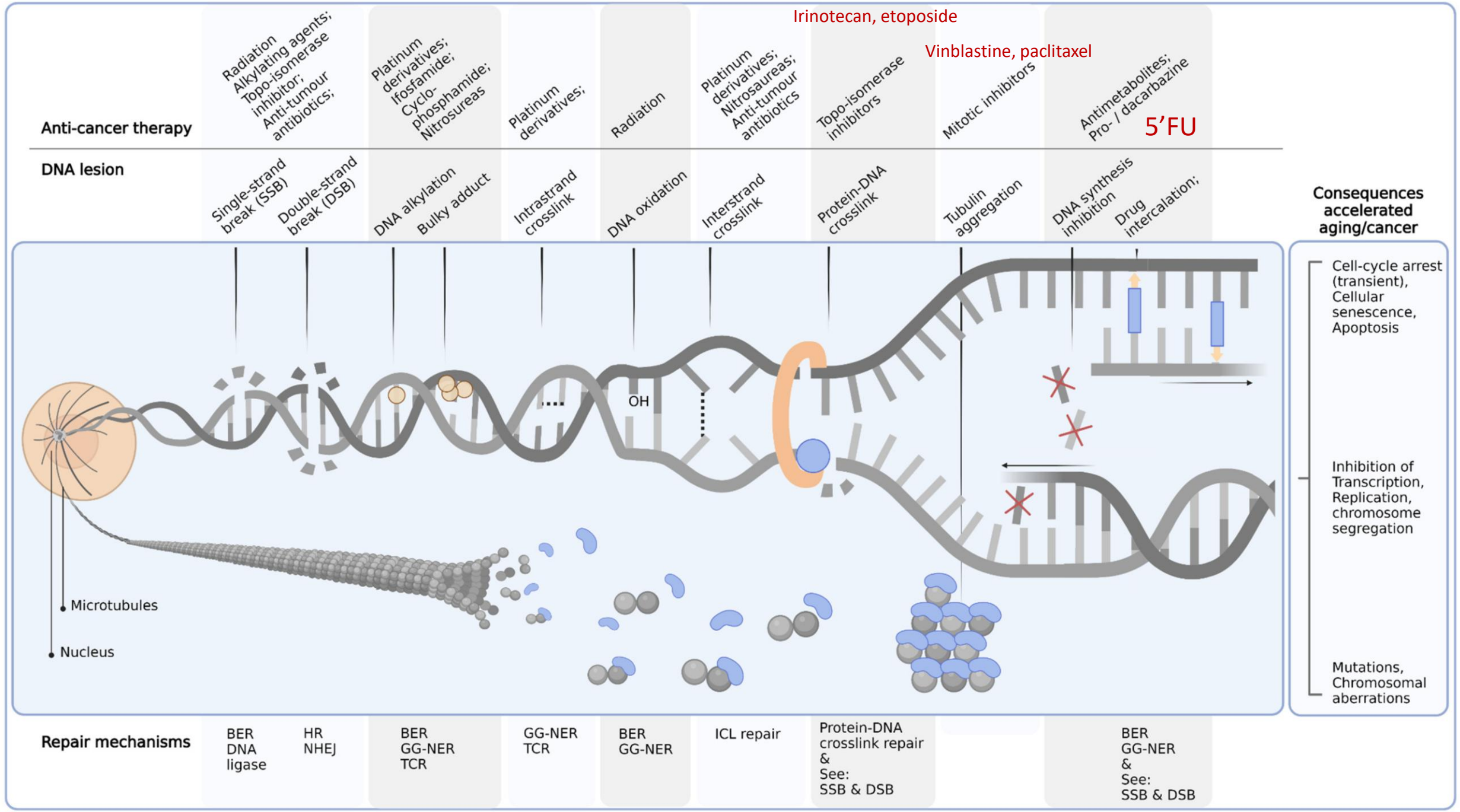
Clinical results with PARP inhibitors:

1) “talazoparib significantly improved patient survival from 5.6 to 8.6 months”

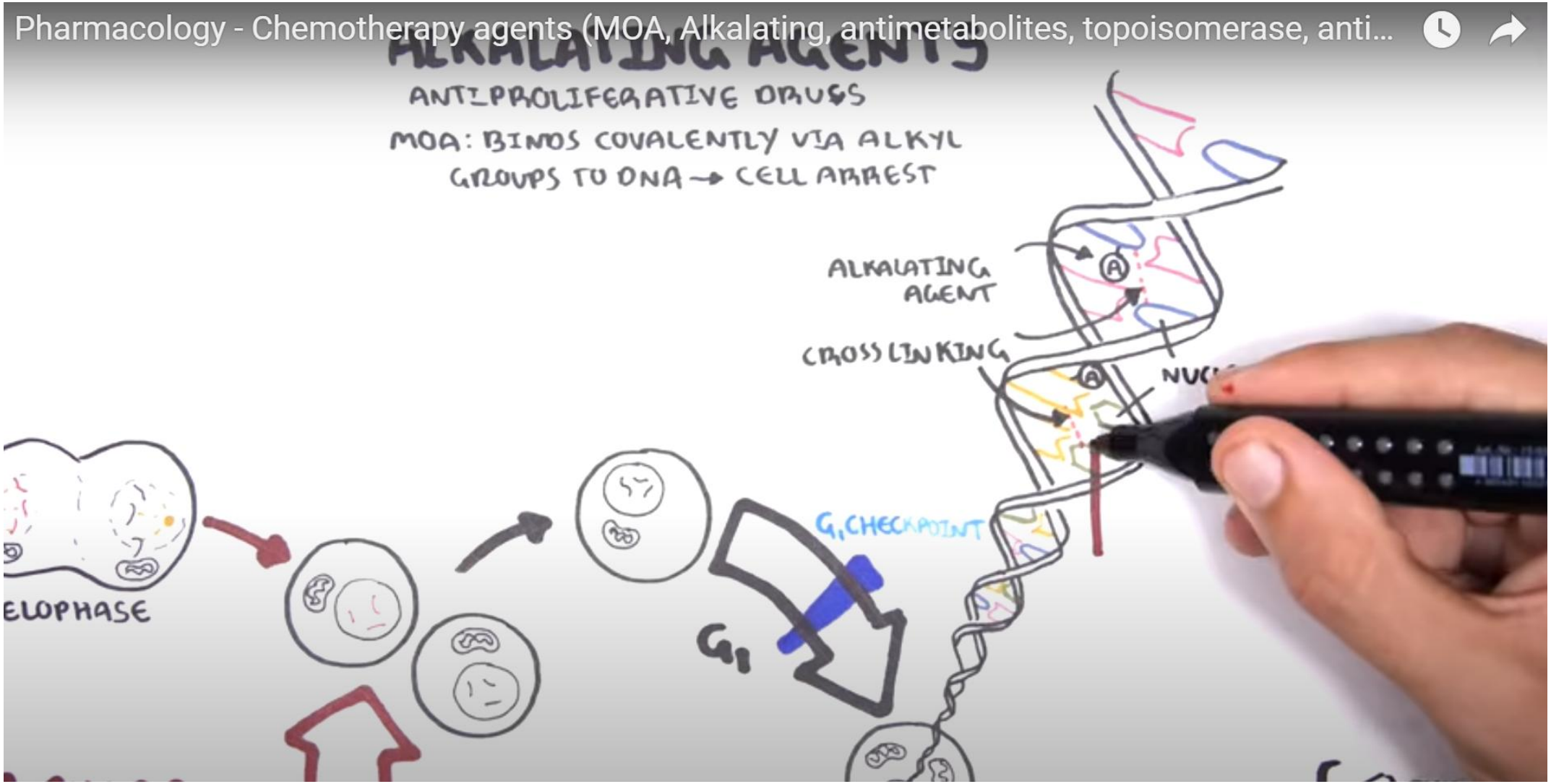
(Litton et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 2018; 37988:753-763.)

2) Treatment resistance observed (reviewed in doi: 10.1038/s41388-024-03227-6):

(selection of secondary mutations that reactivate the HRR or avoid replication fork colapse; selection of PARP mutations that affect inhibitor binding; increase in drug efflux from cancer cells)


Irinotecan, etoposide
Vinblastine, paclitaxel
5'FU
Anti-cancer therapy
Radiation
Alkylating agents;
Topo-isomerase
inhibitor;
Anti-tumour
antibiotics;
Platinum
derivatives;
Ifosfamide;
Cyclo-
phosphamide;
Nitrosureas
Platinum
derivatives;
Radiation
Platinum
derivatives;
Nitrosaureas;
Anti-tumour
antibiotics
Topo-isomerase
inhibitors
Mitotic inhibitors
Antimetabolites;
Pro- / dacarbazine
DNA lesion
Single-strand
break (SSB)
Double-strand
break (DSB)
DNA alkylation
Bulky adduct
Intrastrand
crosslink
DNA oxidation
Interstrand
crosslink
Protein-DNA
crosslink
Tubulin
aggregation
DNA synthesis
inhibition
Drug
intercalation;
Consequences
accelerated
aging/cancer
Cell-cycle arrest
(transient),
Cellular
senescence,
Apoptosis
Inhibition of
Transcription,
Replication,
chromosome
segregation
Mutations,
Chromosomal
aberrations
OH
Microtubules
Nucleus
Repair mechanisms
BER
DNA
ligase
HR
NHEJ
BER
GG-NER
TCR
GG-NER
TCR
BER
GG-NER
ICL repair
Protein-DNA
crosslink repair
&
See:
SSB & DSB
BER
GG-NER
&
See:
SSB & DSB

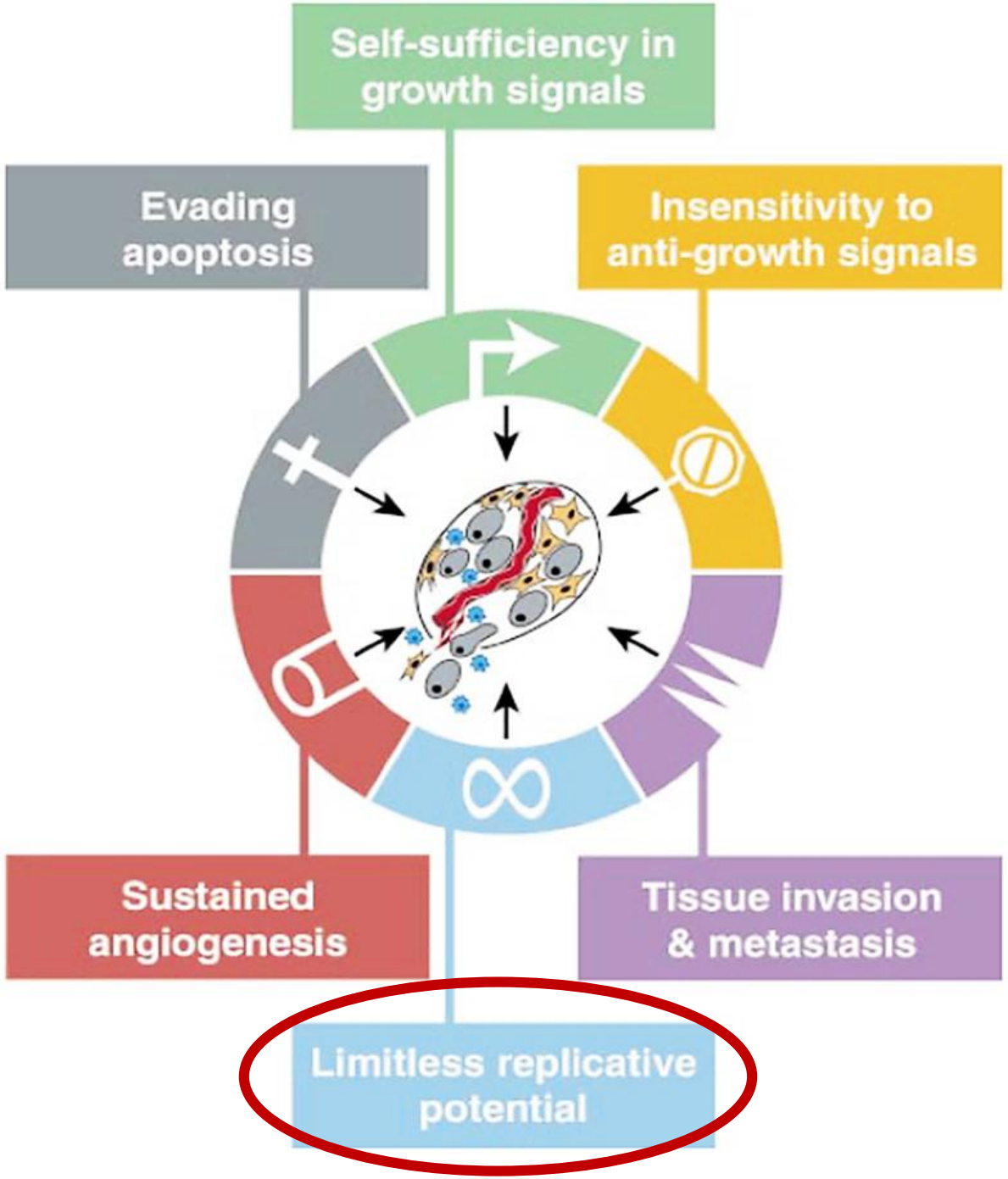
14 min-video animation- DNA damage exploited by chemotherapy agents

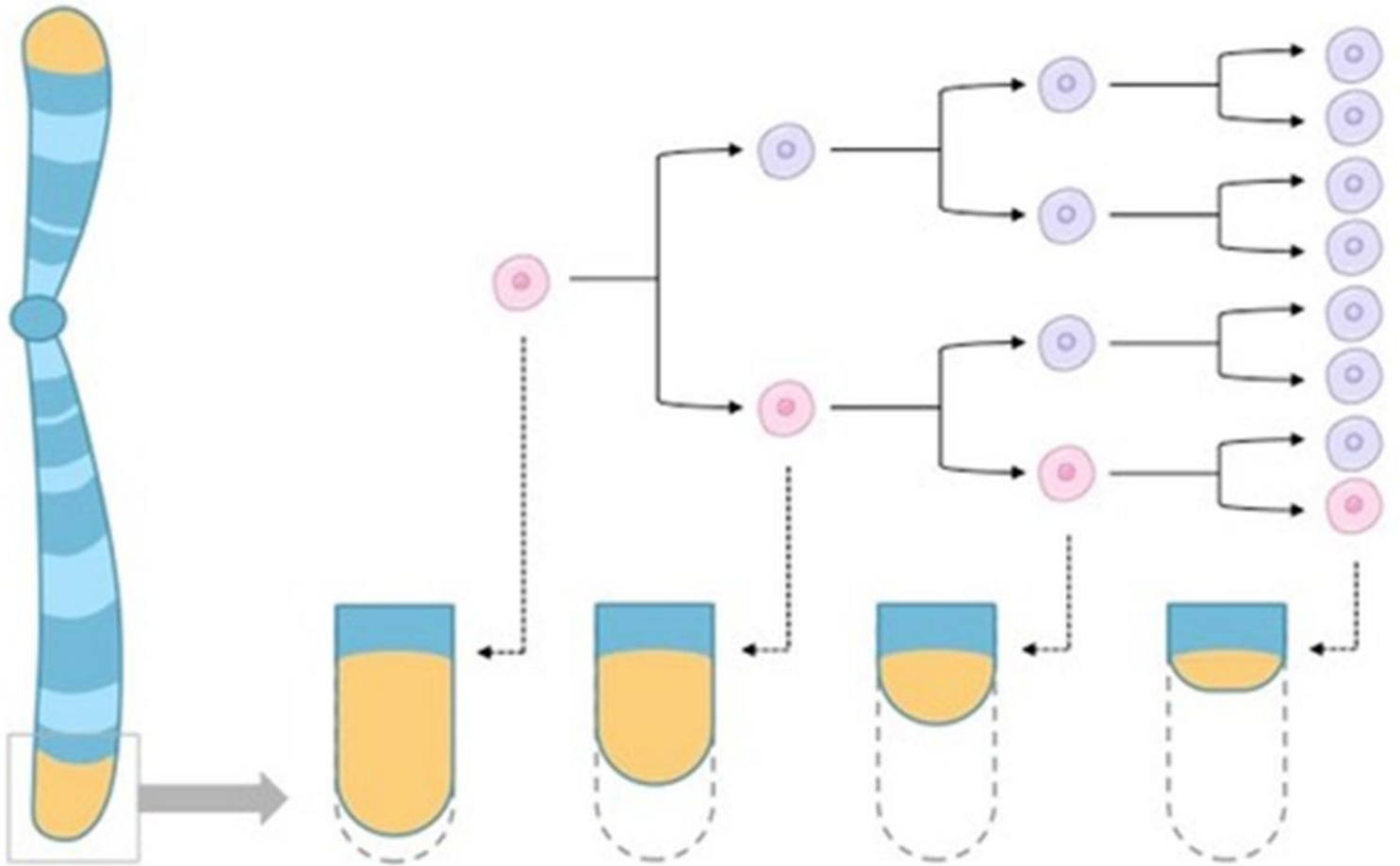


https://armandoh.org/video/pharmacology-chemotherapy-agents-moa-cell-cycle/

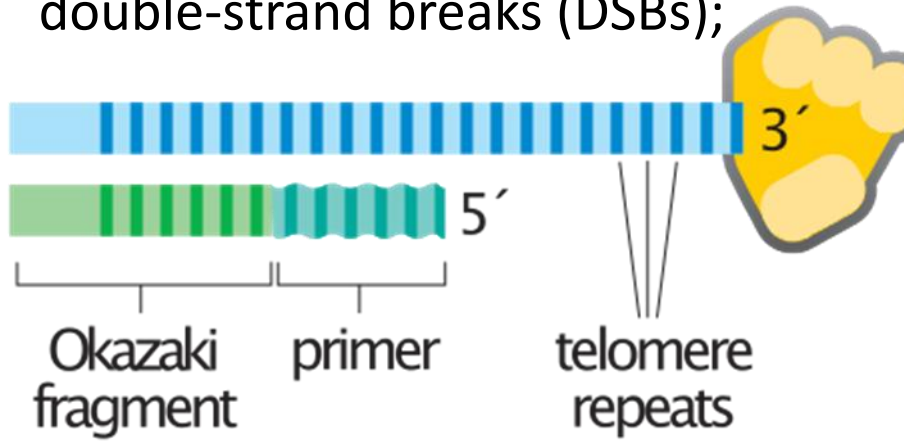
The Hallmarks of Cancer

Cell 100, 57–70 (2000)
Hanahan & Weinberg





- **Telomeres** are composed of 5-15 kb of TTAGGG tandem repeats and a G-rich single-stranded overhang of 12–400 nt; Telomere-associated proteins bind the overhang into a loop and prevent recognition of telomeres as double-strand breaks (DSBs);

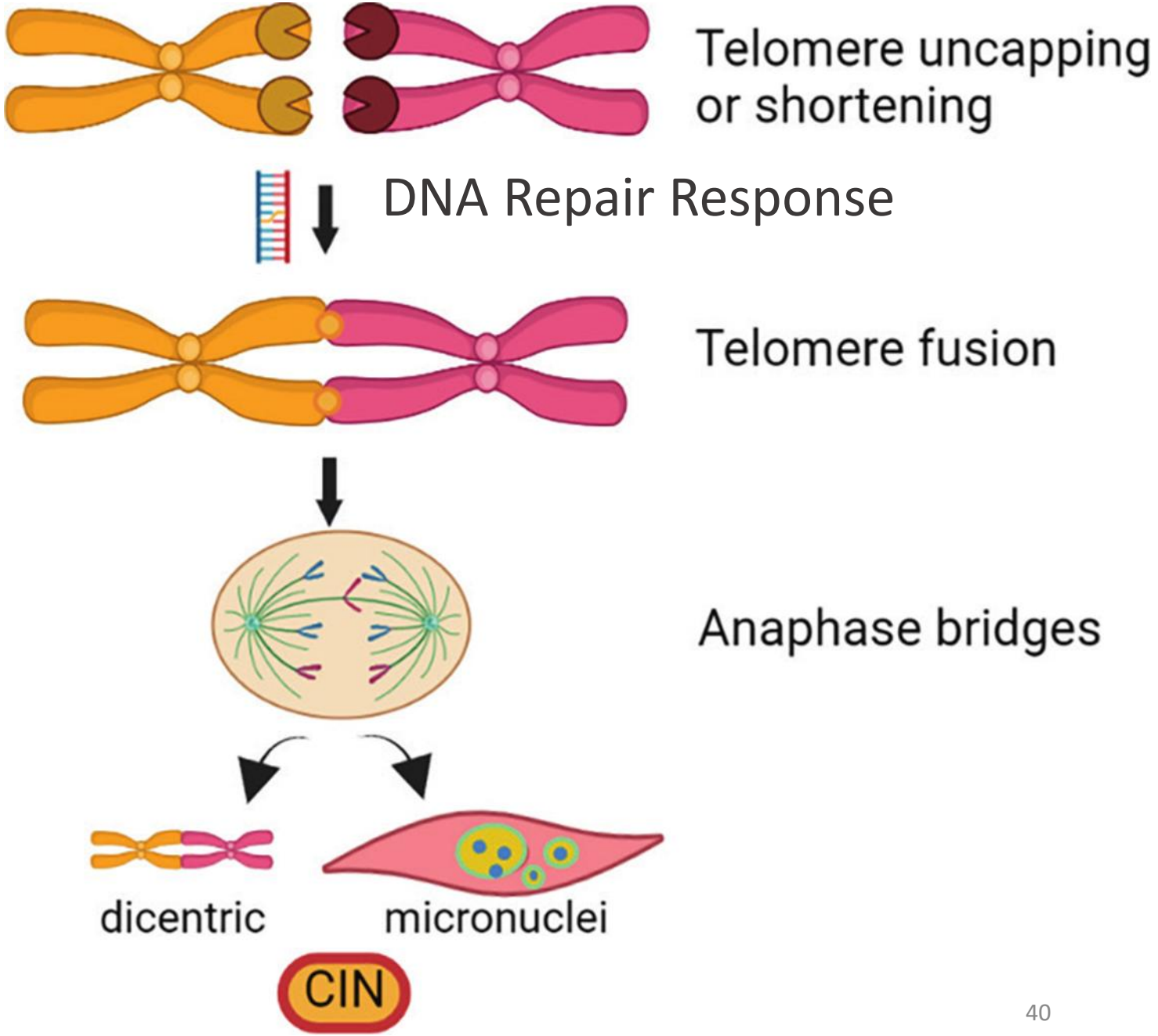


- During each DNA replication, the extreme ends of the telomere cannot be copied (**End replication problem**: terminal RNA primer on the lagging strand cannot be replaced)

- The progressive shortening of telomeres is associated with ageing (senescence, age-related diseases), as normal cells have a limited capacity for cellular division (***Hayflick limit*** = ~ 40 – 60 divisions)

Abnormalities in telomere replication can activate a **p53-dependent DNA damage response** leading to programmed senescence or apoptosis

Severe telomere shortening can lead to chromosome end fusions, mitotic abnormalities and chromosomal instability

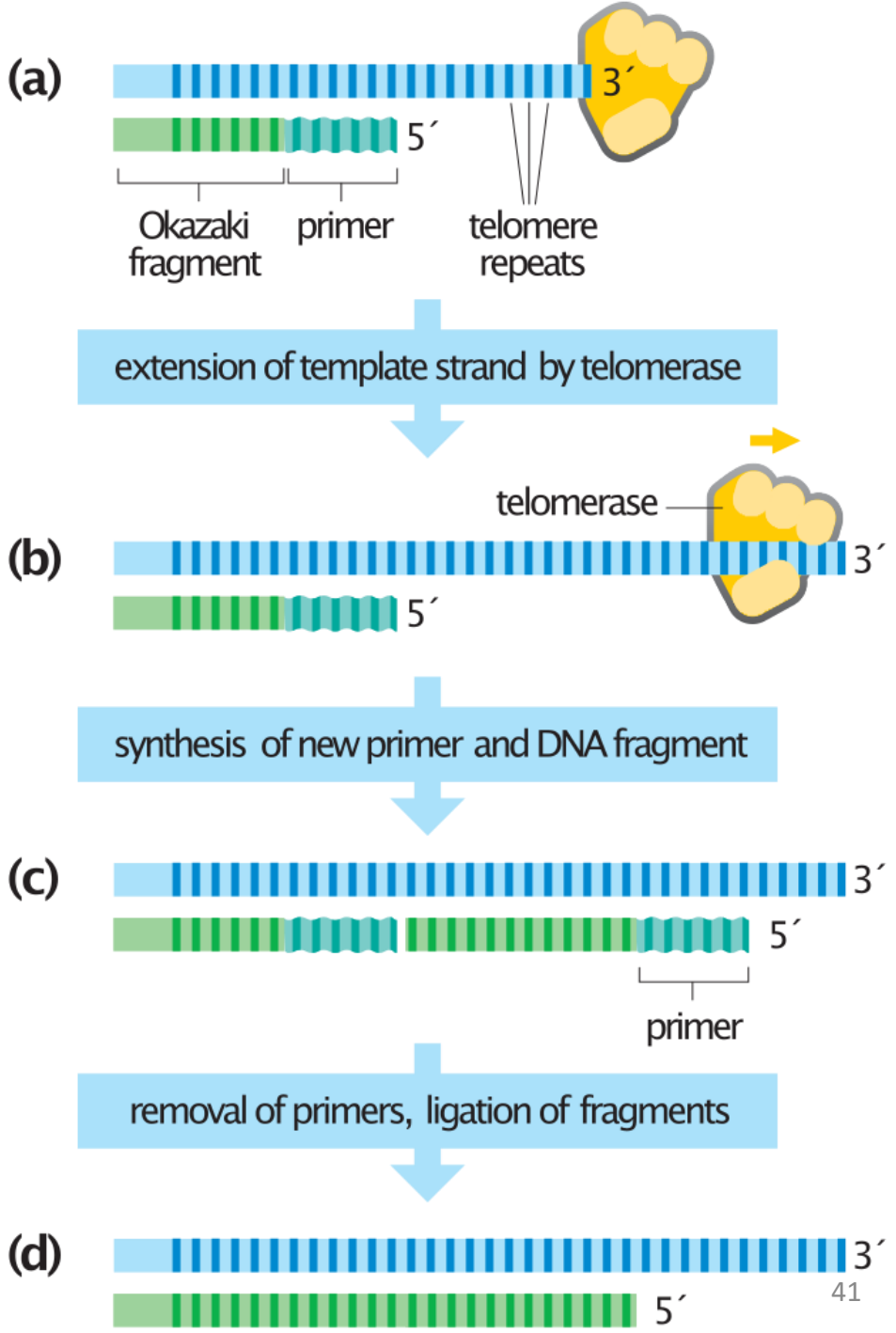


Telomeres can be lengthened by the RNA-containing enzyme **telomerase**, allowing for continued cell division past the Hayflick limit **in germ, embryonic or stem cells**.

Reactivation of telomerase in cancer cells can allow them to become immortal.

Cancer hallmark
'limitless replicative potential':

- Reactivation of telomerase;
- TP53 inactivation;
- Escape from apoptosis



Lecture 7- Some take-home concepts

- **Four different DNA damage repair pathways exist in cells (base excision, nucleotide excision, mismatch and double strand repair) and many tumours cells show genetic inactivation or mutation of repair factors;**

- **Inactivation of the mismatch repair pathway leads to high tumour mutation burden throughout the genome. This can be measured in non-coding microsatellite markers, but also affects repetitive sequence stretches in coding regions of tumour suppressor genes;**

- **Double-strand repair is affected by mutations in BRCA1 or -2, which are required for assembly of homologous recombination repair factors. This can be exploited therapeutically by increased sensitivity to PARP inhibitors;**

- **Tumours cells can gain 'limitless replicative potential' by reactivating telomerase transcription, or TP53 inactivation, or escape from apoptosis-inducing mechanisms.**