Recent advances in clinical practice

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DNA damage repair as a target in pancreatic cancer: state-of-the-art and future perspectives

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Journal club presentation

25-11-1400

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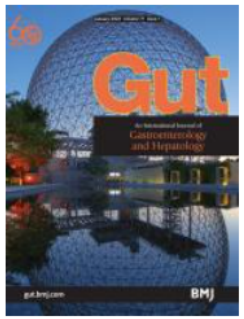

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COVID-19 Gastroenterology Collection

AG Prof. Dr. Seufferlein

Cancer represents one of the deadliest diseases of our century. Tumor cells are characterized by the ability to grow uncontrollably, change their shape, acquire invasive properties and to spread through the blood stream to other parts of the body where they form metastases. Our lab is committed to investigate various aspects of tumorigenesis and tumor progression of pancreatic cancer (PDAC) and aims at characterizing the role of crucial signaling pathways controlled by several protein kinases.

General interests

Group: Tumor angiogenesis, Metastasis and Cancer metabolism

AG Eiseler: Exosomes in metastasis and inflammation

AG Armacki: In Vivo and Ex Vivo Models

Staff

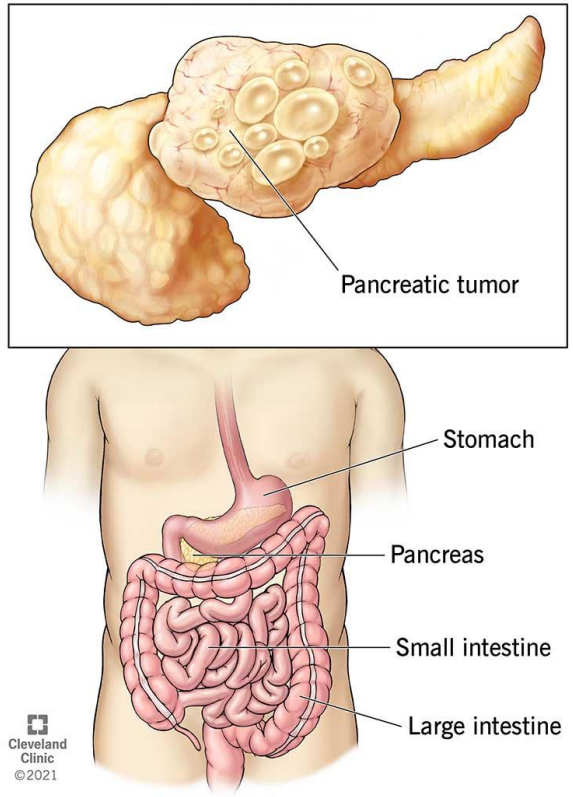
Department Director

Prof. Dr. Thomas Seufferlein
Ärztlicher Direktor der Klinik für Innere Medizin I (Speiseröhre, Magen, Darm, Leber und Niere sowie Stoffwechselerkrankungen) und Sprecher des Darmzentrums

Kontakt
Details

Pancreatic ductal adenocarcinoma (PDAC)

- Pancreatic ductal adenocarcinoma (PDAC) is an exceptional malignancy with a distinct biology and epidemiology.
- It is nowadays **the fourth leading cause** of cancer-related deaths in the Western world and one of the few cancers with a rising incidence.
- Median overall survival (mOS) in advanced disease rarely exceeds **1 year,** resulting in a 5-year OS of <10% for all stages.
- PDAC has shown the least therapeutic progress out of all major GI cancers over the last decades.



PDAC has very poor prognosis

The poor prognosis of PDAC is mainly caused by a unique molecular complexity including:

- (i) a desmoplastic and immunosuppressive stroma
- (ii) a high level of intratumoural and intertumoural heterogeneity
- (iii) early metastasis
- (iv) high chemoresistance

Numerous, but not all clinical trials on PDAC have failed over the last decade.

Adjuvant treatment options:

- Single gemcitabine
- Gemcitabine/ capecitabine
- Folinic acid, fluouracil, irinotecan, oxaliplatin (FOLFIRINOX)

- **Neoadjuvant** treatment concepts are currently being investigated.

The standard of care in advanced PDAC:

- FOLFIRINOX
- Nanosized albumin-bound paclitaxel (nab-PTX) + gemcitabine

Driver mutations

- Activating KRAS mutations
- Loss of distinct tumour suppressor gene pathways (eg, *TP53*, *CDKN2A*, *SMAD4*).

(https://portal.gdc.cancer.gov/)

Oncogenic KRAS may synergise with additional tumour suppressor mutations to deregulate **double-strand break (DSB)** repair and subsequently induce genomic instability.

- High number of **passenger mutations** causes the high intratumoural and intertumoural **heterogeneity**.

- To approach this heterogeneity, **transcriptional profiling** of purified pancreatic cancer epithelial cells allowed a certain degree of subgrouping, having led to various partly overlapping classifications according to Collisson *et al*,18 Moffitt *et al*19 and Bailey *et al*.20

- However, **transcriptional phenotypes** could be recently integrated with **genomic alterations** by using whole-genome analysis from purified epithelium.

- **Single-cell RNA sequencing**: molecular subtypes are specifically linked to copy number aberrations, for example, in the *KRAS* gene.

- To genomically classify PDAC according to patterns of variation in chromosomal structure, four subtypes have been defined and also allowed predictions in terms of a given treatment:

(i) '*stable*', (ii) '*locally rearranged*', (iii) '*scattered*' and (iv) '*unstable*' (Waddell et al 2015)

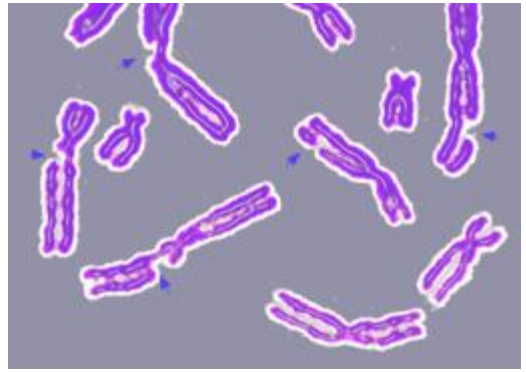
- The **Unstable** accounts for around 14% of PDACs and harbours mutations in **the genes responsible for DNA repair** (*BRCA1/2*, *PALB2* and *ATM)*.

- Mutations in DNA Repair genes also cluster in inherited forms of PDAC (Roberts et al 2016)

- Waddell N, Pajic M, Patch A-M, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015;518:495–501.
- Roberts NJ, Norris AL, Petersen GM, et al. Whole genome sequencing defines the genetic heterogeneity of familial pancreatic cancer. Cancer Discov 2016;6:166–75.

A significant proportion of human PDACs with either somatic or germline mutations in DDR genes might benefit from tailored, targeted therapies. These aspects form the focus of the current review.

DNA Damage Repair (DDR) [From Bruce Alberts]



Maintaining the **genetic stability** that an organism needs:

- An extremely accurate mechanism for **replicating DNA**
- Mechanisms for repairing the many accidental lesions that DNA continually suffers.

Spontaneous changes in DNA are immediately corrected by a set of processes that are collectively called **DNA damage repair.**

Tens of thousands of random changes are created every day in the DNA of a human cell by:

- Heat
- Metabolic accidents
- Radiation of various sorts (sun light, etc)
- Exposure to substances in the environment

Only a few changes **(less than 0.02%)** accumulate as permanent mutations in the DNA sequence. The rest are eliminated with remarkable efficiency.

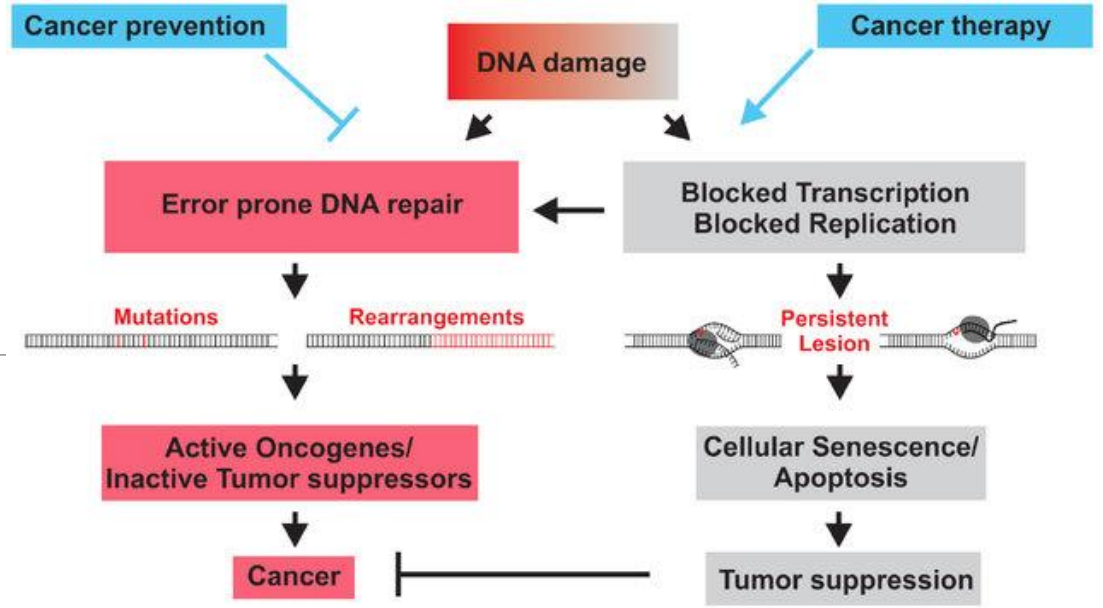
The importance of DNA repair is evident from:

- The large investment of the cells: **Several hundred genes** of most genomes are devoted to DNA repair.
- Increased rate of mutations that follows the inactivation of a DNA repair gene.

Inability to repair DNA leads to:

- Cancer
- Aging

DNA DAMAGE REPAIR (DDR) AND CANCER



DNA damage is **a common event** and **must undergo immediate repair** in order to ensure the exact transfer of genetic information during cell division.

In carcinogenesis:

The **impariment of DDR** can lead to an accumulation of genomic defects and subsequent malignant transformation, cancer progression and further impairment of the DNA repair capacity.

In cancer therapy:

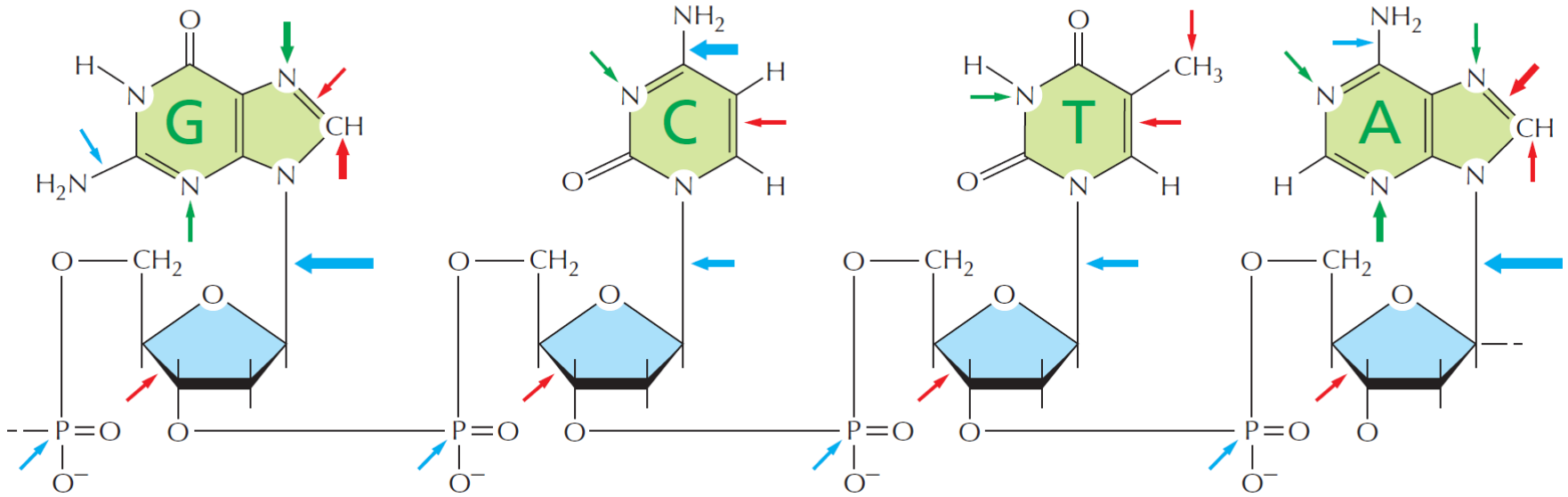
By contrast, the **DDR function** gives growth advantages to tumor clones with disturbed genomic integrity.

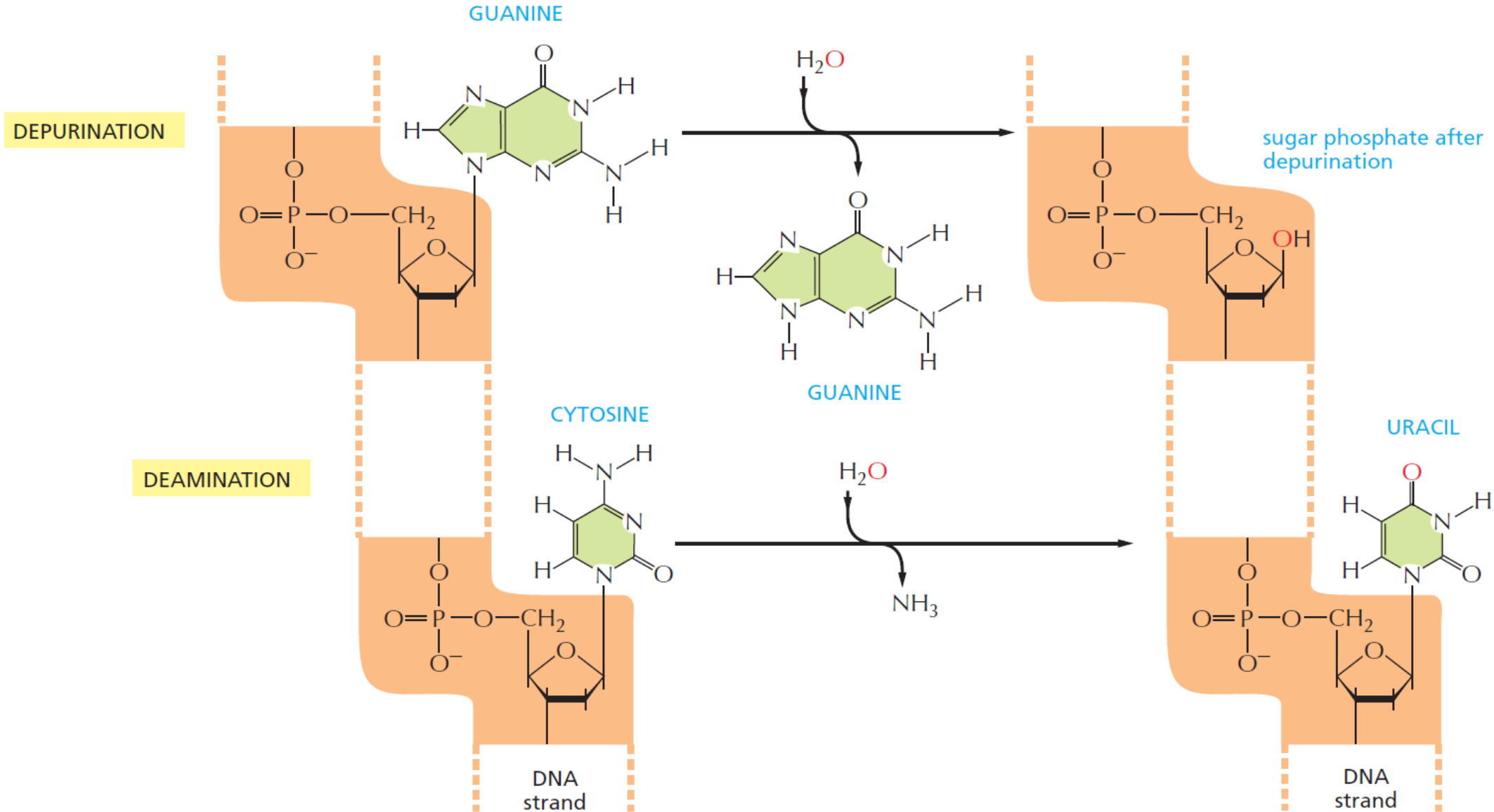
Endogenous DNA lesions

TABLE 5–3 Endogenous DNA Lesions Arising and Repaired in a Diploid Mammalian Cell in 24 Hours

DNA lesion	Number repaired in 24 h
Hydrolysis	
Depurination	18,000
Depyrimidination	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxo G	1500
Ring-saturated pyrimidines (thymine glycol, cytosine hydrates)	2000
Lipid peroxidation products (M1G, etheno-A, etheno-C)	1000
Nonenzymatic methylation by *S*-adenosylmethionine	
7-Methylguanine	6000
3-Methyladenine	1200
Nonenzymatic methylation by nitrosated polyamines and peptides	
O^6-Methylguanine	20–100

normal chemical reactions that take
icals and radiation suffer greater and more
.E. Barnes, *Cold Spring Harb. Symp.*




GUANINE
DEPURINATION
H_2O
sugar phosphate after depurination
GUANINE
CYTOSINE
URACIL
DEAMINATION
H_2O
NH_3
DNA strand
DNA strand

Major DNA lesions can occur in two ways:

- Single-strand break (SSB) including mono-adducts
- Double-strand breaks (DSB) including interstrand crosslinks

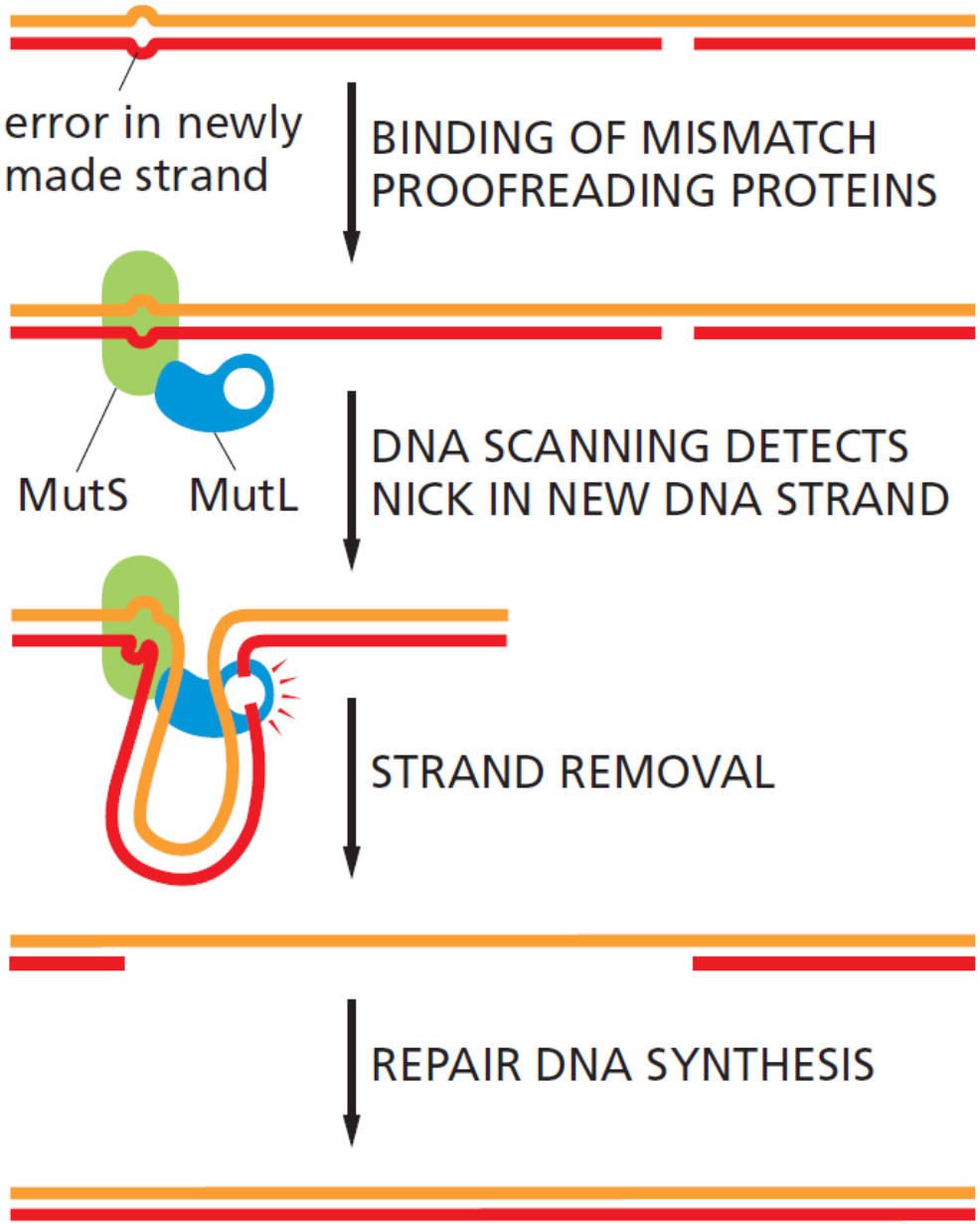
Damage affecting a single DNA strand (including SSB) can be repaired by:

- Base excision repair
- Mismatch repair (MMR)
- Nucleotide excision repair (NER)

- Accumulation of unresolved SSBs induces DSB formation, namely when encountering a replication fork.

Two main mechanisms repairing DSB repair:

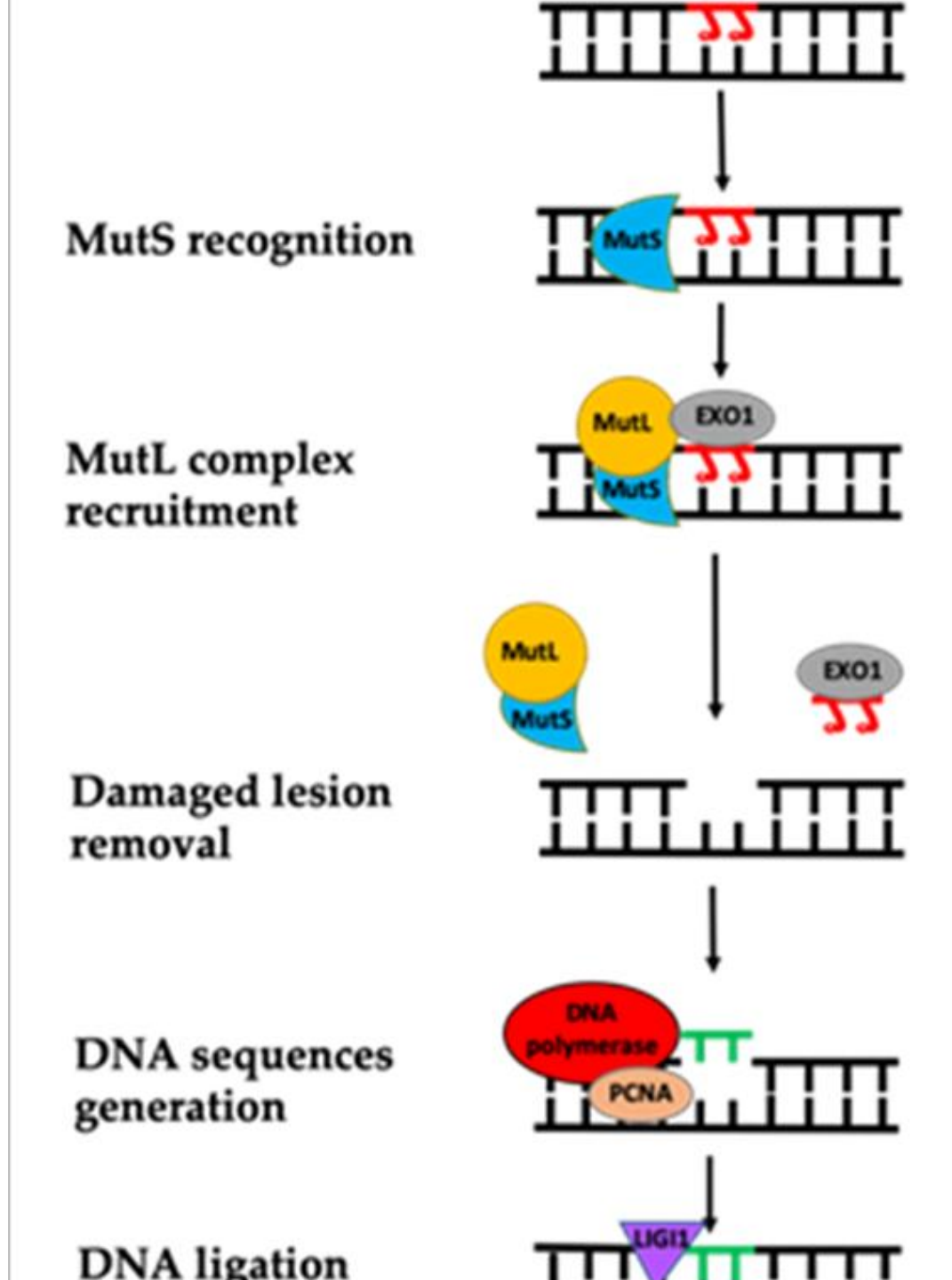
- Non-homologous end joining (NHEJ)
- Homologous recombination (HR)



Strand-directed mismatch repair

- **MutS** binds specifically to a mismatched base pair, while **MutL** scans the nearby DNA for a nick.
- Once MutL finds a nick, it triggers the degradation of the **nicked strand all the way back through the mismatch**.
- Because **nicks are largely confined to newly replicated strands** in eukaryotes, replication errors are selectively removed.
- In bacteria, an additional protein in the complex (MutH) nicks unmethylated (and therefore newly replicated) GATC sequences, thereby beginning the process .

- MutS protein **scans the DNA for mismatches** by testing for sites that **can be readily kinked**, which are those with an abnormal base pair.


MutS recognition
MutS
MutL complex recruitment
MutL
EXO1
MutS
MutL
MutS
EXO1
Damaged lesion removal
DNA sequences generation
DNA polymerase
PCNA
DNA ligation
LIG1

Nucleotide excision repair (NER)

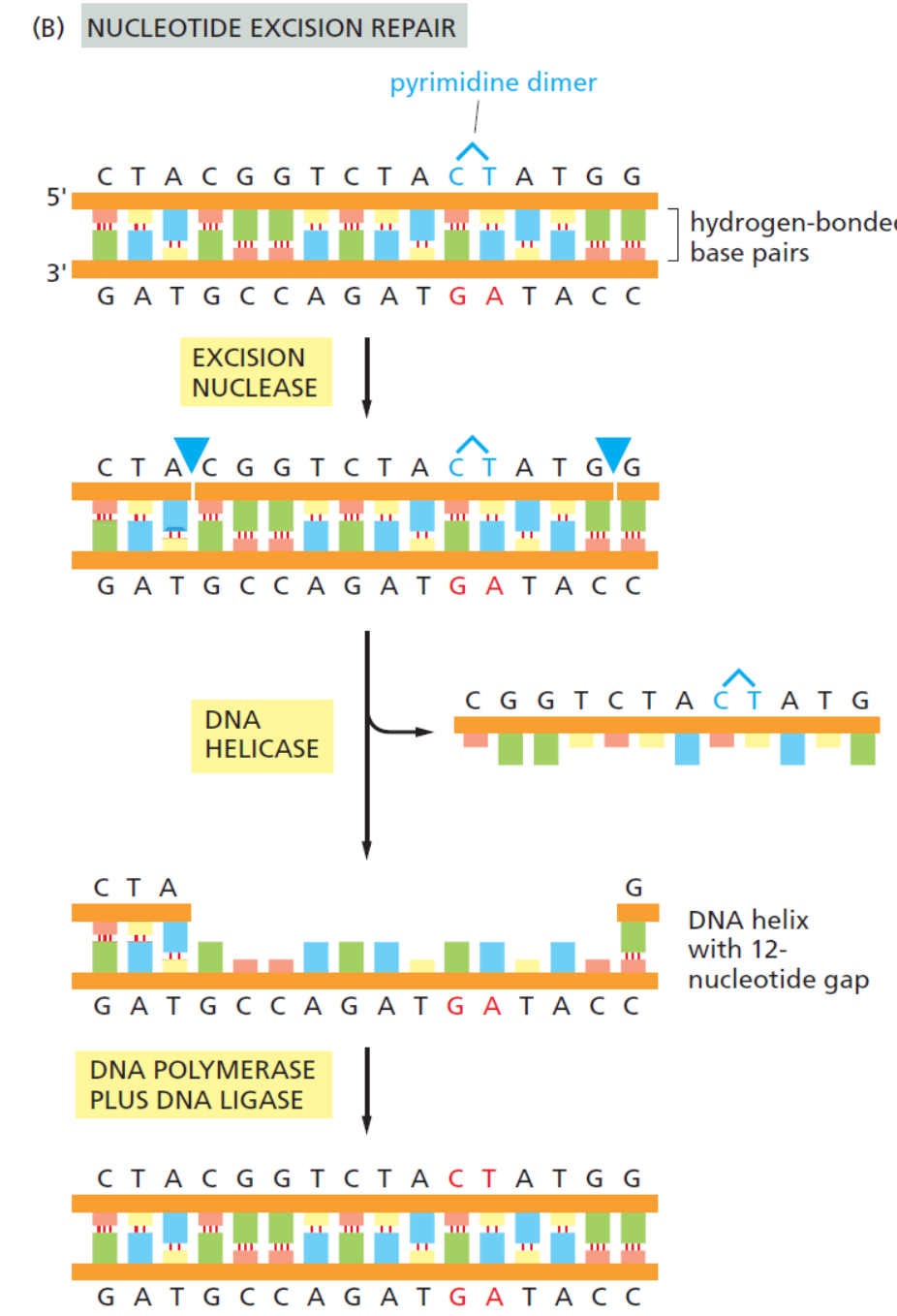
Repairs the damage caused by almost any large change in the structure of the DNA double helix.

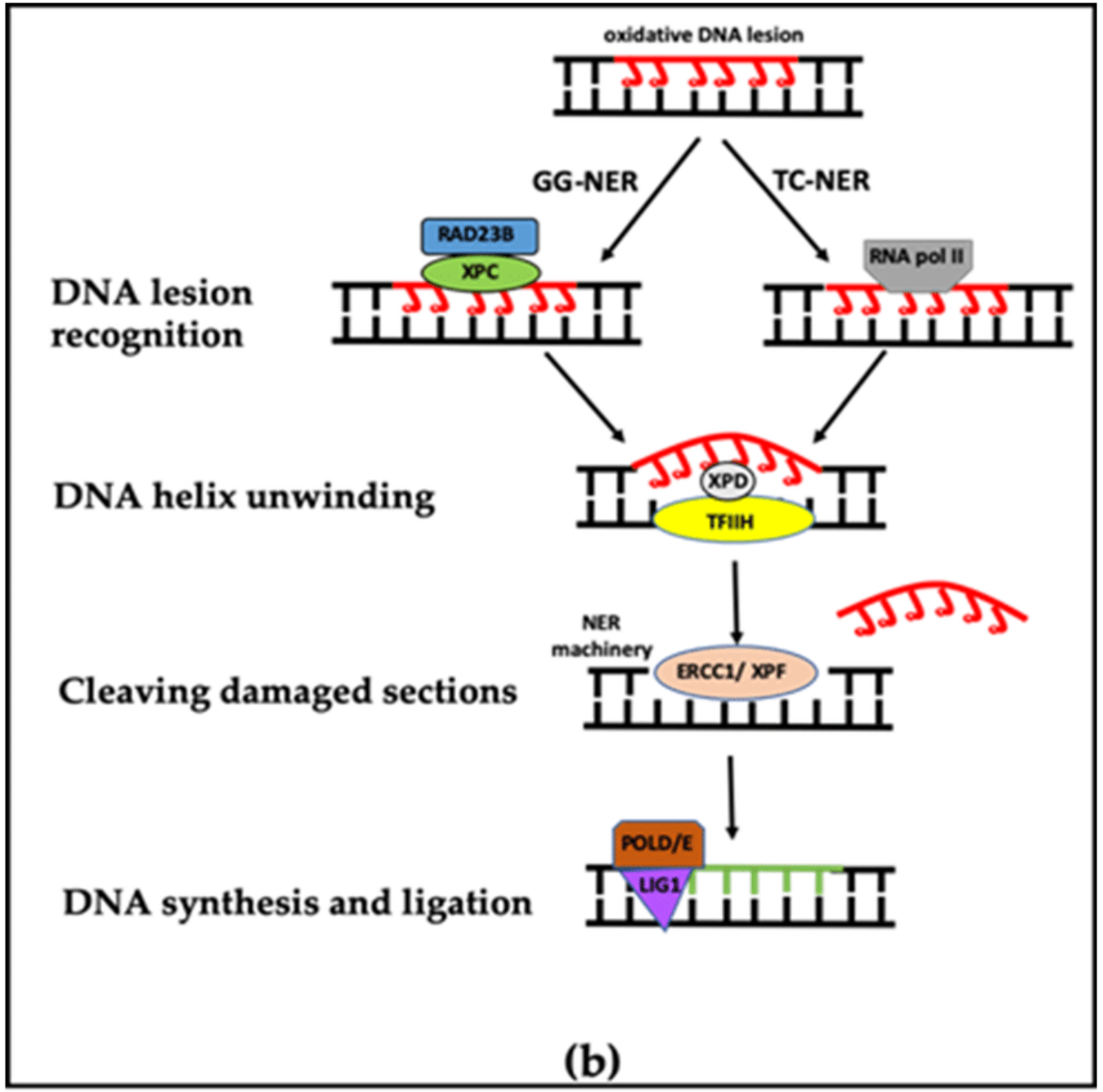
Such “bulky lesions” include:

- Covalent reaction of DNA bases with large hydrocarbons (the carcinogen benzopyrene, found in **tobacco smoke**, coal tar, and diesel exhaust)
- **Pyrimidine dimers** (T-T, T-C, and C-C) caused by **sunlight**.

A large multienzyme complex scans the DNA for a **distortion in the double helix**, rather than for a specific base change. It **cleaves the phosphodiester backbone of the abnormal strand on both sides of the distortion**, and a **DNA helicase removes** the single-strand oligonucleotide containing the lesion.

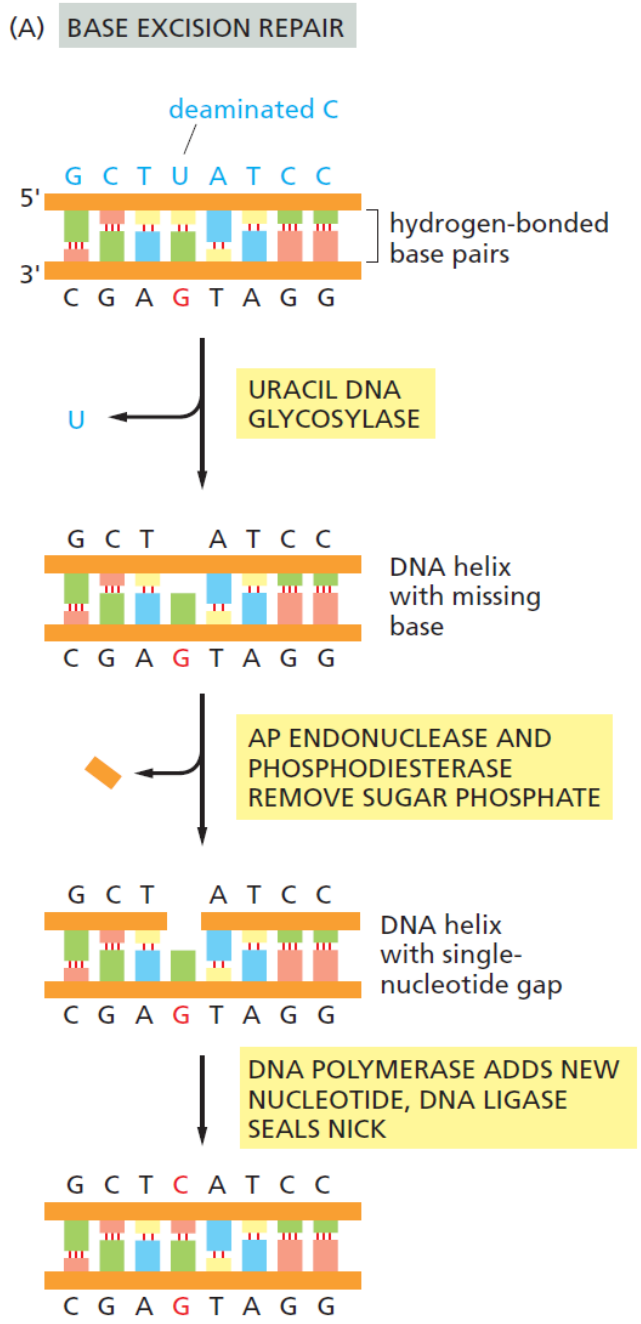
The large gap produced in the DNA helix is then repaired by DNA polymerase and DNA ligase

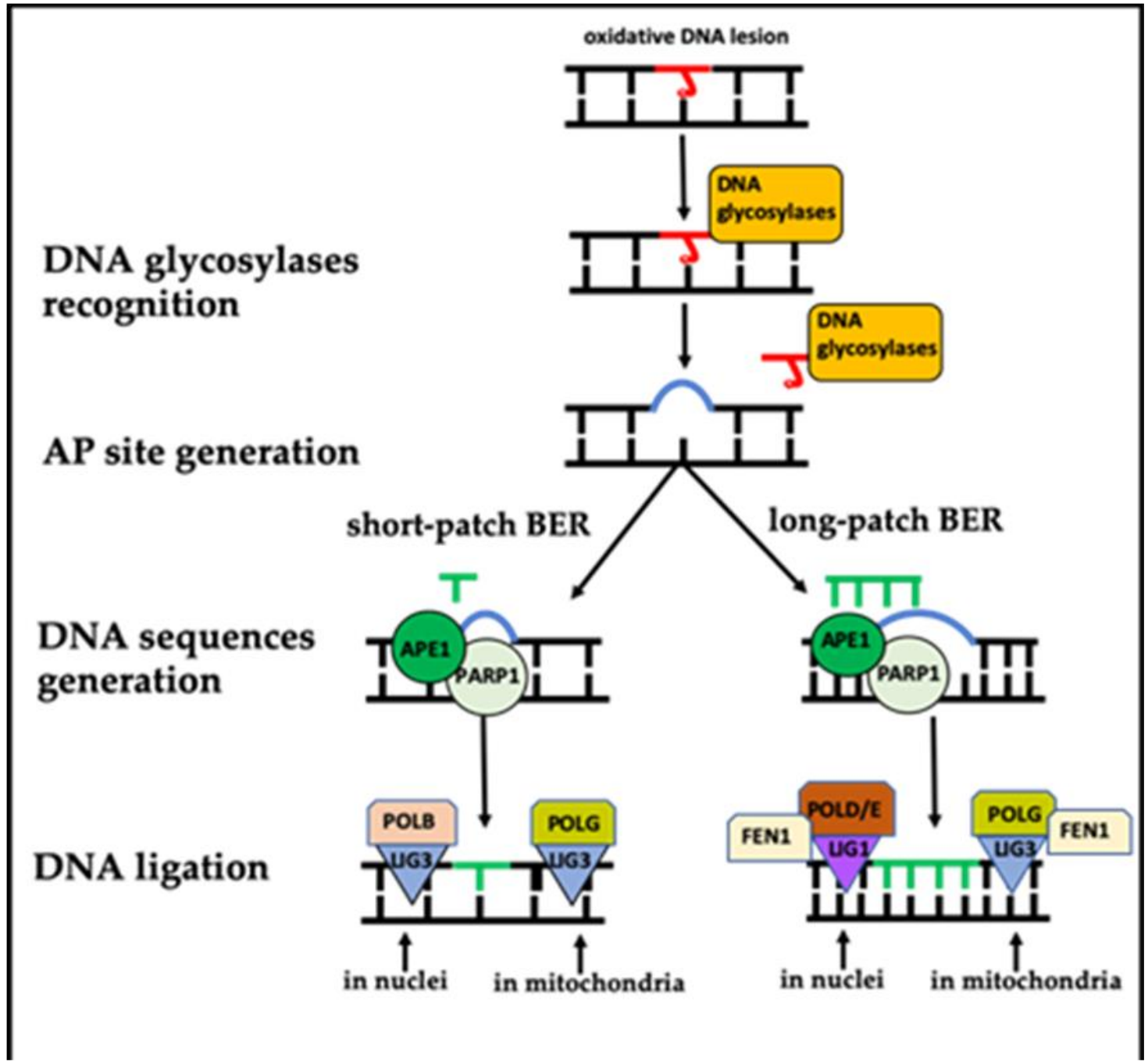



oxidative DNA lesion
GG-NER
TC-NER
RAD23B
XPC
RNA pol II
DNA lesion recognition
XPD
TFIIH
DNA helix unwinding
NER machinery
ERCC1/ XPF
Cleaving damaged sections
POLD/E
LIG1
DNA synthesis and ligation
(b)

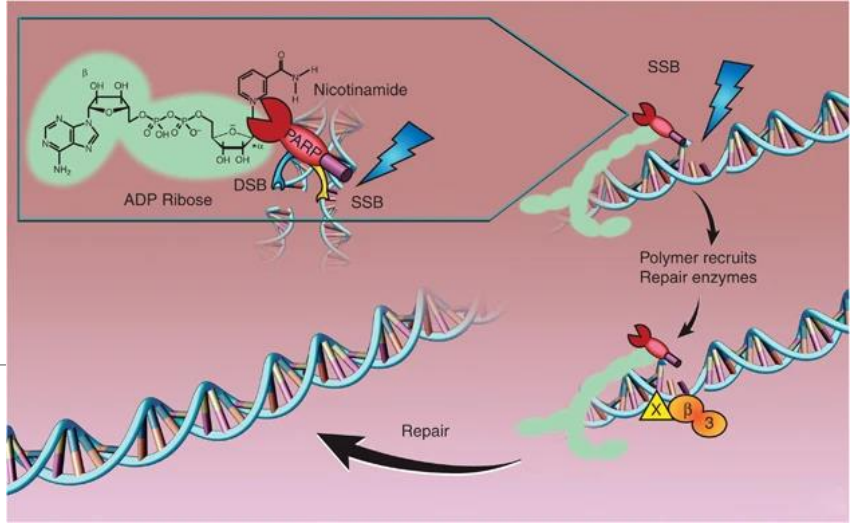
Base excision repair (BER)

- **DNA glycosylases:** catalyze the **hydrolytic removal** of altered base from its sugar.
- There are at least six types of these enzymes (remove deaminated Cs, deaminated As, different types of alkylated or oxidized bases, etc.)
- **The "missing tooth"** created by DNA glycosylase action is recognized by an enzyme called **AP endonuclease** (AP for apurinic or apyrimidinic, endo to signify that the nuclease cleaves within the polynucleotide chain), which cuts the phosphodiester backbone, after which the resulting gap is repaired.
- **Depurination**, which is by far the most frequent type of damage suffered by DNA, also leaves a deoxyribose sugar with a missing base. (directly repaired beginning with AP endonuclease)
- The gap of a single nucleotide is then filled by DNA polymerase and DNA ligase.




oxidative DNA lesion
DNA glycosylases recognition
DNA glycosylases
DNA glycosylases
AP site generation
short-patch BER
long-patch BER
DNA sequences generation
APE1
PARP1
APE1
PARP1
DNA ligation
POLB
LIG3
POLG
LIG3
POLD/E
FEN1
LIG1
POLG
LIG3
FEN1
in nuclei
in mitochondria
in nuclei
in mitochondria

Poly (ADP-ribose) polymerase (PARP)



Poly (ADP-ribose) polymerase (PARP) is a family of proteins involved in a number of cellular processes:

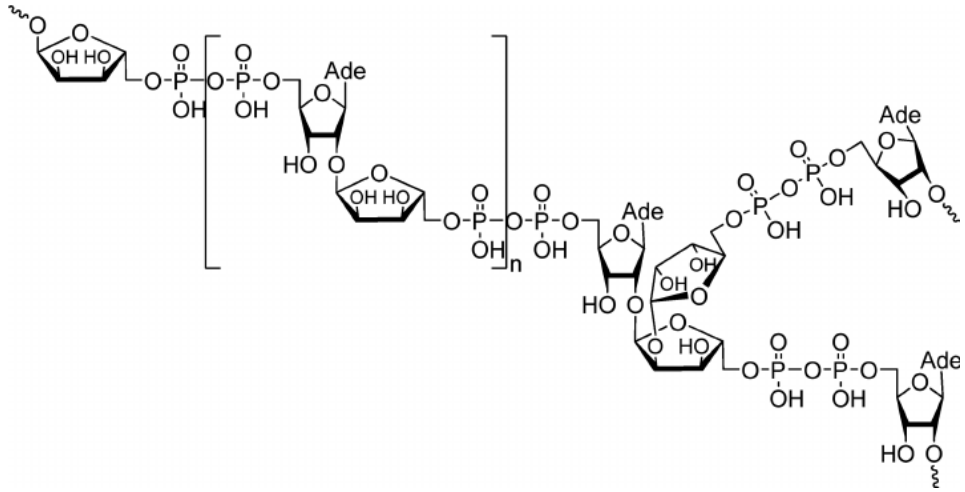
DNA repair

Programmed cell death

Chromatin modifications

The PARP family comprises 17 members. They vary greatly in structure and function within the cell. Some of them like PARP1, PARP2 have a confirmed PARP activity.

Poly (ADP-ribose) or PAR polymer



- PAR polymer can reach lengths of up to **200 nucleotides.** It is formed from nucleoside triphosphates.

- Normal DNA/RNA: a **single phosphate** group linking deoxyribose/ribose sugars.
- PAR: a **pyrophosphate** is the linking group between ribose sugars rather than single phosphate groups. This creates some special bulk to a PAR bridge, which may have an additional role in cell signaling.

- PARP activity (which is mainly due to PARP1) measured in the mononuclear leukocyte blood cells of thirteen mammalian species correlates with maximum lifespan of the species.
- These findings suggest that PARP-mediated DNA repair capability contributes to mammalian longevity.

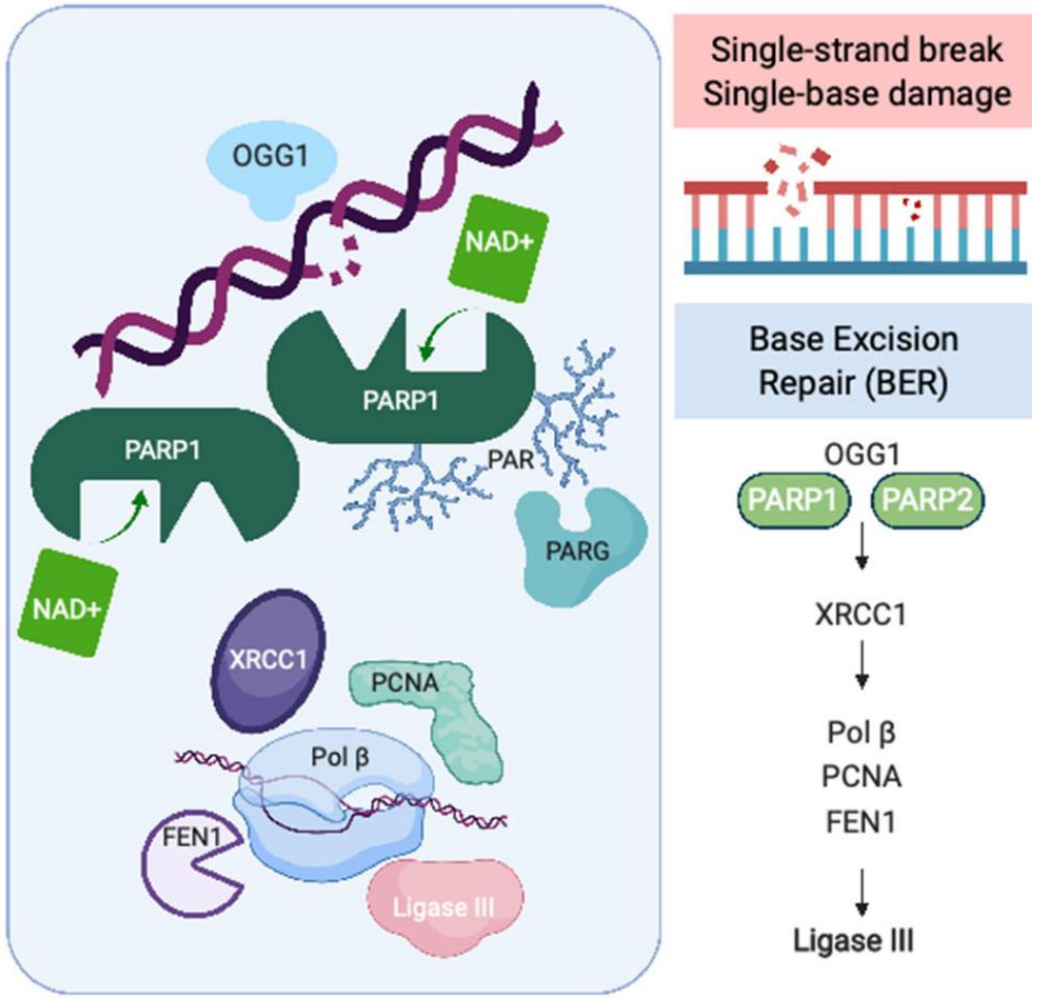
- PARP (found in the cell nucleus) detects single-strand **DNA breaks (SSB)** and initiates cellular response.

- Once PARP detects a SSB, it binds to the DNA, undergoes a structural change, and begins the synthesis of a **polymeric adenosine diphosphate ribose (poly (ADP-ribose) or PAR) chain**, which acts as a signal for the other DNA-repairing enzymes.

Target enzymes include:

- DNA ligase III (LigIII)
- DNA polymerase beta (polβ)
- Scaffolding proteins such as X-ray cross-complementing gene 1 (XRCC1).

After repairing, the PAR chains are degraded via Poly(ADP-ribose) glycohydrolase (PARG).



DNA Damage Delays Progression of the Cell Cycle

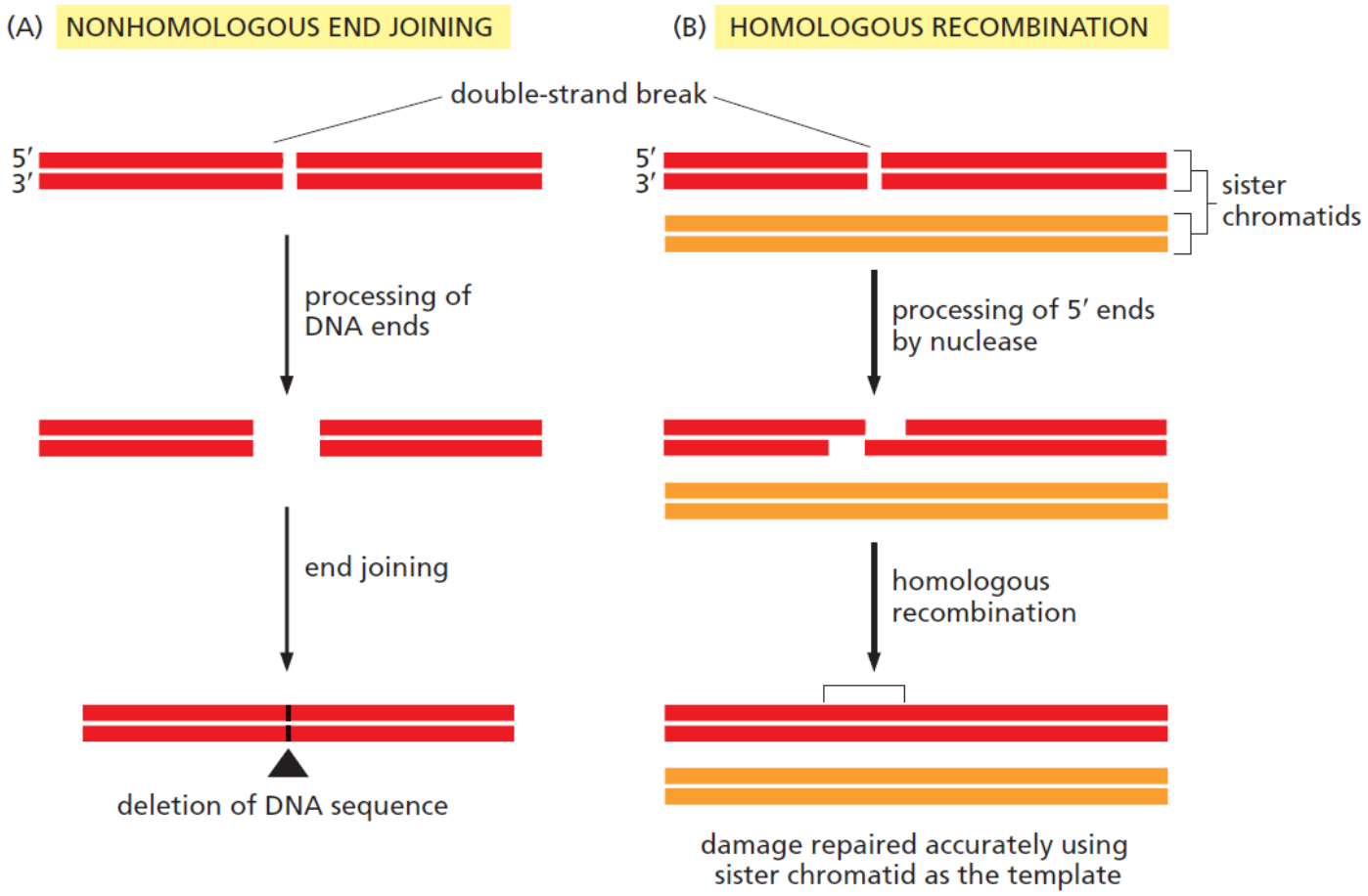
- Because of the importance of maintaining DNA intact, eukaryotic cells have an additional mechanism that maximizes the effectiveness of their DNA repair enzymes: they **delay progression of the cell cycle** until DNA repair is complete.

- The orderly progression of the cell cycle is stopped if damaged DNA is detected, and it restarts when the damage has been repaired (P53 and other pathways)

In mammalian cells, the presence of DNA damage can block:

- Entry from G1 into S phase,
- Slow S phase once it has begun
- Block the transition from G2 phase to M phase.

Two ways to repair double strand breaks.



A) **Nonhomologous end joining:** alters the original DNA sequence when repairing a broken chromosome.

The initial degradation of the broken DNA ends is important because the nucleotides at the site of the initial **break are often damaged and cannot be ligated**.

Nonhomologous end joining usually takes place when cells **have not yet duplicated** their DNA.

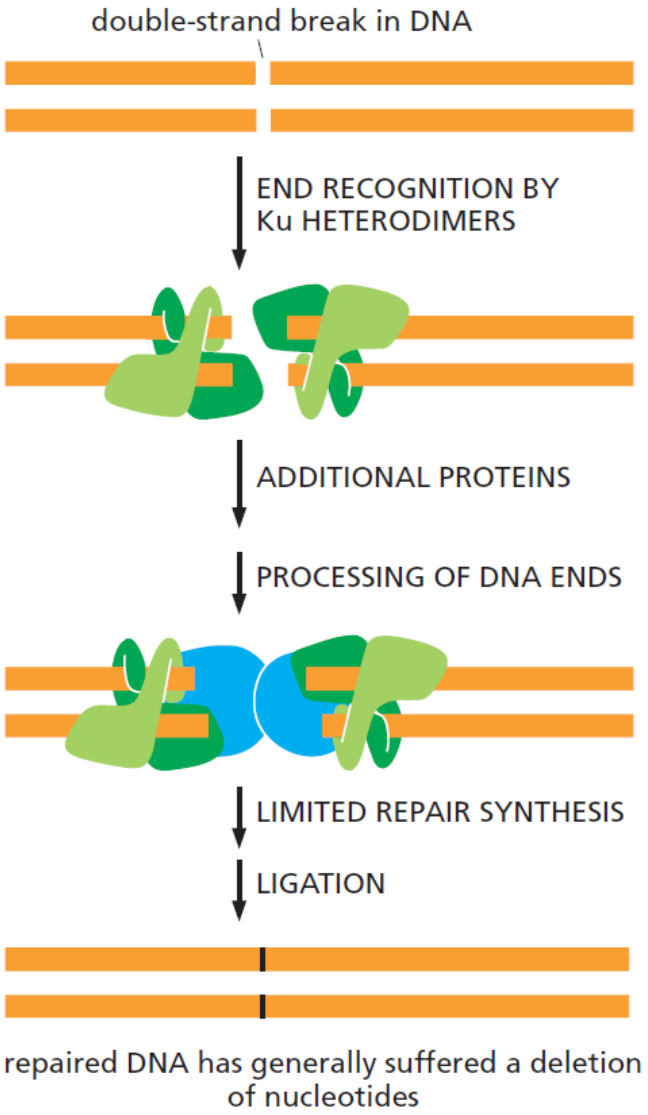
(B) **Homologous recombination:** is more difficult to accomplish but **restores the original DNA sequence**.

It typically takes place after the **DNA has been duplicated** (when a duplex template is available) but before the cell has divided.

- Most organisms employ both nonhomologous end joining and homologous recombination to repair double-strand breaks in DNA.

- Nonhomologous end joining predominates in humans; homologous recombination is used only during and shortly after DNA replication (in S and G2 phases), when sister chromatids are available to serve as templates.

Nonhomologous end joining

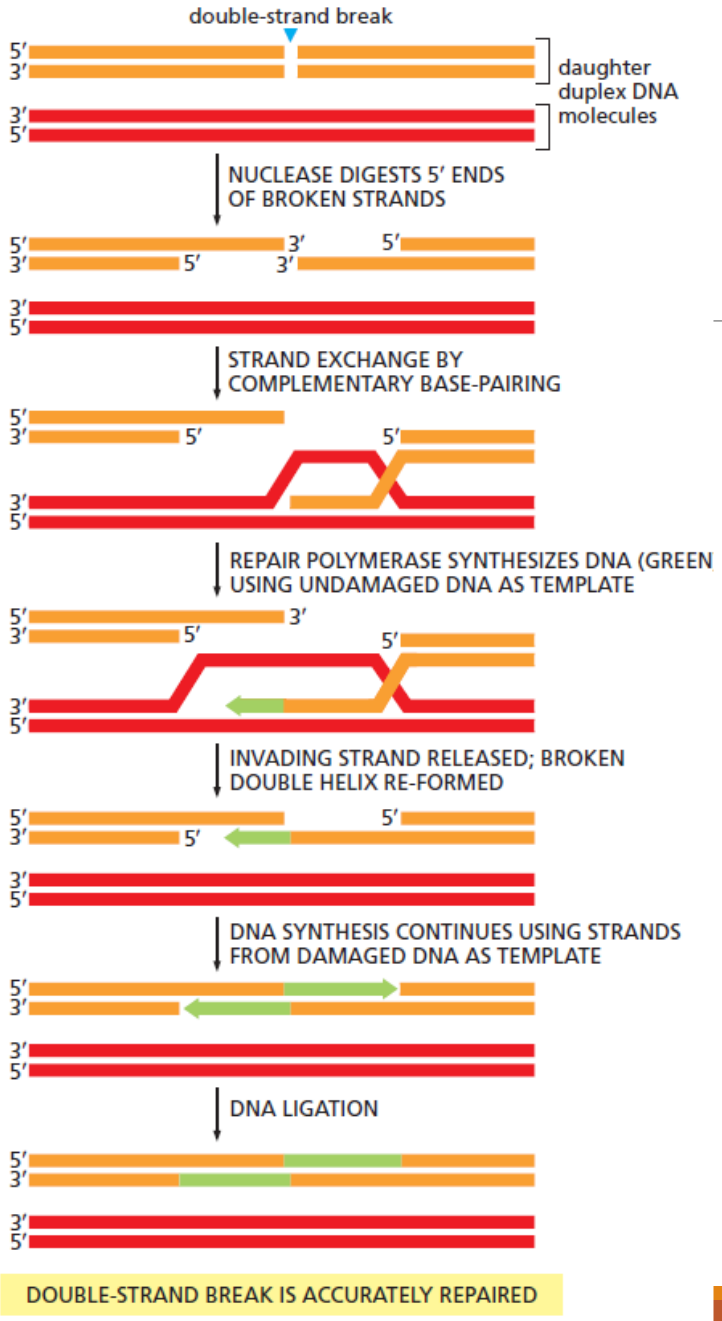


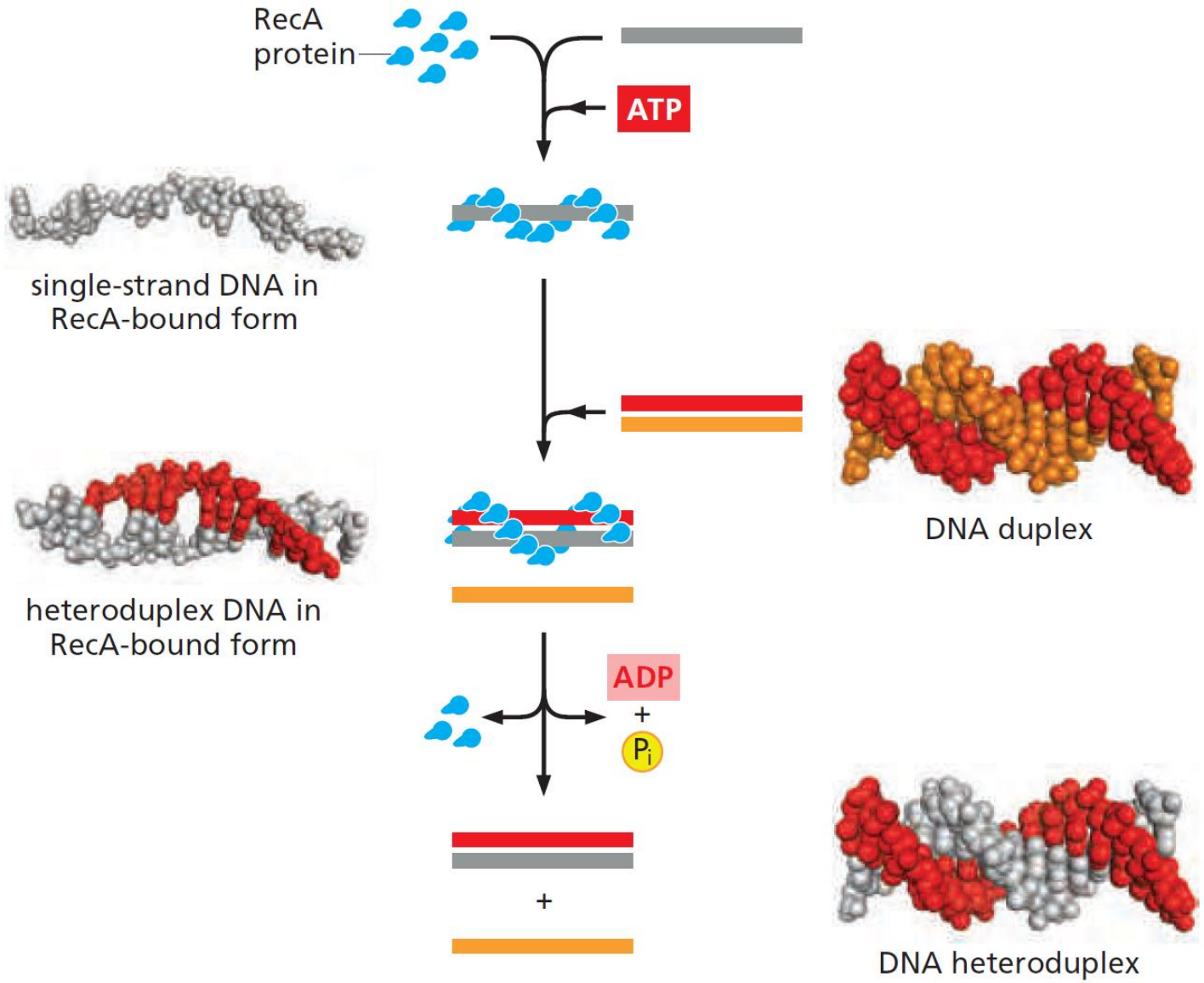
- A central role is played by the Ku protein, a heterodimer that grasps the broken chromosome ends.
- The additional proteins shown are needed to hold the broken ends together while they are processed and eventually joined covalently.

HOMOLOGOUS RECOMBINATION

- Because the template for repair is not limited to the strand complementary to that containing the damage, homologous recombination can **repair many types of DNA damage**.
- Double-strand breaks can result from radiation and reactive chemicals, but most of the time they arise from **DNA replication forks** that become stalled or broken independently of any such external cause.
- Homologous recombination **accurately** corrects these accidents and, because they occur during nearly every round of DNA replication, this repair mechanism is essential for every proliferating cell.
- Because of the "all-purpose" nature of recombinational repair it is conserved in virtually all cells on Earth.

Mechanism of double strand break repair by homologous recombination.



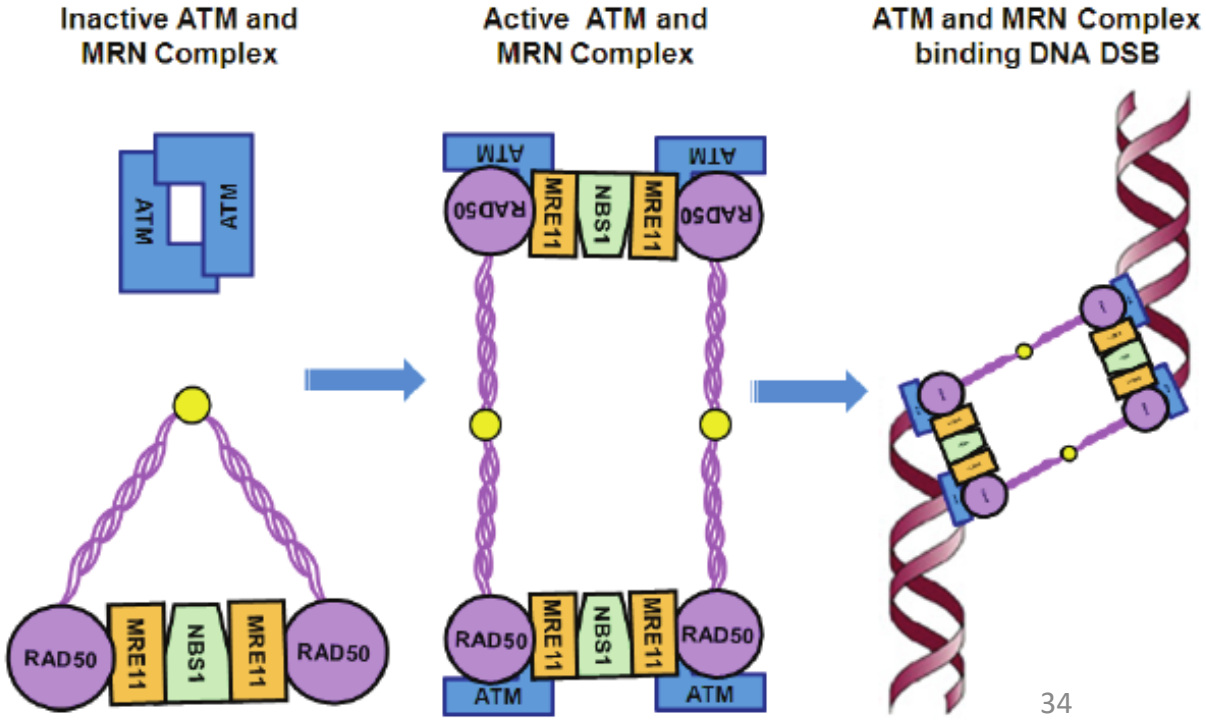

RecA
protein
ATP
single-strand DNA in
RecA-bound form
DNA duplex
heteroduplex DNA in
RecA-bound form
ADP
+
Pi
+
DNA heteroduplex

DSB detection and repair

- **MRN (Mre11, Rad50 and Nbs1 proteins)** complex makes up the core of the initial DSB repair machinery as an upstream effector of **HR** and partially **NHEJ**.

This complex crucially participates in all DSB repair events such as:

- (i) DNA damage sensing (Together with ATM)
- (ii) DDR protein recruitment to the damaged site
- (iii) cell cycle checkpoints activation
- (iv) damage repair

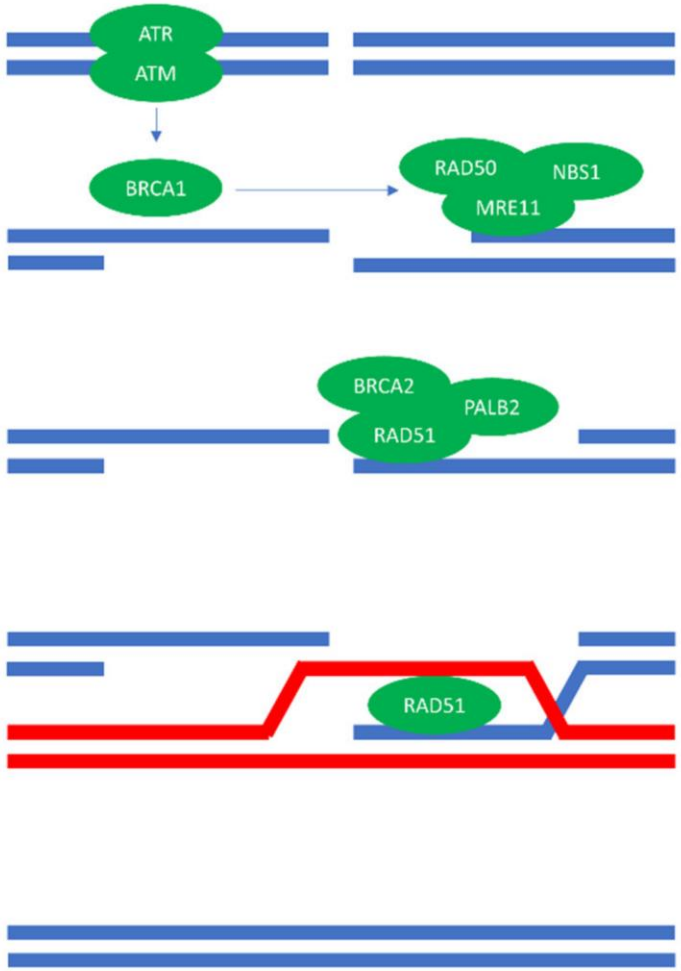


- DSBs detected by the MRN complex activate the cell cycle regulatory serine/threonine kinases **ataxia telangiectasia mutated (ATM**) and **Rad3-related protein (ATR**) to allow the formation of **protruding 3' ends** at both sides of the break.

- Subsequently, **ATM** activates **CHK2**, which arrests cell cycle progression, interacts with **TP53** that is responsible for cell cycle and apoptosis control.

- The **MRN complex also attracts BRCA1** to the DNA damage site, supporting DNA resection, forming the adjoining 3' ends and recruiting **PALB2** and **BRCA2**.

- This formed complex of **BRCA1, PALB2 and BRCA2** finally activates **RAD51**, that is responsible for binding single-stranded DNA segments and invading the homologous sequences in the sister chromatid.

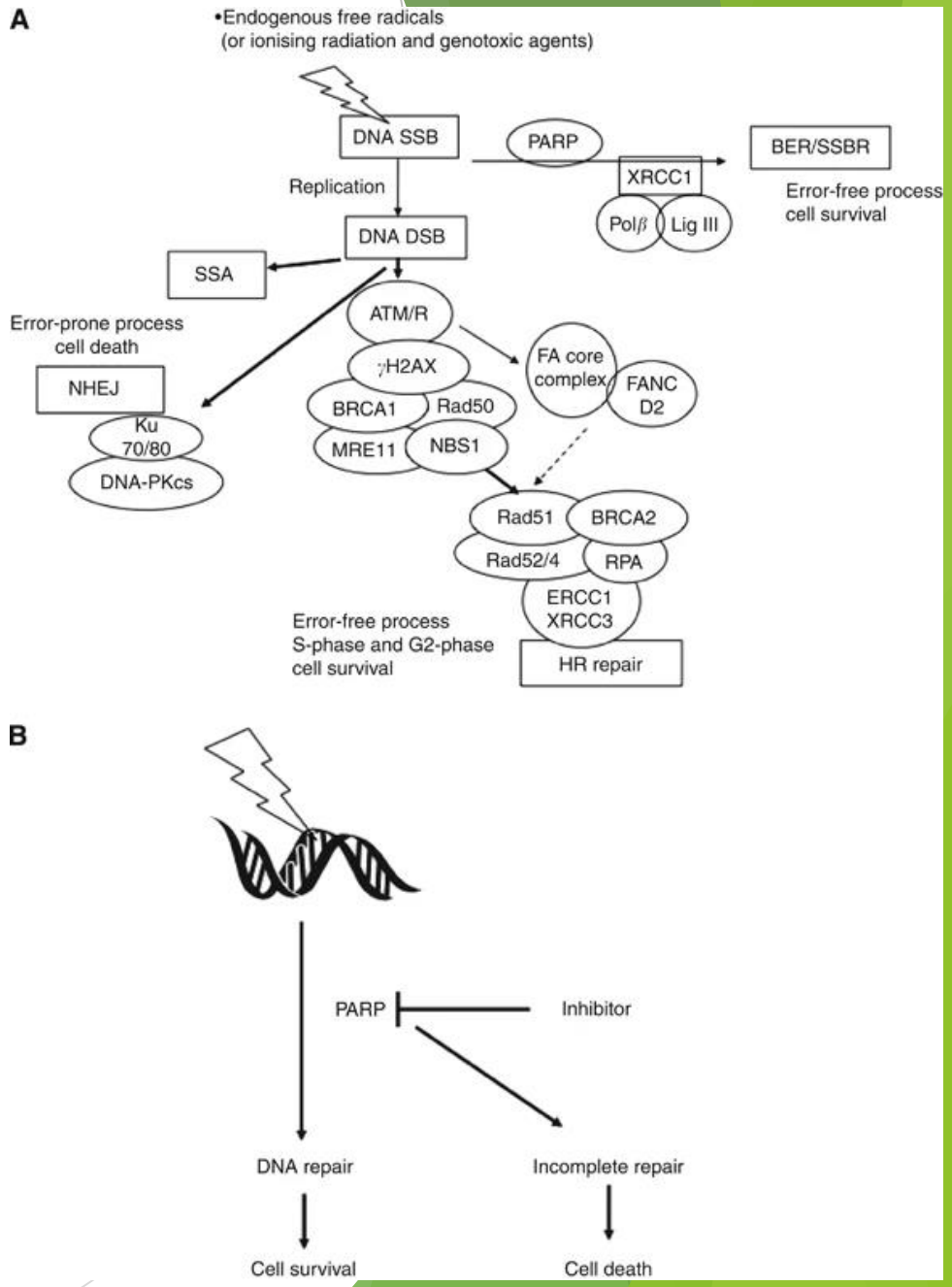


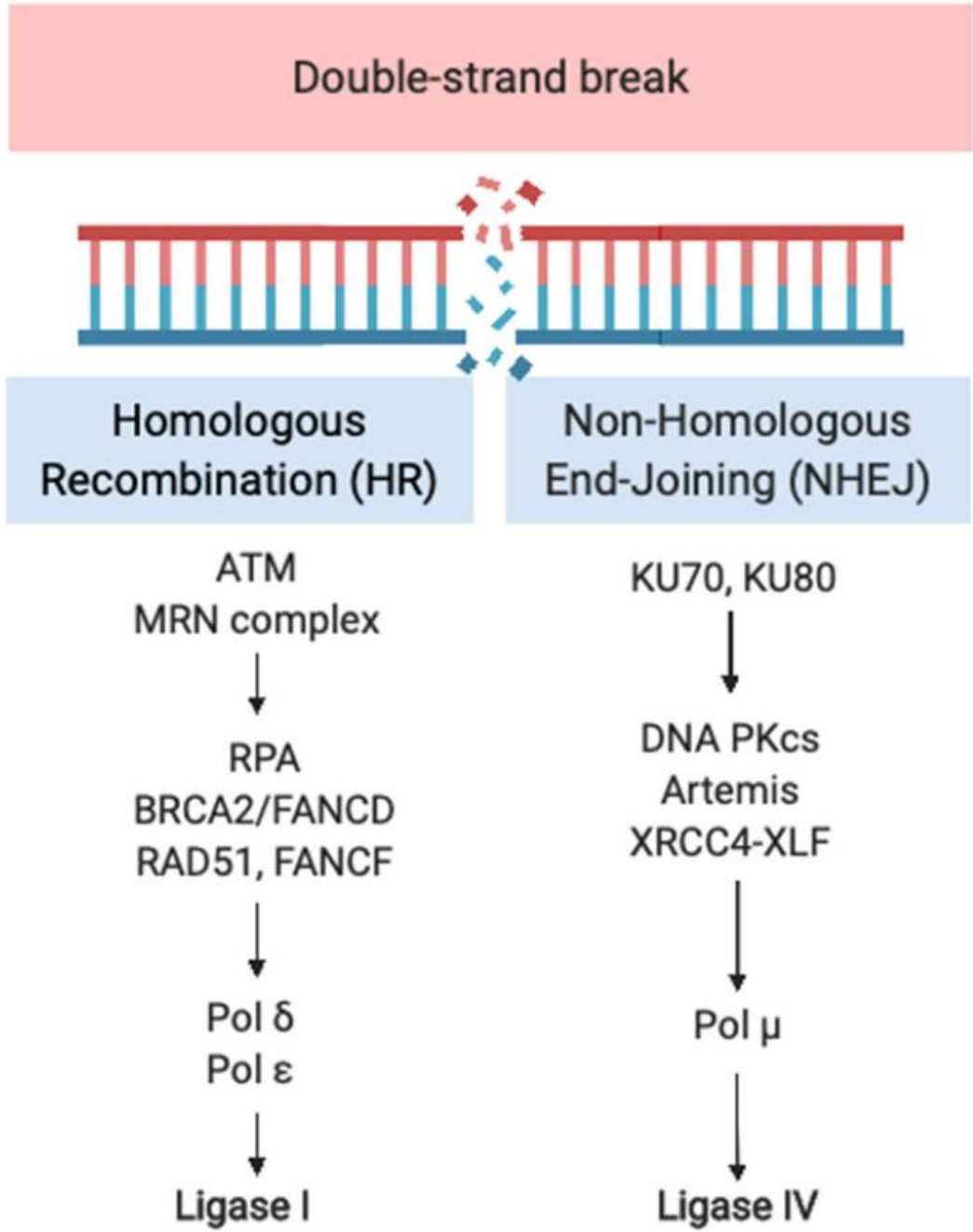
- Alternatively, **ATR** is activated by the presence of a DNA crosslinking adduct phosphorylating the **Fanconi anaemia (FA)** core complex, which helps to excise the defect.

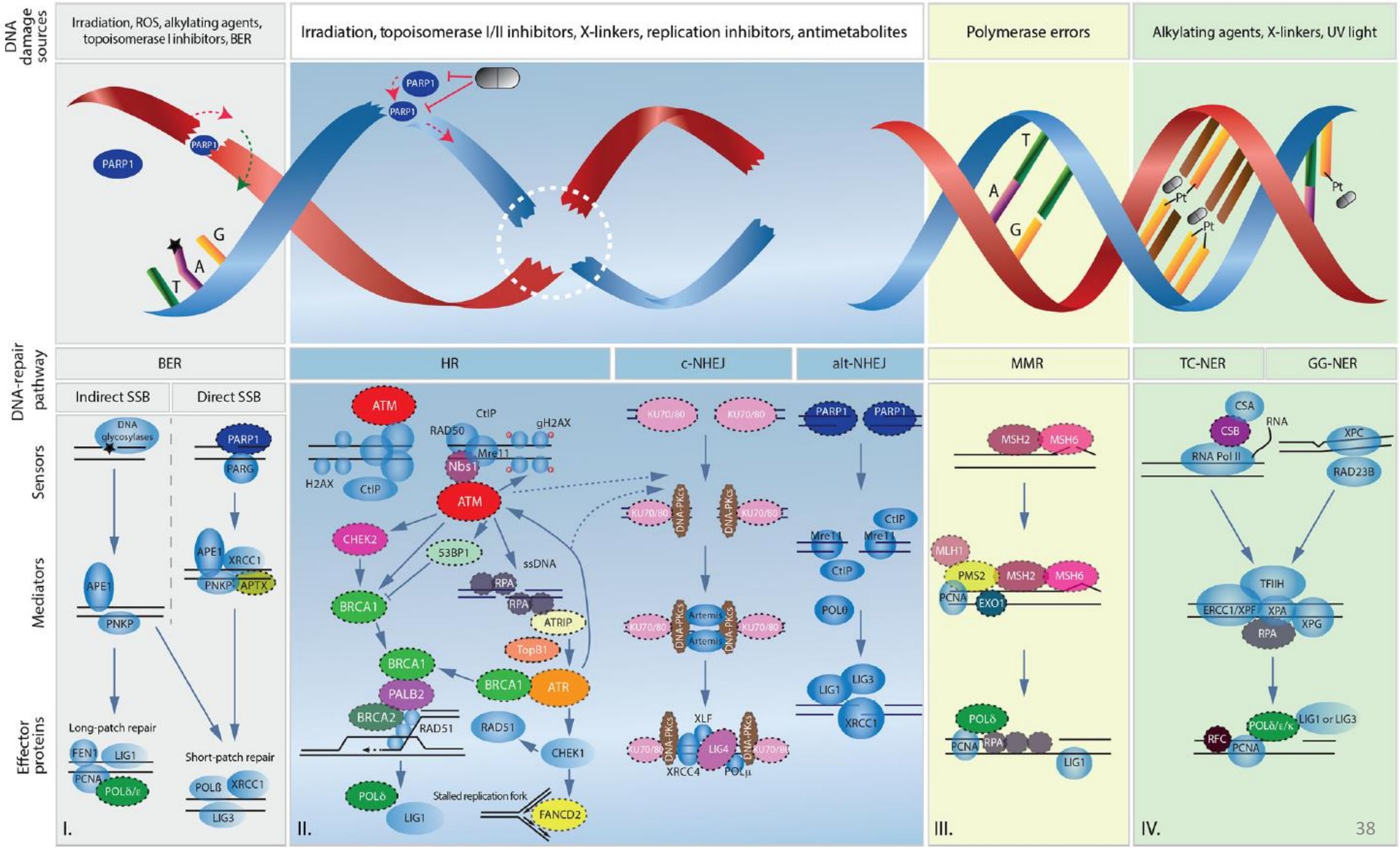
- During this DNA crosslink repair process involving NER, DSBs are generated in the proximity of the incised oligonucleotide.

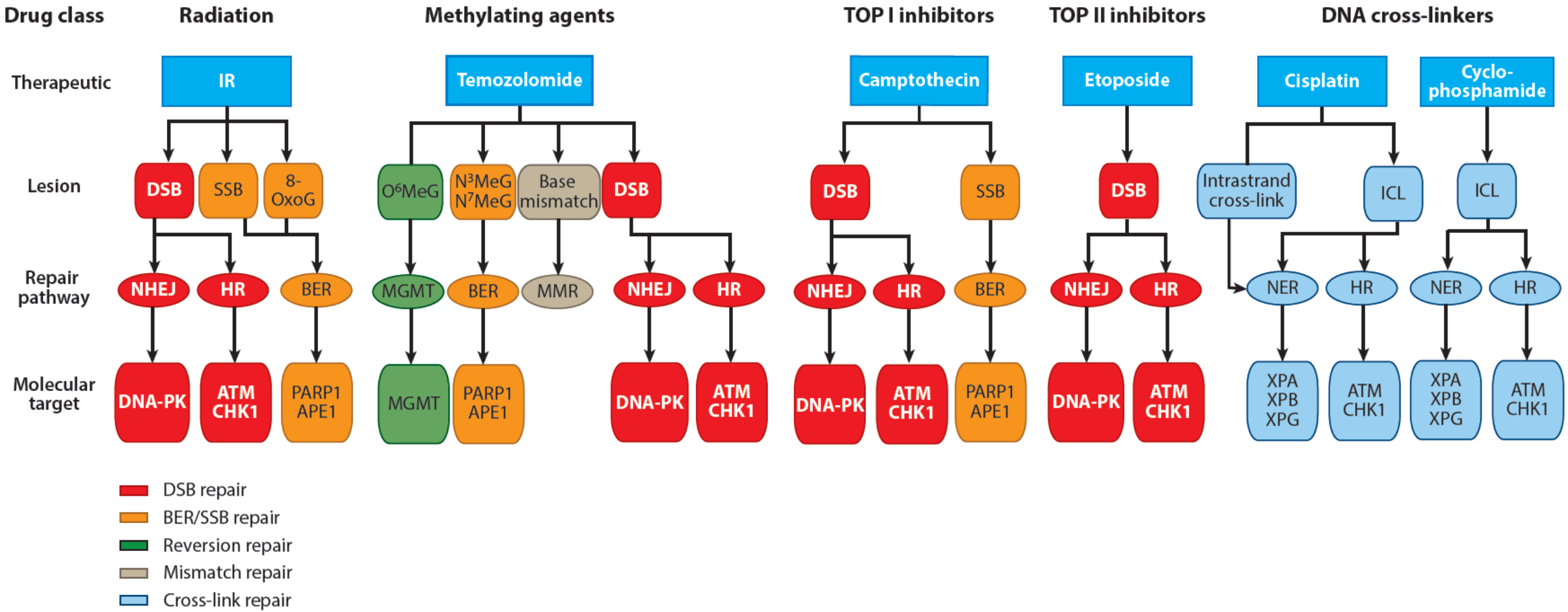
- Subsequently, this accumulation of DSBs requires repair, particularly by HR and not NHEJ.

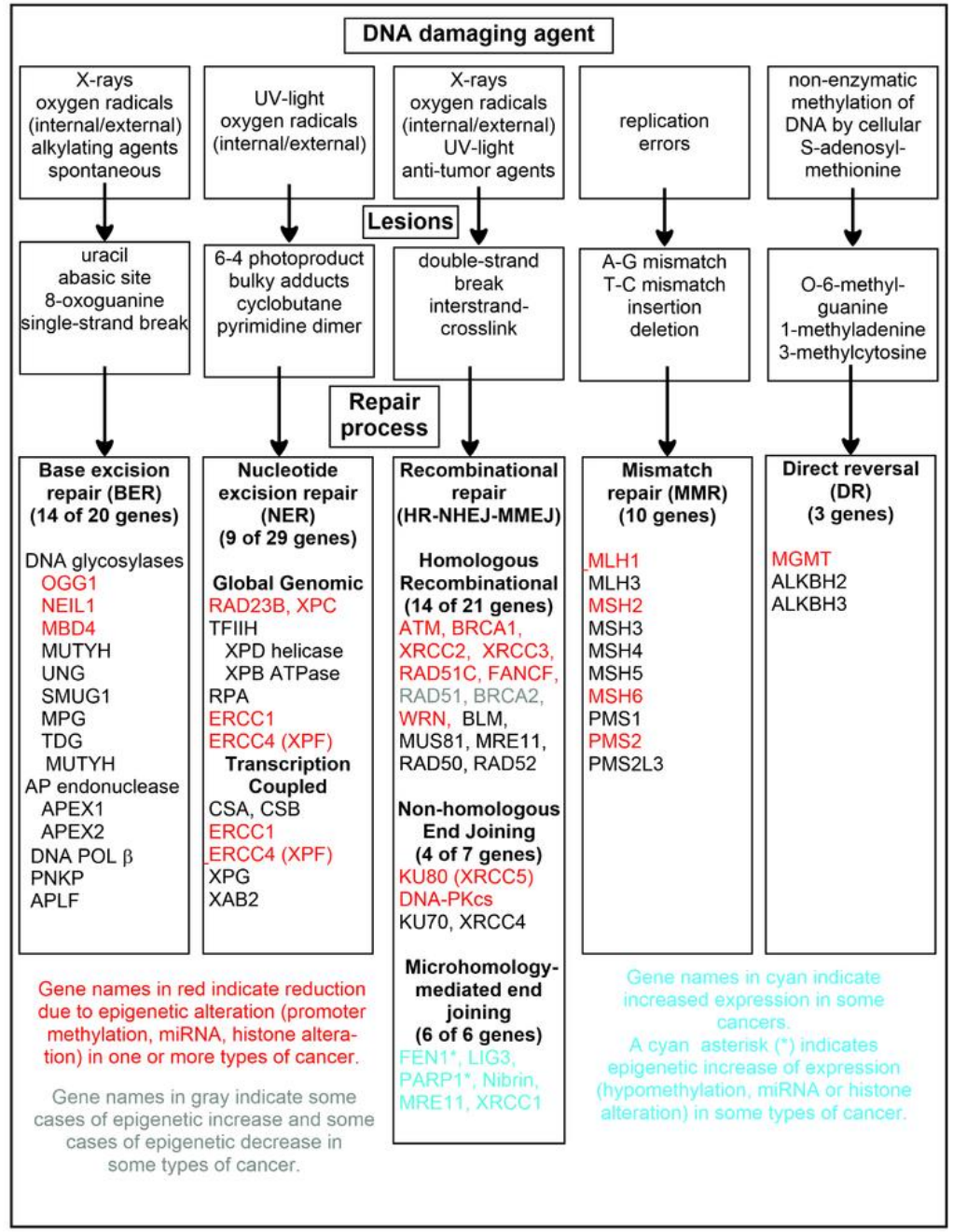
- Consequently, mutations in HR genes (such as *BRCA1*, *BRCA2*, *XRCC2* and *XRCC3*) can further exhibit hypersensitivity to crosslinking agents.




Double-strand break
Homologous Recombination (HR)
Non-Homologous End-Joining (NHEJ)
ATM
MRN complex
RPA
BRCA2/FANCD
RAD51, FANCF
Pol δ
Pol ε
Ligase I
KU70, KU80
DNA PKcs
Artemis
XRCC4-XLF
Pol μ
Ligase IV


DNA damage sources
Irradiation, ROS, alkylating agents, topoisomerase I inhibitors, BER
Irradiation, topoisomerase I/II inhibitors, X-linkers, replication inhibitors, antimetabolites
Polymerase errors
Alkylating agents, X-linkers, UV light
DNA-repair pathway
BER
HR
c-NHEJ
alt-NHEJ
MMR
TC-NER
GG-NER
Indirect SSB
Direct SSB
Sensors
Mediators
Effector proteins
PARP1
DNA glycosylases
PARG
APE1
PNKP
XRCC1
APTX
Long-patch repair
Short-patch repair
FEN1
LIG1
PCNA
POLδ/ε
POLß
LIG3
ATM
CtIP
RAD50
Mre11
Nbs1
gH2AX
H2AX
CHEK2
53BP1
ssDNA
RPA
ATRIP
BRCA1
TopB1
PALB2
BRCA2
RAD51
ATR
CHEK1
POLδ
Stalled replication fork
FANCD2
KU70/80
DNA-PKcs
Artemis
XLF
LIG4
XRCC4
POLμ
POLθ
MSH2
MSH6
MLH1
PMS2
EXO1
CSA
CSB
RNA
RNA Pol II
XPC
RAD23B
TFIIH
ERCC1/XPF
XPA
XPG
RFC
POLδ/ε/κ
LIG1 or LIG3
I.
II.
III.
IV.


Drug class
Radiation
Methylating agents
TOP I inhibitors
TOP II inhibitors
DNA cross-linkers
Therapeutic
Lesion
Repair pathway
Molecular target
IR
Temozolomide
Camptothecin
Etoposide
Cisplatin
Cyclo-phosphamide
DSB
SSB
8-OxoG
O6MeG
N3MeG N7MeG
Base mismatch
Intrastrand cross-link
ICL
NHEJ
HR
BER
MGMT
MMR
NER
DNA-PK
ATM CHK1
PARP1 APE1
XPA XPB XPG
DSB repair
BER/SSB repair
Reversion repair
Mismatch repair
Cross-link repair


DNA damaging agent
X-rays
oxygen radicals
(internal/external)
alkylating agents
spontaneous
UV-light
oxygen radicals
(internal/external)
X-rays
oxygen radicals
(internal/external)
UV-light
anti-tumor agents
replication
errors
non-enzymatic
methylation of
DNA by cellular
S-adenosyl-
methionine
Lesions
uracil
abasic site
8-oxoguanine
single-strand break
6-4 photoproduct
bulky adducts
cyclobutane
pyrimidine dimer
double-strand
break
interstrand-
crosslink
A-G mismatch
T-C mismatch
insertion
deletion
O-6-methyl-
guanine
1-methyladenine
3-methylcytosine
Repair
process
Base excision
repair (BER)
(14 of 20 genes)
DNA glycosylases
OGG1
NEIL1
MBD4
MUTYH
UNG
SMUG1
MPG
TDG
MUTYH
AP endonuclease
APEX1
APEX2
DNA POL β
PNKP
APLF
Nucleotide
excision repair
(NER)
(9 of 29 genes)
Global Genomic
RAD23B, XPC
TFIIH
XPD helicase
XPB ATPase
RPA
ERCC1
ERCC4 (XPF)
Transcription
Coupled
CSA, CSB
ERCC1
ERCC4 (XPF)
XPG
XAB2
Recombinational
repair
(HR-NHEJ-MMEJ)
Homologous
Recombinational
(14 of 21 genes)
ATM, BRCA1,
XRCC2, XRCC3,
RAD51C, FANCF,
RAD51, BRCA2,
WRN, BLM,
MUS81, MRE11,
RAD50, RAD52
Non-homologous
End Joining
(4 of 7 genes)
KU80 (XRCC5)
DNA-PKcs
KU70, XRCC4
Microhomology-
mediated end
joining
(6 of 6 genes)
FEN1*, LIG3,
PARP1*, Nibrin,
MRE11, XRCC1
Mismatch
repair (MMR)
(10 genes)
MLH1
MLH3
MSH2
MSH3
MSH4
MSH5
MSH6
PMS1
PMS2
PMS2L3
Direct reversal
(DR)
(3 genes)
MGMT
ALKBH2
ALKBH3
Gene names in red indicate reduction due to epigenetic alteration (promoter methylation, miRNA, histone alteration) in one or more types of cancer.
Gene names in gray indicate some cases of epigenetic increase and some cases of epigenetic decrease in some types of cancer.
Gene names in cyan indicate increased expression in some cancers.
A cyan asterisk (*) indicates epigenetic increase of expression (hypomethylation, miRNA or histone alteration) in some types of cancer.