Лекции N°1-4

«**Повреждения клеточной ДНК и биологические последствия химической модификации нуклеиновых кислот**»

НАЦИОНАЛЬНЫЙ ЦЕНТР НАУЧНЫХ ИССЛЕДОВАНИЙ ФРАНЦИЯ
Centre National de la Recherche Scientifique
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Research Director, заведующий лабораторией
САПАРБАЕВ Мурат Калиевич

Types of DNA damage

1.Spontaneous

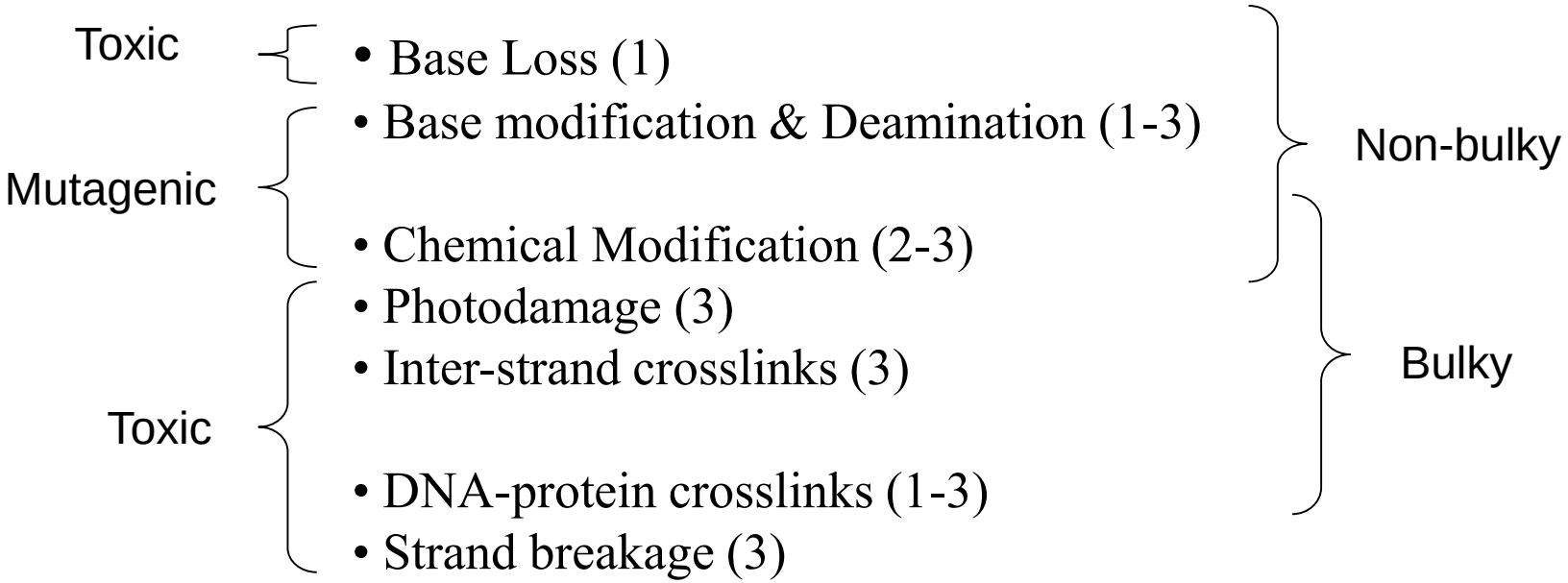
Intrinsic instability of DNA

2. Endogenous

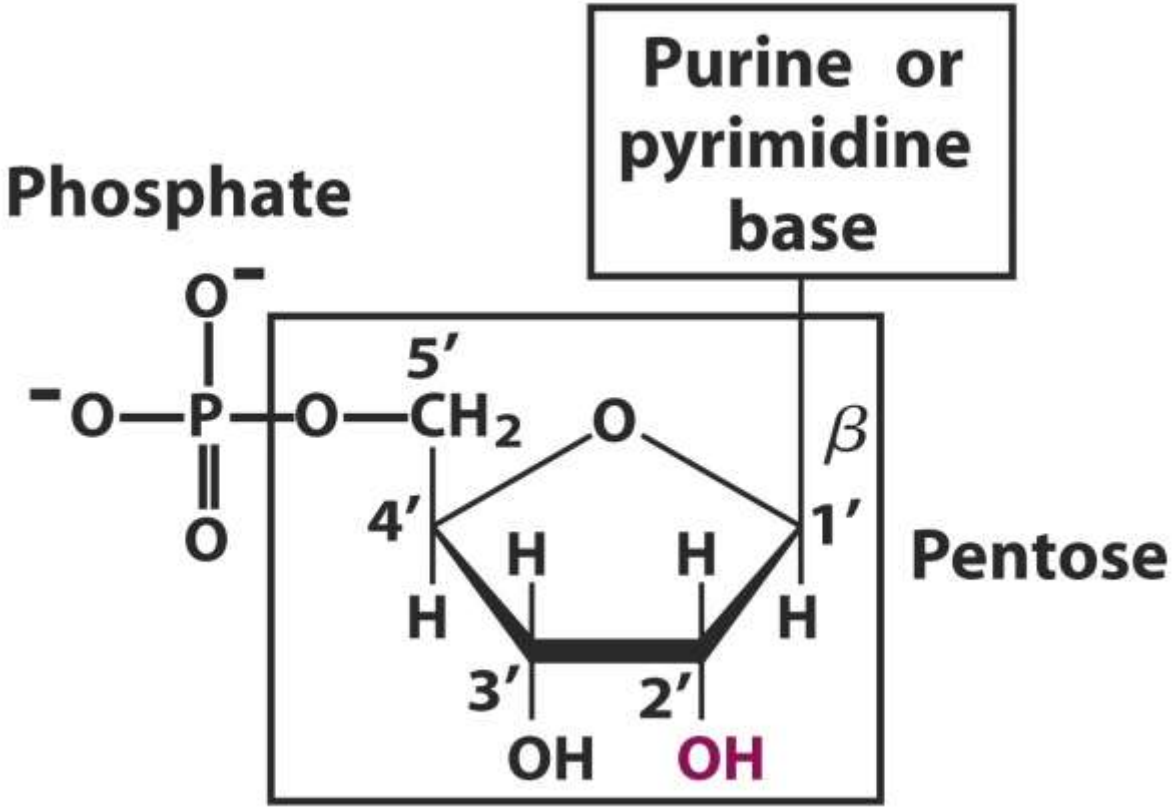
Oxidative metabolism

3. Exogenous

Ultraviolet
Ionizing radiation
Industrial pollutants
Drugs



Nucleotides are the building blocks of **nucleic acids**



Damage can occur at the base, sugar or phosphate

Sources of DNA damage: spontaneous and induced.

1) Spontaneous chemical changes in the DNA

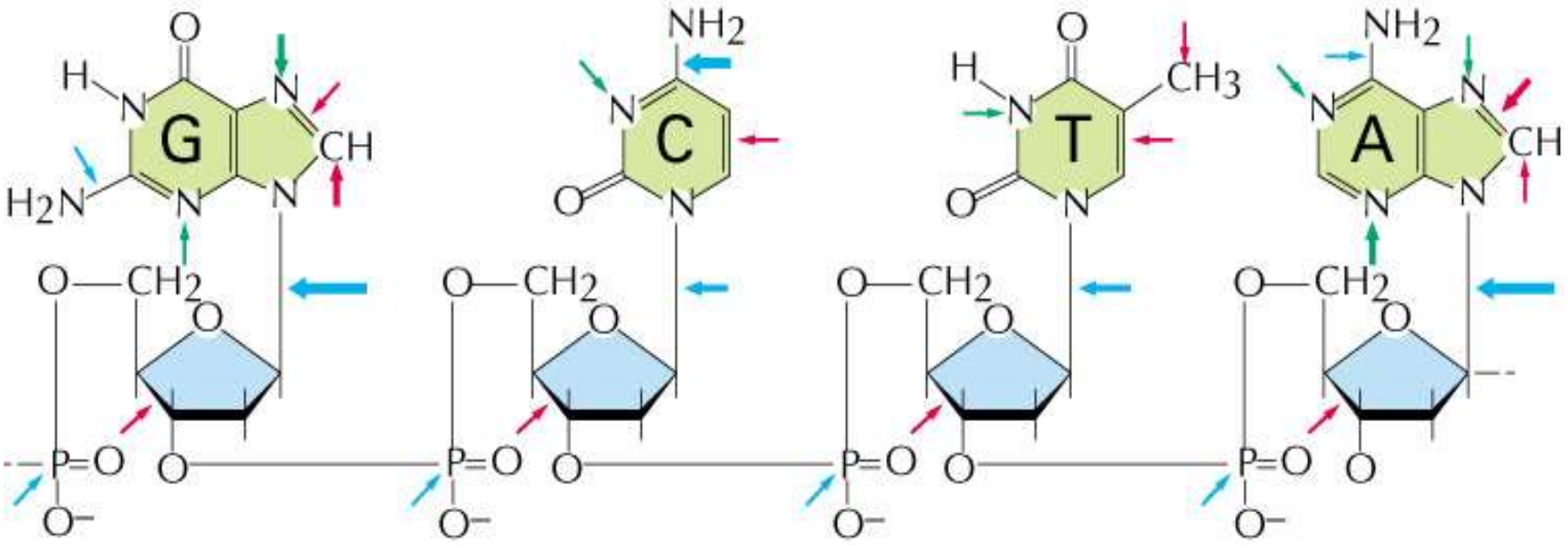
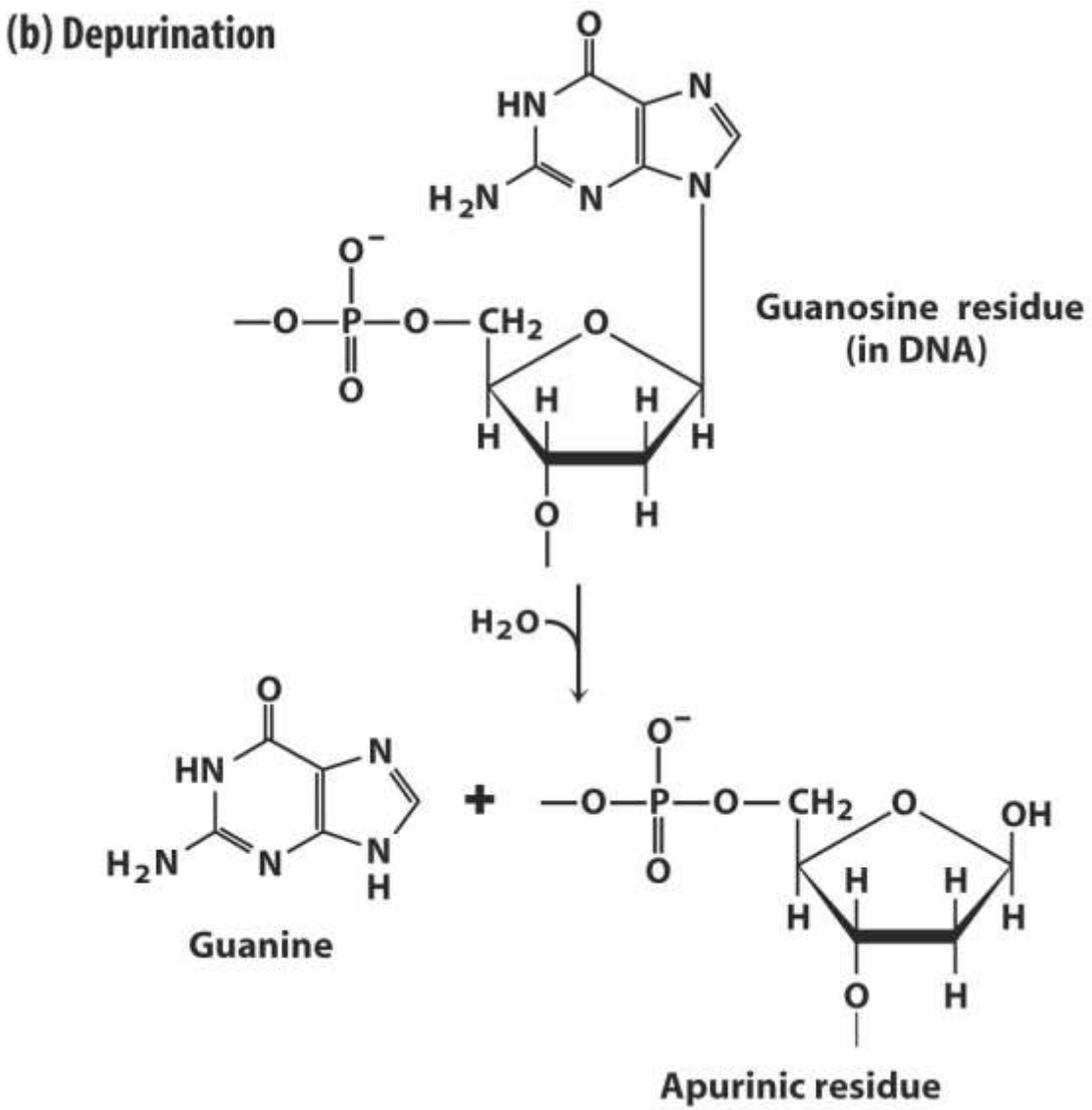


Figure 5–46. Molecular Biology of the Cell, 4th Edition.

Oxidation (red arrows) Hydrolysis (blue arrows) Methylation (green arrows

Depurination

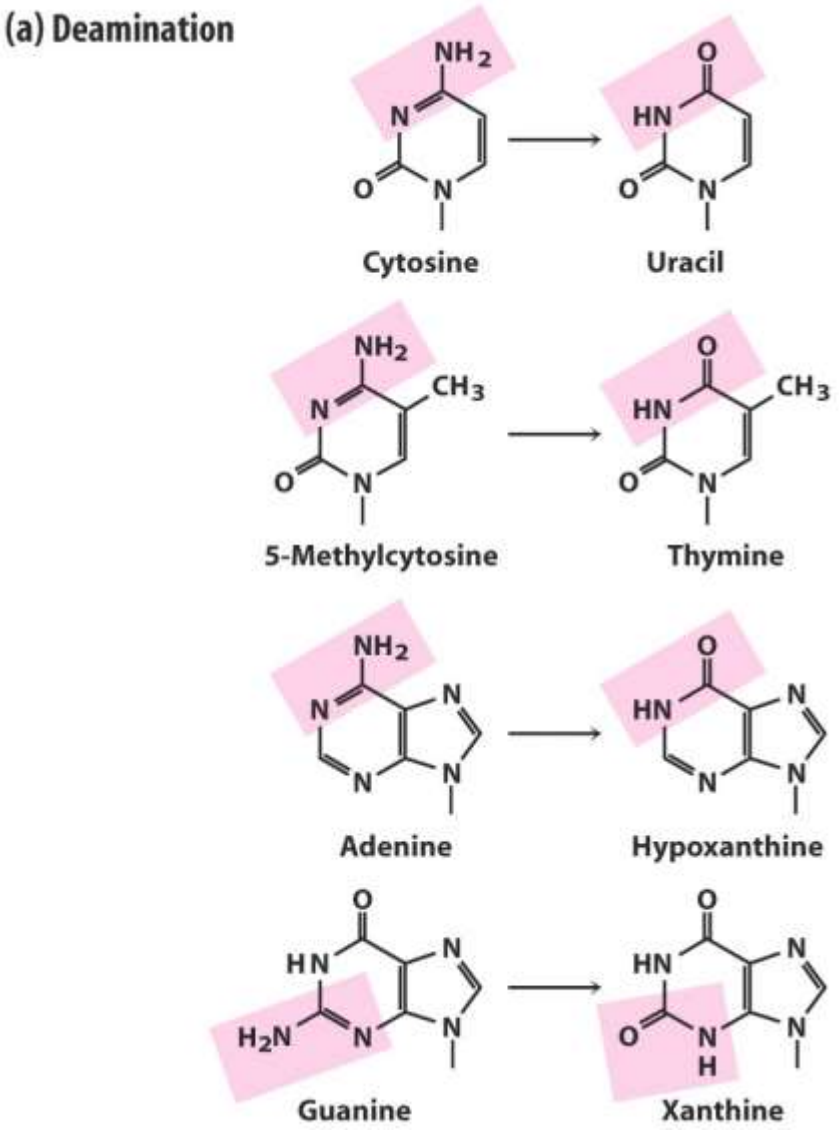


- The *N*-glycosyl bond between the base and the pentose can undergo hydrolysis.
- The rate is faster for purines than for pyrimidines.
- 1/100,000 purines are lost from DNA every 24 hours
- Depurination of RNA is much slower.

- dATP is likely to be inserted opposite abasic site

Base deamination

- Nucleotide bases can undergo spontaneous loss of their exocyclic amino groups (deamination).
- Under typical cellular conditions, deamination off cytocine in DNA to uracil occurs in about one of every 10^7 cytidine residues in 24 hours.
- A and G deamination occurs at 1/10 of this rate.



Failure to repair a deaminated base leads to point mutation

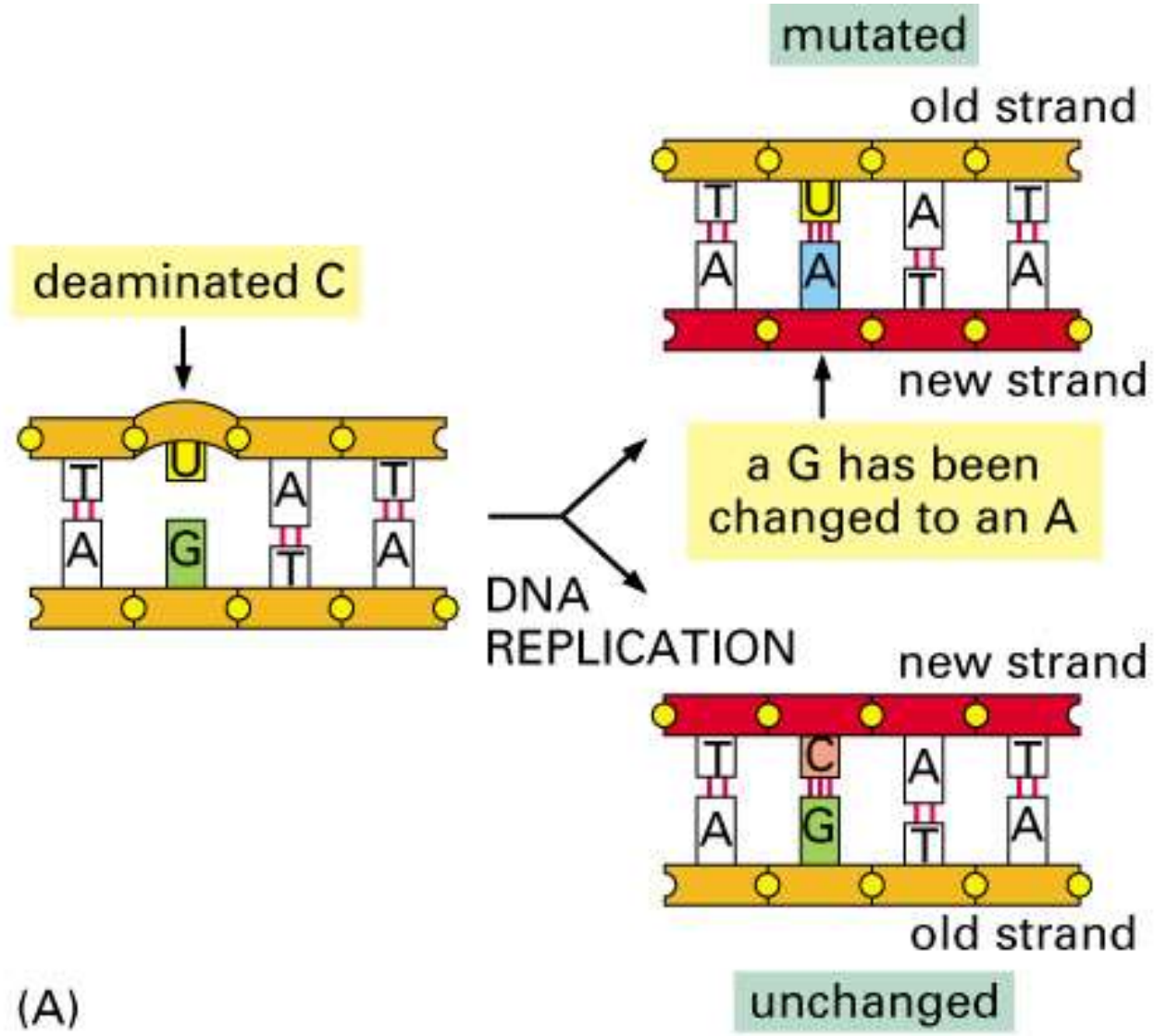


Figure 5–49 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Reactive Oxygen Species (ROS)

- Have unpaired electron (most)
- Very-to-Extremely reactive
- Non-specific
- Self propagating
- Referred to as ROS or free radicals
- Usually damaging

Major ROS

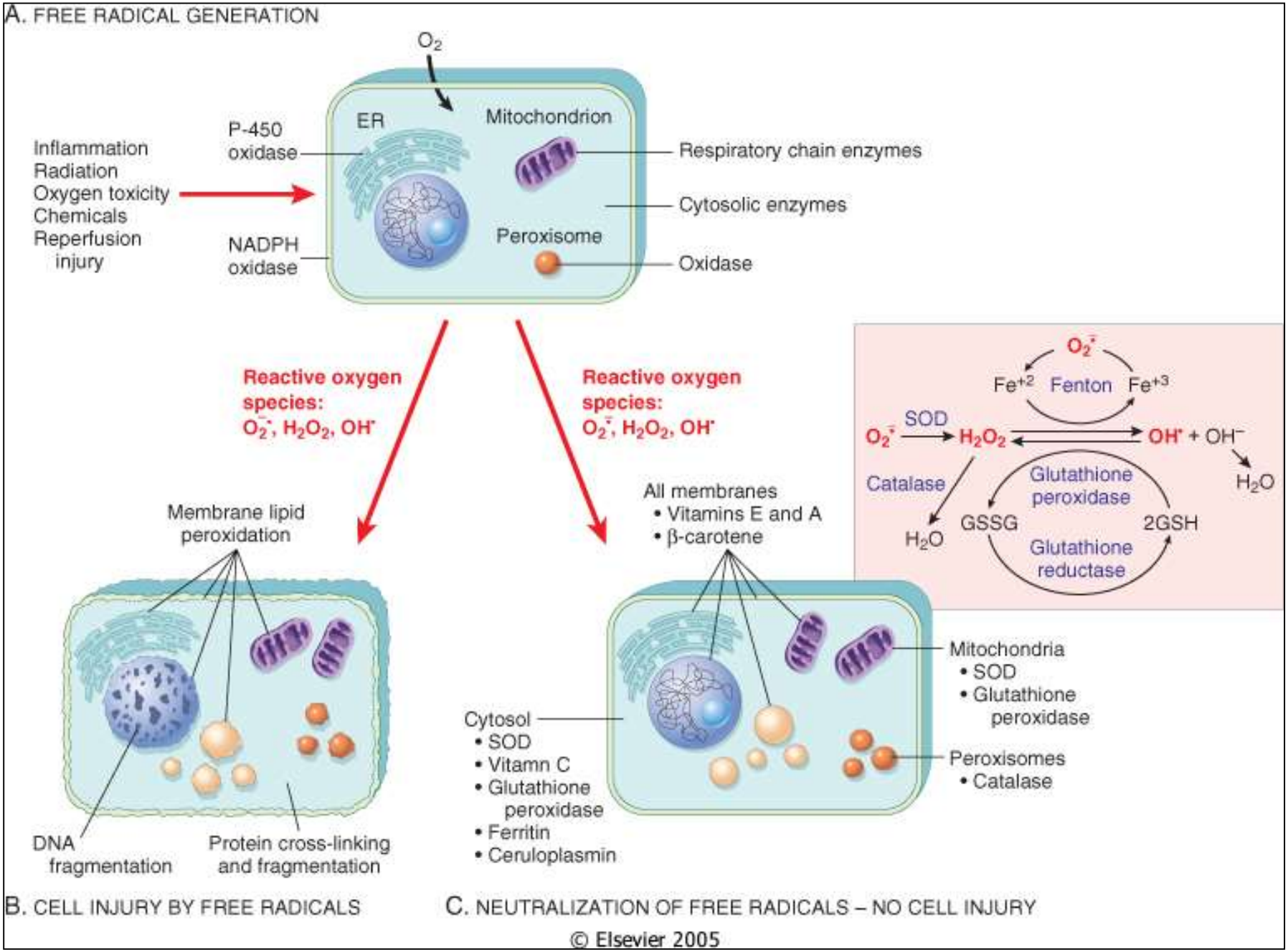
- **Superoxide ($O_2^{\cdot -}$)**
- **Hydrogen peroxide (H_2O_2)**
- **Hydroxyl radical ($HO^{\cdot}$)**
- **Nitric oxide ($NO^{\cdot}$)**

Sources of ROS

- UV light, ionizing radiation
- Mitochondrial ETS
- Enzymes (P450, XO, NADPH oxidase)
- Reduced metals (Fe, Cu, etc)
  - NB: Fenton reaction

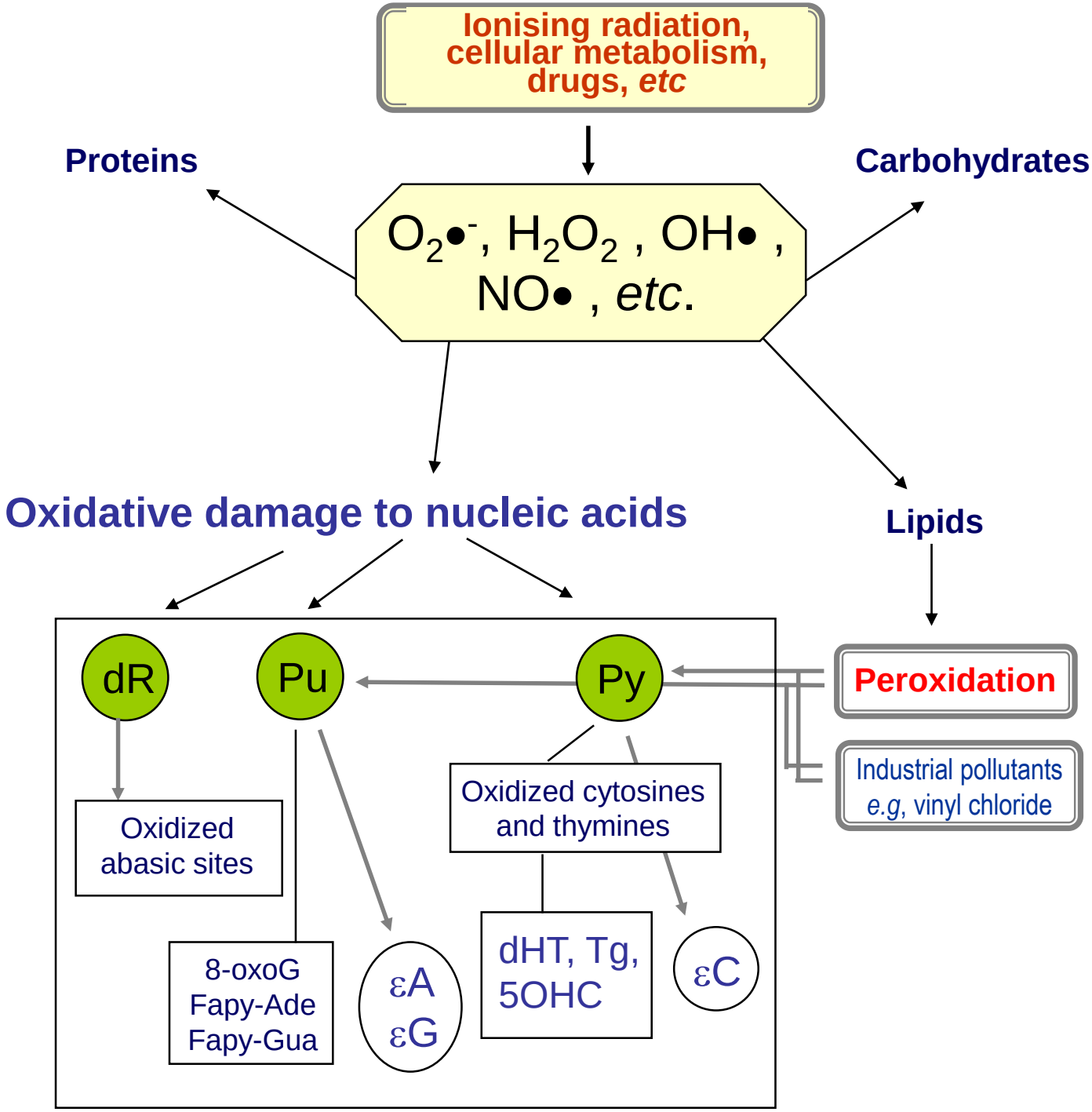
Effects/Targets of ROS

- Membranes (lipid peroxidation)
- Proteins (via SH, TRP and TYR ox, etc)
  - Cross linking; fragmentation
- DNA damage
  - Base damage



Cellular defenses against ROS (Antioxidants)

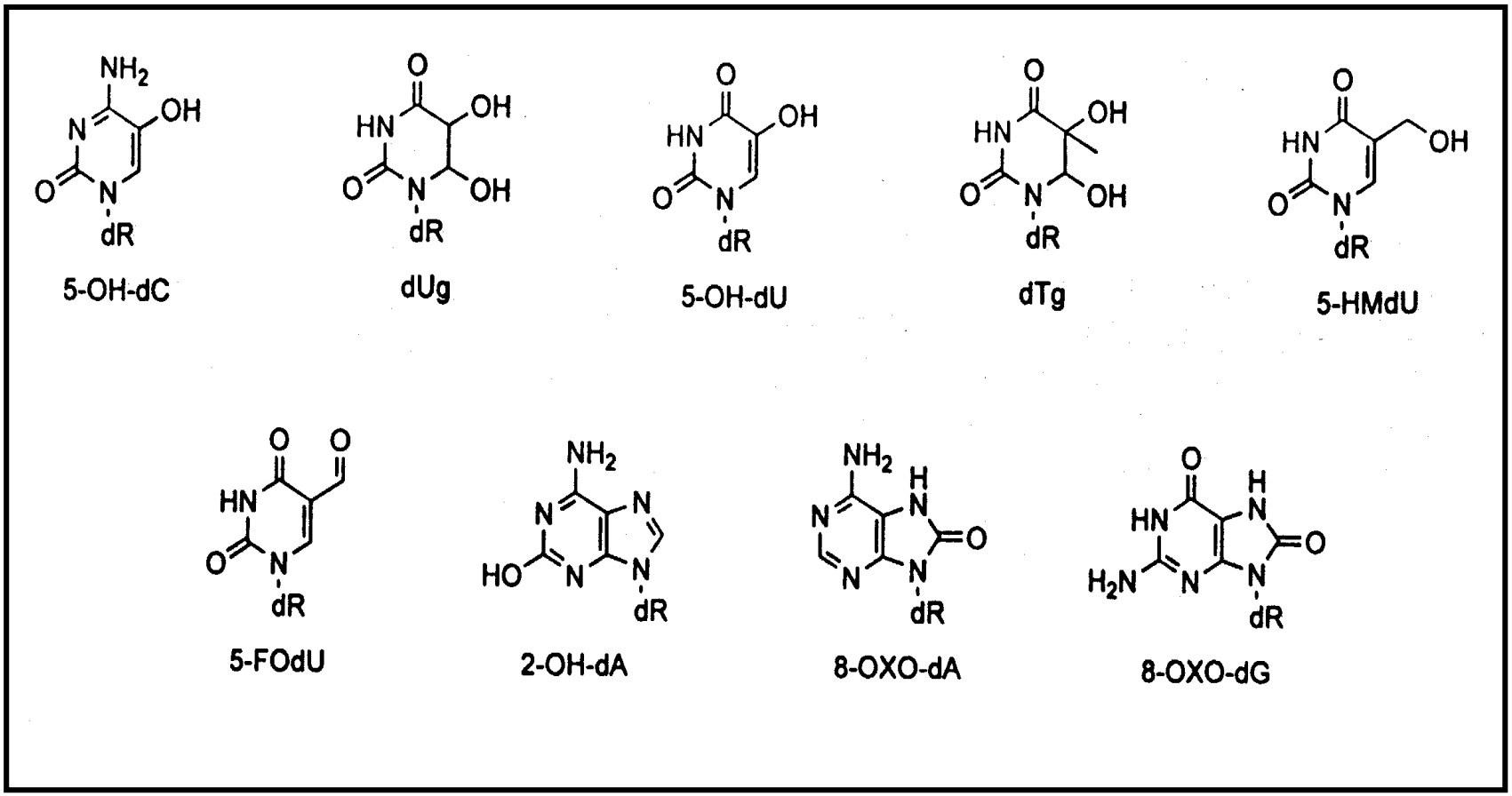
- Enzymatic
  - SOD, catalase, GPX
- Non-enzymatic
  - Vitamins A, C, E
  - Glutathione (GSH)
  - Metal binding proteins (transferrin, ceruloplasmin, etc)
  - NB: lipid and water soluble species


Ionising radiation, cellular metabolism, drugs, etc
Proteins
Carbohydrates
O2•-, H2O2 , OH• , NO• , etc.
Oxidative damage to nucleic acids
Lipids
dR
Pu
Py
Peroxidation
Industrial pollutants e.g, vinyl chloride
Oxidized abasic sites
Oxidized cytosines and thymines
8-oxoG
Fapy-Ade
Fapy-Gua
εA
εG
dHT, Tg, 5OHC
εC

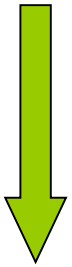
Oxidative Damage

- Variety of base lesions produced

  Typically small damage can potentially fit in polymerase active site, like mispair



Ionising radiation

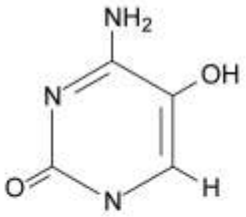


Nucleic acids

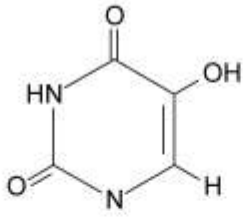
Type of damage	Number per Gy
1. Base damage	1000-2000
2. Sugar damage	800-1000
3. Single-strand breaks	1000
4. Double-strand breaks	40
5. Clustered damage	100-120

CHEMICAL STRUCTURES OF THE MAJOR DNA DAMAGE INDUCED BY IR

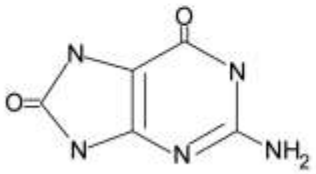
Miscoding



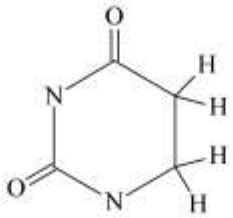
5-hydroxycytosine
C*•G⇒ T•A



5-hydroxyuracil
U*•G⇒ T•A

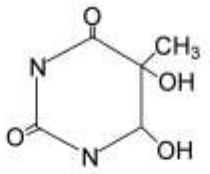


7,8-dihydro-8-oxoguanine
G*•C⇒ T•A

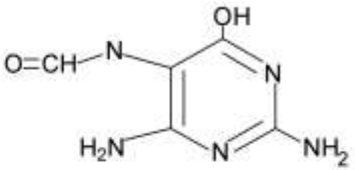


5,6-dihydrouracil
U*•G⇒ T•A

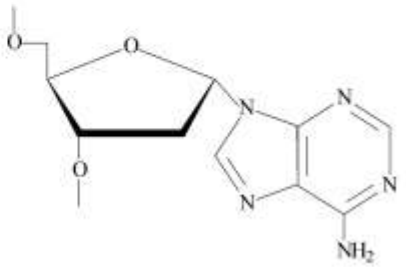
Replication block



Thymine glycol

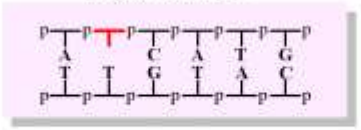


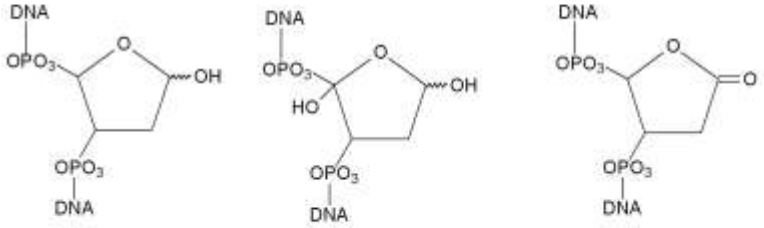
2,6-diamino-4-hydroxy-5-formamidopyrimidine



Alpha -2´-deoxyadenosine*

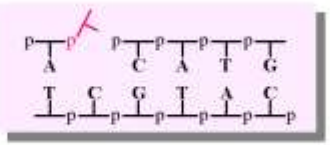
Sugar damage

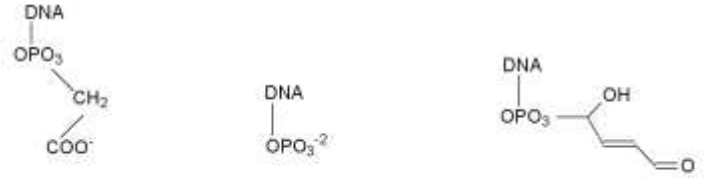




Abasic site | 2-deoxypentos-4-ulose | 2-deoxyribonolactone (dL)

Single-strand and double-strand breaks with 3'-blocking groups





3'-phosphoglycolate | 3'-phosphate | 3'-α,β-unsaturated aldehyde β-elimination product

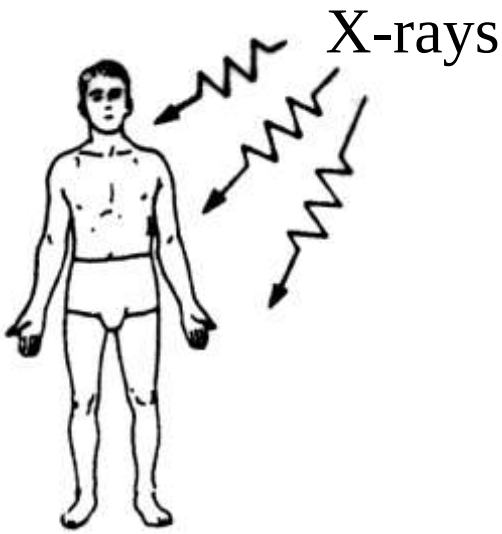
Energy equivalent of lethal radiation dose

Total body irradiation

Mass = 70 kg

LD50 dose = 4 Gy

4 Gy = 67 calories



67 calories equivalent to energy in one sip (3 ml) of hot coffee

Lethal dose is relatively modest amount of energy

Sequence of Radiation Effects

Incident X-ray photon

↓

Photon absorption via Compton effect

↓

Formation of free radicals

↓

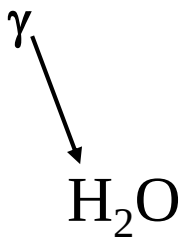
Radical induced DNA damage

↓

Biologic effect (mutation, cancer, etc.)

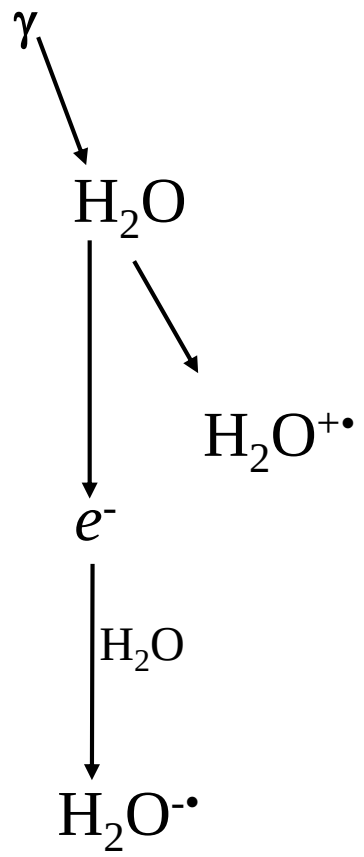
Radiation interaction with water

Initial reaction with cells



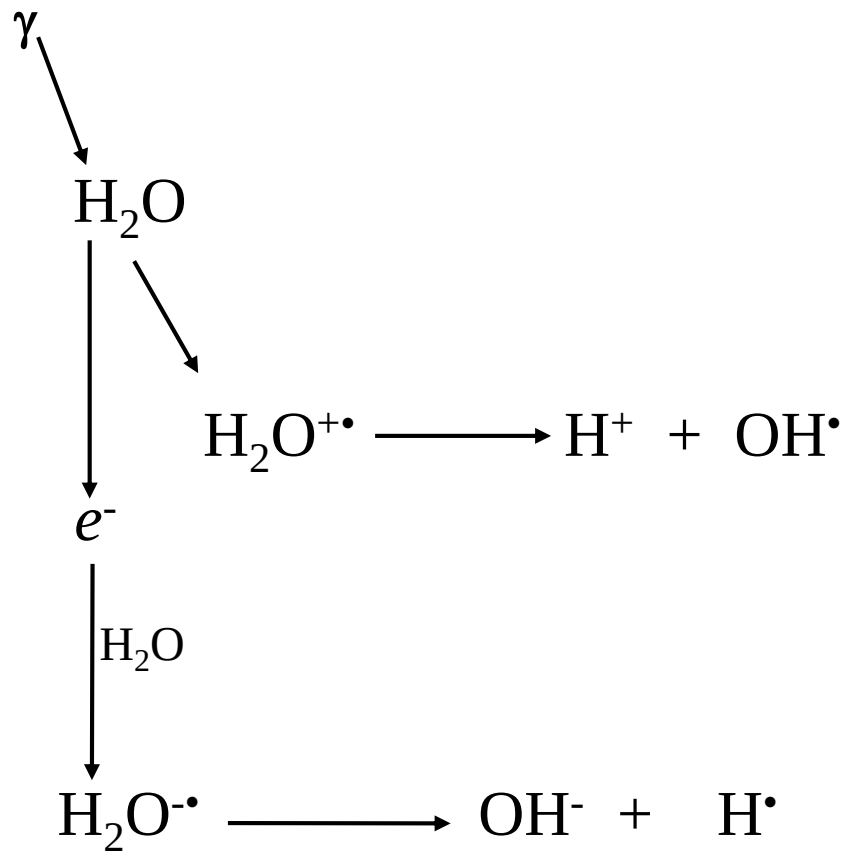
Radiation interaction with water

Initial reaction with cells



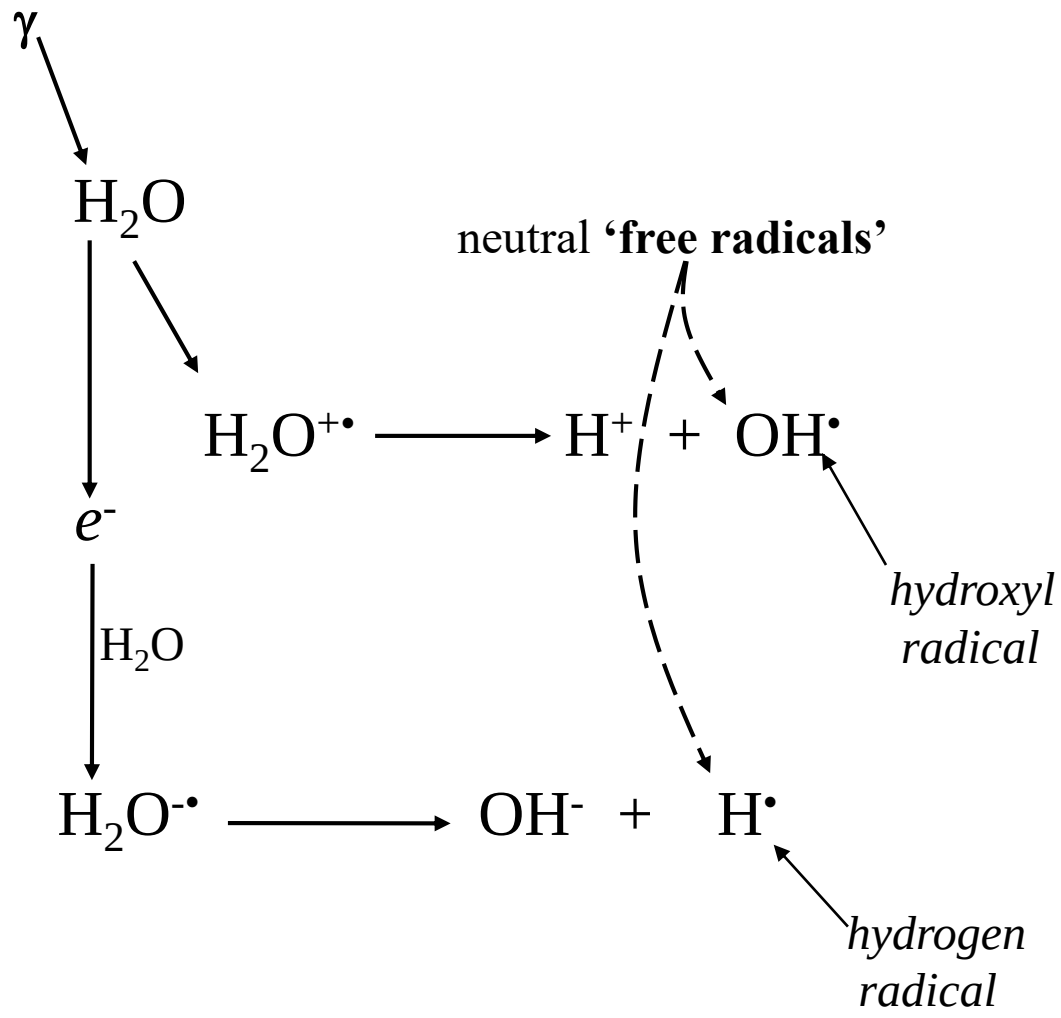
Radiation interaction with water

Initial reaction with cells



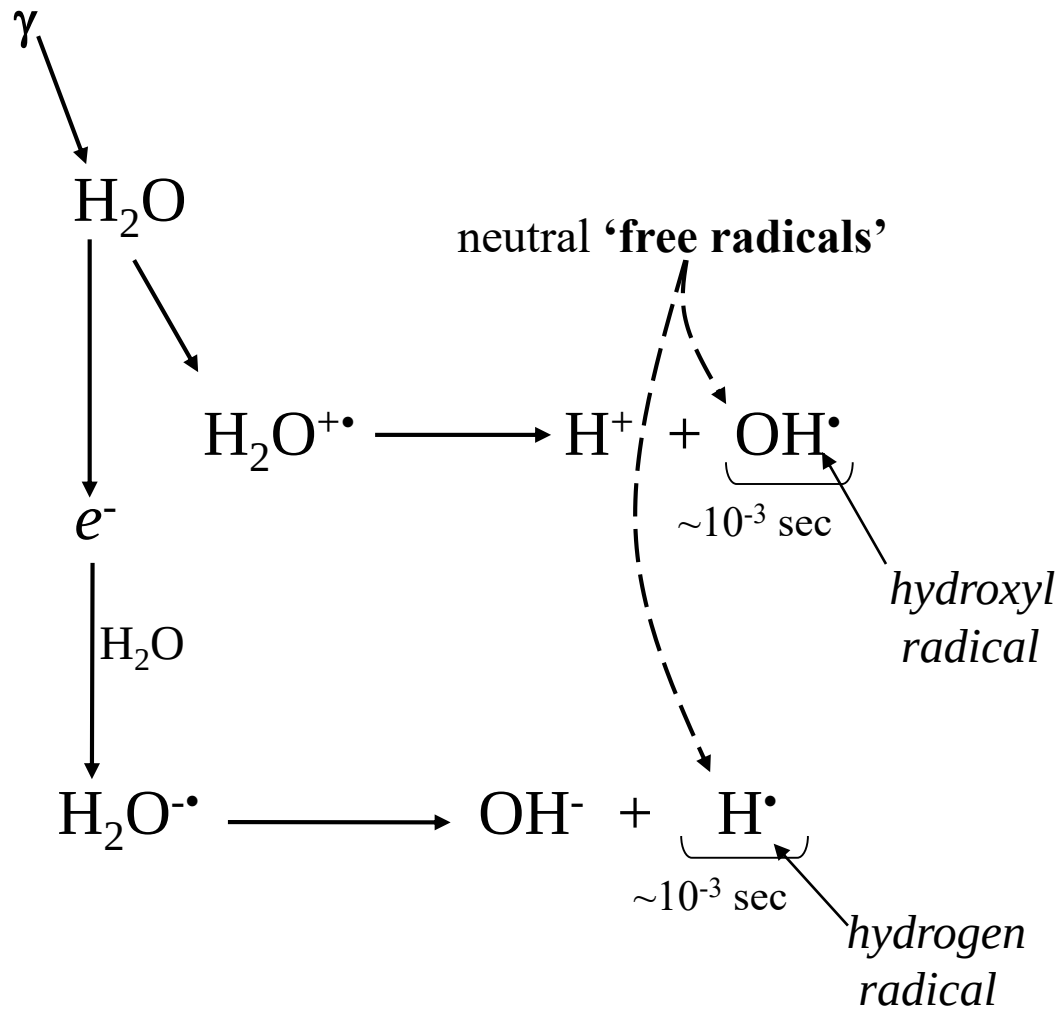
Radiation interaction with water

Initial reaction with cells



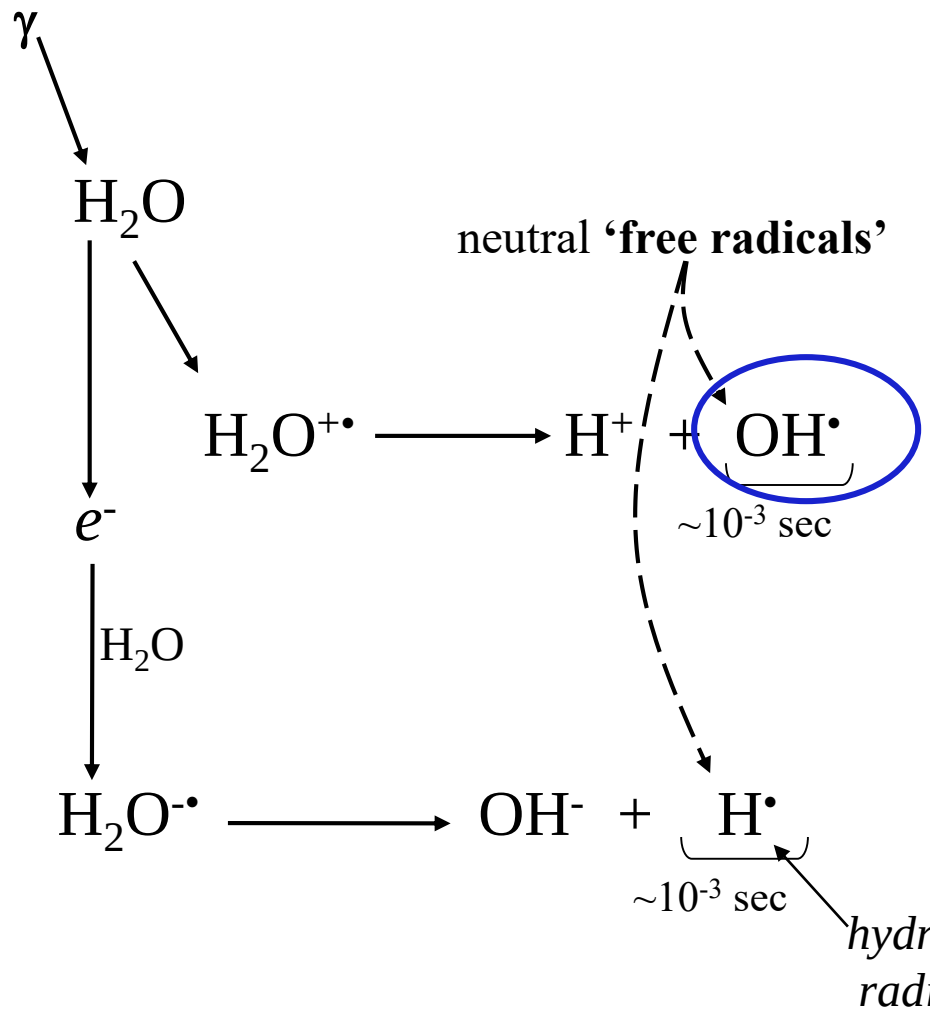
Radiation interaction with water

Initial reaction with cells



Radiation interaction with water

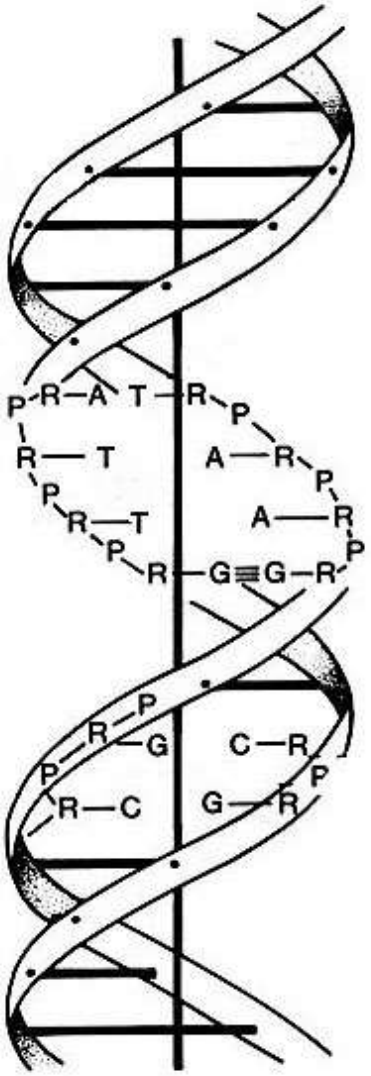
Initial reaction with cells



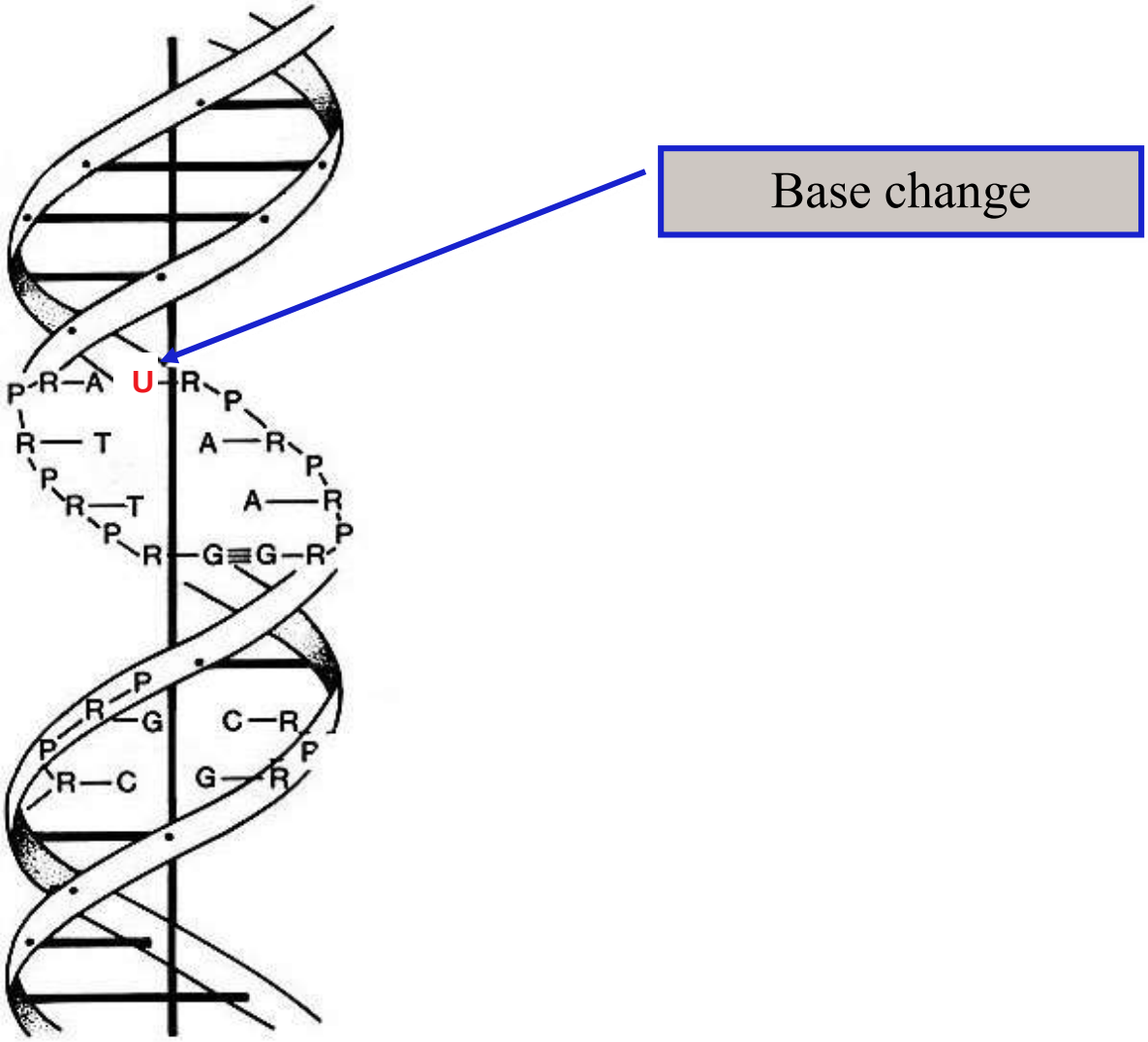
The highly reactive hydroxyl radical is particularly damaging to DNA

The presence of oxygen amplifies the effects of hydroxyl radical

Examples of DNA lesions induced by Radiation



Examples of DNA lesions induced by Radiation



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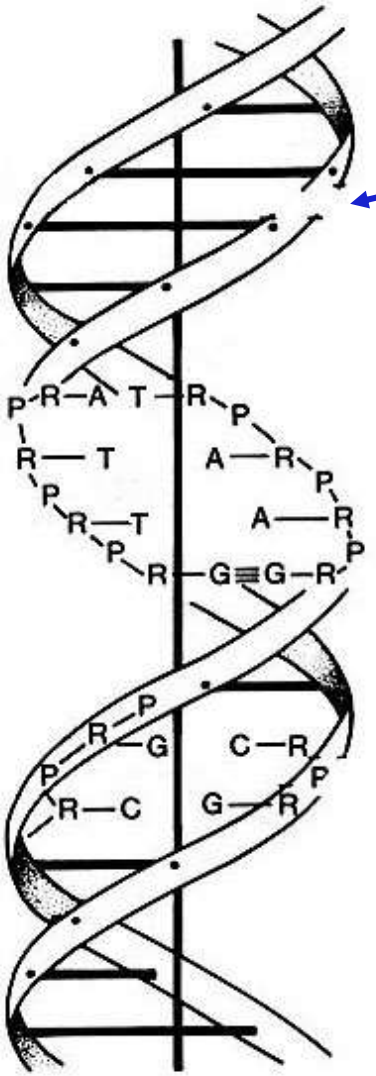
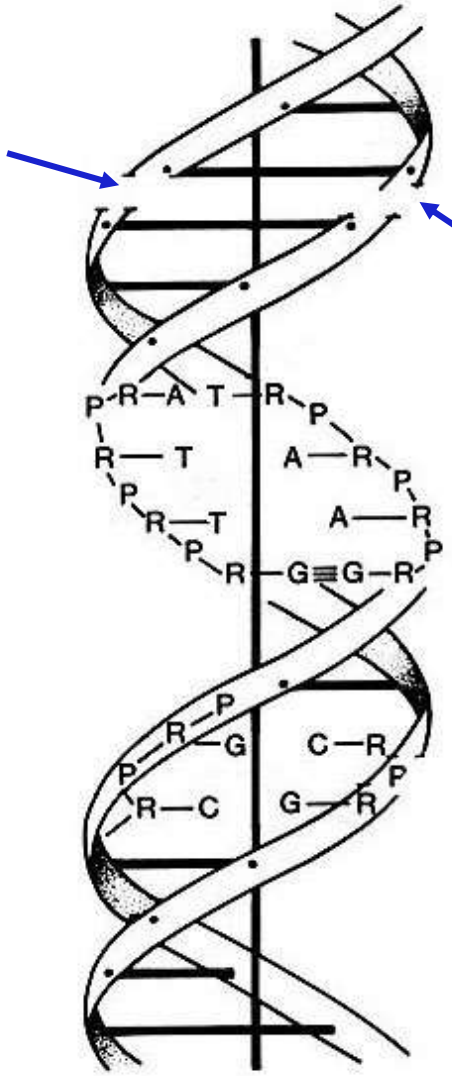


Single strand break

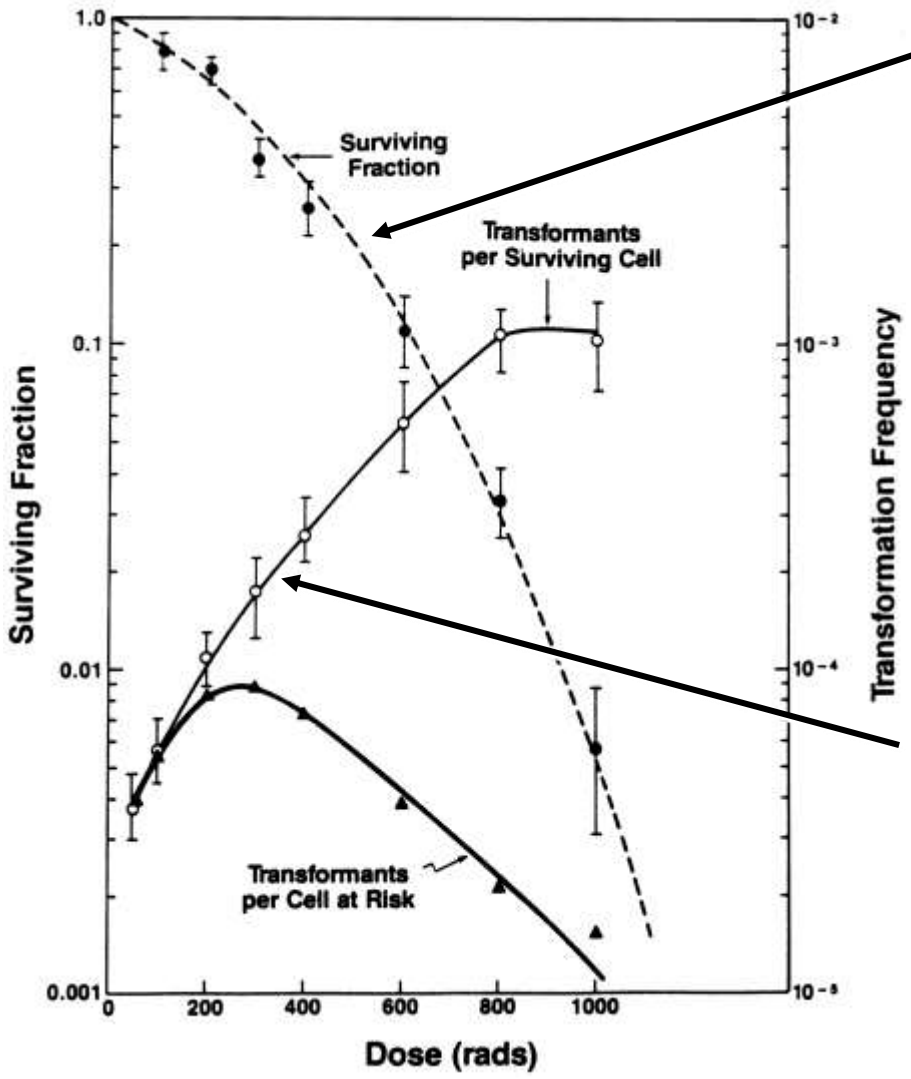
Examples of DNA lesions induced by Radiation



Double strand break

Most severe type of damage

Radiation induced DNA lesions cause cell death & transformation



Decreased cell survival

Surviving cells transformed and become cancer cells

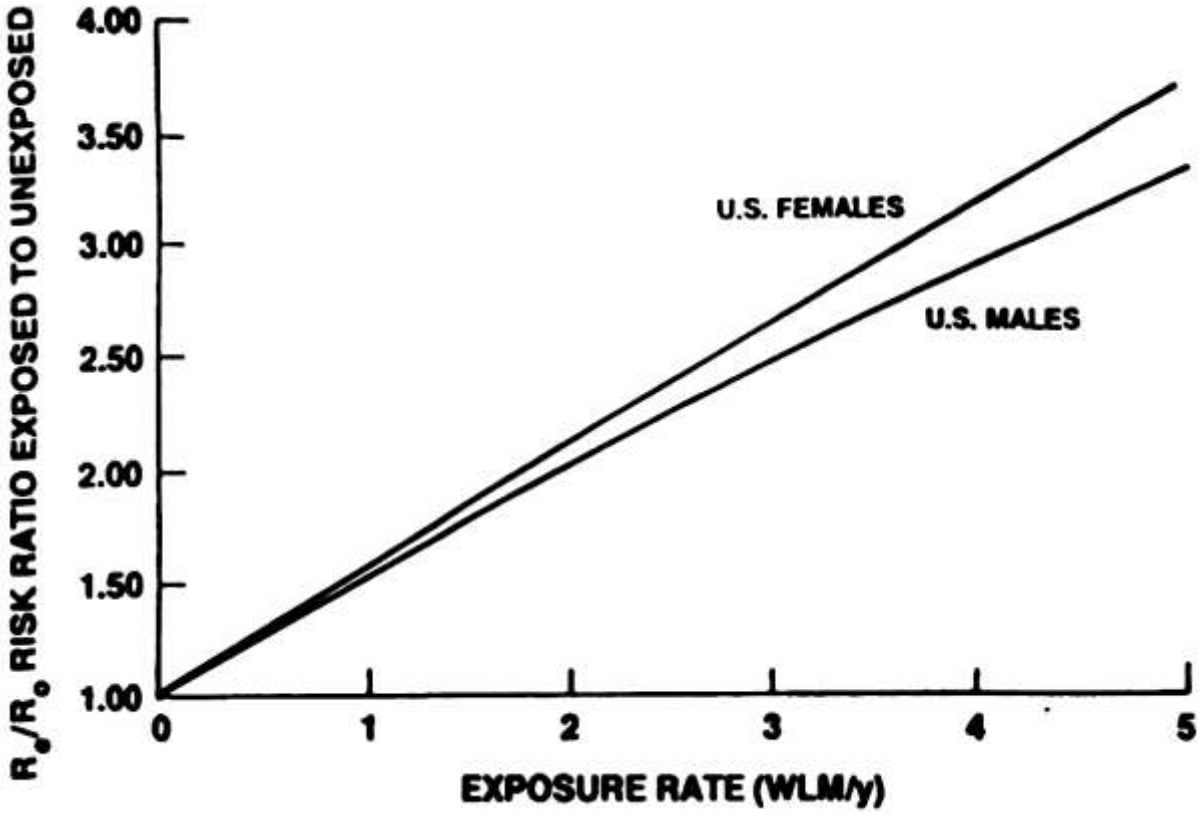
Radiation and Cancer

There is a link between radiation exposure and cancer.

Some examples of the evidence of a link:

- Bone cancers in radium dial painters
- Women given multiple breast fluoroscopies
- Radon and lung cancer

Radon and Lung Cancer Risk

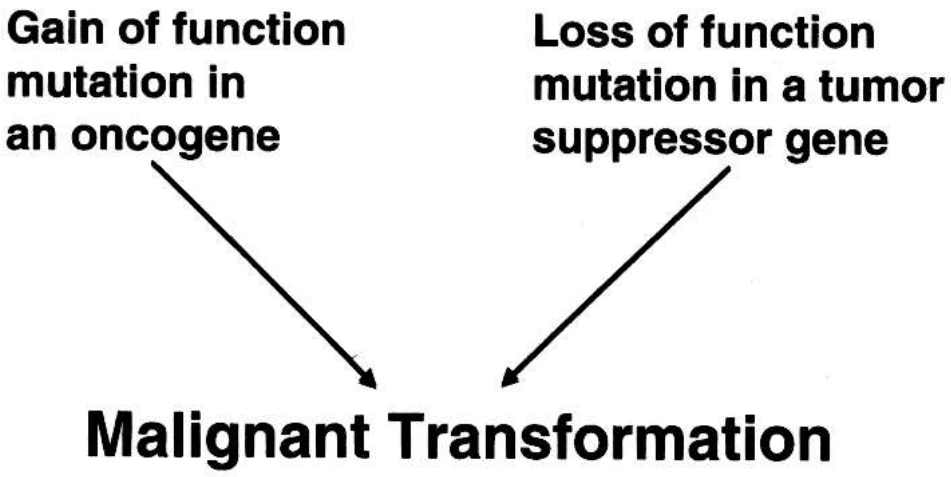


High correlation on cancer with radon exposure

How does radiation cause cancer?

A multi-step induction over 10-20 yrs

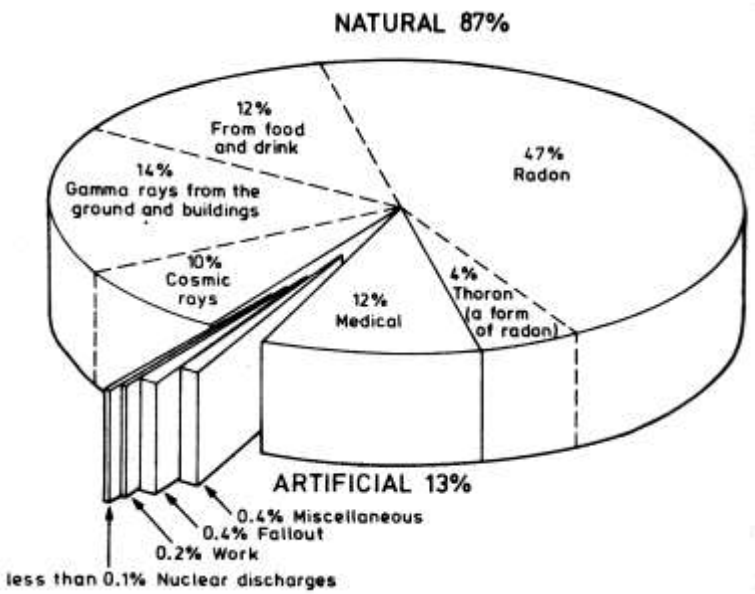
- The transformation of a cell from normal to malignant may result from activation of **oncogene**s
- The transformation of a cell from normal to malignant may also result from loss of **tumor-suppressor genes**



How much Radiation is received?

(U.S. annual values)

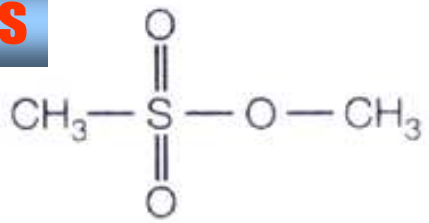
Source	Exposure (mrem/yr)
External radiation from:	
• cosmic rays	30
• radioactive ores, etc.	30
• radon gas	200
• med X-rays/CAT scan	55
Internal exposure from:	
• radioactive material (ingested into body)	45
• smoking	~150
Total:	**360** (non-smoker) **510** (smoker)



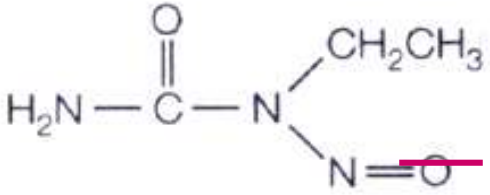
Alkylation DNA damage

1. **Electrophilic chemicals adds alkyl groups to various positions on nucleic acids**
2. **Distinct from those methylated by normal methylating enzymes.**

alkylating agents



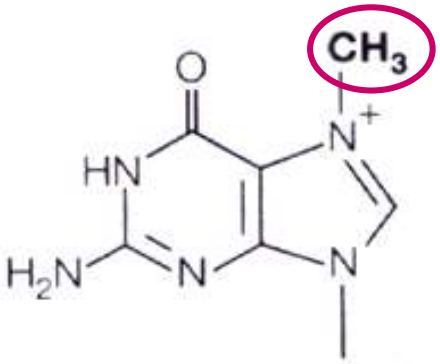
Methylmethane sulfonate



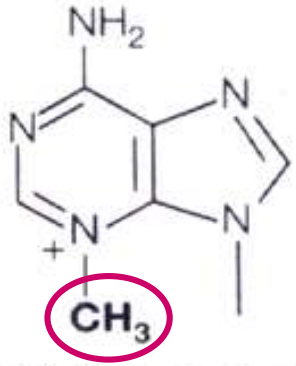
Ethylnitrosourea

Alkylated bases

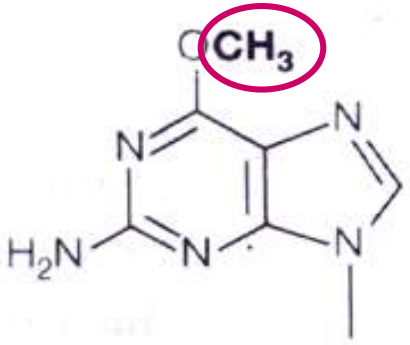
(b)



7-Methylguanine



3-Methyladenine



O^6-Methylguanine

Innocuous

Highly toxic

Highly mutagenic

Lipid peroxidation

Initiation $LH \xrightarrow{OH \text{ or } M=O} L^{\bullet}$

Propagation

$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$

$LOO^{\bullet} + LH \longrightarrow L^{\bullet} + LOOH$ Hydroperoxide

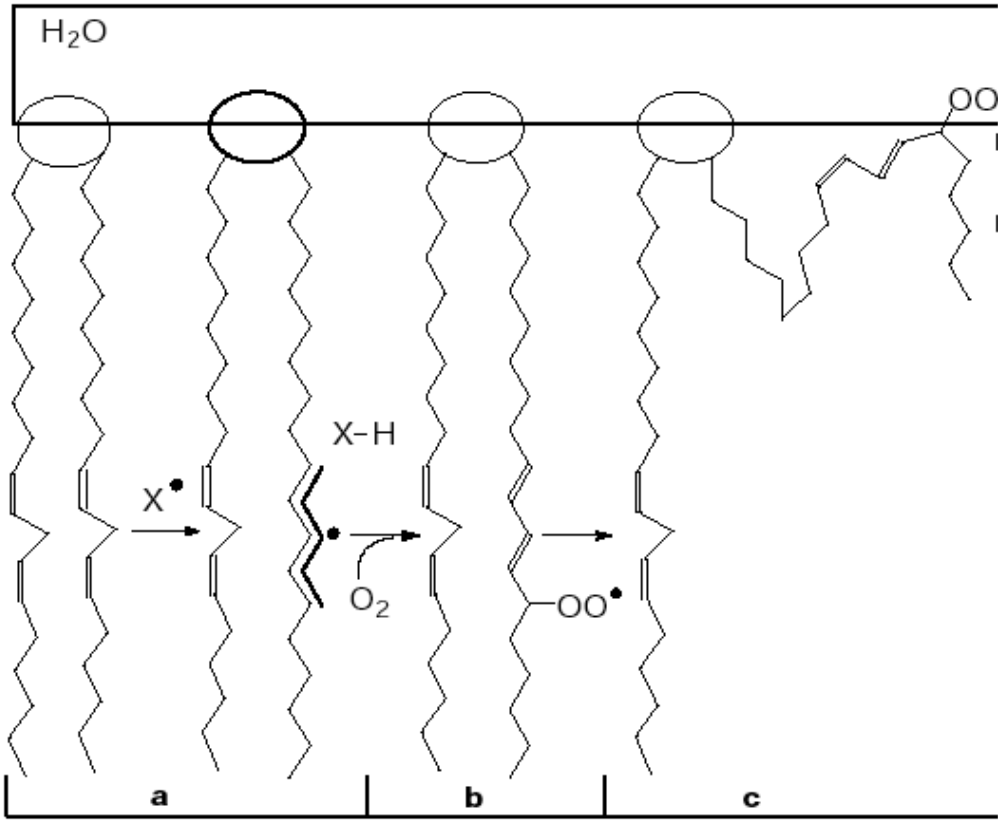
$LOOH + \text{metal} \longrightarrow LO^{\bullet}$ Alkoxy radical

$LOO^{\bullet}$ Peroxy radical

Commonly measured decomposition products

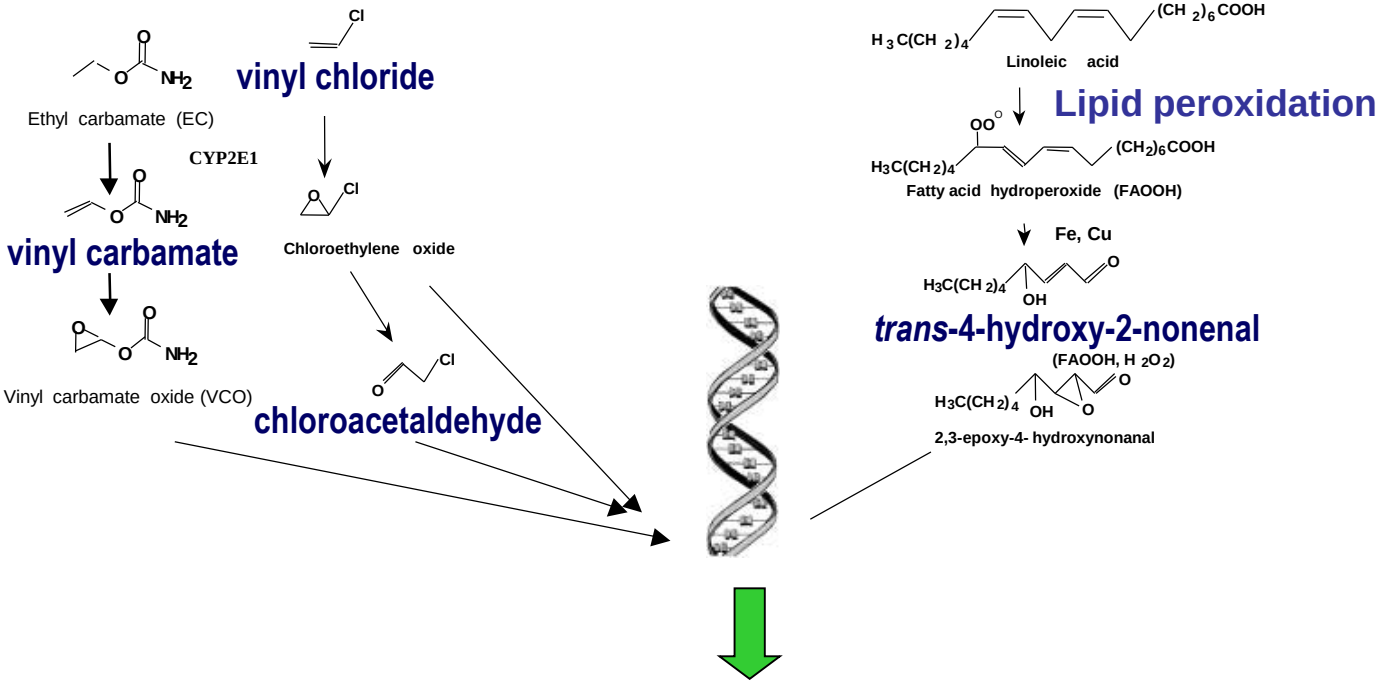
- Alkanes
- Malondialdehyde
- 4 hydroxynananol
- 8-isoprostanes

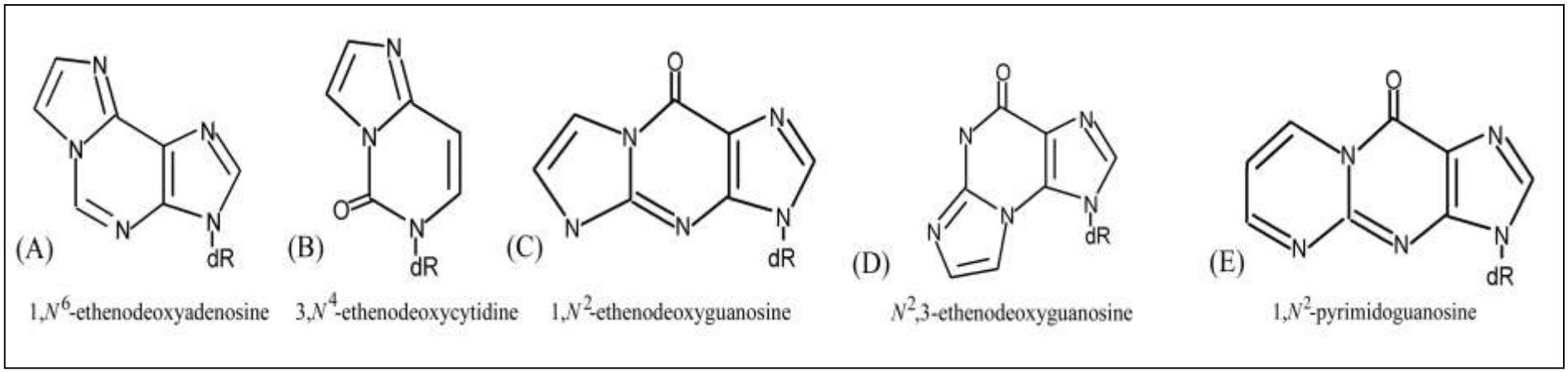
Lipid Peroxidation



Destruction of the Membrane

FORMATION OF EXOCYCLIC DNA ADDUCTS





1,N^6-εA → G 3,N^4-εC → A 1,N^2-εG → A,T,C N^2,3-εG → A M_1G → Δ

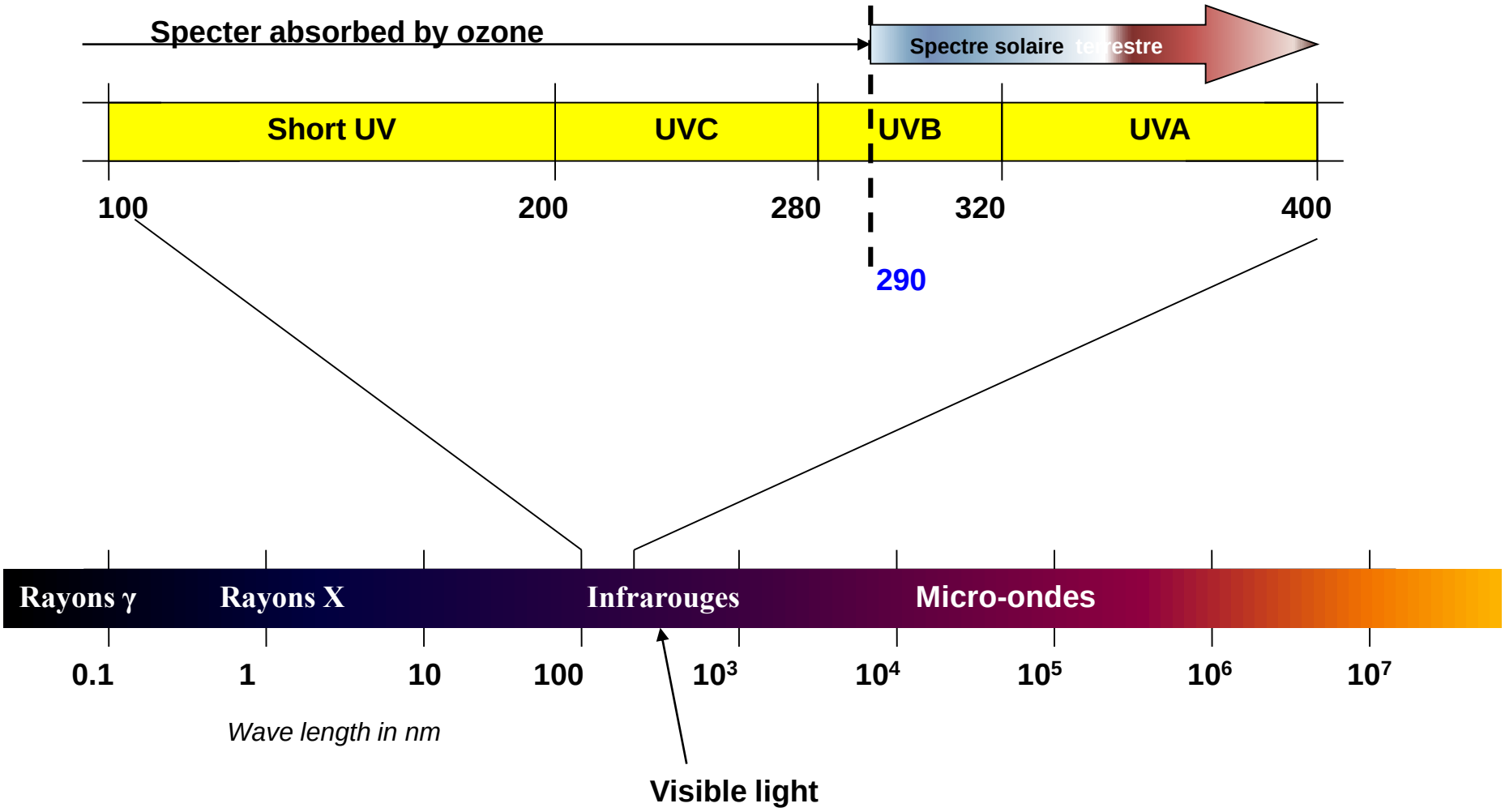
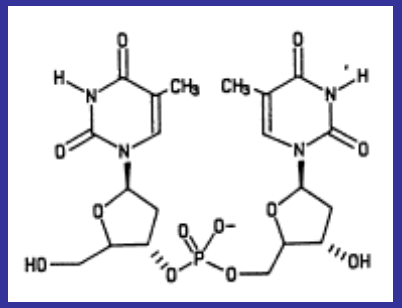
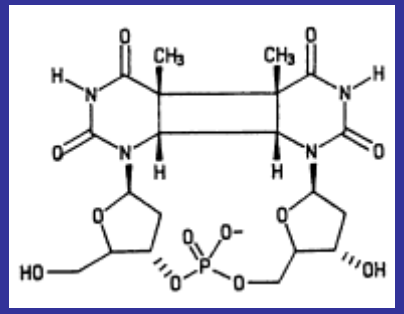


Figure : Spectra of electromagnetic radiation emited by sun.

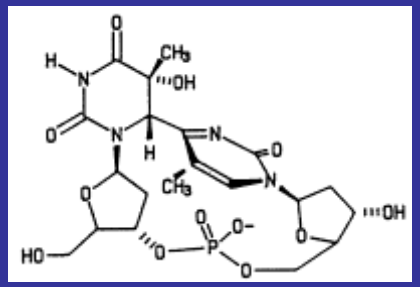
DNA lesions induced by UV light



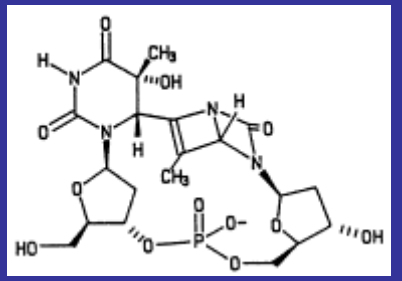
Bi-pyrimidinique sequence (TT)



Cyclobutane dimer of pyrimidines



Pyrimidine (6-4) pyrimidone

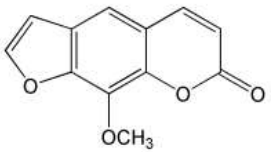


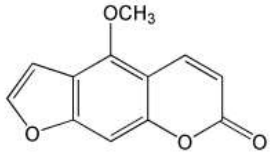
Dewar isomer

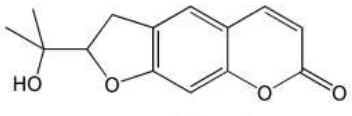
DNA crosslinking agents

Furanocoumarins,
or furocoumarins, are a class of organic chemical compounds produced by a variety of plants

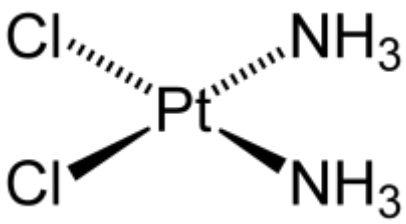

Angelicin


Xanthotoxin


Bergapten


Nodakenetin

Cisplatin



Cisplatin, cisplatinum or cis-diamminedichloroplatinum(II) (CDDP) is a platinum-based chemotherapy drug used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas and germ cell tumors.

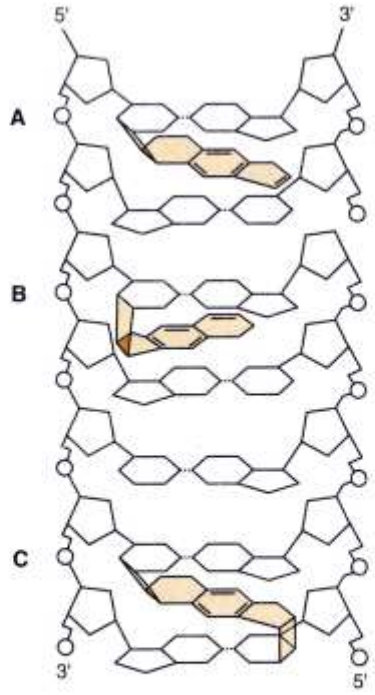
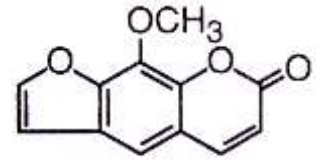
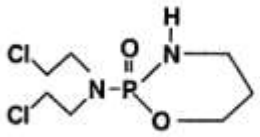
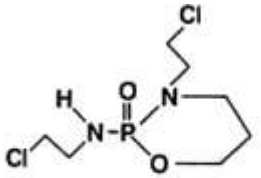


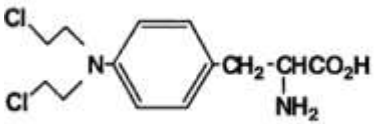
Figure 2–35 Intercalation of psoralen with DNA to form two types of monoadducts (A and B) or a diadduct (interstrand DNA cross-link) (C). Two types of monoadducts can result because the 5,6 double bond of thymine can photoreact with psoralen at either its 3,4 double bond or its 4′,5 double bond (see Fig 2–34). The formation of the cross-link requires independent UV absorption events at each reactive end.

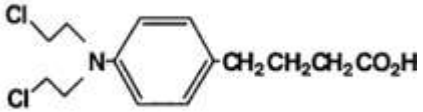

8-Methoxypsoralen

Nitrogen Mustards


Cyclophosphamide


Ifosfamide


Melphalan


Chlorambucil

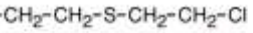
Лекции N°5-6

«Клеточный ответ на повреждения ДНК. Изменения клеточного цикла при повреждении генома и основные молекулярные механизмы участвующие в этом процессе».

НАЦИОНАЛЬНЫЙ ЦЕНТР НАУЧНЫХ ИССЛЕДОВАНИЙ ФРАНЦИЯ
Centre National de la Recherche Scientifique
ИНСТИТУТ ГУСТАВА РОЗИ, Департамент CNRS UMR 8126
Лаборатория «Репарации ДНК»
Research Director, заведующий лабораторией
САПАРБАЕВ Мурат Калиевич

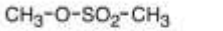
Alkylation DNA damage

Chemical structures of several representative simple alkylating agents that react with DNA.

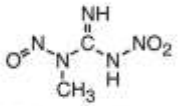


di-(2-chloroethyl) sulfide
(mustard gas or sulfur mustard)

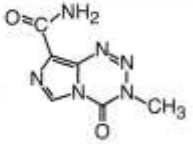
ethyl methane sulfonate
(EMS)



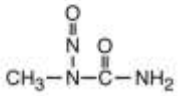
methyl methane sulfonate
(EMS)



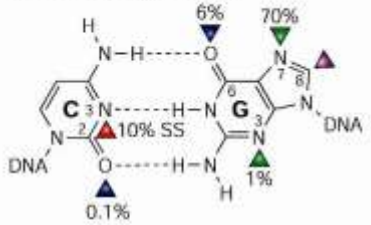
***N*-methyl-*N*'-nitro-*N*-nitrosoguanidine**
(MNNG)



Temozolomide



***N*-methyl-*N*-nitrosourea**
(MNU)

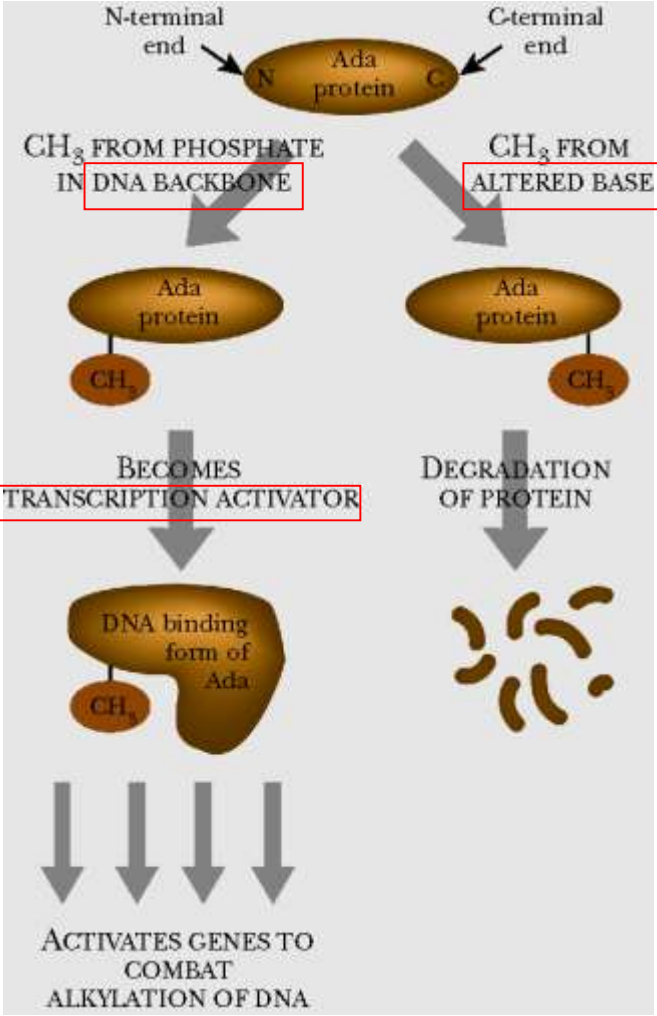


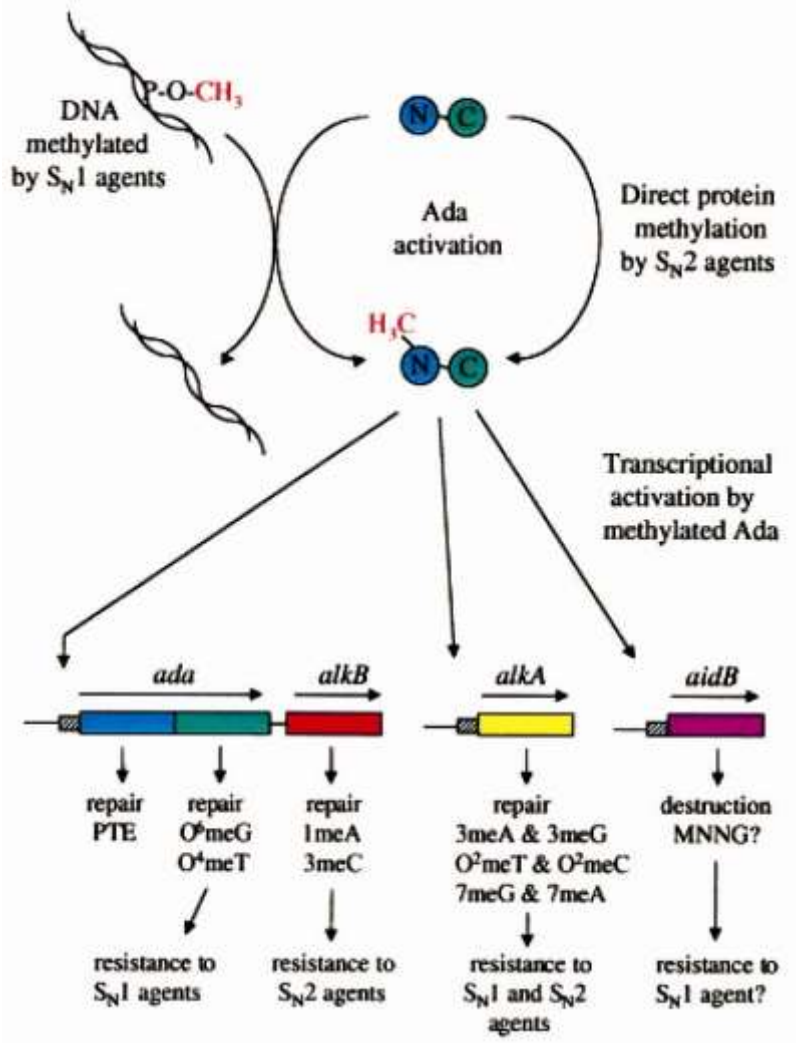
Sites of methylation on the bases and sugar–phosphate backbone of DNA.
Blue arrows indicate oxygen atoms in DNA that are most frequently methylated by S_N1 agents, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). Red arrows indicate sites in single-stranded (ss)DNA that are methylated by S_N2 agents, such as methylmethane sulphonate (MMS). The pink arrow is a site that is methylated by methyl radicals. Green arrows indicate sites that are methylated by most agents. The percentages indicate the relative abundance of each modification[13].

Relative proportions of alkylated bases by carcinogenic alkylating agents

	% of total alkylation after reaction with:	
	S_N2 type agent	S_N1 type agent
Adduct	Methyl methane-sulfonate	*N*-ethyl-*N*-nitrosourea
N1-Alkyladenine	1.2	0.3
N3-Alkyladenine	11	4
N7-Alkyladenine	1.9	0.4
N3-Alkylguanine	0.7	0.6
N7-Alkylguanine	83	12
O^6-Alkylguanine	0.3	8
N3-Alkylcytosine		0.2
O^2-Alkylcytosine		3
N3-Alkylthymine		0.8
O^2-Alkylthymine		7
O^4-Alkylthymine		1–4
Alkylphosphates	1	53

Adaptive response in *E. coli* following alkylation DNA damage



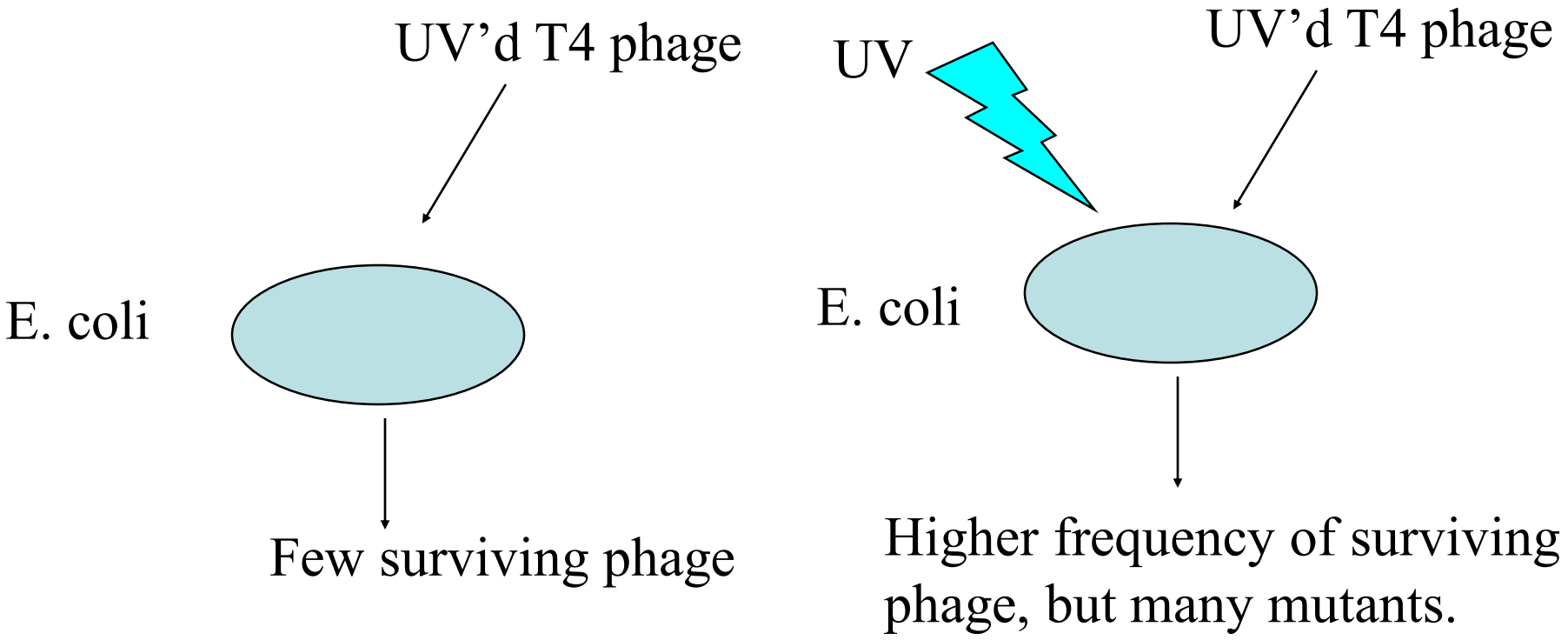


Diagrammatic representation of the E. coli Ada response. The Ada protein is activated as a positive regulator by methylation of its Cys-38 residue in the amino-terminal half of the protein. This activation occurs by repair of methylphosphotriesters (PTE) in DNA or, less efficiently, by direct protein methylation. The activated Ada protein induces expression of several genes resulting in increased DNA repair and probable destruction of certain alkylating agents

Ada = Adaptation to alkylation

Note! ~CH3 at N- and C- has different effects.

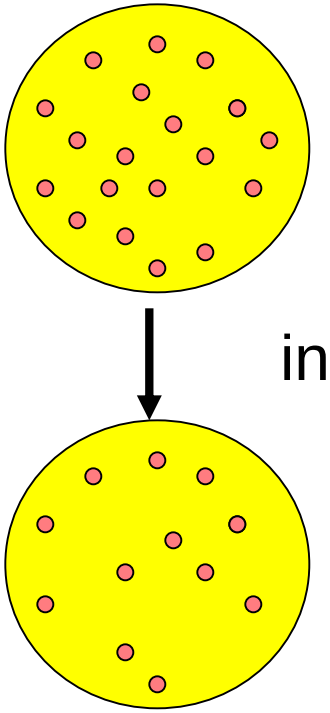
SOS response in *E. coli*



- Irradiation of bacteria before virus infection enhanced repair of damaged viral genes but led to mutations. This has an evolutionary advantage for the viral population since it increases the probability that some members will survive albeit in altered form

SOS response in *E. coli*

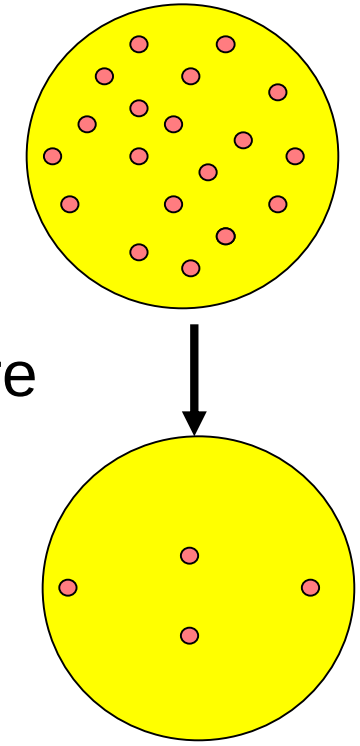
E. coli ***recA***$^{+}$ strain



intensive UV exposure

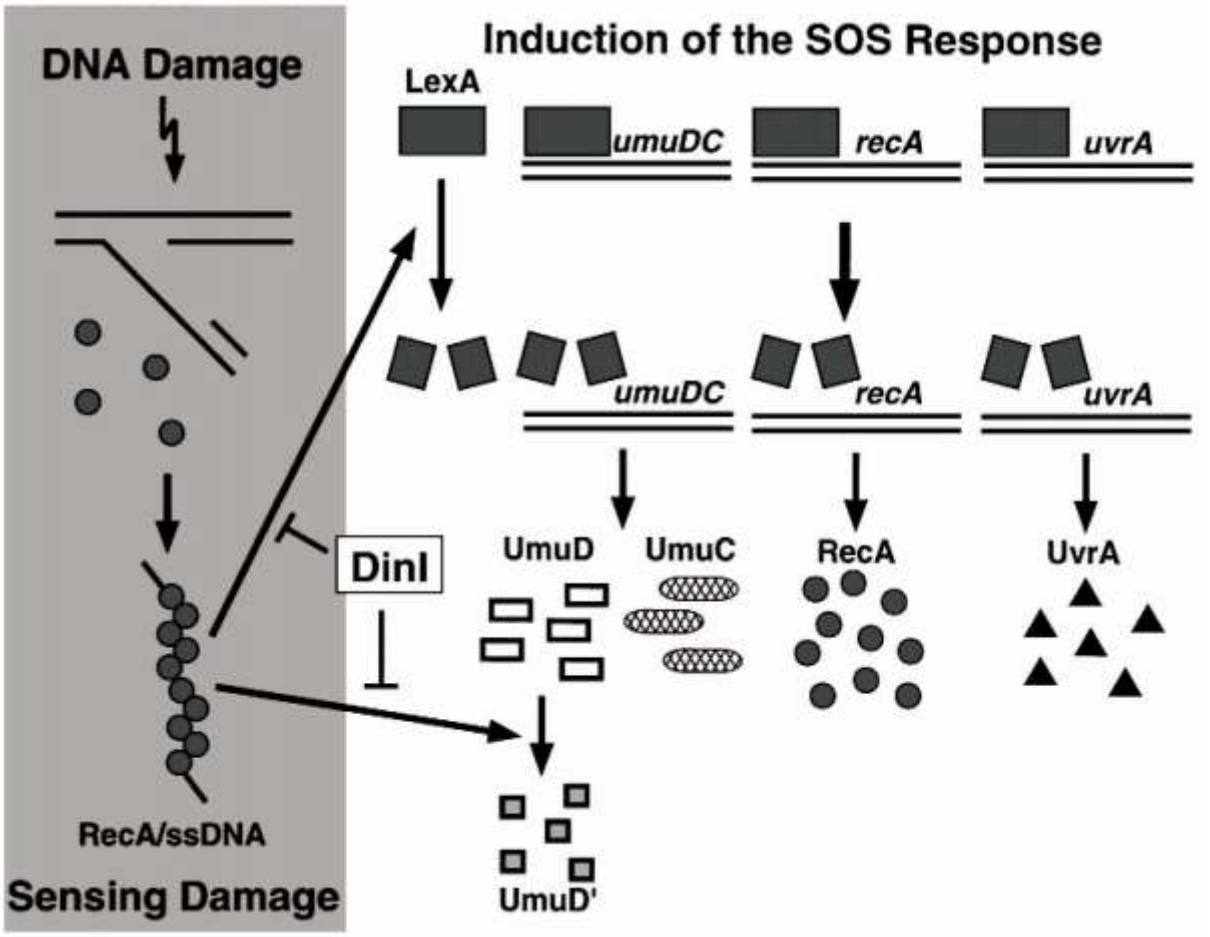
Numerous survivors
High mutation rates

E. coli ***recA***$^{-}$ strain

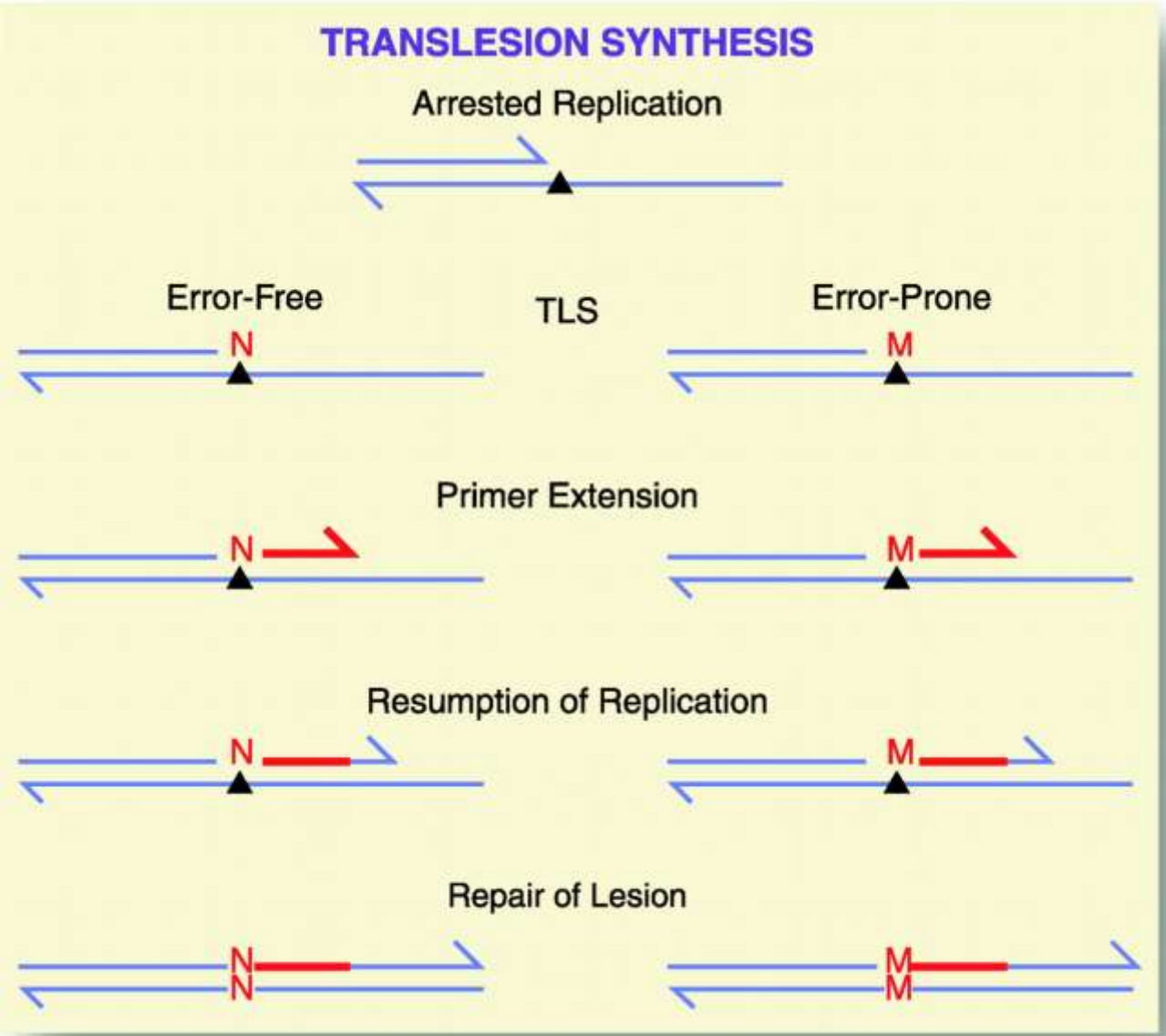


Few survivors
Low mutation rates

SOS response in *E. coli*



Following DNA damage, RecA becomes activated for its role in SOS induction when it forms a nucleoprotein filament by binding to ssDNA generated by the cell's failed attempts to replicate damaged DNA. The RecA/ssDNA nucleoprotein filament then functions as a co-protease that mediates LexA cleavage by stimulating the latent ability of the LexA repressor to cleave itself in two via a proteolytic autodigestion mechanism. The resulting decrease of LexA results in the induction of the SOS regulon. Translesion DNA synthesis, the mechanistic basis of SOS mutagenesis, endows the cell with an increased capacity to recover from DNA damage by allowing it to replicate past lesions that would normally block continued polymerization by E. coli's replicative polymerase (DNA Pol III). In exchange for increased survival, the cell pays the cost of an elevated mutation rate resulting from translesion DNA synthesis. This process requires the products of the SOS-regulated recA gene and the similarly regulated umuDC operon, which was originally identified by screening for E. coli mutants that were not mutable by UV light and other agents. TLS requires not the full-length UmuD protein, but rather a posttranslationally processed form called UmuD'. The biochemical nature of this processing is similar to that of LexA autodigestion (Figure): Interaction of UmuD with the RecA/ssDNA nucleoprotein filament stimulates a latent ability of UmuD to autodigest, resulting in the removal of the amino-terminal 24 amino acids.



- Replication-blocking lesions such as UV photodimers can be repaired by NER but pose a serious problem if they are in ssDNA
- As a last resort, cells employ “bypass” polymerases with loosened specificity
- In E. coli: DinB (PolIV) and UmuD’C (Pol V); homologs in eukaryotes; mutated in XPV
- These polymerases are “error-prone” and are responsible for UV-induced mutation
- Expression and function highly regulated: dependent on DNA damage

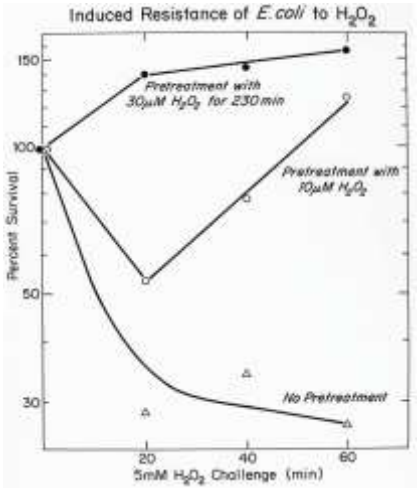
Characteristics of lesion bypass polymerases

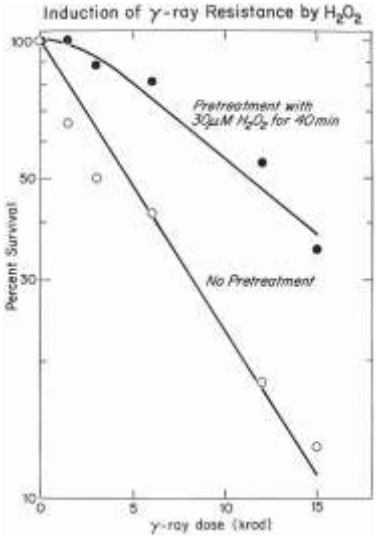
- Error rate 100-10,000 x higher on undamaged templates
- Lack 3’ to 5’ proofreading exonuclease activity
- Exhibit distributive rather than processive polymerization (nt. incorporated per binding event)
- Support translesion DNA synthesis in vitro

Table 1. Low-fidelity copying of undamaged DNA by specialized DNA polymerases from human cells. [Adapted from P. J. Gearhart and R. D. Wood, *Nature Rev. Immunol*. **1**, 187 (2001)]

DNA polymerase	Gene	Infidelity on undamaged DNA templates (relative to pol ε = ~1)
β	*POLB*	~50
ζ	*REV3L*	~70
κ	*POLK*	~580
η	*POLH*	~2,000
ι	*POLI*	~20,000
λ	*POLL*	?
μ	*POLM*	?
θ	*POLQ*	?
Rev1	*REV1L*	?

Oxidative stress response in *E. coli*. OxyS system.





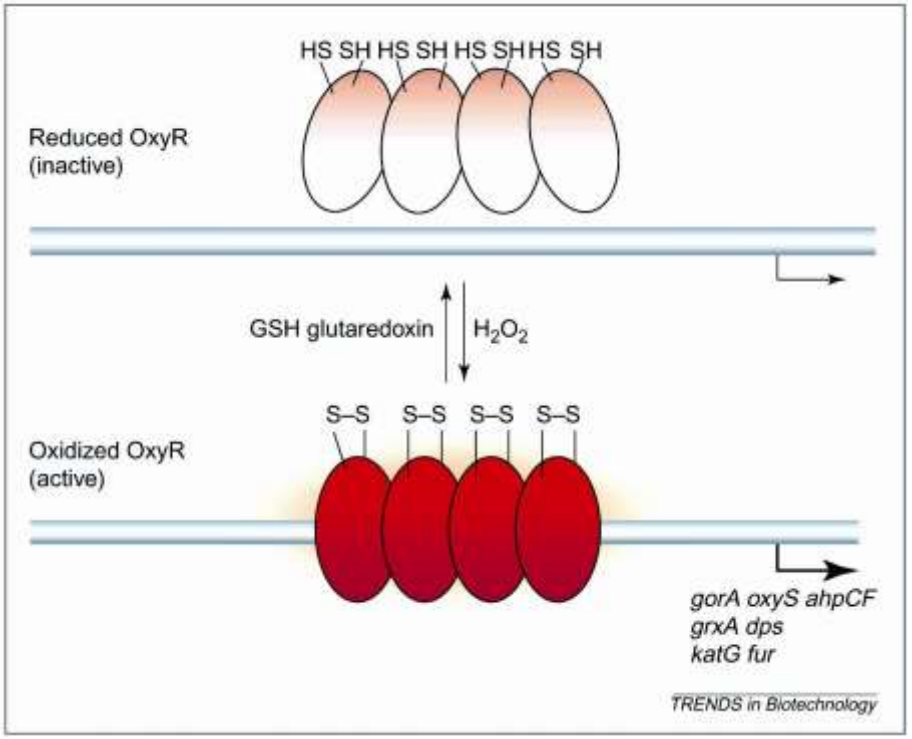


Fig. The oxyR regulon. The OxyR protein is produced constitutively and is oxidized by hydrogen peroxide (H2O2). The oxidized form of OxyR binds to promoter regions of target genes and activates transcription by protein–protein contact with RNA polymerase. OxyR-activated genes have direct and indirect antioxidant functions. Each subunit of the OxyR tetramer contains two cysteine residues that form intramolecular disulfide bonds upon exposure to H2O2. The disulfide bonds are re-reduced by glutathione, which in turn is re-reduced by glutathione reductase. The expression of the glutathione reductase and glutaredoxin genes is under transcriptional control of OxyR, and thus the response is selfregulated. Abbreviation: GSH, glutathione.

Oxidative stress response in *E. coli*. SoxRS system.

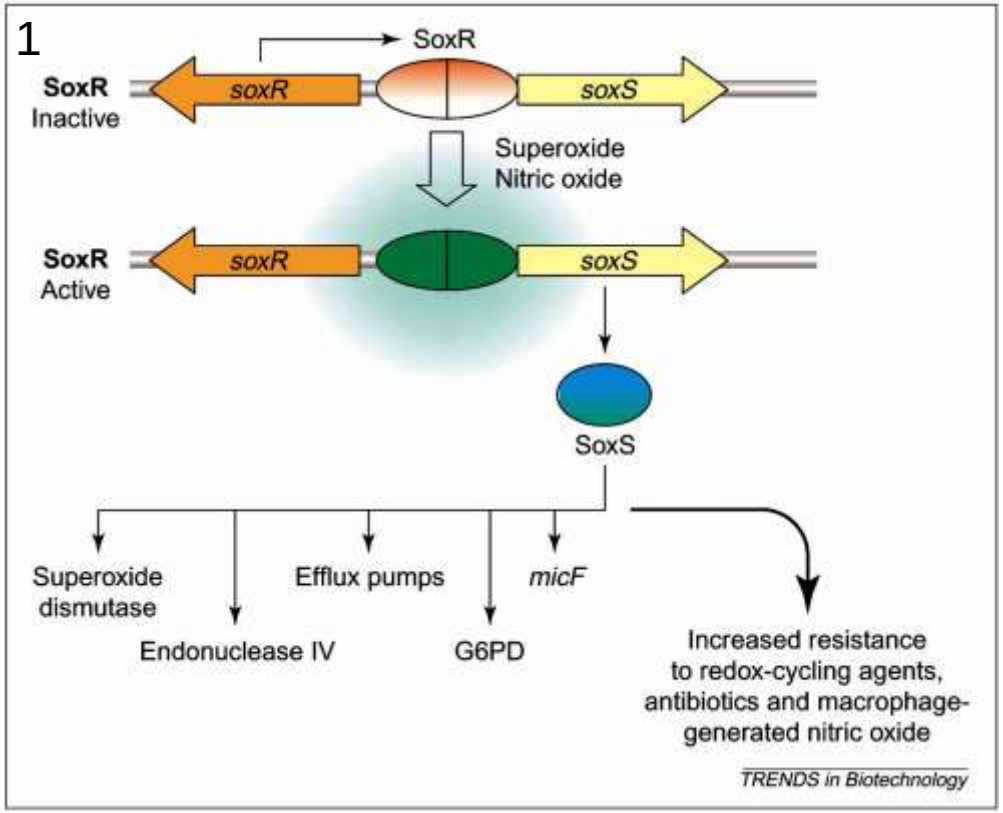


Fig. 1. The soxRS regulon. The soxRS locus is composed of the divergently transcribed soxR and soxS genes. The SoxR protein is produced constitutively and is activated upon exposure to superoxide-generating agents or nitric oxide (NO). The oxidized form of SoxR enhances the transcription of the soxS gene, the product of which is also a transcriptional activator. The SoxS protein activates transcription of genes that increase the resistance to oxidants. Additionally, activation of the SoxS-regulated genes increases the resistance to antibiotics and macrophage-generated NO. Abbreviation: G6PD, glucose-6-Pdehydrogenase.

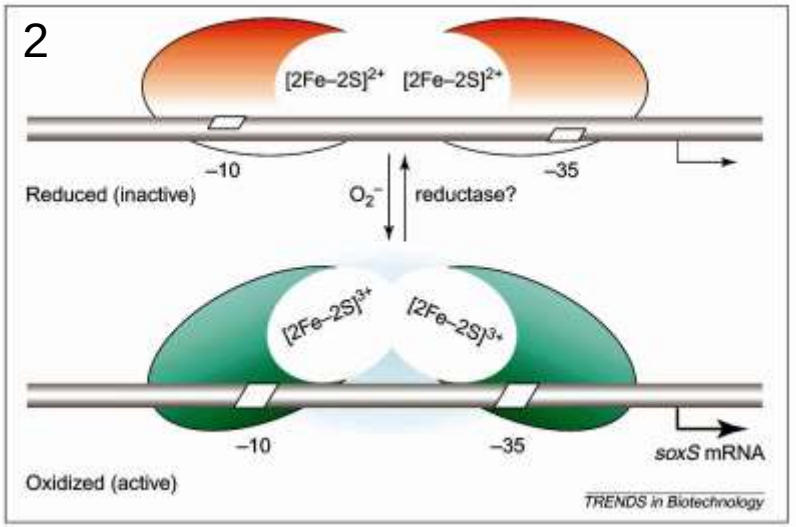
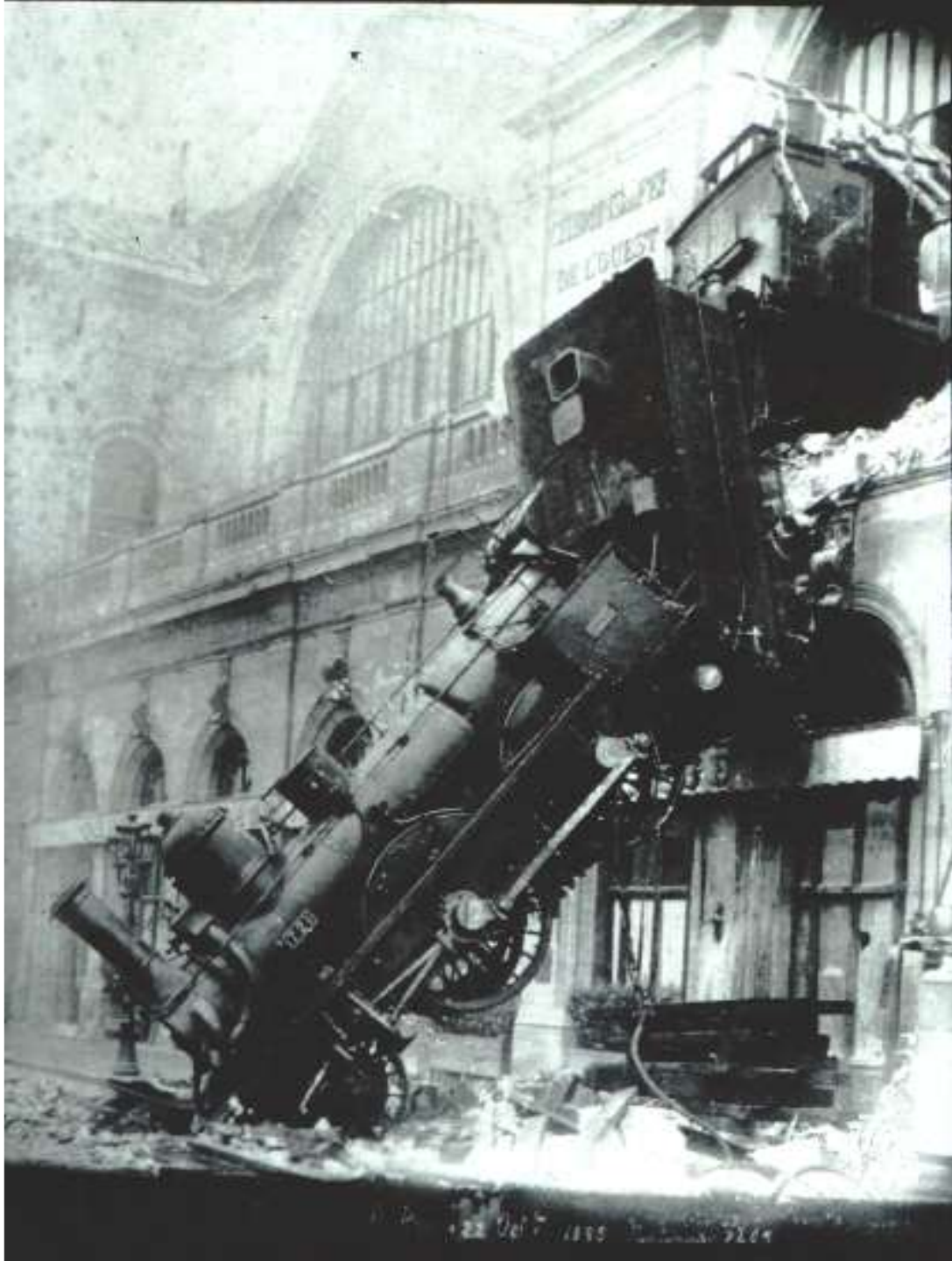


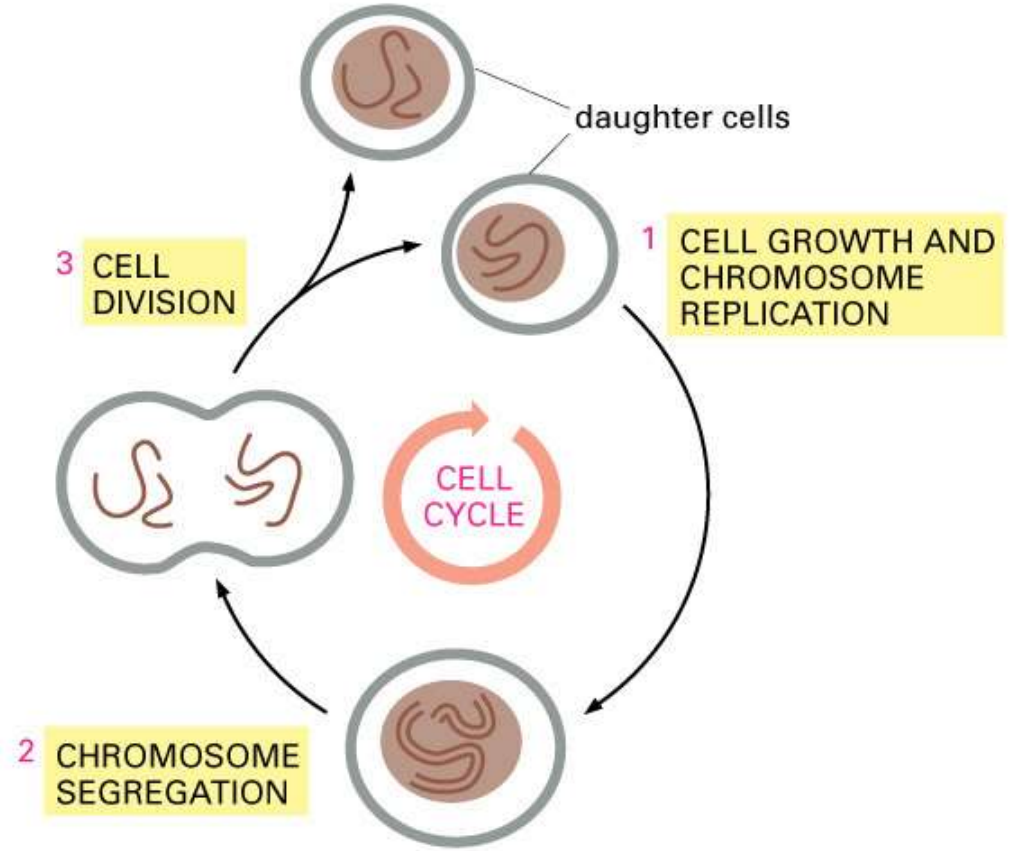
Fig. 2. Mechanism of SoxR activation by superoxide. The SoxR dimer can bind DNA in either the reduced or the oxidized form. However, only oxidized SoxR activates transcription of soxS. After exposure to superoxide-generating agents, the iron in the Fe–S clusters is oxidized. The model for the activation of soxS proposes a conformational change in SoxR that modifies the local DNA topology at the promoter and compensates for a dysfunctional spacing between promoter elements. The oxidation of SoxR is rapid and transient: after cessation of the superoxide stress, SoxR is completely re-reduced in a few minutes. The mechanism of SoxR re-reduction remains elusive.

The DNA damage checkpoint

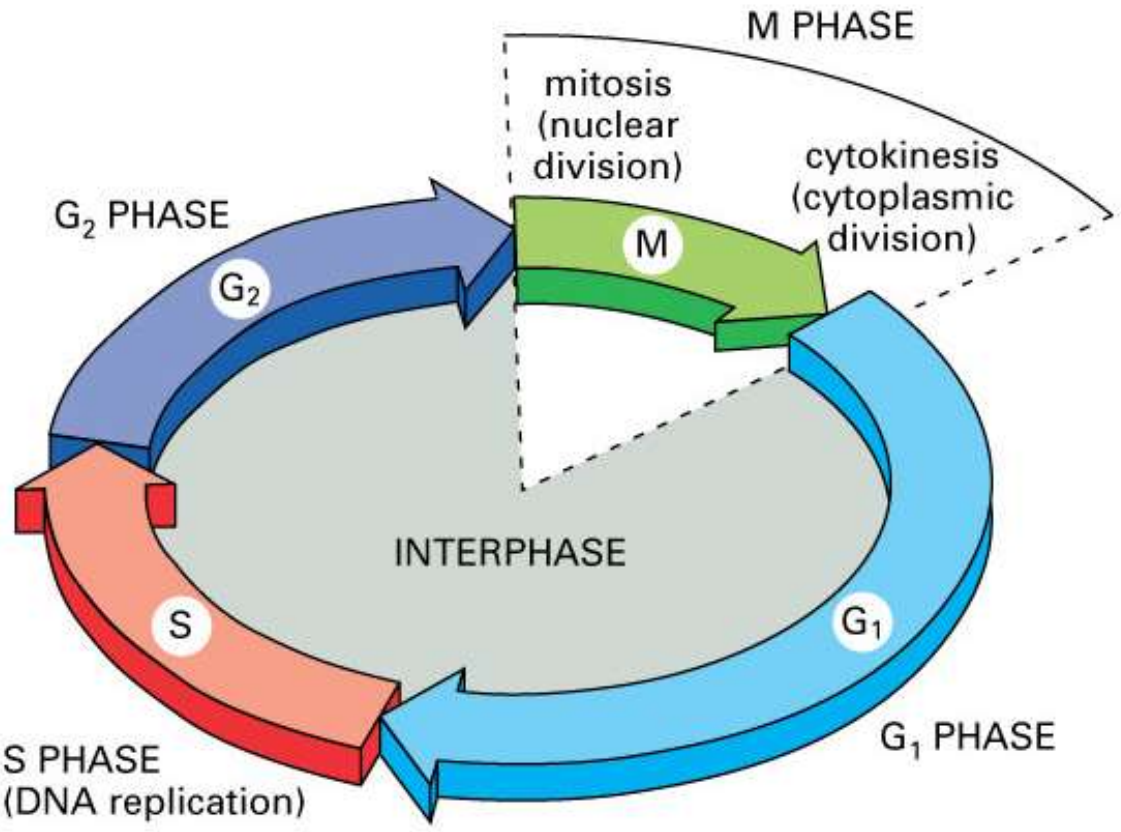
Preventing cell cycle progression to allow more time for DNA repair



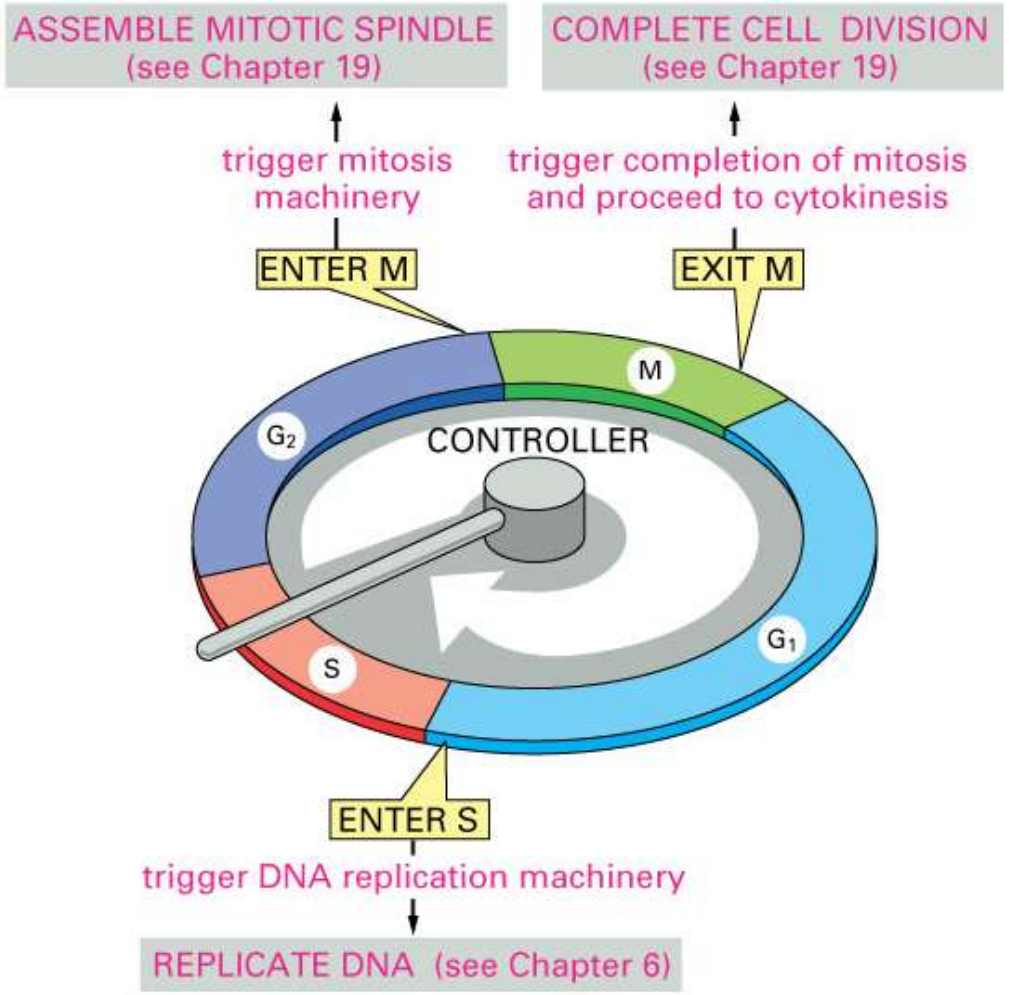
The cell cycle: cells duplicate their contents and divide



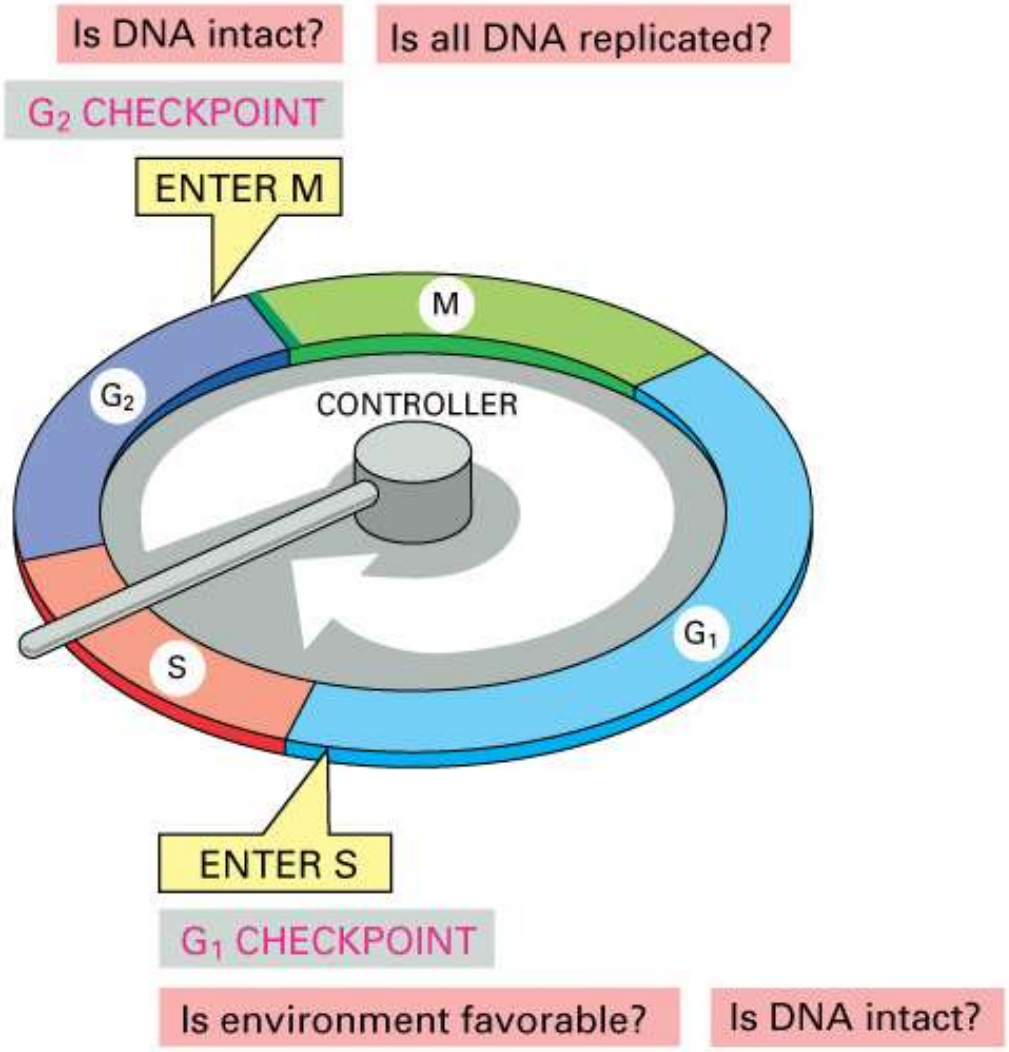
The cell cycle may be divided into 4 phases



The cell cycle triggers essential processes (DNA replication, mitosis)

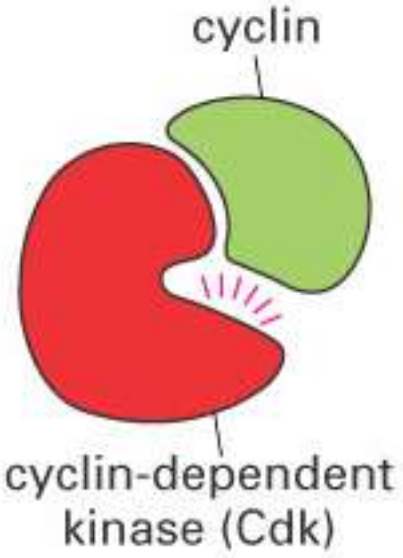


Progression of the cell cycle is regulated by feedback from intracellular events

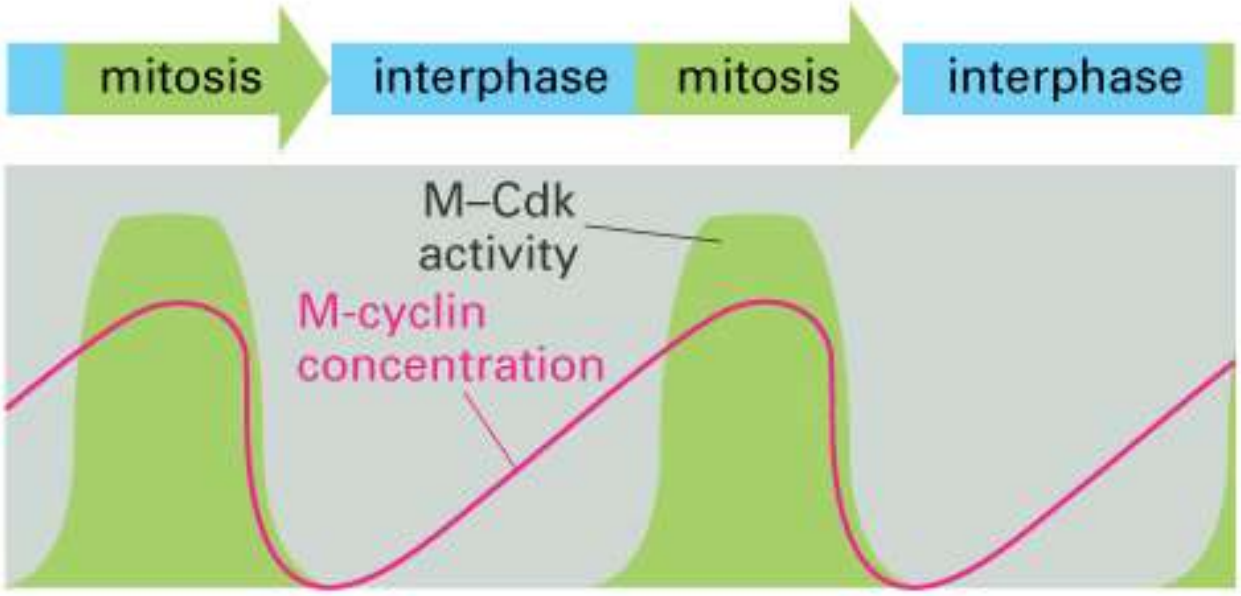


Cyclin-dependent protein kinases drive progression through the cell cycle

- Cyclin-dependent kinases (Cdks) are inactive unless bound to cyclins
- Active complex phosphorylates downstream targets
- Cyclin helps to direct Cdks to the target proteins



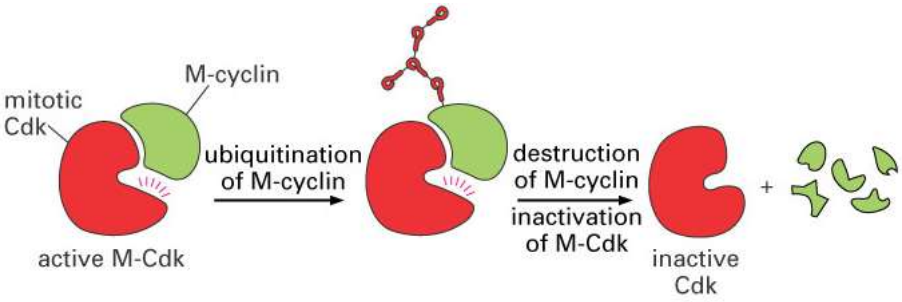
Cellular levels of (mitotic) M-cyclin rises and falls during the cell cycle



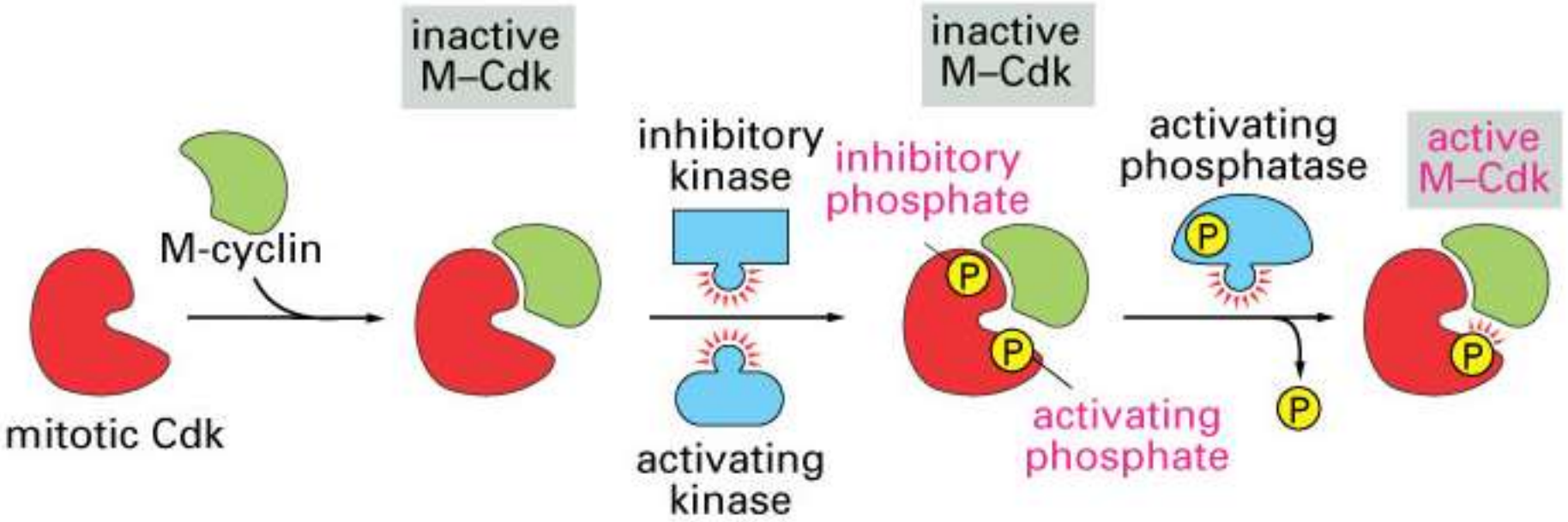
- M-cyclin levels are low during interphase but gradually increases to a peak level during mitosis
- M-cdk activity is, likewise, low in interphase but increases in mitosis

The abundance of cyclins (and the activity of Cdks) is regulated by protein degradation

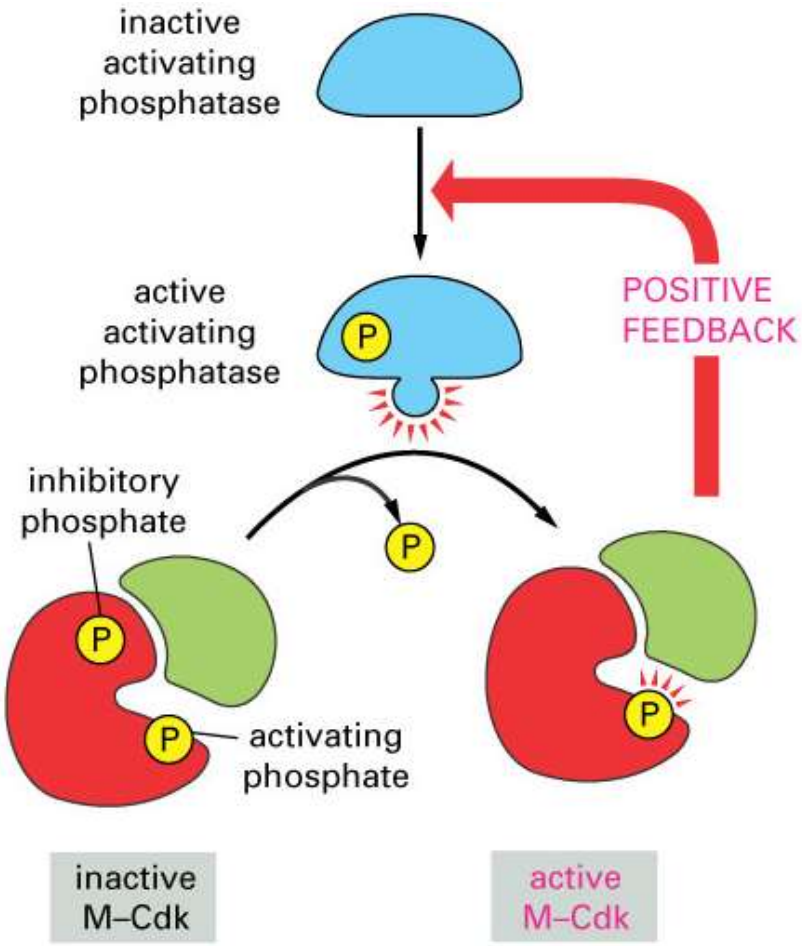
- M-cyclin becomes covalently modified by addition of multiple copies of ubiquitin at the end of mitosis
- Ubiqutination is mediated by the anaphase promoting complex (APC)
- Ubiquitination marks cyclins for destruction by large proteolytic machines called proteasome



Cdks are also regulated by cycles of phosphorylation and dephosphorylation



Cdk activates itself indirectly via a positive feedback loop



Distinct cyclins partner with distinct Cdks to trigger different events of the cell cycle

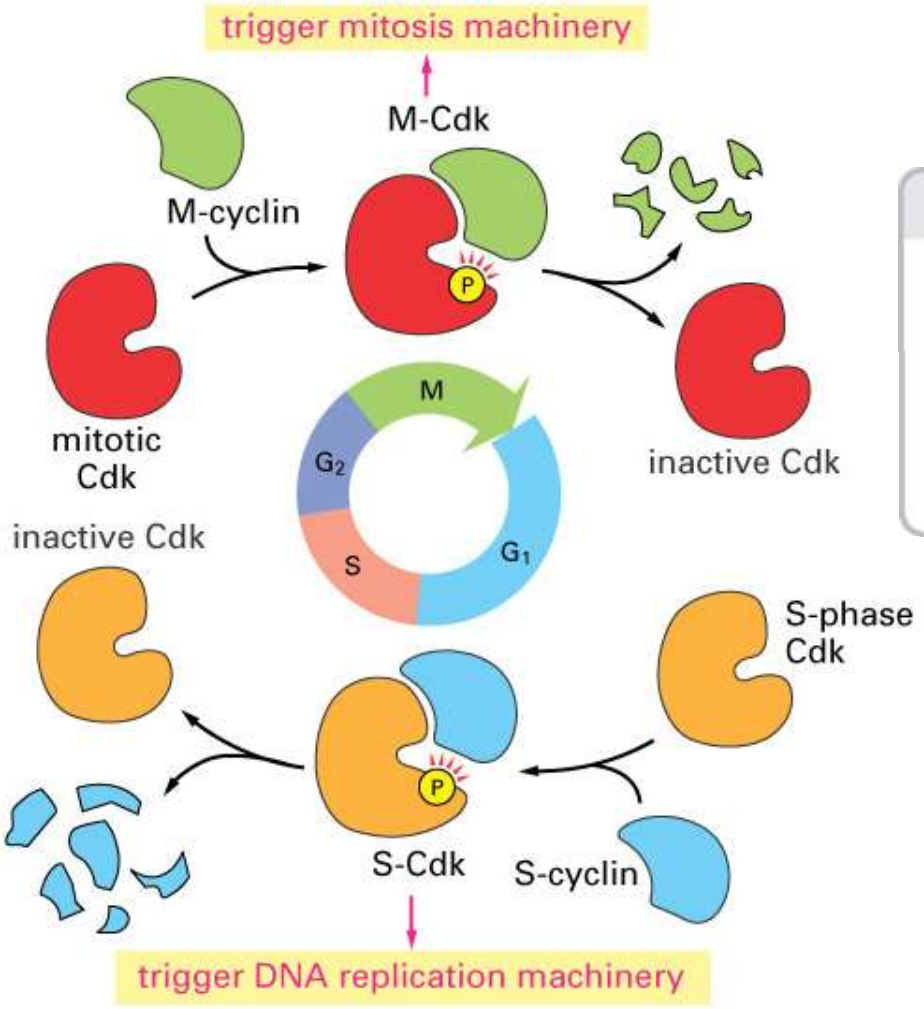
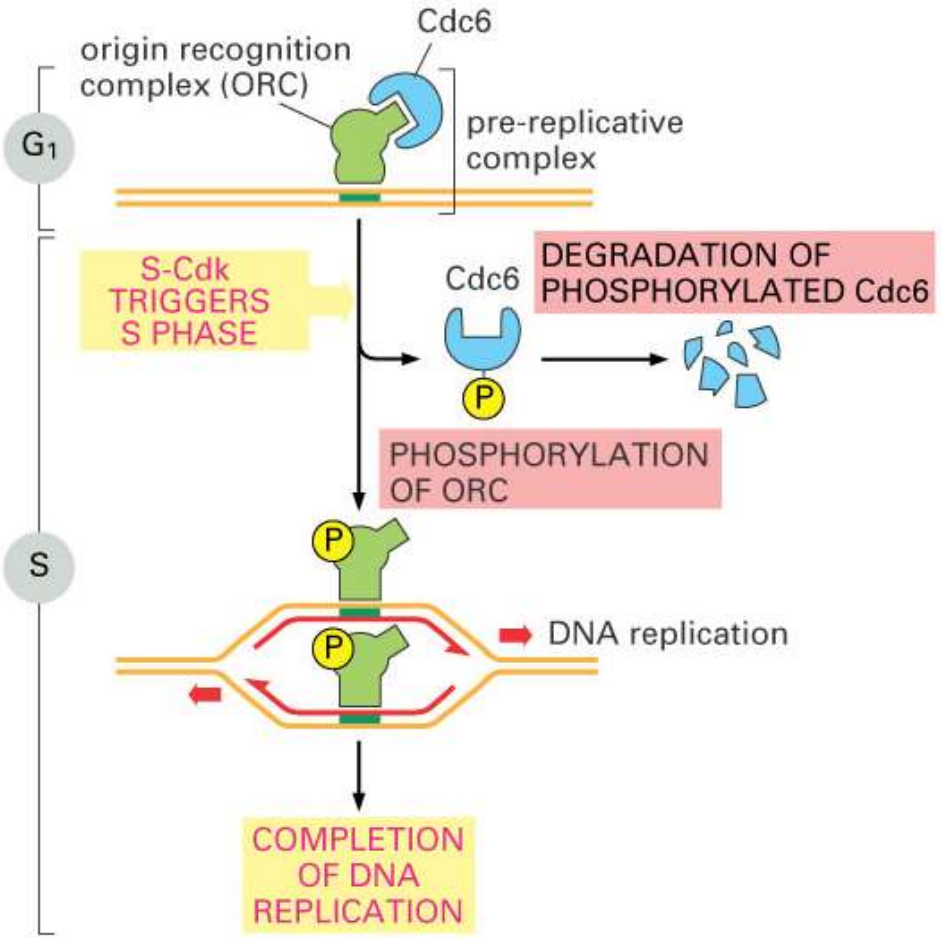


Table 18–2 The Major Cyclins and Cdks of Vertebrates

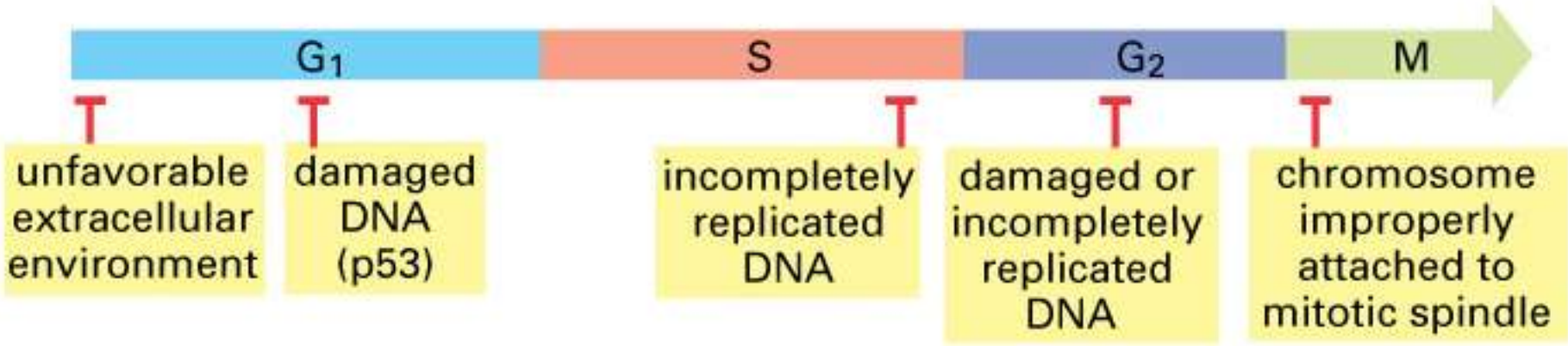
Cyclin–Cdk Complex	Cyclin	Cdk Partner
G1-Cdk	cyclin D*	Cdk4, Cdk6
G1/S-Cdk	cyclin E	Cdk2
S-Cdk	cyclin A	Cdk2
M-Cdk	cyclin B	Cdk1**

*There are three D cyclins in mammals (cyclins D1, D2, and D3).
**The original name of Cdk1 was Cdc2 in vertebrates.

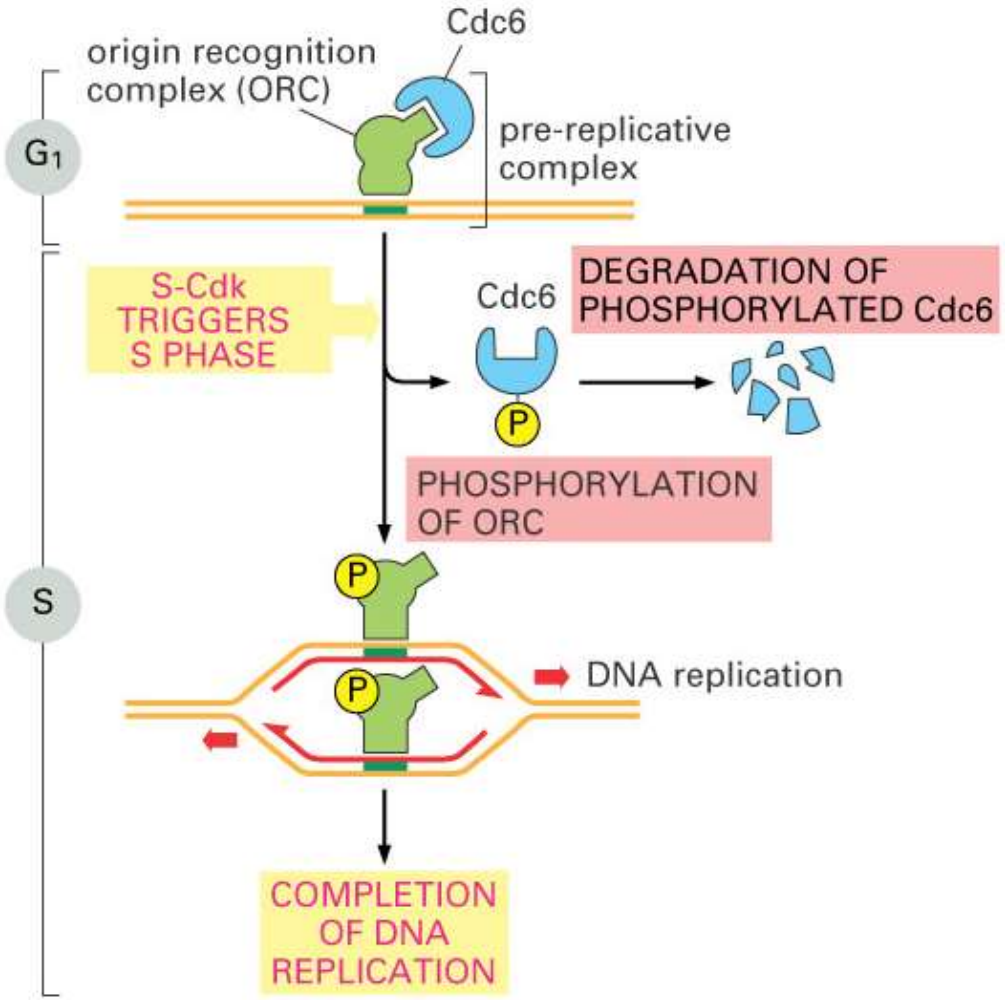
S-Cdk triggers DNA replication - its destruction ensures this happens once per cell cycle



Checkpoints ensure the cell cycle proceeds without errors



Checkpoint: DNA damage arrests the cell cycle in G_1

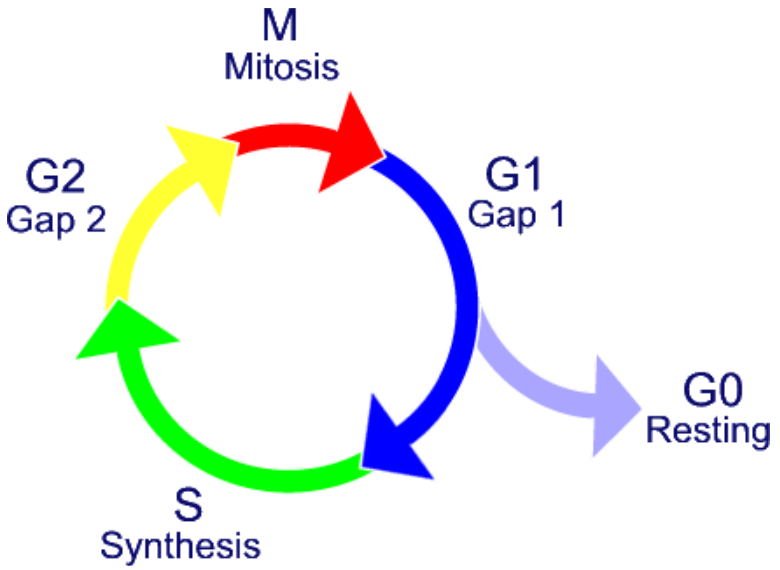


Checkpoint: spindle assembly

- Mitosis must not complete unless all the chromosomes are attached to the mitotic spindle
- Mitotic checkpoint delays metaphase to anaphase transition until all chromosomes are attached
- Prolonged activation of the checkpoint -->cell death
- Mechanism of many anti-cancer drugs

Cells can withdraw from the cell cycle and dismantle the regulatory machinery

- G_0 is a quiescent state
- Cdks and cyclins disappear
- Some cells enter G_0 temporarily and divide infrequenty (I.e. hepatocytes)
- Other differentiated cell types (neurons) spend their life in G_0



Damage & Repair

- Multiple forms of DNA damage occur
- These are repaired constantly by several mechanisms
- Failure to repair damage leads to mutations
- Often defects in damage sensing machinery or DNA repair processes can be correlated with increased incidence of diseases such as cancer

ATM response to double-strand break as a model for cellular response to DNA damage

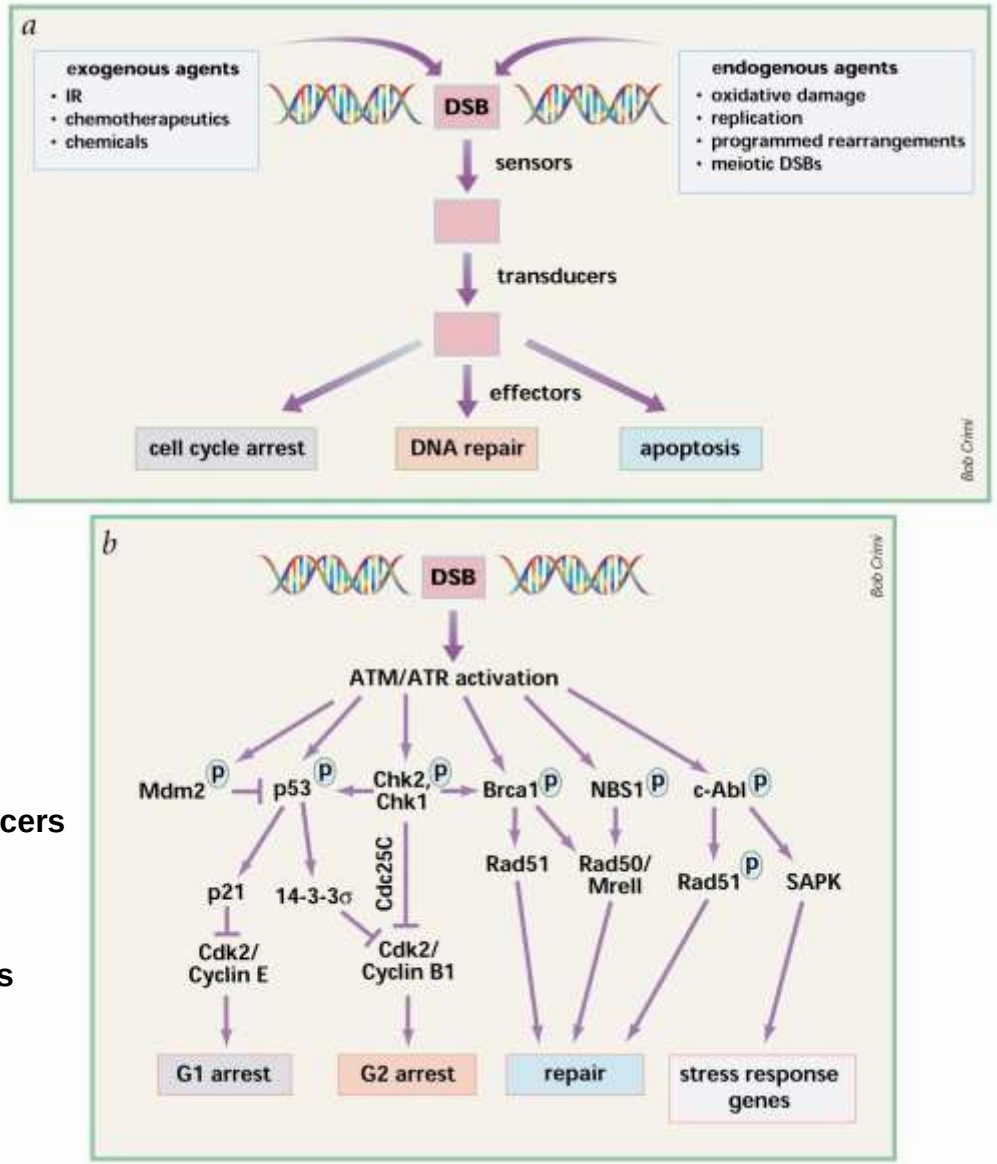


Fig. Signaling of DSBs. a, The general organization of the DNA-damage response pathway. The presence of DSBs is recognized by a sensor, which transmits the signal to a series of downstream effector molecules through a transduction cascade, to activate signaling mechanisms for cell-cycle arrest and induction of repair, or cell death if the damage is irreparable. b, A central role for ATM in the cellular response to DSBs. ATM is activated in response to DSBs by an unknown mechanism. Activated ATM signals the presence of DNA damage by phosphorylating targets involved in cell-cycle arrest, DNA repair and stress response. In addition to those discussed in the text, we also show downstream effectors of p53, notably p21/Cip1 and 14-3-3σ. p21 inhibits the activity of cdk2/cyclinE and 14-3-3σ inhibits the activity of cdc2/cyclin B for effecting cell-cycle arrest. We also show that c-Abl activates stress-activated protein kinase (SAPK) for transcriptional regulation of stress-response genes.

Factors involved in Damage Sensing

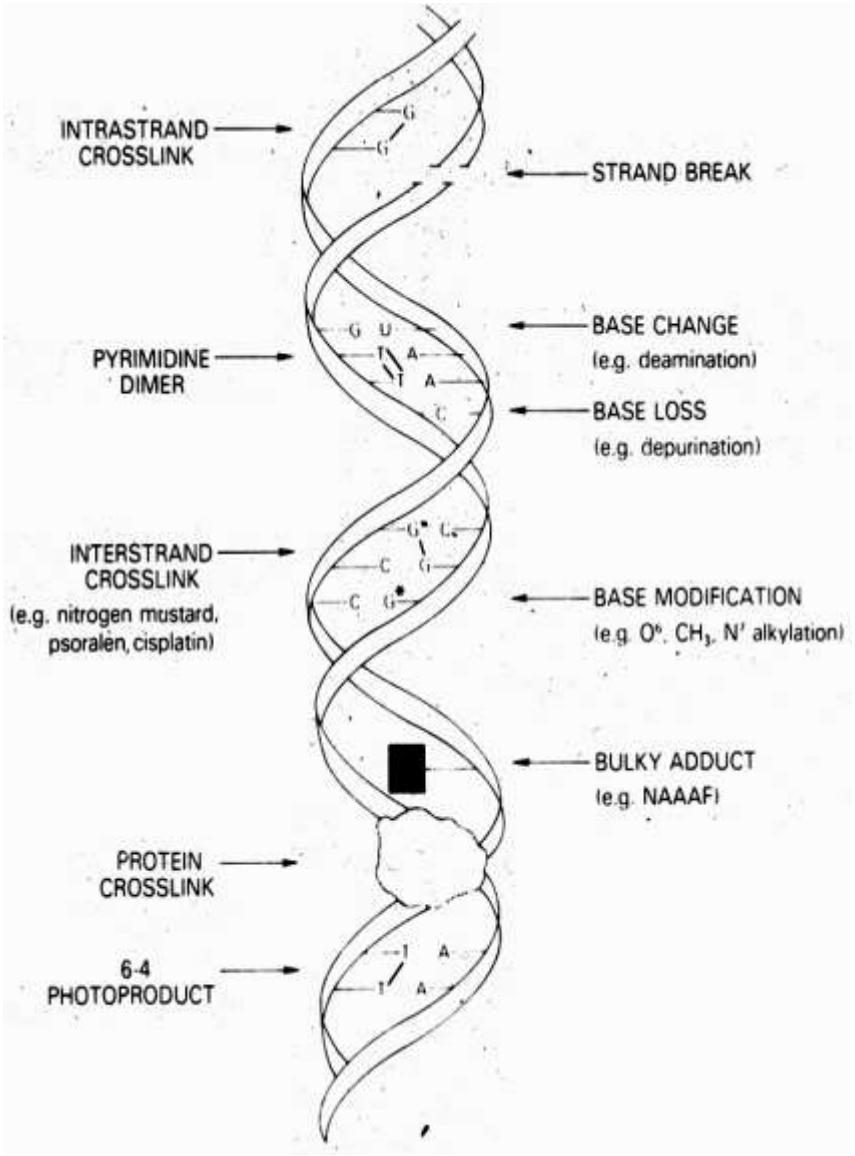
Protein function	*S. cerevisiae*	*S. pombe*	Mammals
ATM/ATR-kinases	Mec1	Rad3	ATR
	Tel1	Tel1	ATM
ATR-interacting proteins	Ddc2	Rad26	ATRIP
RFC-like proteins	Rad24	Rad17	Rad17
	Rfc2-5	Rfc2-5	Rfc2-5
PCNA-like proteins	Ddc1	Rad9	Rad9
	Rad17	Rad1	Rad1
	Mec3	Hus1	Hus1
Mediators	Rad9	Crb2	BRCA1
	Mrc1	Mrc1	Claspin
Replication fork stabilizers	Tof1	Swi1	?
DSB recognition/processing	Mre11	Rad32	Mre11
	Rad50	Rad50	Rad50
	Xrs2	?	Nbs1
Effector kinases	Rad53	Cds1	Chk2
	Chk1	Chk1	Chk1

Лекции N°9-10

«Лекция: «Системы экцизионной репарации ДНК. Часть 1. Экцизионная репарация нуклеотидов, экцизионная репарация оснований и инцизионная репарация нуклеотидов».

НАЦИОНАЛЬНЫЙ ЦЕНТР НАУЧНЫХ ИССЛЕДОВАНИЙ ФРАНЦИЯ
Centre National de la Recherche Scientifique
ИНСТИТУТ ГУСТАВА РОЗИ, Департамент CNRS UMR 8126
Лаборатория «Репарации ДНК»
Research Director, заведующий лабораторией
САПАРБАЕВ Мурат Калиевич

Common Types of DNA Damage and Spontaneous Alterations



Exogneous Sources

UV (sunlight)
Pollution (hydrocarbons)

Smoking
Foodstuffs

Radiotherapy
Ionizing Radiation
X-rays

Chemotherapy
(Alkylating agents)
Cisplatin
Mitomycin C
Cyclophosphamide
Psoralen
Melphalan

Endogenous Sources

Oxidative damage by free radicals
(oxygen metabolism)
Replicative errors
Spontaneous alterations in DNA
Alkylating agents (malondialdehyde)

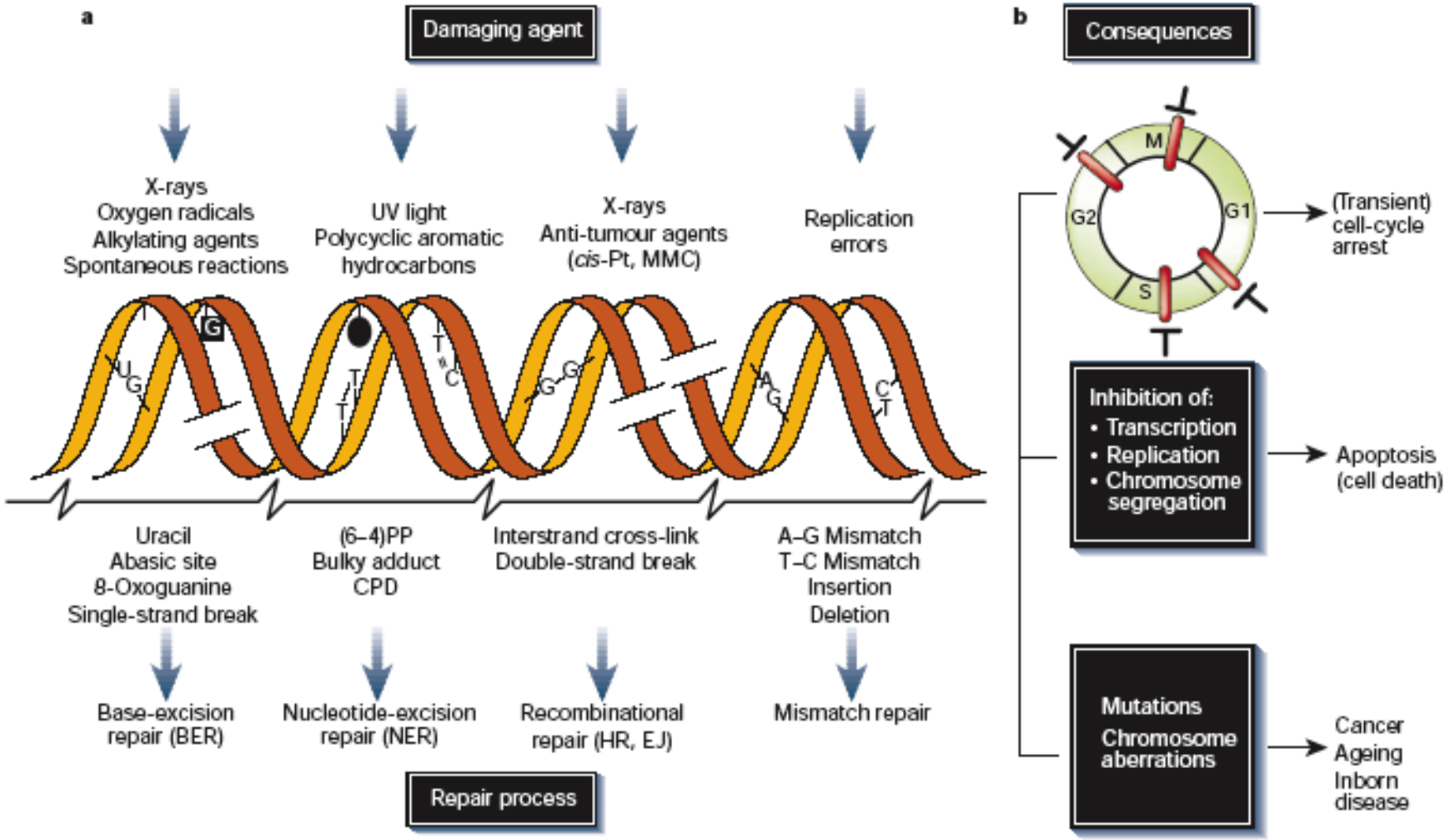
DNA Repair Pathways

1. Direct reversals
2. Excision repair
    a. Base excision repair (BER)
    b. Nucleotide excision repair (NER)
    c. Nucleotide incision repair (NIR)

3. Mismatch repair
    - replication errors
4. Recombinational repair
    - multiple pathways
    - double strand breaks and interstrand cross-links

5. Tolerance mechanisms
    - lesion bypass
    - recombination

DNA Damage, Repair, and Consequences



Human Syndromes Related to DNA Repair Defects

Table 1 Human syndromes with defective genome maintenance

Syndrome	Affected maintenance mechanism	Main type of genome instability	Major cancer predisposition
Xeroderma pigmentosum	NER (± TCR)	Point mutations	UV-induced skin cancer
Cockayne syndrome	TCR	Point mutations	None*
Trichothiodystrophy	NER / TCR	Point mutations	None*
Ataxia telangiectasia (AT)	DSB response/repair	Chromosome aberrations	Lymphomas
AT-like disorder	DSB response/repair	Chromosome aberrations	Lymphomas
Nijmegen breakage syndrome	DSB response/repair	Chromosome aberrations	Lymphomas
BRCA1/BRCA2	HR	Chromosome aberrations	Breast (ovarian) cancer
Werner syndrome	HR?/TLS?	Chromosome aberrations	Various cancers
Bloom syndrome	HR?	Chromosome aberrations (SCE↑)	Leukaemia, lymphoma, others
Rothmund–Thomson syndrome	HR?	Chromosome aberrations	Osteosarcoma
Ligase IV deficiency†	EJ	Recombination fidelity	Leukaemia(?)
HNPCC	MMR	Point mutations	Colorectal cancer
Xeroderma pigmentosum variant	TLS‡	Point mutations	UV-induced skin cancer

*Defect in transcription-coupled repair triggers apoptosis, which may protect against UV-induced cancer.
†One patient with leukaemia and radiosensitivity described with active-site mutation in ligase IV.
‡Specific defect in relatively error-free bypass replication of UV-induced cyclobutane pyrimidine dimers.
Abbreviations: BER, base-excision repair; DSB, double-strand break; HNPCC, hereditary non-polyposis colorectal cancer; HR, homologous recombination; MMR, mismatch repair; NER, nucleotide-excision repair; SCE, sister-chromatid exchange; TCR, transcription-coupled repair; TLS, translesion synthesis.

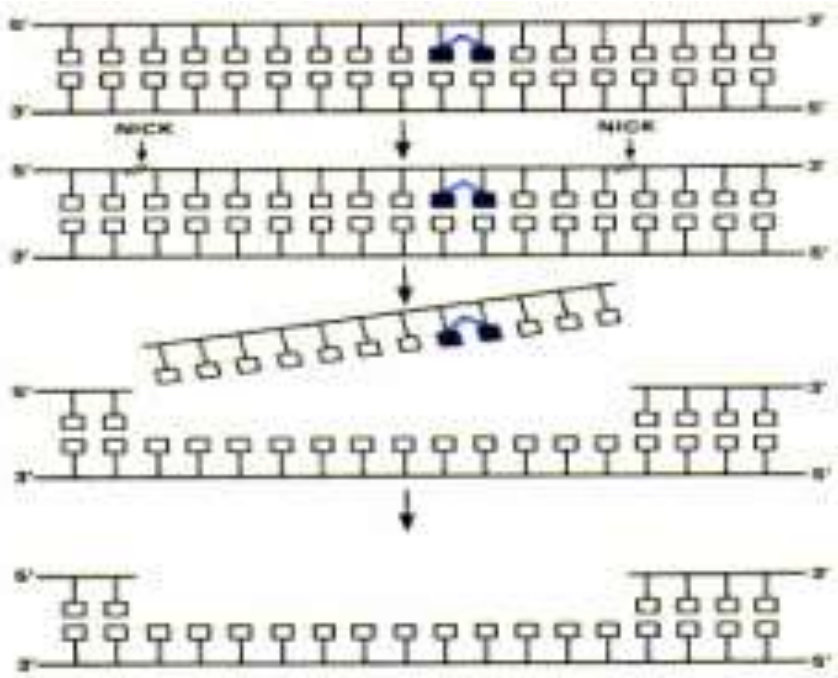
Excision Repair Pathways

Nucleotide Excision Repair
- damaged bases are removed as oligonucleotides
- primarily responsible for removal of UV-induced damage and bulky adducts
- several NER proteins are involved in other pathways
- deficient in human disorders

Base Excision Repair
- damaged bases are removed as free bases
- primarily responsible for removal of oxidative and alkylation damage
- most genes in pathway are not essential
- thought to have an important role in aging

Nucleotide Incision Repair
- damaged bases are removed as oligonucleotides
- primarily responsible for removal of oxidative damage
- most genes in pathway are essential
- thought to have an important role in cellular resistance

Mechanism of Incision by the NER Pathway



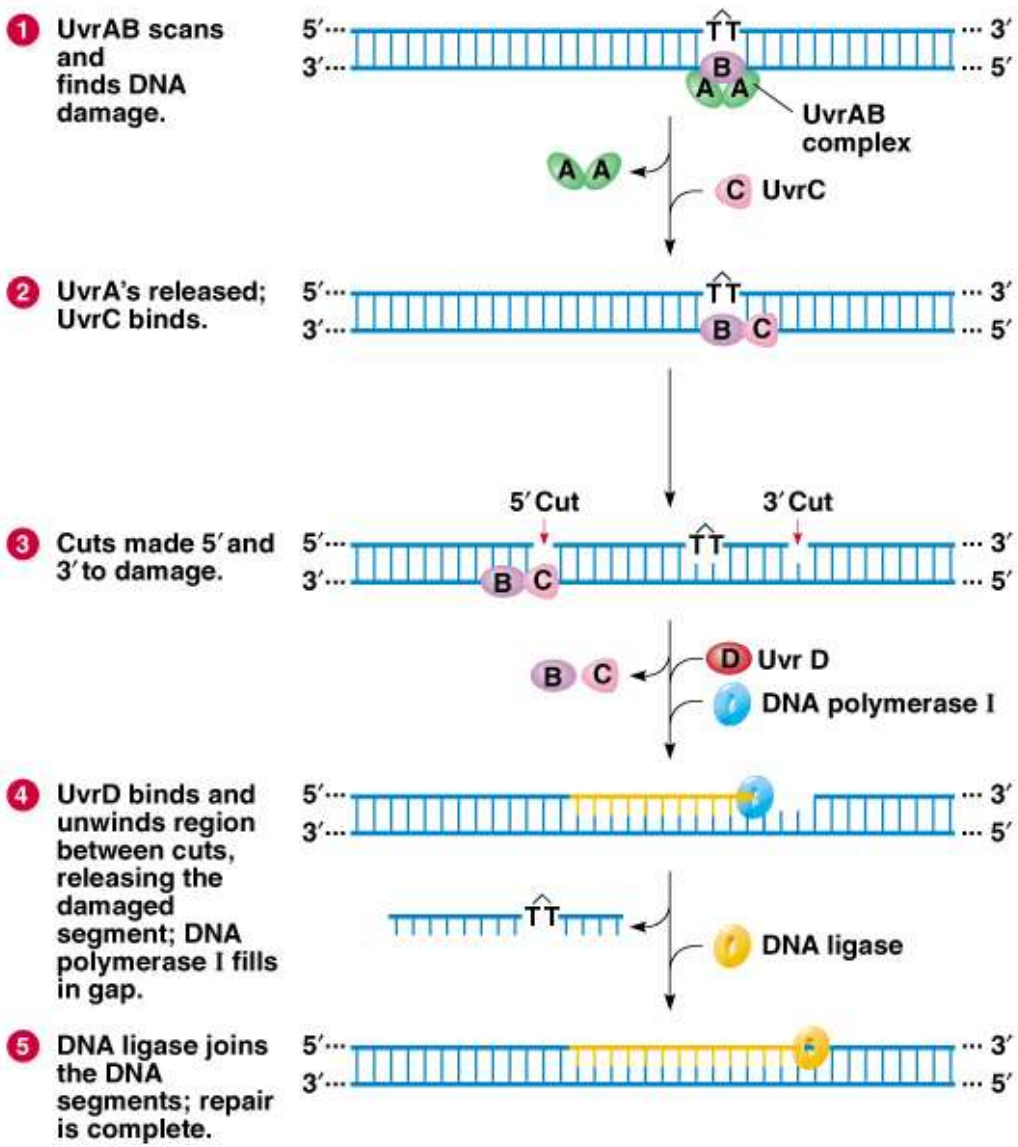
E. coli

5’ incision is 8 nuc. from lesion
3’ incision is 4 nuc. from lesion

Mammals

5’ incision is 22 nuc. from lesion
3’ incision is 6 nuc. from lesion

Nucleotide excision repair (NER) of pyrimidine dimmer and other damage-induced distortions of DNA in E. coli



Six genes

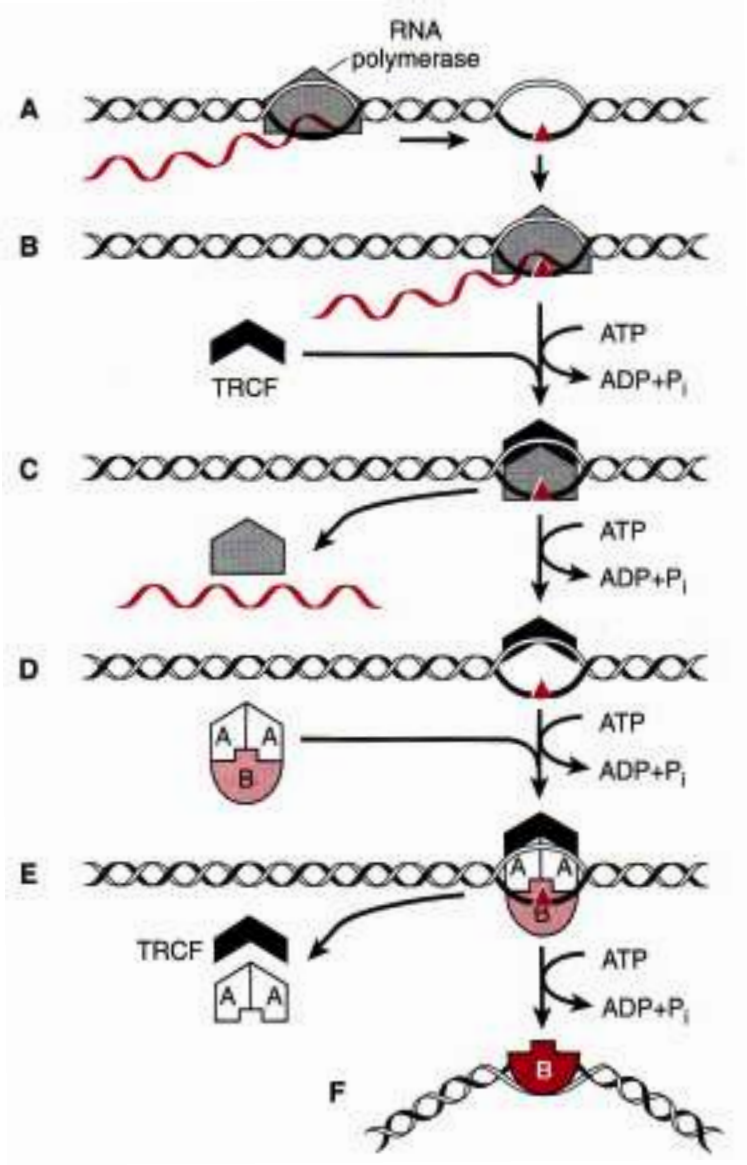
UvrA (helicase)
UvrB (endonuclease)
UvrC (endonuclease)
UvrD (helicase)
Polymerase I
DNA ligase

Transcription-Coupled Repair in the E. coli NER Pathway

Two pathways

1. Global genome repair (GGR)
2. Transcription-coupled repair (TCR)

TRCF - transcription repair coupling factor



Genetics of NER in Humans

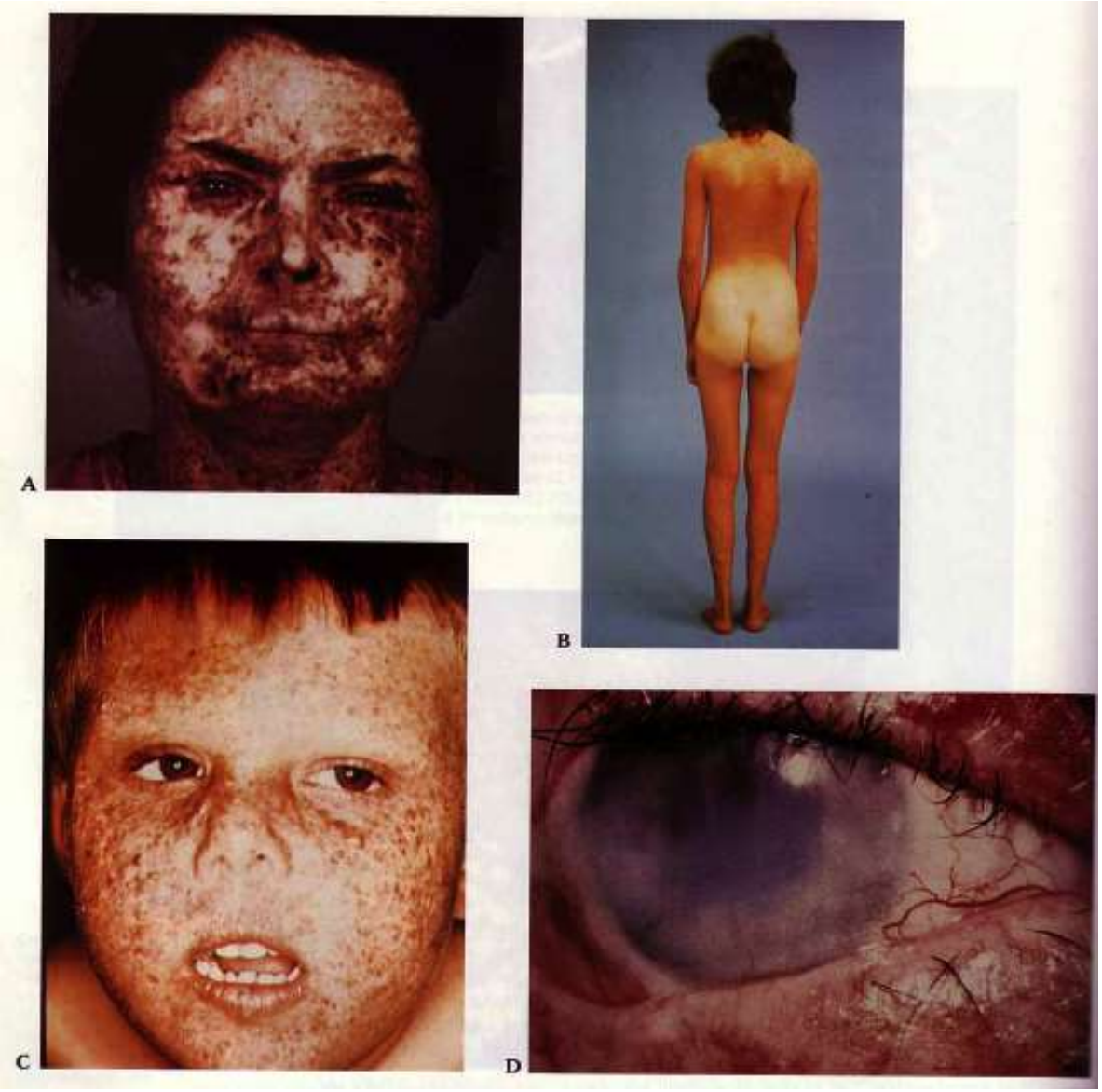
Xeroderma Pigmentosum (classical)

- Occurrence: 1-4 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: multiple skin disorders; malignancies of the skin; neurological and ocular abnormalities
- Biochemical: defect in early step of NER
- Genetic: autosomal recessive, seven genes (A-G)

Xeroderma Pigmentosum (variant)

- Occurrence: same as classical
- Sensitivity: same as classical
- Disorder: same as classical
- Biochemical: defect in translesion bypass

Xeroderma Pigmentosum

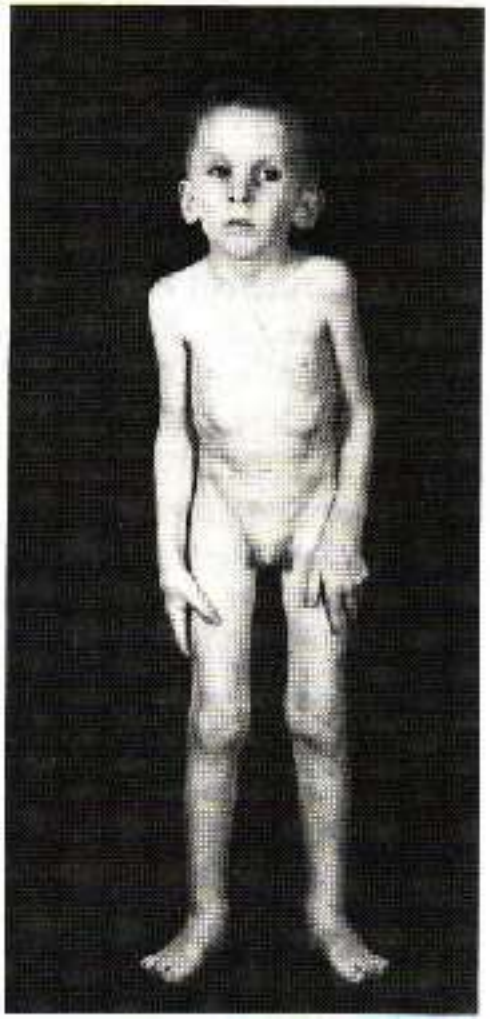


Genetics of NER in Humans

Cockayne's Syndrome

- Occurrence: 1 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: arrested development, mental retardation, dwarfism, deafness, optic atrophy, intracranial calcifications; (no increased risk of cancer)
- Biochemical: defect in NER
- Genetic: autosomal recessive, five genes (A, B and XPB, D & G)

Cockayne's Syndrome



Genetics of Nucleotide Excision Repair

ERCC	CLONED	YEAST	FUNCTION
1	+	RAD10	endonuclease
2	+ (XP-D)	RAD3	helicase
3	+ (XP-B)	RAD25	helicase
4	+ (XP-F)	RAD1	endonuclease
5	+ (XP-G)	RAD2	endonuclease
6	+ (CS-B)	RAD26	helicase
7	-	?	?
8	+ (CS-A)	RAD28	WD-40 repeat
9-10	-	?	?
XP			
A	+	RAD14	DNA binding
B (CS,TTD)	+ (ERCC3)	RAD25	helicase
C	+	RAD4	DNA binding
D (CS,TTD)	+ (ERCC2)	RAD3	helicase
E	+ (p48)	?	DNA binding?
F	+ (ERCC4)	RAD1	endonuclease
G (CS)	+ (ERCC5)	RAD2	endonuclease
V	+	RAD30	polymerase eta
CS			
A	+ (ERCC8)	RAD28	WD-40 repeat
B	+ (ERCC6)	RAD26	helicase
TTD (PIBIDS)			
A	-	?	TFIIH ?

Twenty Five Known Genes Involved in NER

Factors required for excision

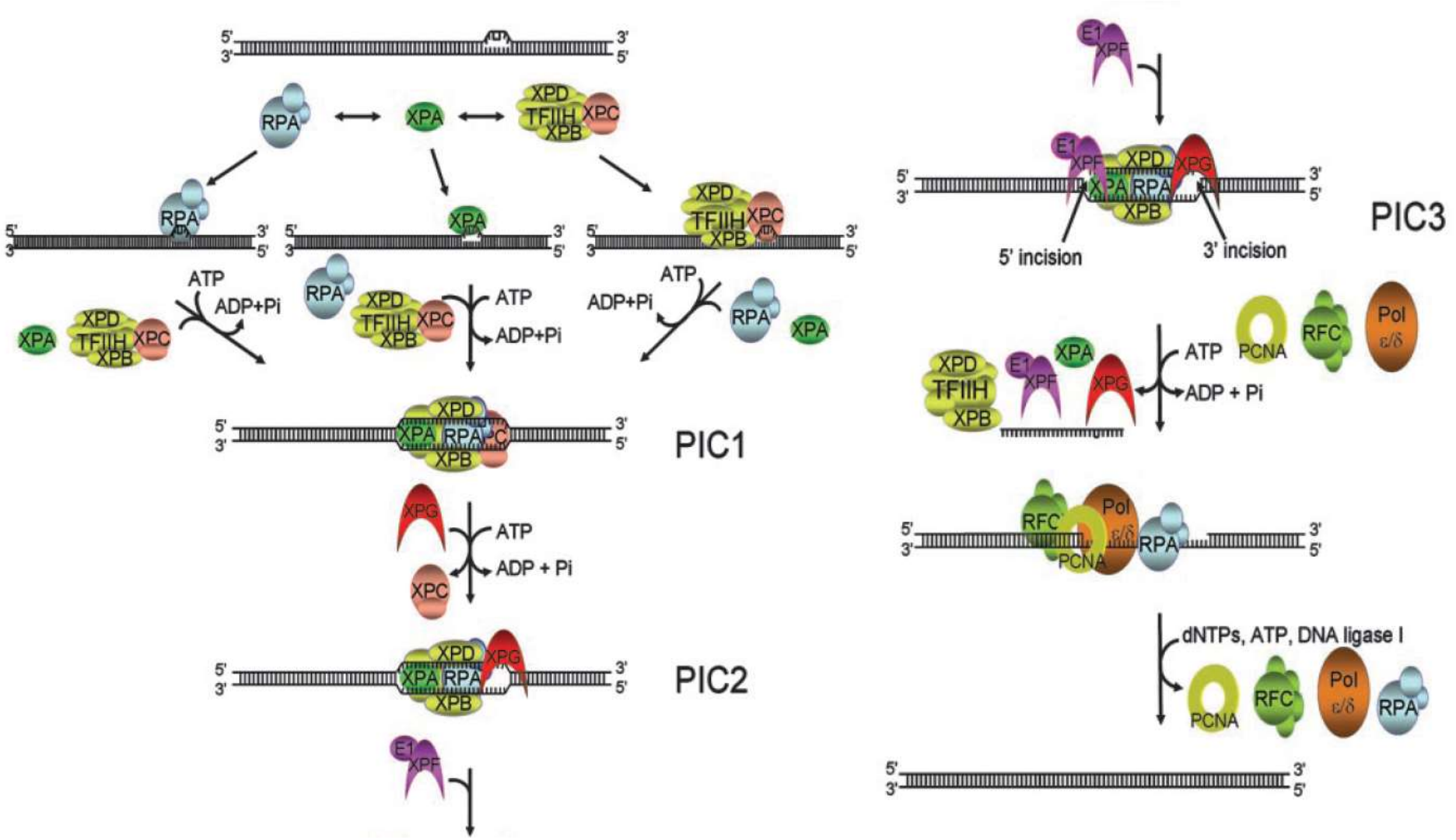
XPC-HHR23B
XPA
Replication Protein A (RPA)
    p70, p32, p14
TFIIH
    XPB, XPD, p62, p44, p34
XPG
ERCC1-XPF

Factors required for repair synthesis

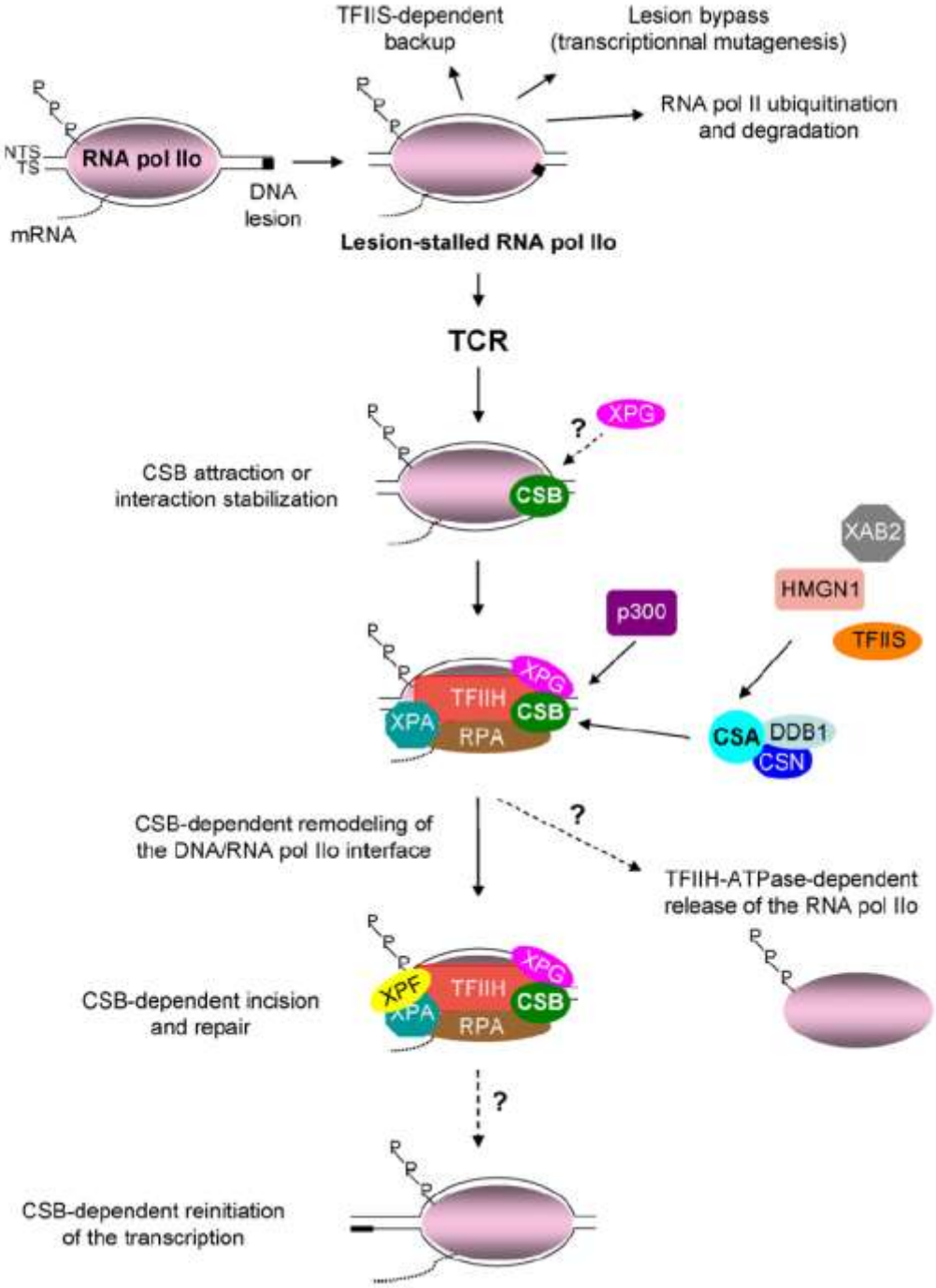
Replication Factor C (RFC)
    5 subunits
Proliferating Cell Nuclear Antigen (PCNA)
DNA polymerases δ, ε
DNA ligase I

Addition factors required for transcription-coupled repair

CSA
CSB



Nucleotide excision repair in human cells. The DNA damage is recognized by the cooperative binding of RPA, XPA, and XPC-TFIIH, which assemble at the damage site in a random order. The four repair factors form a complex at the binding site, and if the binding site is damage-free, ATP hydrolysis by the XPB and XPD helicases dissociates the complex (kinetic proofreading). If the site contains a lesion, ATP hydrolysis unwinds the duplex by about 25 bp around the lesion, making a stable preincision complex 1 (PIC1) at the damage site. XPG then replaces XPC in the complex to form a more stable preincision complex 2 (PIC2). Finally, XPFERCC1 is recruited to the damage site to form preincision complex 3 (PIC3). The damaged strand is incised at the 6th 3 phosphodiester bond, 3 to the damage by XPG, and the 20th 5 phosphodiester bond 5 to the damage by XPFERCC1. The resulting 24–32 oligomer is released, and the gap is filled by Pol/ with the aid of replication accessory proteins PCNA and RFC.



Current view of transcription-coupled repair.

When elongating RNA pol IIo is blocked by DNA damage located on the transcribed strand of an active gene, several pathways can occur to allow a fast recovery of RNA synthesis. The most studied pathway is TCR. Stalled RNA pol IIo at sites of UV damage attracts CSB [5] and eventually XPG [4]. At stalled RNA pol II complex one can see the sequential arrival of TFIIH, RPA and XPA proteins. It seems that THIIH and XPA may stabilize each other onto the RNA pol IIo [3]. XPF join later the complex formed around RNA pol IIo. TFIIH is likely to be partially responsible for the release of RNA pol IIo driven by an ATP-dependent reaction [3]. CSB together with RNA pol IIo promote dual incision [3] but how and when CSB joins RNA pol IIo is not clear. Wild-type CSB is a prerequisite factor to assemble the functional CSA-DDB1 E3-ubiquitin ligase/CSN complex [5]. The latter (in addition to CSB) is required for recruitment of the nucleosomal binding protein HMGN1, and the XAB2 and TFIIS proteins [5]. The exact fate of RNA pol II is not known yet.

Mammalian NER Pathways

XPC - damage recognition

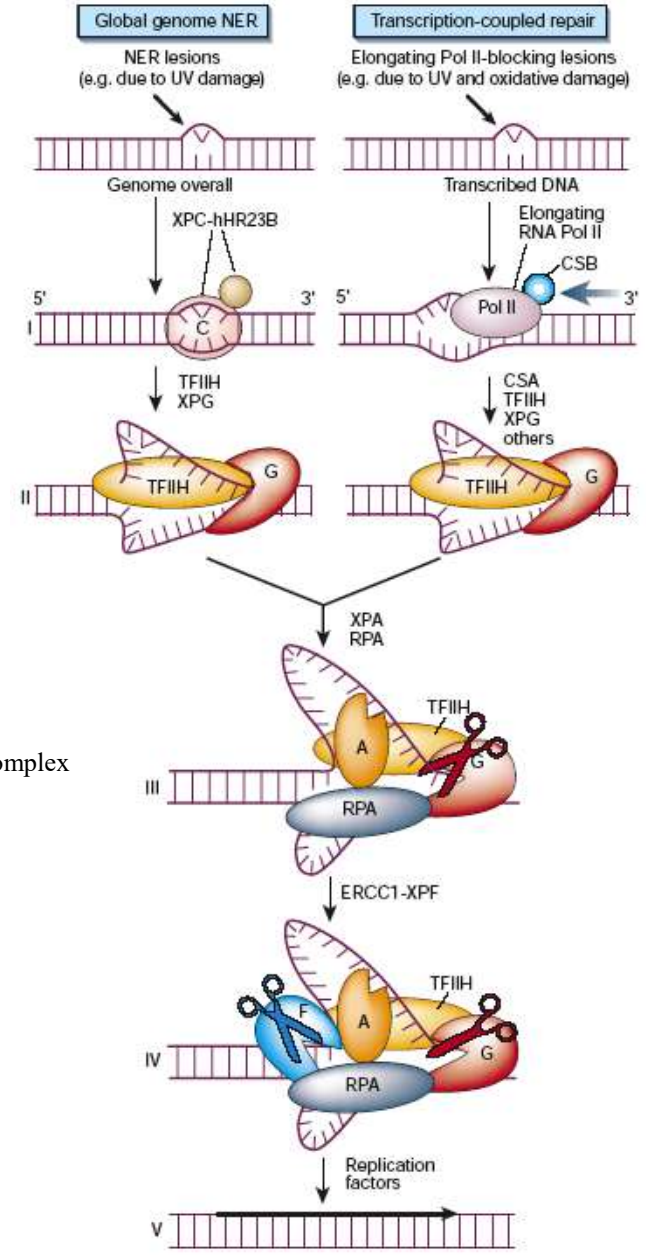
XPB & XPD - DNA helicases

XPA & RPA - damage validation & complex stabilization

ERCC1-XPF - 5' incision

XPG - 3' incision

(junction specific endonucleases)



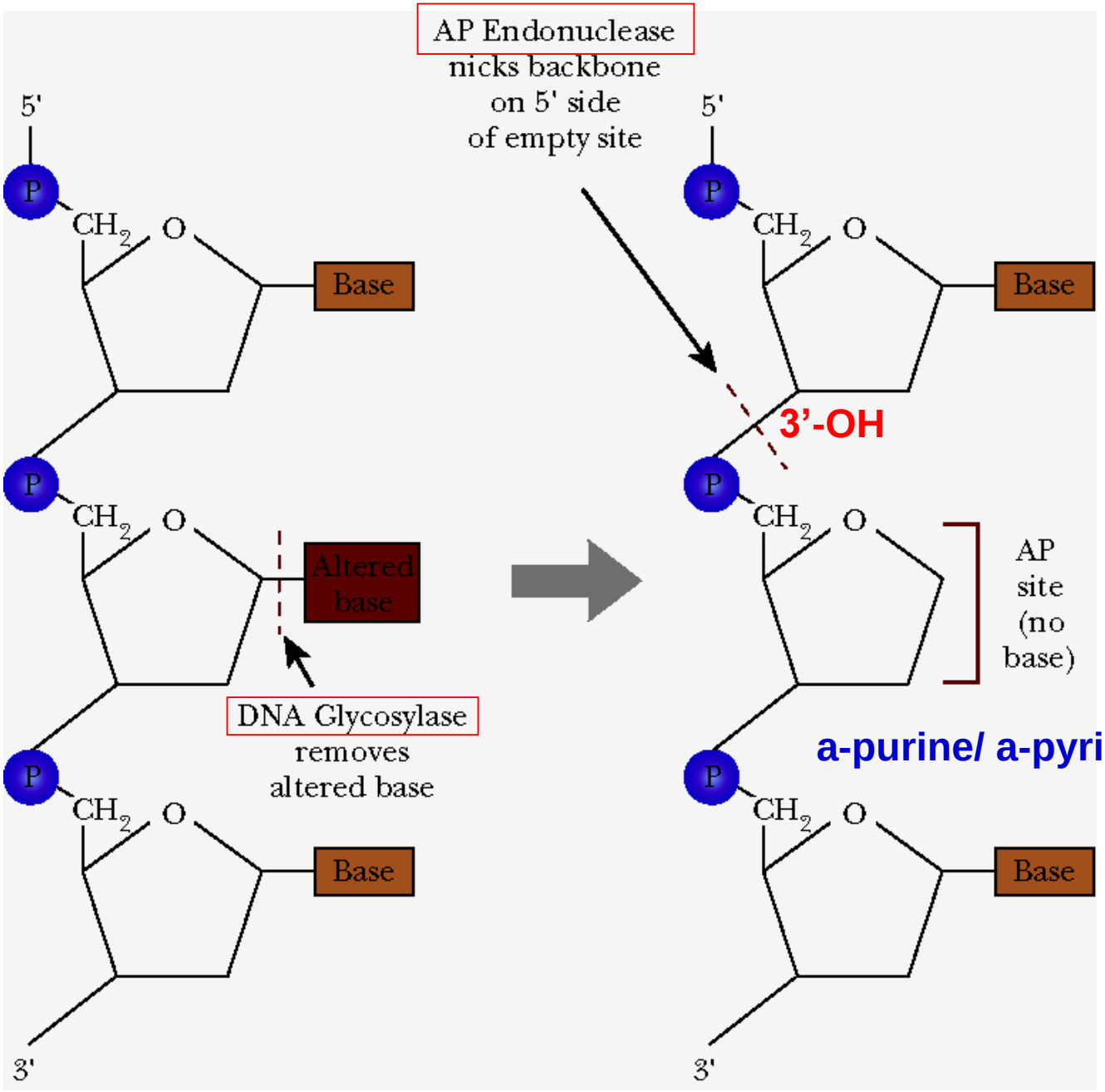
CSA & CSB - role in processing RNAP II?

Summary for Mammalian NER

1. Global Genomic Repair (GGR)
   - repairs all regions of the genome
   - repairs all types of bulky adducts
   - apparently down regulated in some rodent cells
   - requires XPC and all other NER factors except CSA and CSB

2. Transcription-Coupled Repair (TCR)
   - repair of template strand during transcription by Pol II
   - primary repair pathway in some rodent cells; faster repair in human cells
   - dependent on type of lesion, e.g., cyclobutane dimers but not 6-4 photoproducts
   - requires CSA, CSB and all other NER factors, except XPC

Base excision repair pathway



The Base Excision Repair Pathway

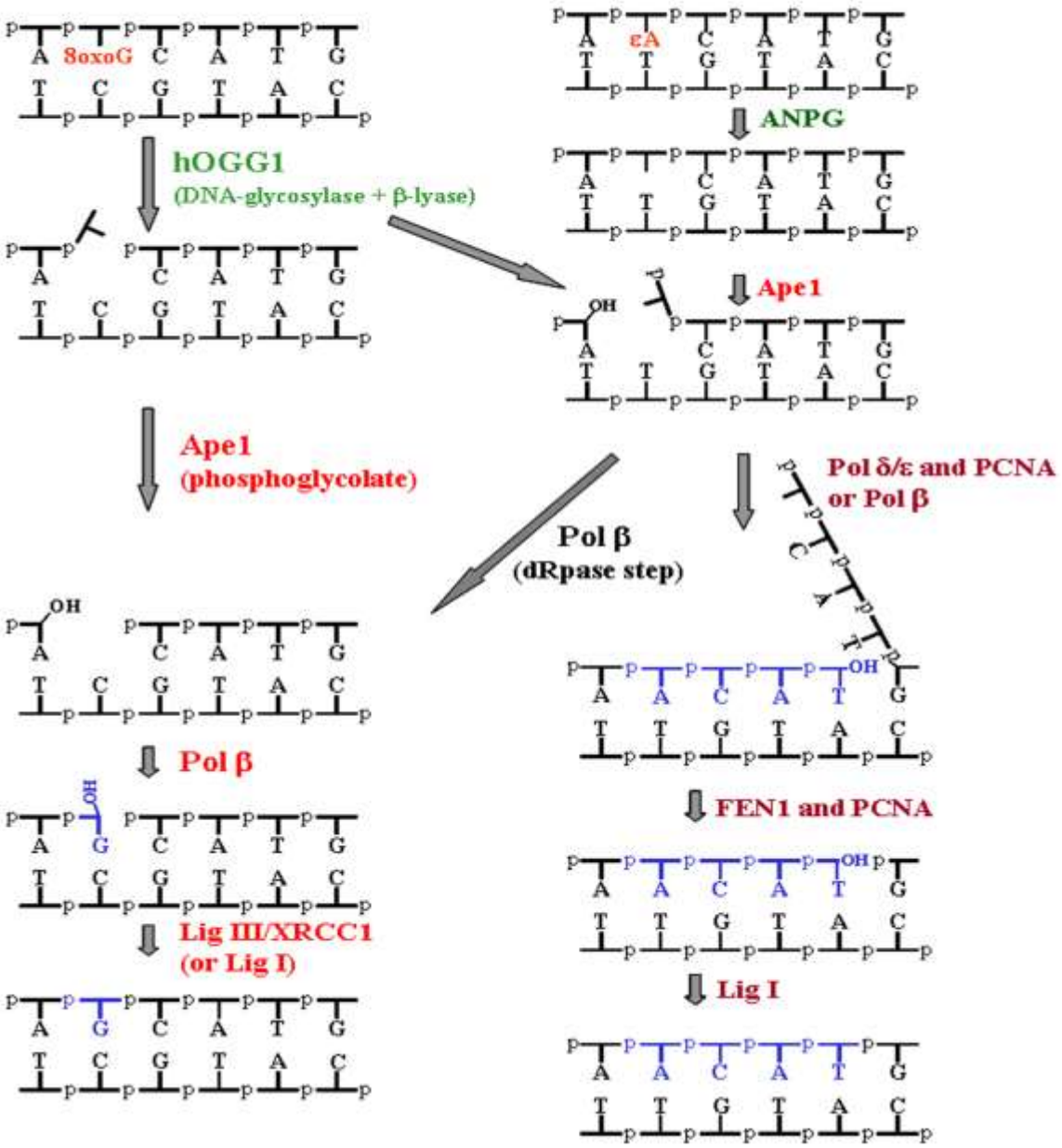
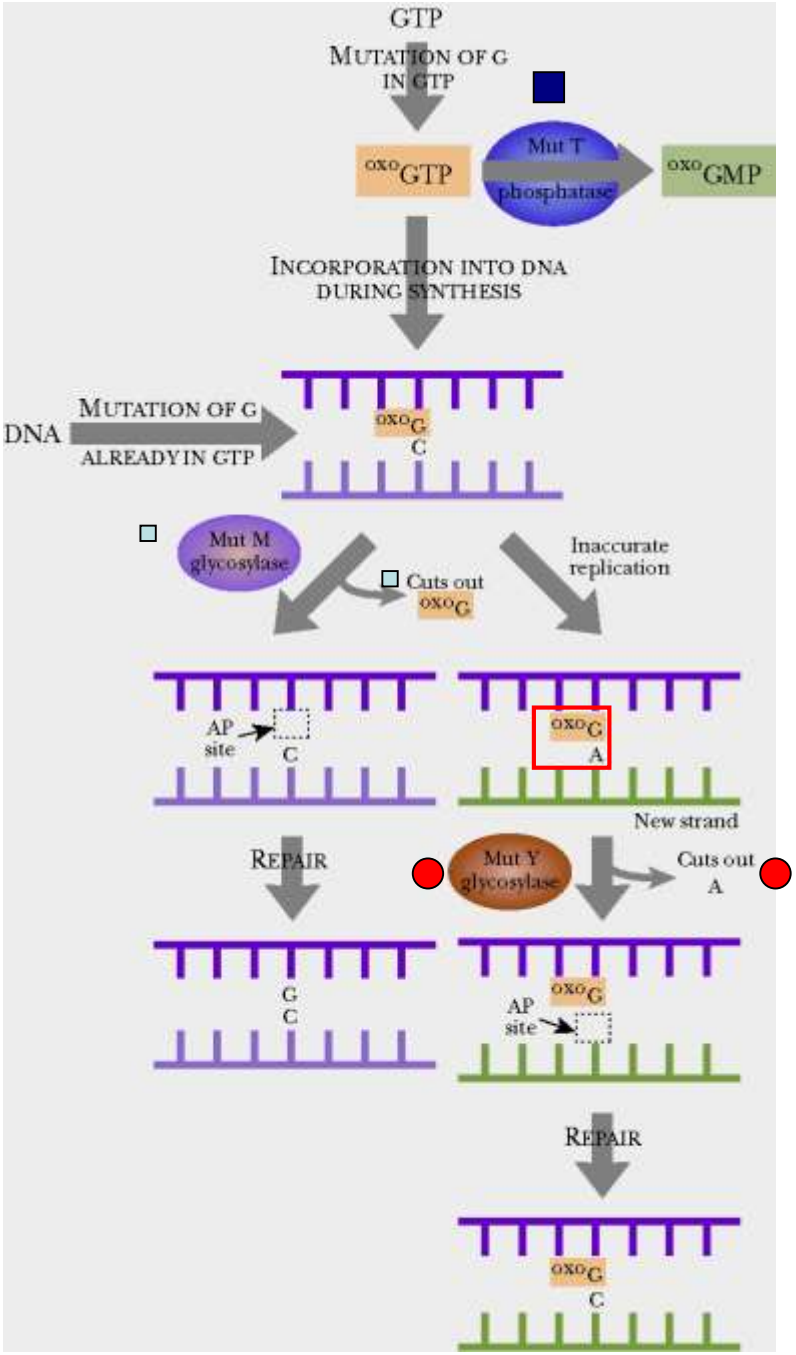


TABLE 1 Mammalian DNA glycosylases

Enzyme	Chromosomal location (human)	Cellular localization (nuclear or mitochondrial)	Major or significant substrates[a]
UNG	12q23–24.1	N and M	U in single- or double-strand DNA
SMUG1	12q13.3–11	N	U in single- or double-strand DNA, 5-OH-meU
TDG	12q24.1	N	T, U or ethenoC opposite G (preferably CpG sites)
MBD4	3q21–22	N	T or U opposite G at CpG, T opposite O^6-meG
MYH	1p32.1–34.3	N and M	A opposite 8-oxoG, 2-OH-A opposite G
OGG1	3p26.2	N and M	8-oxoG opposite C, fapyG
NTH1	16p13.3	N and M	Tg, DHU, fapyG, 5-OHU, 5-OHC
NEIL1	15q22–24	N	As NTH1; also fapyA, 5S, 6R Tg isomer, 8-oxoG
NEIL2	8p23	N	Overlap and some differences with NTH1/NEIL1
NEIL3	4q34.2	N	To be determined
MPG	16p13.3	N	3-meA, hypoxanthine, ethenoA

For comprehensive updated information see: http://www.cgal.icnet.uk/DNA_Repair_Genes.html

[a]For abbreviations, see the text.

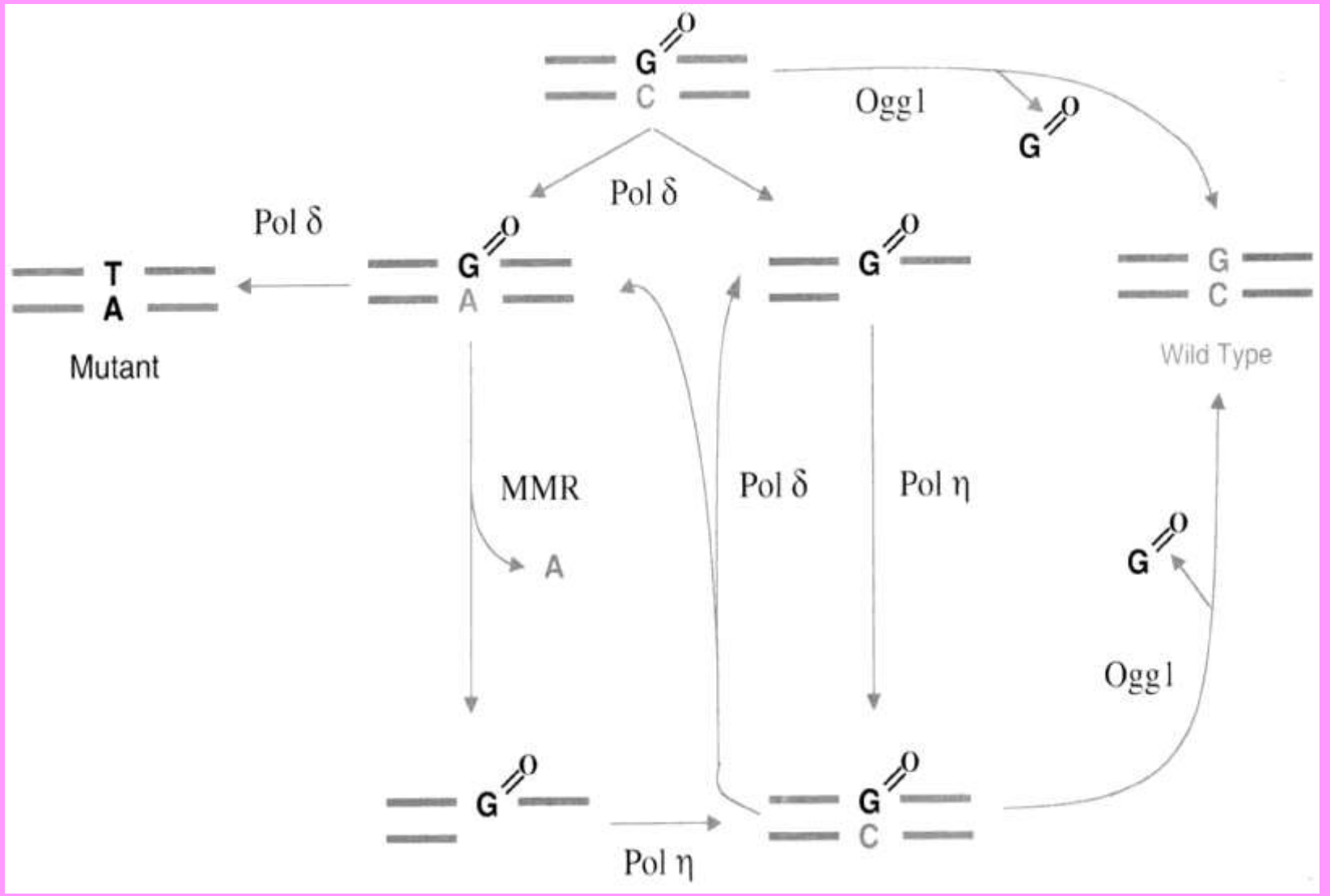


GO – repair system in E. coli prevent mutagenesis of 8-oxoG

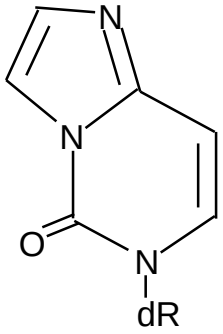
MutT, MutM, MutY

Dealing with oxidized guanine.

GO – repair system in eukaryots



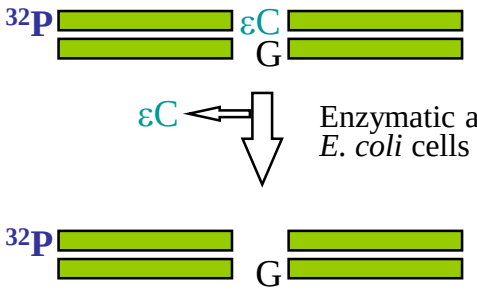
Repair Of Ethenocytosine Residues: Identification Of The Enzymes Excising These Residues.

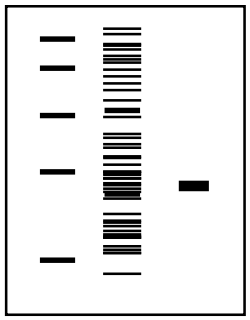


3,*N*⁴-ethenodeoxycytidine (εdC)

MUTATION BY TRANSVERSION

εCG ⟶ AT



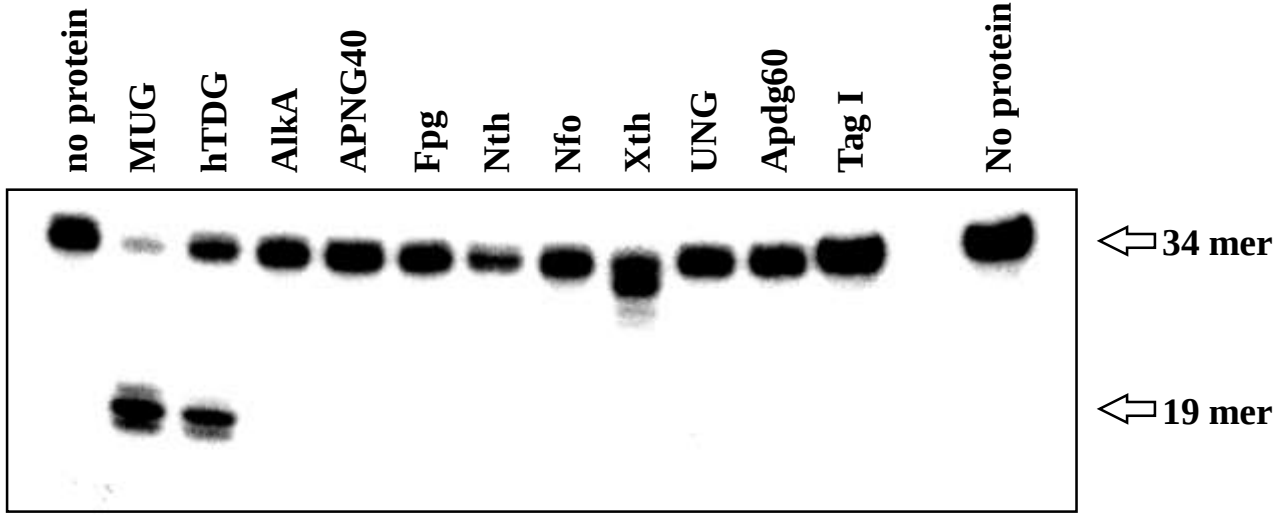


Microsequencing of Protein

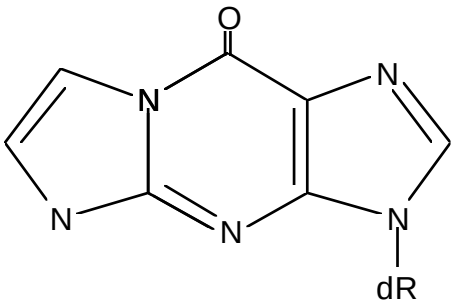
QLKPQEAHLLDYR
and VIYQAGFTDR

Data bank search. Protein of 168 aa M.W. 18673 Da
Mismatch-specific Uracil-DNA Glycosylase (MUG) Gallinari & Jiricny, (1996)

human homologue is a
Mismatch-Specific Thymine-DNA Glycosylase (hTDG)
Neddermann *et al*, (1996)

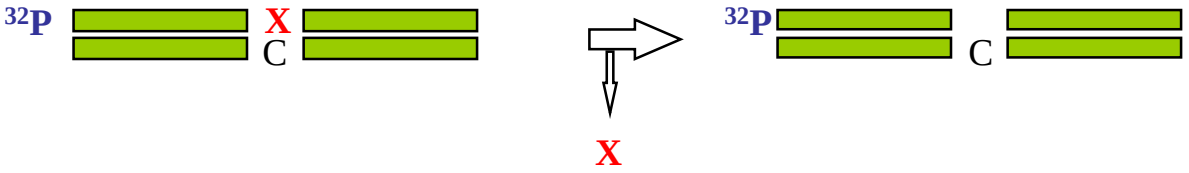


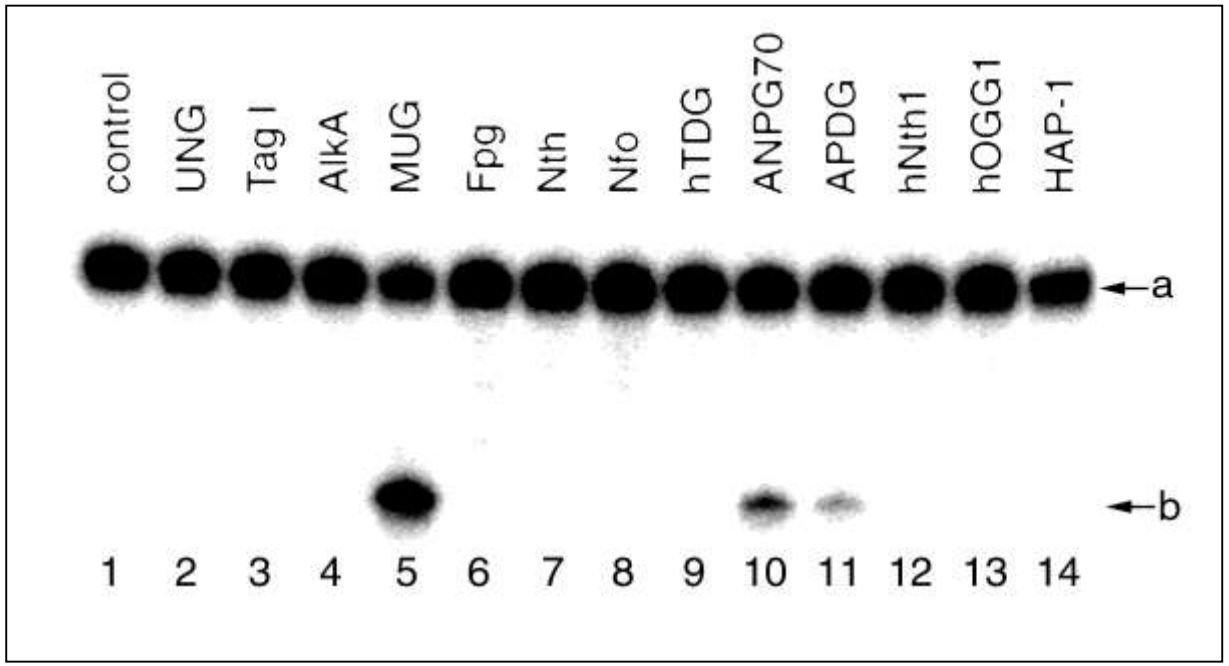
Identification of the Enzyme(s) Involved in the Repair of 1,N^2-Ethenoguanine Residues.

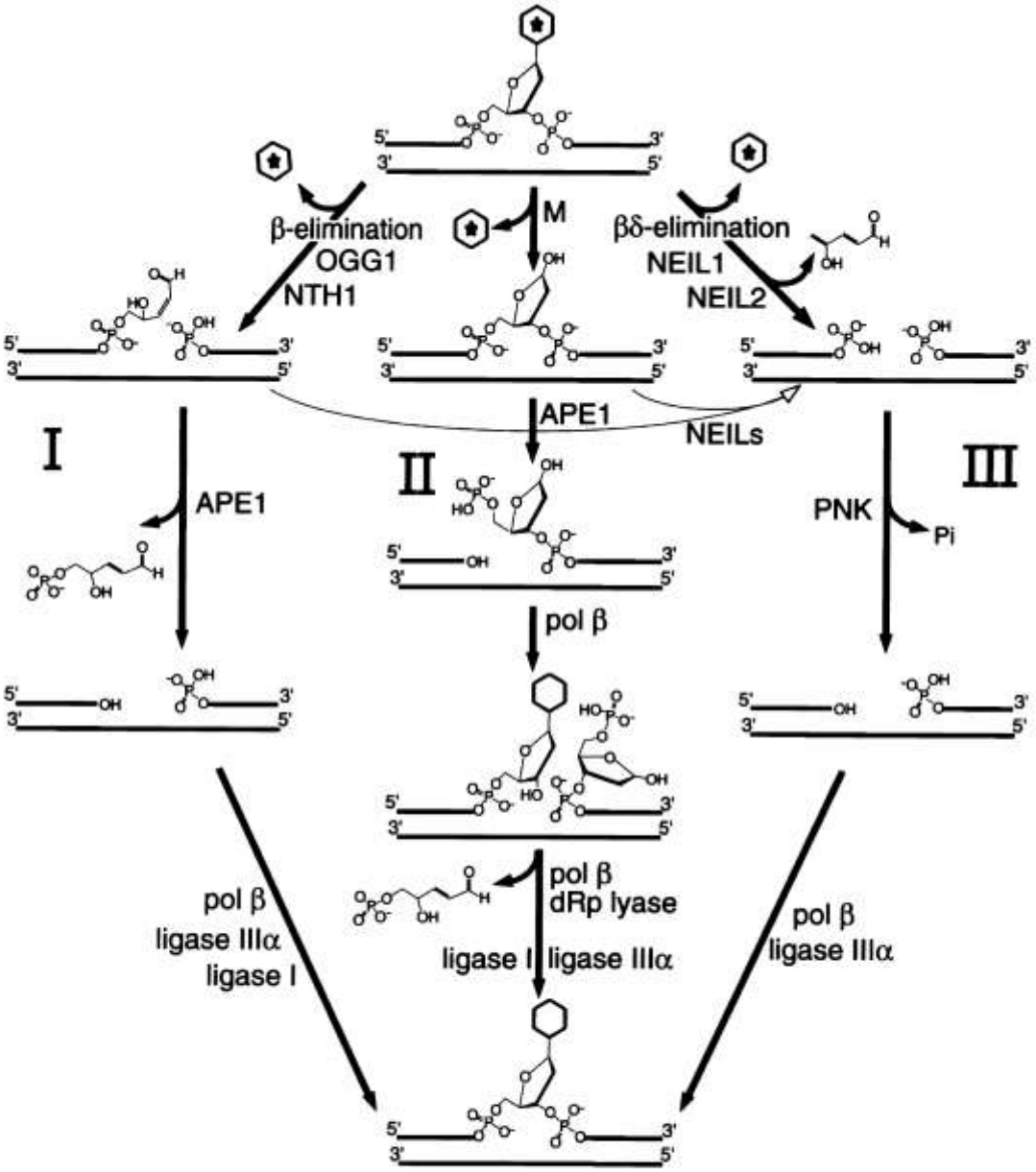


1,N^2-ethenoguanosine (1,N^2-εG)

1,N^2-εG ⟶ A



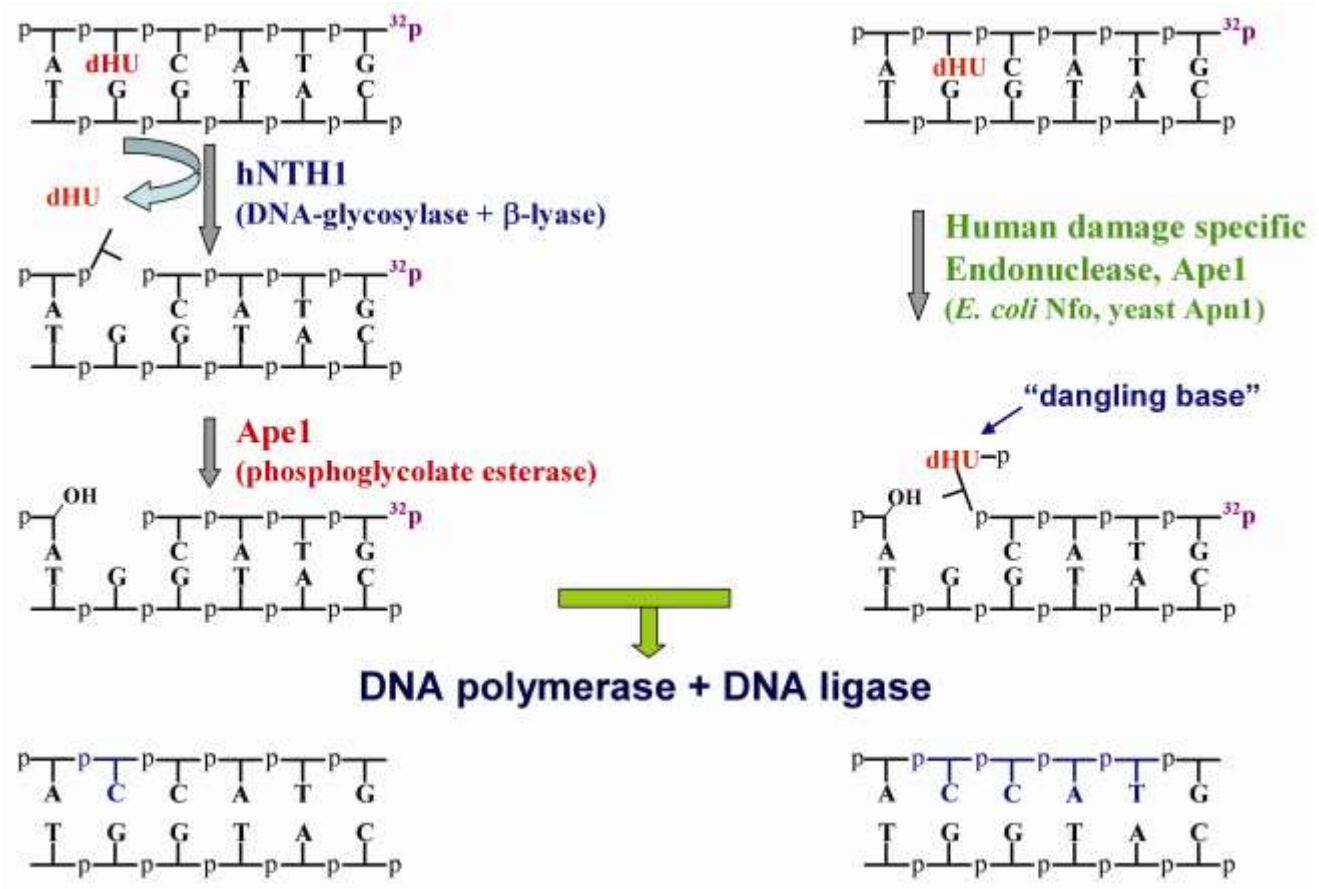




A Model for APE and PNK-Dependent BER Pathways in Mammalian Cells. Three BER subpathways (I, II, and III) defined by the type and reaction mechanism of DNA glycosylases are shown. Monofunctional glycosylases (M) generate AP sites which are cleaved by APE1 to leave a 5'-deoxyribosephosphate terminus. It is removed by pol beta producing a singlenucleotide gap necessary for nucleotide addition (pathway II). When NTH1 and OGG1 carry out beta-elimination, APE1 removal of the resulting 3'-dRP generates a single nucleotide gap with a 3'-OH (pathway I). With NEILs as the initial glycosylase, a 3'-phosphate terminus is generated which is then removed by PNK (pathway III).

The Base Excision Repair Pathway (BER)

The Nucleotide Incision Repair Pathway (NIR)



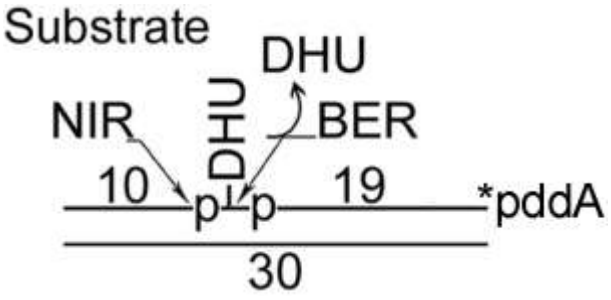
Previous observations described in the literature:

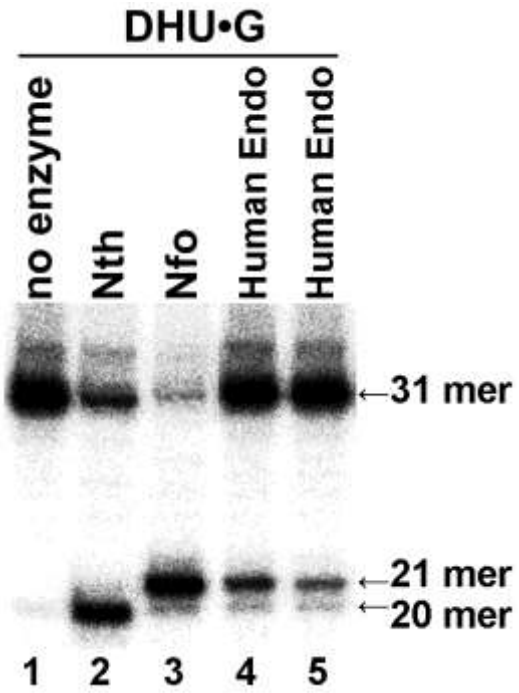
1. Kow, Y.W. and Wallace, S.S. (1985) Exonuclease III recognizes urea residues in oxidized DNA. *Proc. Natl. Acad. Sci. U. S. A.*, **82**, 8354.

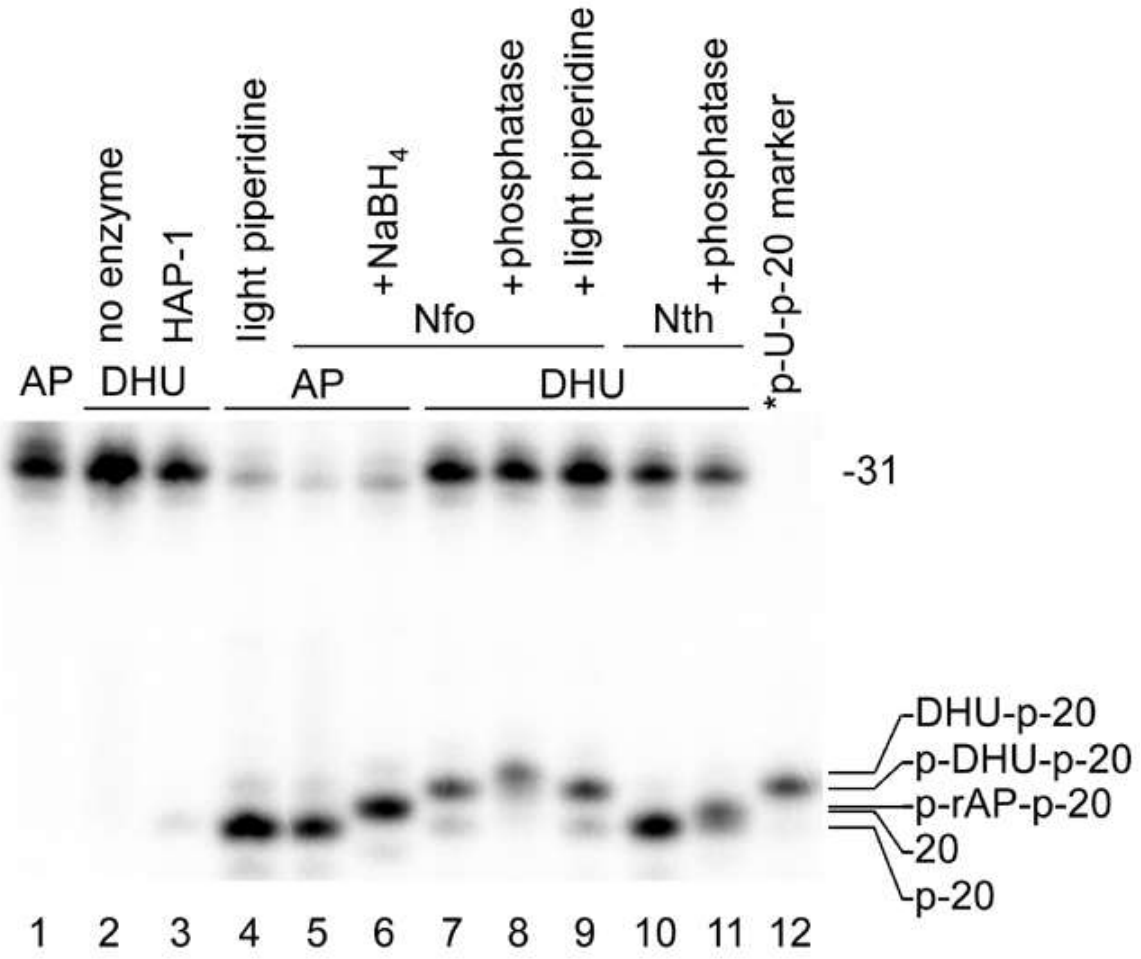
2. Hang, B., Chenna, A., Fraenkel-Conrat, H. and Singer, B. (1996) An unusual mechanism for the major human apurinic/apyrimidinic (AP) endonuclease involving 5' cleavage of DNA containing a benzene-derived exocyclic adduct in the absence of an AP site. *Proc. Natl. Acad. Sci. U. S. A.*, **93**, 13737.

3. Yajima, H., Takao, M., Yasuhira, S., Zhao, J.H., Ishii, C., Inoue, H. and Yasui, A. (1995) A eukaryotic gene encoding an endonuclease that specifically repairs DNA damaged by ultraviolet light. *Embo J*, **14**, 2393

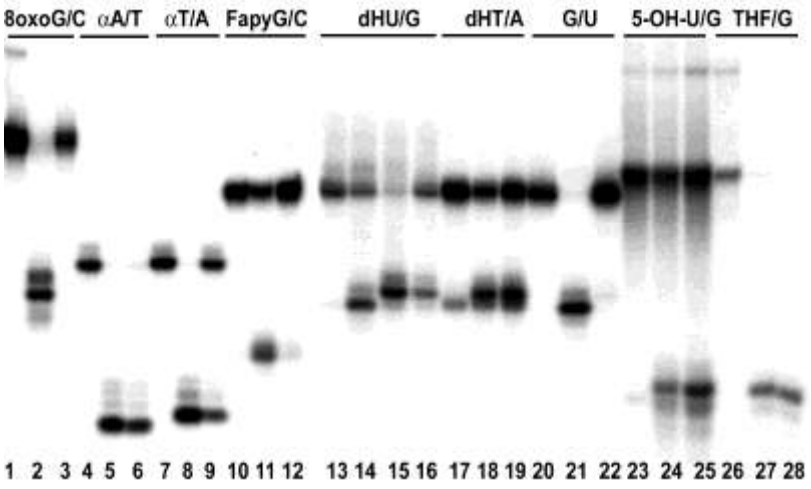
Reaction products following oligonucleotide incision by DNA glycosylase / lyase or AP endonuclease action.

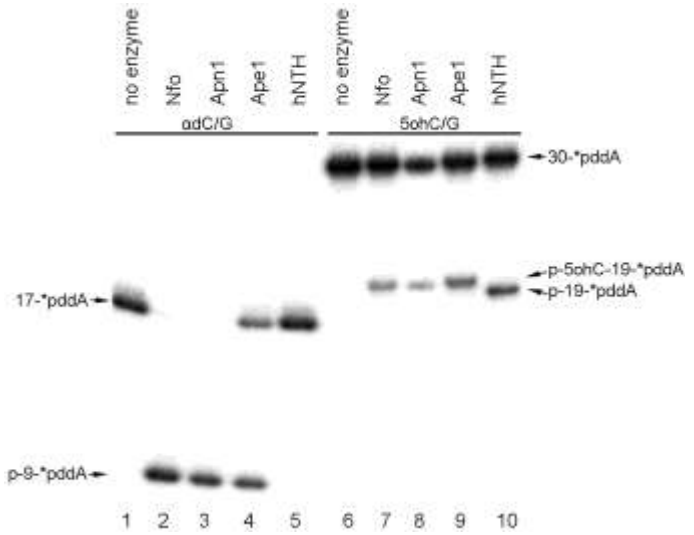


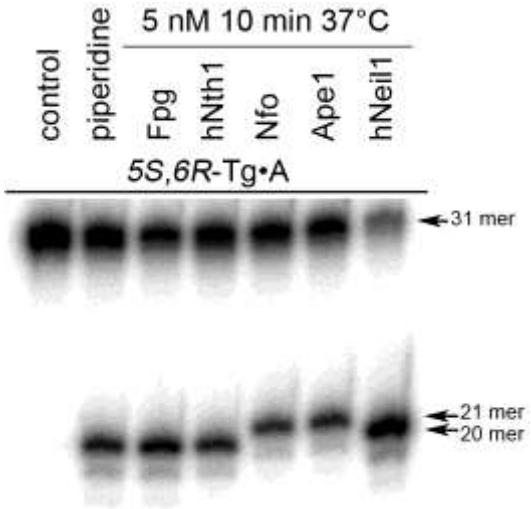




Substrate specificity of human major apurinic/apyrimidinic endonuclease (Ape1/Hap-1/Ref-1).

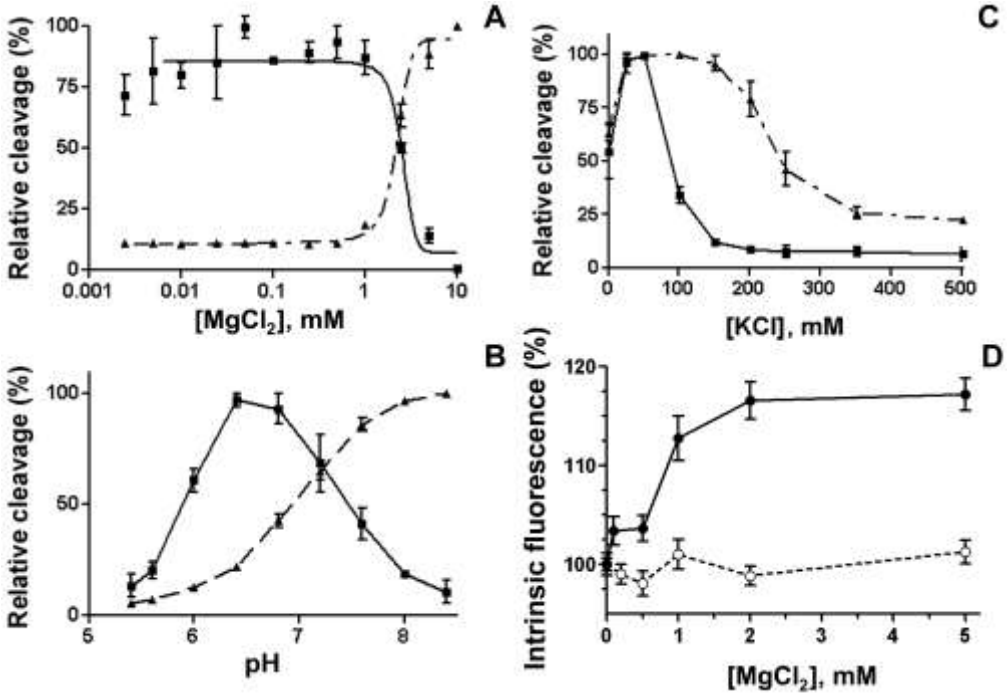




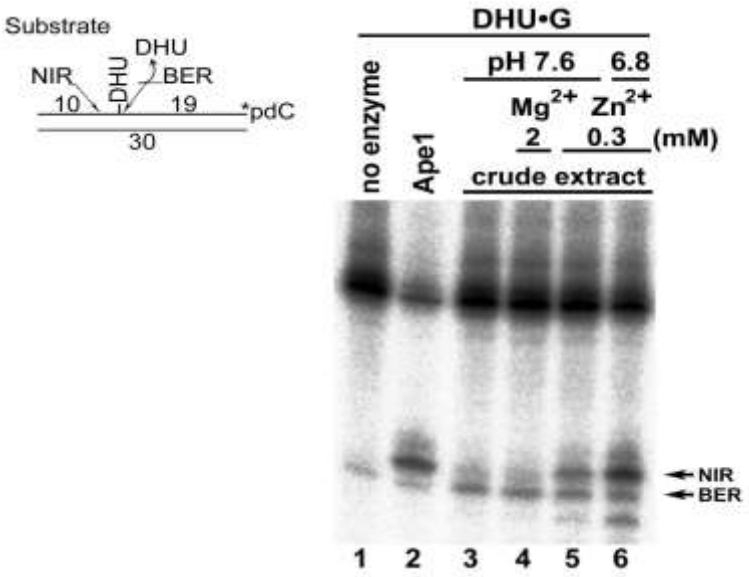


DNA damage	Incision activity	
	Ape1	Nfo and Apn1
Tetrahydrofuran	+++	+++
5,6-dihydrothymine	+++	+++
5,6-dihydrouracil	+++	+++
5-hydroxyuracil	++−	+++
5-hydroxycytosine	++−	++−
Alpha -2′-deoxyadenosine	+++	+++
Alpha -2′-thymidine	+++	+++
Alpha -2′-deoxycytidine	+++	+++
2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (meFapyG)	−(?)	+++
Thymine glycol (*5S,6R*-Tg)	+++	+++
8-oxoguanine	−−−	−−−
Uracil	−−−	−−−
1,N^6-ethenoadenine	−−−	−−−

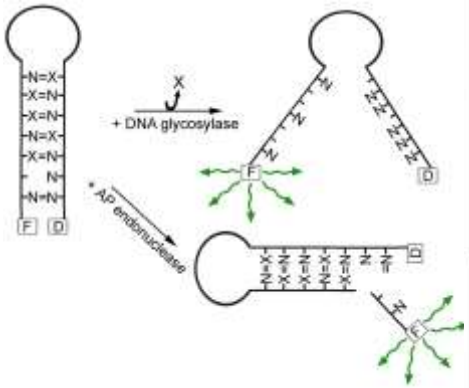
Activity profiles and conformational changes of Ape1

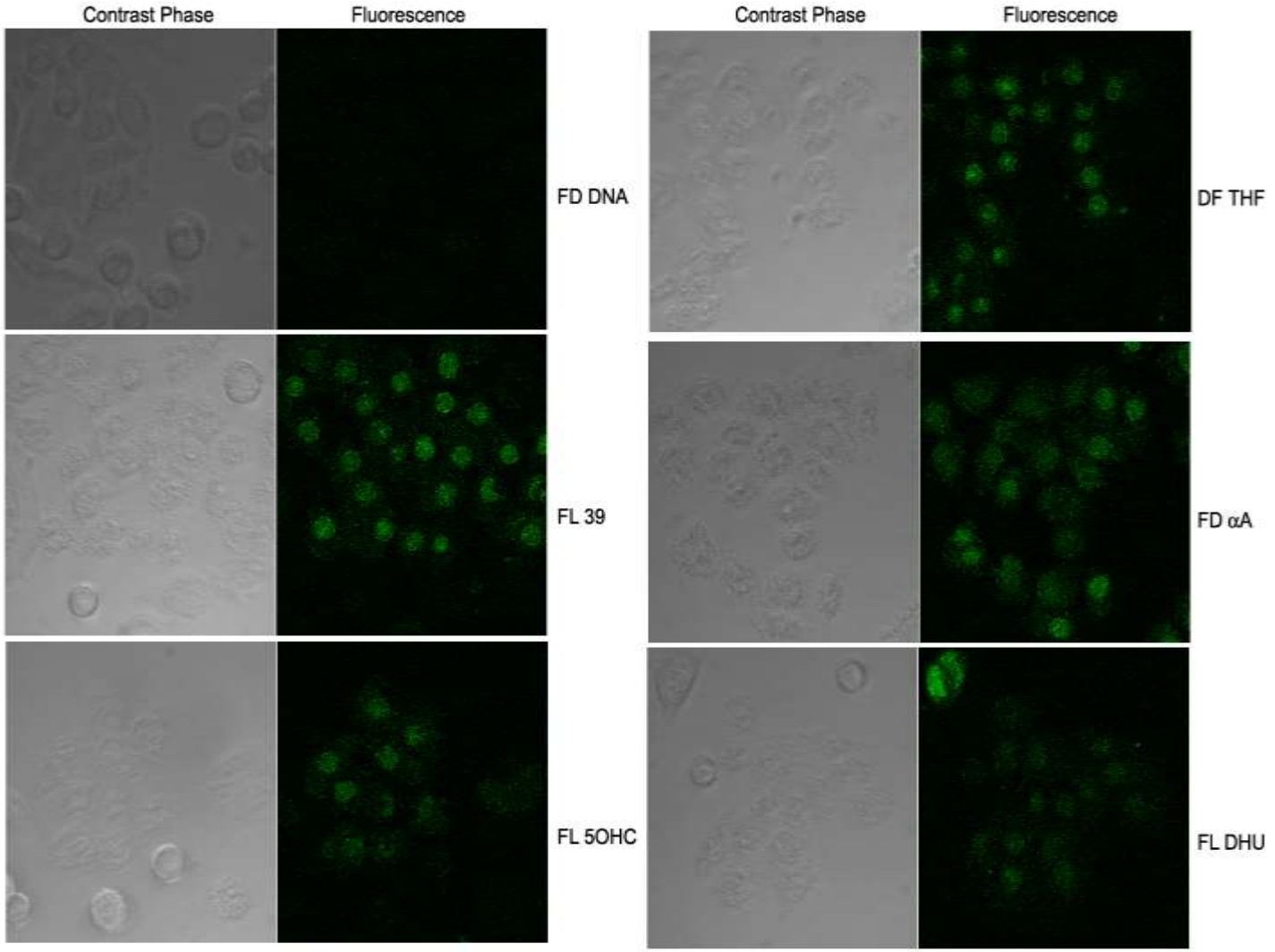


DNA repair assay using whole whole-cell Extracts from HeLa cells

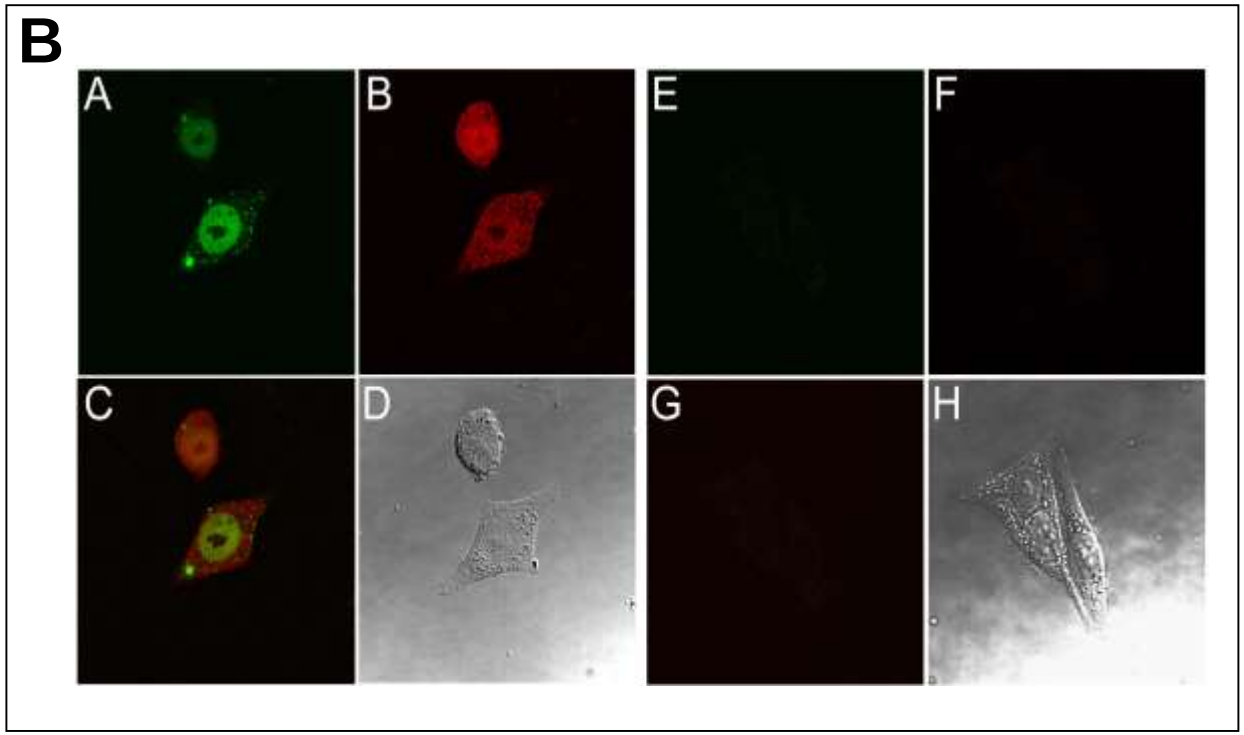


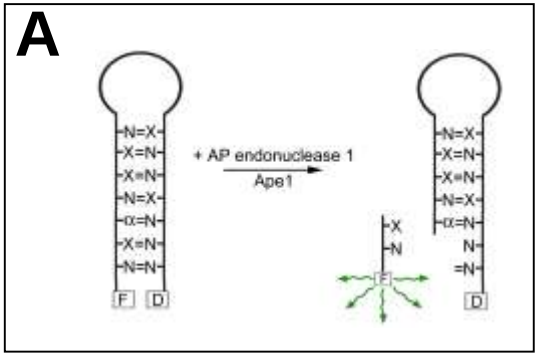
A molecular beacon assay for measuring DNA excision repair activities *in vivo*.

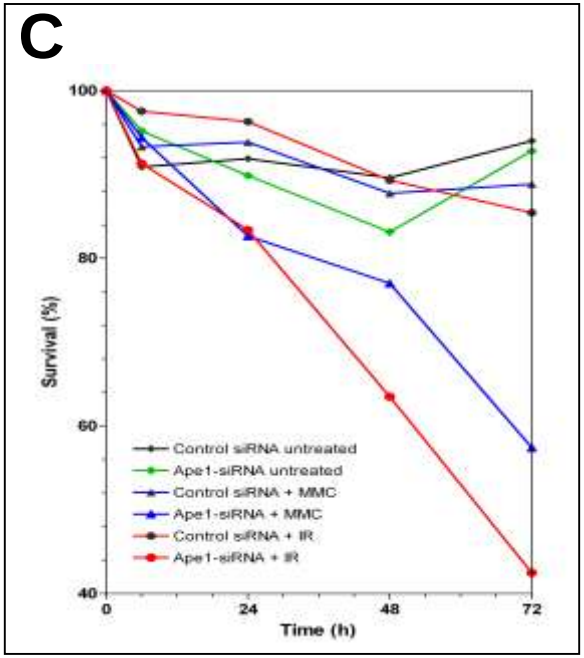




Alternative nucleotide incision repair (NIR) pathway for IR & MMC induced base damage in human cells. DNA glycosylase-independent repair of alpha-2'-deoxynucleotide (αA) by the human major AP endonuclease Ape1.

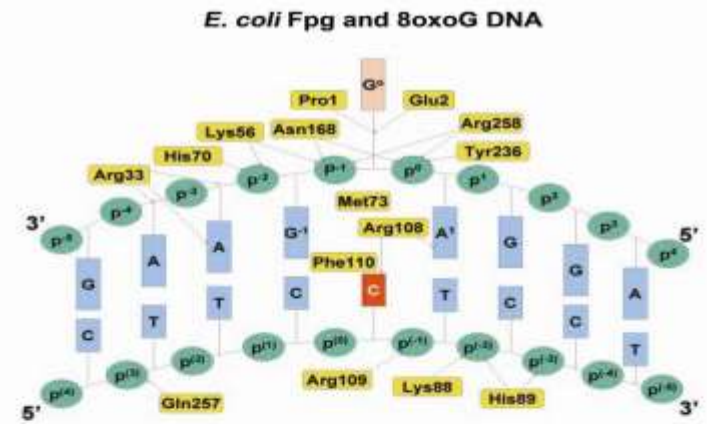




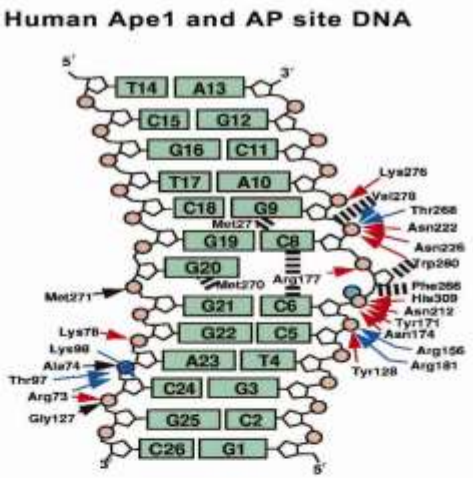


A. Fluorescence quenching mechanism of molecular beacons (MB).
B. Nucleotide incision repair in cultured HeLa cells transfected with FD-αA.
Control siRNA (A-D) and Ape1-siRNA (E-H). (A,E) fluorescence; (B,F) Ape1 immunostaining; (C,G) merge of fluorescence and Ape1 immunostaining; (D,H) phase contrast.
C. Down-regulation of Ape1 expression in HeLa cells using Ape1-siRNA oligonucleotides greatly increase cells sensitivity towards **IR (5Gy)** and mitomycin (MMC). Importantly, MMC generates in DNA bi-stranded clusters suggesting that Ape1 may be involved in removal of this class of DNA damage.

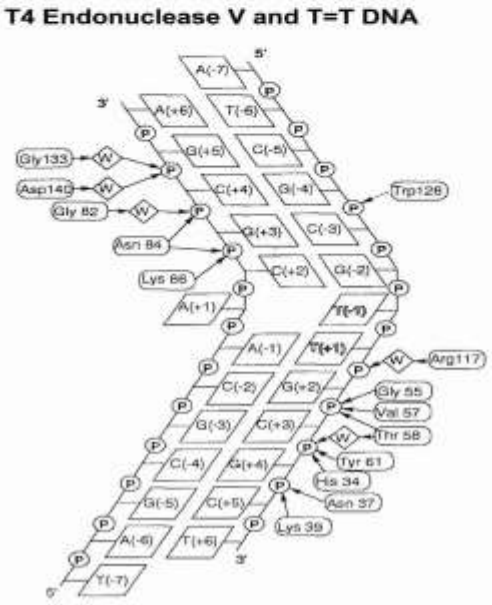
Structural basis for DNA damage recognition by DNA glycosylases and AP endonucleases



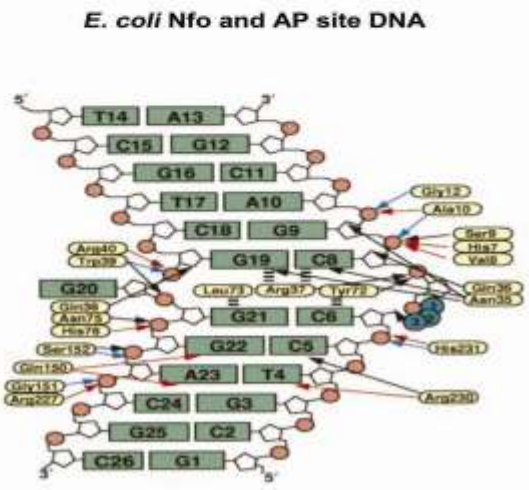
Gilboa et al., *J. Biol. Chem.* **277,** 19811, 2002;



Hosfield et al., *Cell* **98,** 397, 1999.

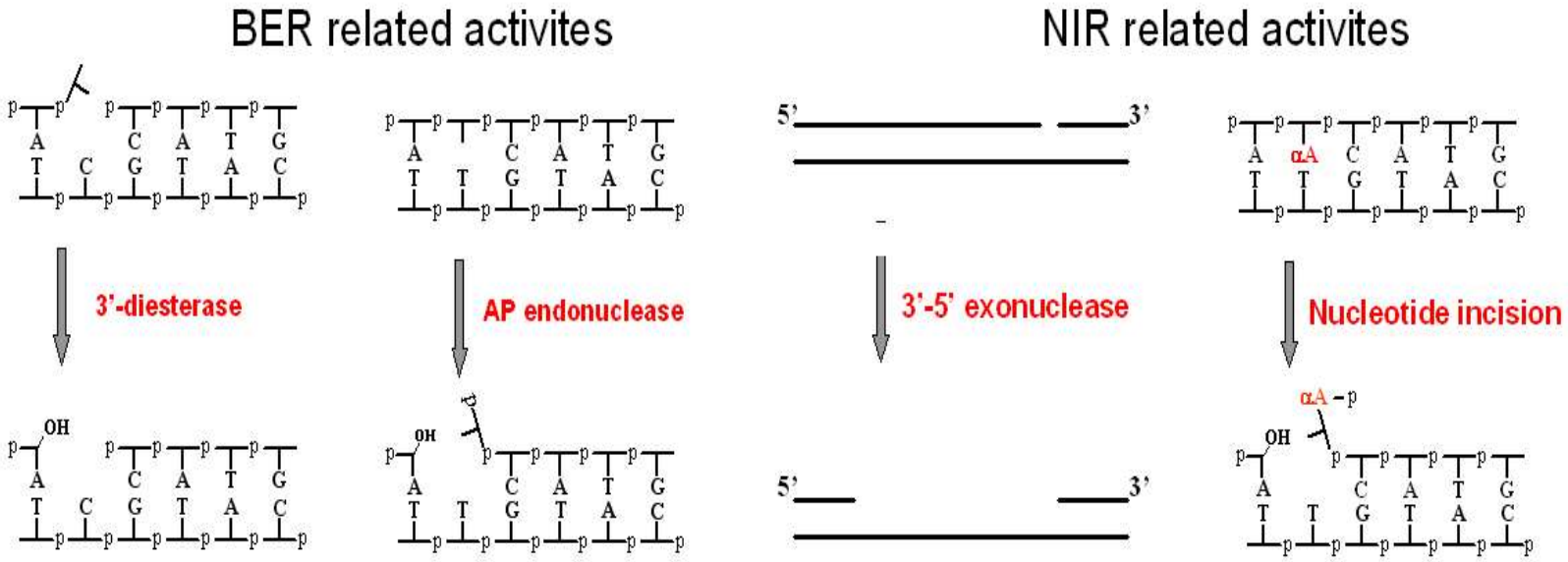


Vassylyev et al., *Cell* **83,** 773, 1995;

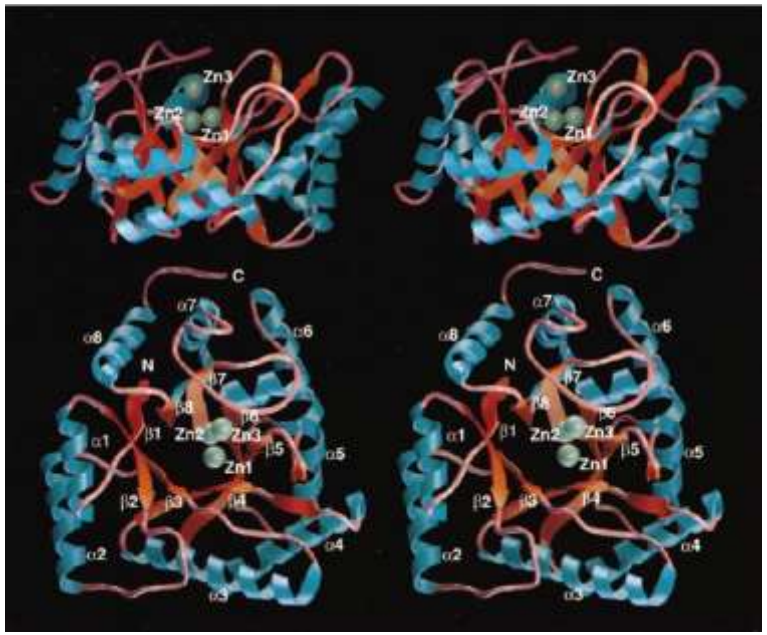


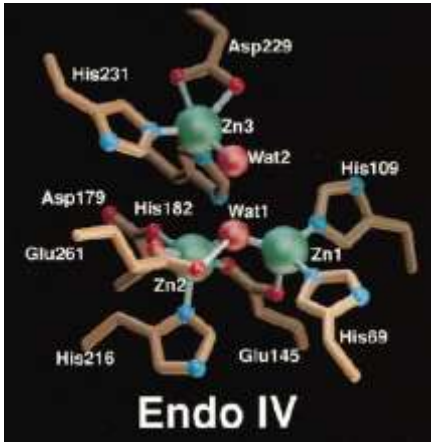
Mol et al., *Mutat. Res.* **460,** 211, 2000.

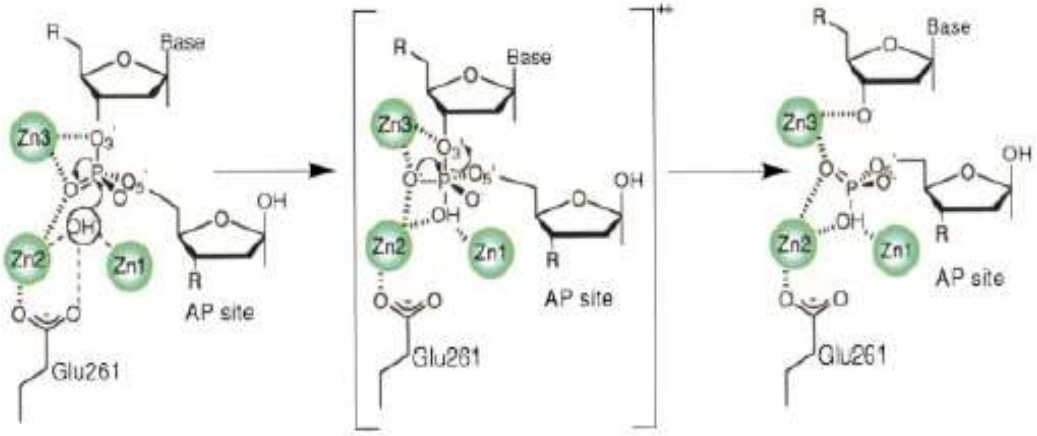
Various DNA repair activities of AP endonucleases (*E. coli* Nfo & human Ape1)



Structure of the DNA Repair Enzyme Endonuclease IV, Nfo



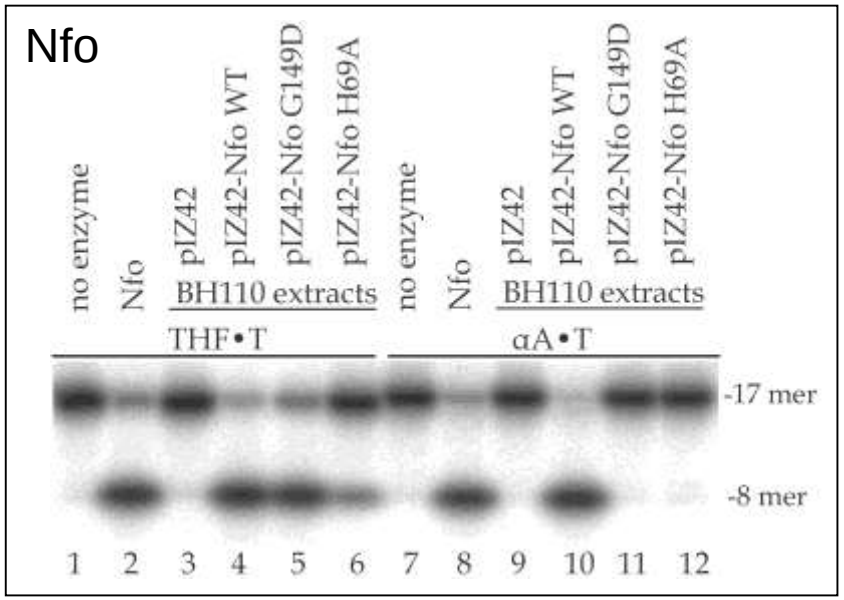




Structure-Based Three-Metal-Ion Mechanism for Nfo Phosphodiester Bond Cleavage

Nucleophilic attack by the bridging hydroxide is facilitated by interaction of the scissile phosphate with all three Zn^{2+} ions that render the phosphorus atom susceptible to nucleophilic attack. As the reaction proceeds through a pentacoordinate transition state that is stabilized by all three metal ions, the unesterified oxygen that bridges Zn2 and Zn3 remains bound to these metal ions and collapse of the transition state inverts the stereochemistry at the scissile phosphate. The developing negative charge at the O39 atom is stabilized by interaction with Zn3.

Mutational separation of DNA Repair Functions of *E. coli* Nfo and human Ape1



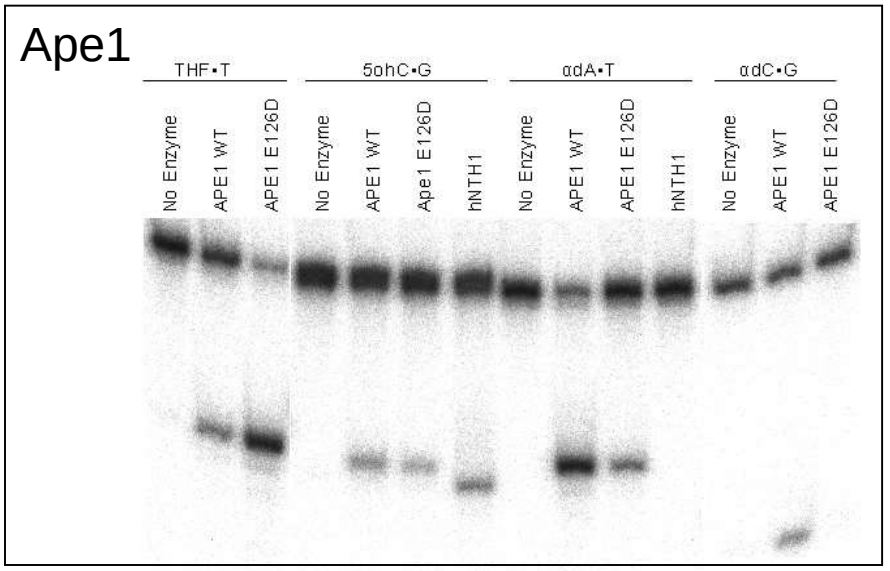
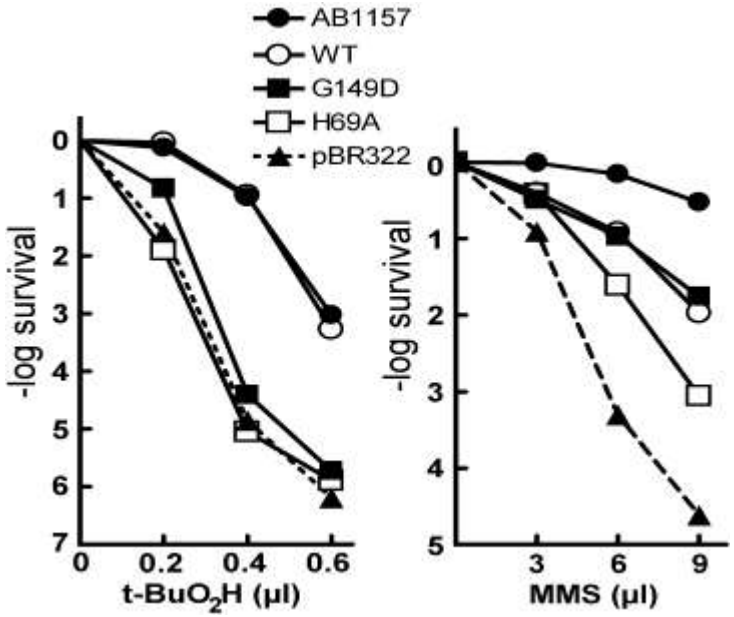


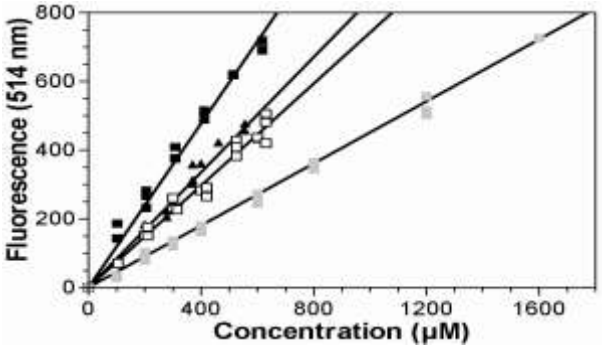
Table 1. Kinetic constants for the AP endonuclease, 3'-diesterase and NIR activities of the wild type and mutant Nfo proteins.

	Nfo		Nfo-H69A			Nfo-G149D	
	k_{cat}/K_M, min^{-1} μM^{-1}	Fold decrease	k_{cat}/K_M, min^{-1} μM^{-1}	Fold decrease +Zn^{2+}	Fold decrease no Zn^{2+}	k_{cat}/K_M, min^{-1} μM^{-1}	Fold decrease
αA•T	1300	1	No activity 19[d]	68	>1000	1.0	1300
THF•T	3100	1	21 450[d]	6.9	147	1300	2.5
3'THF^{NICK}	7500	1	23	-	326	1800	4.2
3'p^{NICK}	6900	1	84	-	82	1300	5.3

Drug sensitivity of *E. coli* Nfo mutants

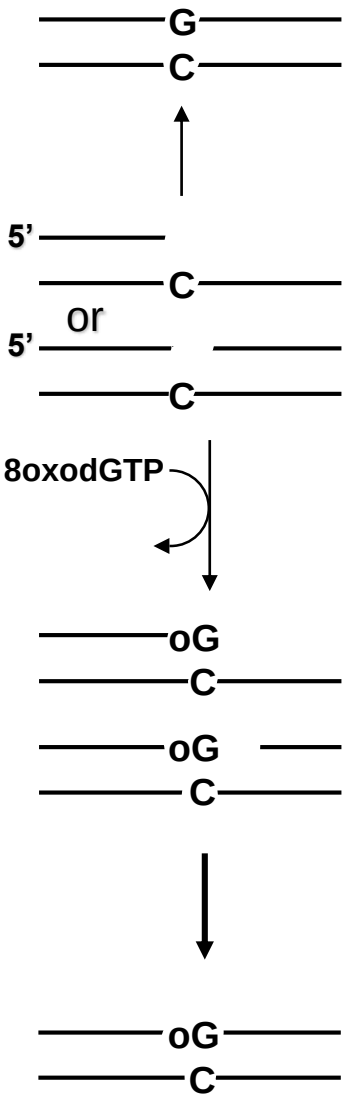


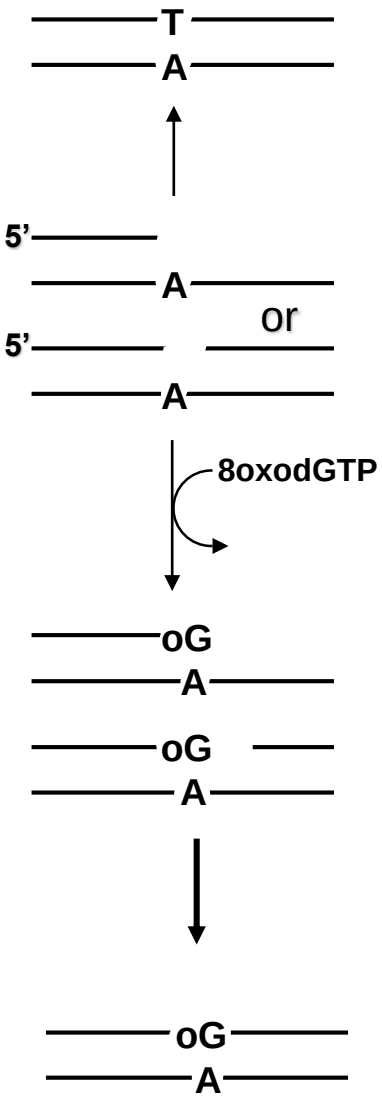
Metal content of the Nfo mutant proteins

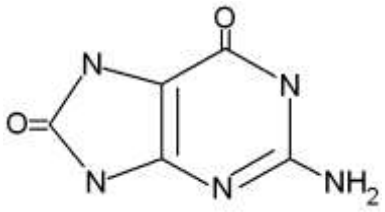


Fluorescence emission of FluoZin-3 (at 514 nm) plotted versus protein concentration of Nfo: WT (■), Nfo-H69A (□) and Nfo-G149D (▲). Calibration experiment using Zn solutions of known concentrations (■).

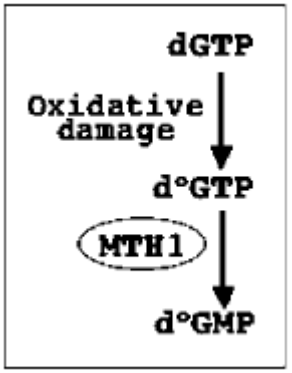
Misincorporation of oxidized dNTPs during DNA replication





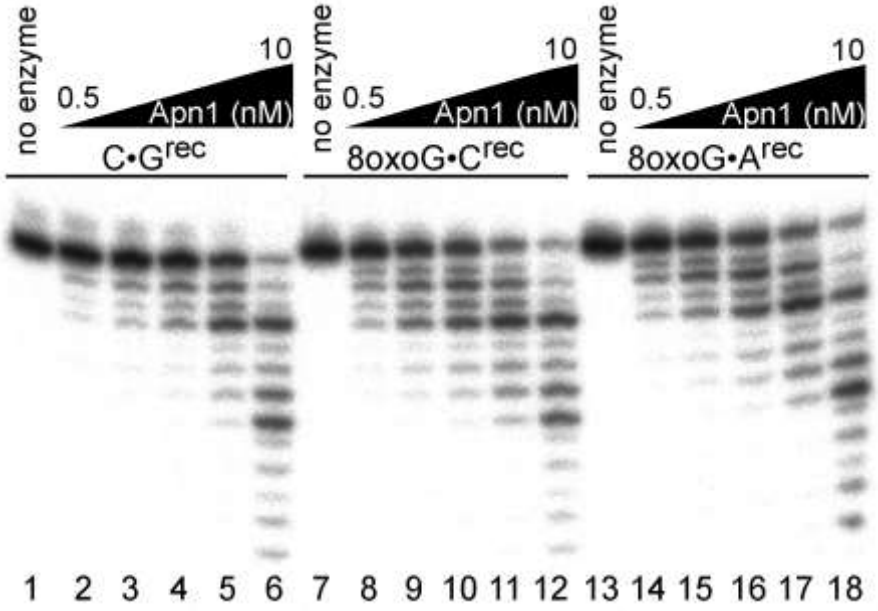


8-oxoguanine

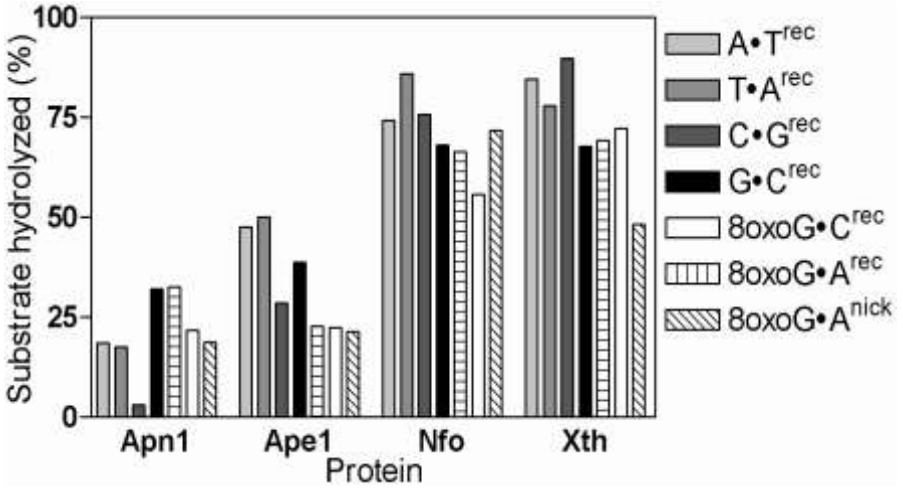


Apn1 and Ape1 remove 3’-terminal modified nucleotides

A



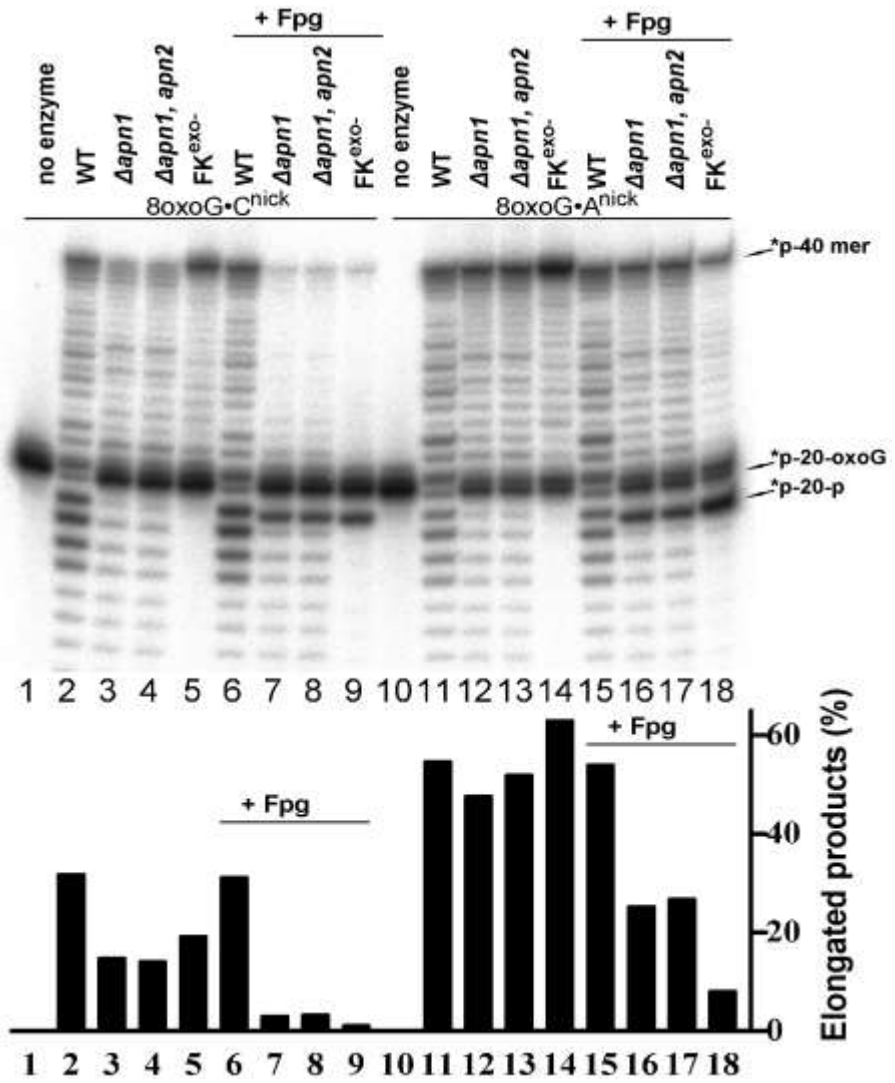
B



***A*,** Apn1 exonuclease activity toward 8oxoG-containing oligonucleotides. The reaction contained 5 nM of 5’-[^{32}P]-labeled recessed duplex oligonucleotide and either 0, 0.5, 1, 2, 5 or 10 nM Apn1.

***B*,** Comparison of the exonuclease activities of Apn1, Ape1, Nfo and Xth on recessed and nicked duplex DNA. The reaction contained 0.5 nM of Apn1 and 1.0 nM of Ape1, Nfo or Xth.

Identification of a new alternative repair pathway for spontaneous and IR-induced clustered lesions in *S. cerevisiae*



Repair of 3'-terminal 8oxoG in yeast cell-free extracts.

Spontaneous rates and spectrum of *Can*^{R} mutations in yeast DNA repair deficient mutants.

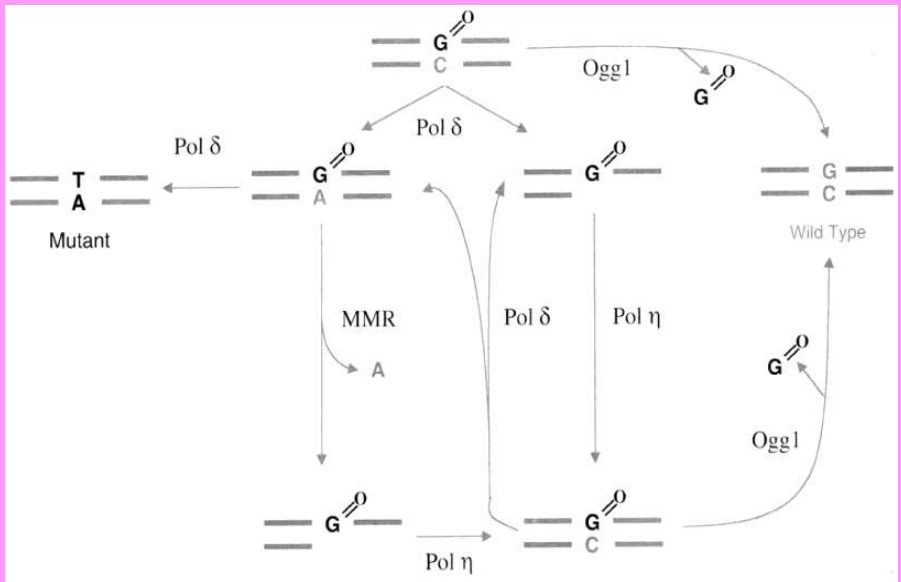
Strains[a]	Experiment	Mutation rate *can*^{R} per cell generation (10^{-8})	Fold increase
FF18733/pYES	1	1.9 ± 0.8	1
	2	2.1 ± 0.6	1
FF18733/pGst-MutT[b]	1	1.7 ± 0.7	
	2	1.8 ± 0.5	
DRY139 (*apn1Δ::LEU2*)/pYES	1	7.7 ± 1.3	4
	2	8.3 ± 1.8	3.9
DRY139 (*apn1Δ::LEU2*)/pGst-MutT	1	6.8± 1.2	3.2
	2	5.7 ± 1.4	3.2
CD138 (*ogg Δ::TRP1*)/pYES	1	20.3 ± 3.1	10.7
	2	23.7 ± 2.8	11.2
CD138 (*ogg1 Δ::TRP1*)/pGst-MutT	1	16.5 ± 2.6	9.7
	2	15.3 ± 2.9	8.5
DRY140 (*apn1Δ::LEU2 ogg1Δ::TRP1*)/pYES	1	89.0 ± 9.3	46.7
	2	93.0 ± 11.3	44.3
DRY140 (*apn1Δ::LEU2 ogg1Δ::TRP1*)/pGst-MutT	1	55.0 ± 6.4	26.2
	2	49.0 ± 5.8	27.2
DRY142 (*apn1Δ::LEU2 ogg1Δ::TRP1 rad30::KAN*)/pYES	1	105.0 ± 12.2	55.3
	2	113.0 ± 9.7	53.8

Mutation type	*APN1 OGG1*			*apn1Δ ogg1Δ*			Fold increase
	No	%	Rate (10^{-8})	No	%	Rate (10^{-8})	
G•C to T•A	11	28.9	0.58	20	50	40.5	70
A•T to C•G	4	10.5	0.21	10	25	22.75	108
A•T to G•C	3	7.9	0.16		-		
A•T to T•A	3	5.3	0.11		-		
G•C to A•T	2	5.3	0.11	3	7.5	6.8	65
G•C to C•G	2	5.3	0.11	3	7.5	6.8	65
Deletions	9	23.7	0.47	2	5	4.55	
Insertions	5	13.2	0.26	1	2.5	2.28	
Complex[a]	-			1	2.5	2.28	
Total	38			40			

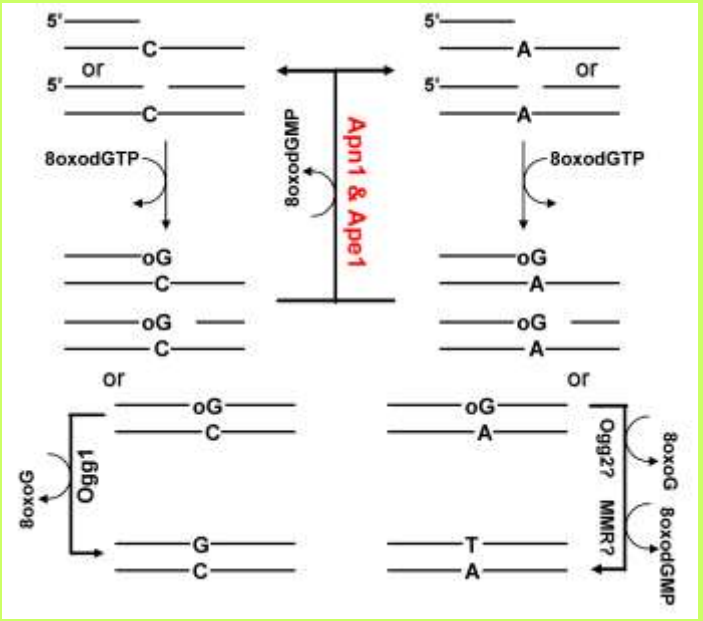
Deletion of both *OGG1* coding for 8oxoG-DNA glycosylase and *APN1* causes a 46-fold synergistic increase in spontaneous mutation rate and this enhanced mutagenesis is shown to be primarily due to G•C to T•A transversions. Taken together, our results indicate that Apn1/Ape1 3'→5' exonuclease activity is involved in DNA glycosylase-independent repair pathway for 8oxoG residues.

DNA repair pathway for 8oxoG residues in *S. cerevisiae*

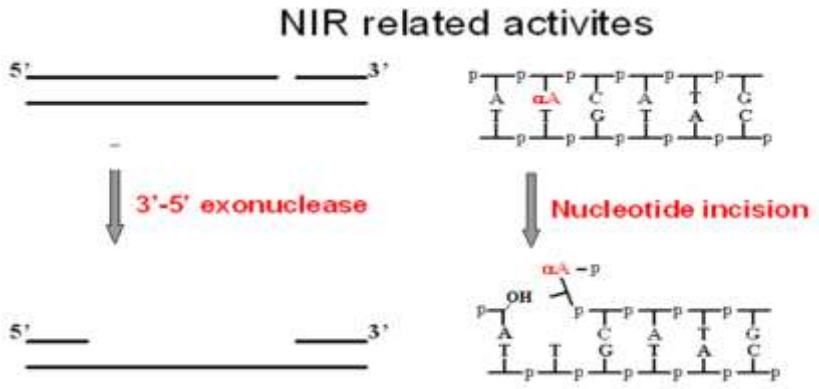
DNA glycosylase-dependent repair

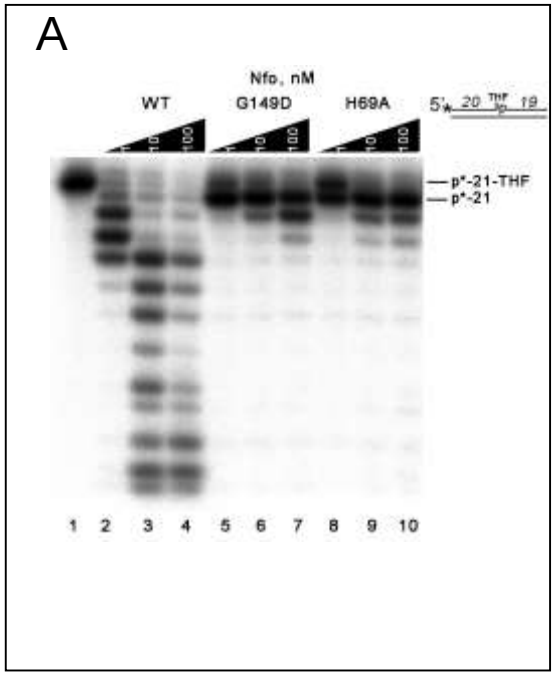


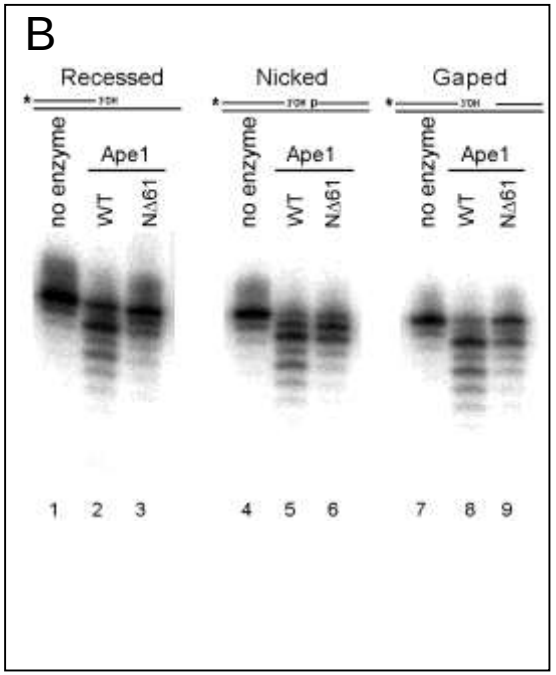
AP endonuclease-dependent repair

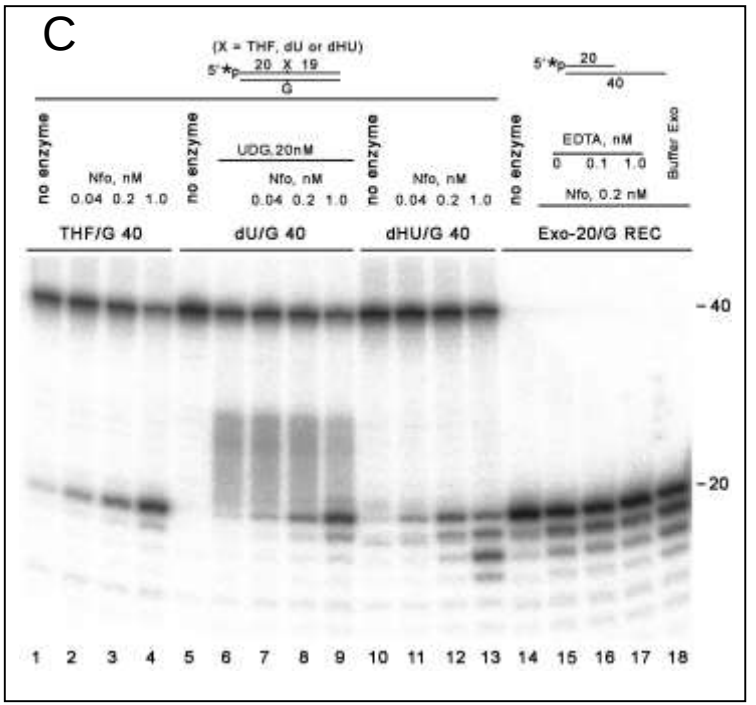


Coupling of the nucleotide incision and 3'-5' exonuclease activities in *E. coli* Nfo and human Ape1 AP endonucleases: structural and genetic evidences





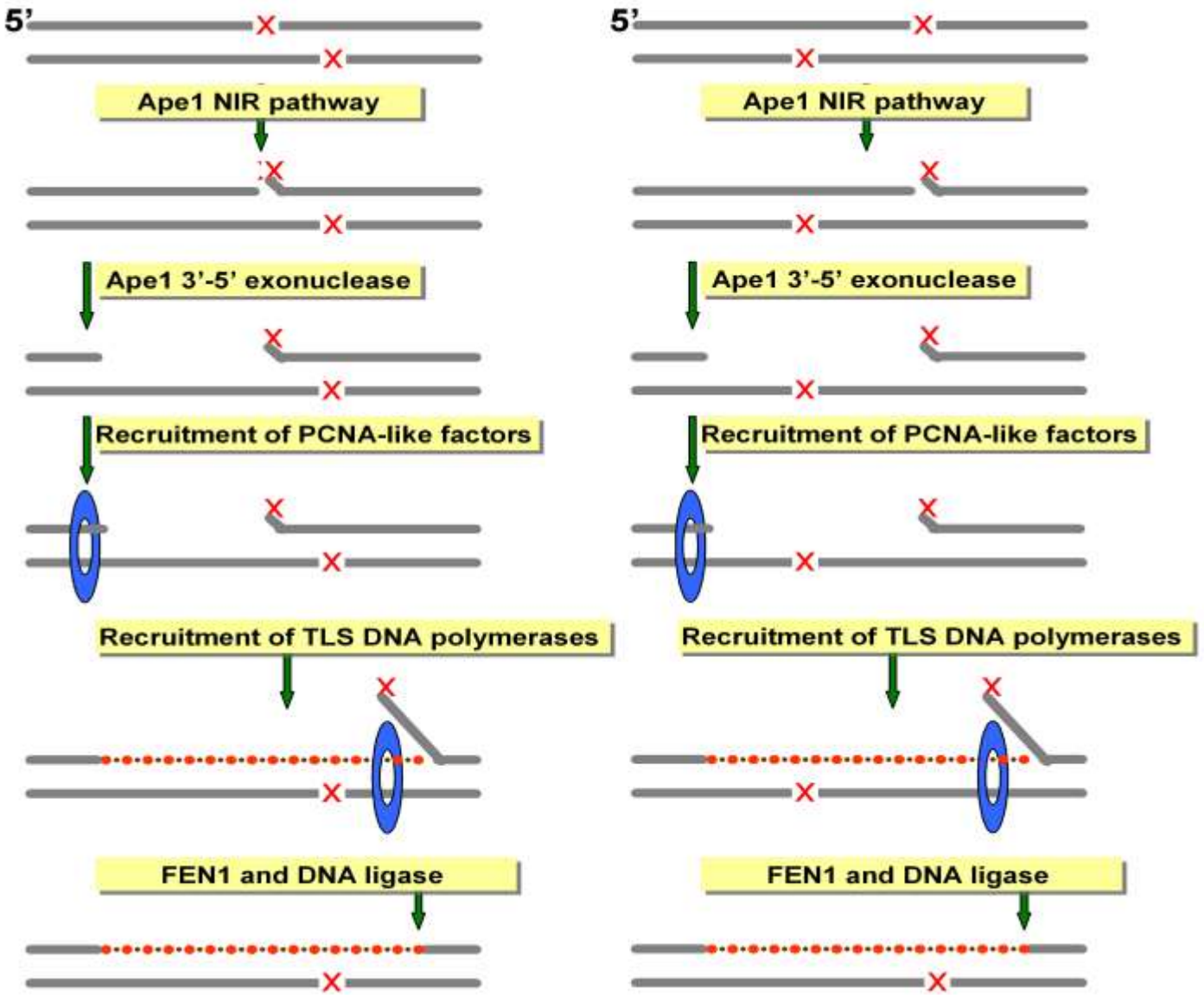




A & B. Comparison of 3'→5' exonuclease activity of Nfo & Ape1 mutants.

C. The 3'→5' exonuclease activity of wild type Nfo on different DNA substrates.

DNA Glycosylase-Independent Repair Pathway for oxidative stress-induced clustered lesions



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Лекции N°9-10
«Системы репарации ДНК. Часть 2. прямая репарация поврежденных оснований, Гомологичная и негомологическая рекомбинация, репарация ошибок репликации».

НАЦИОНАЛЬНЫЙ ЦЕНТР НАУЧНЫХ ИССЛЕДОВАНИЙ ФРАНЦИЯ
Centre National de la Recherche Scientifique
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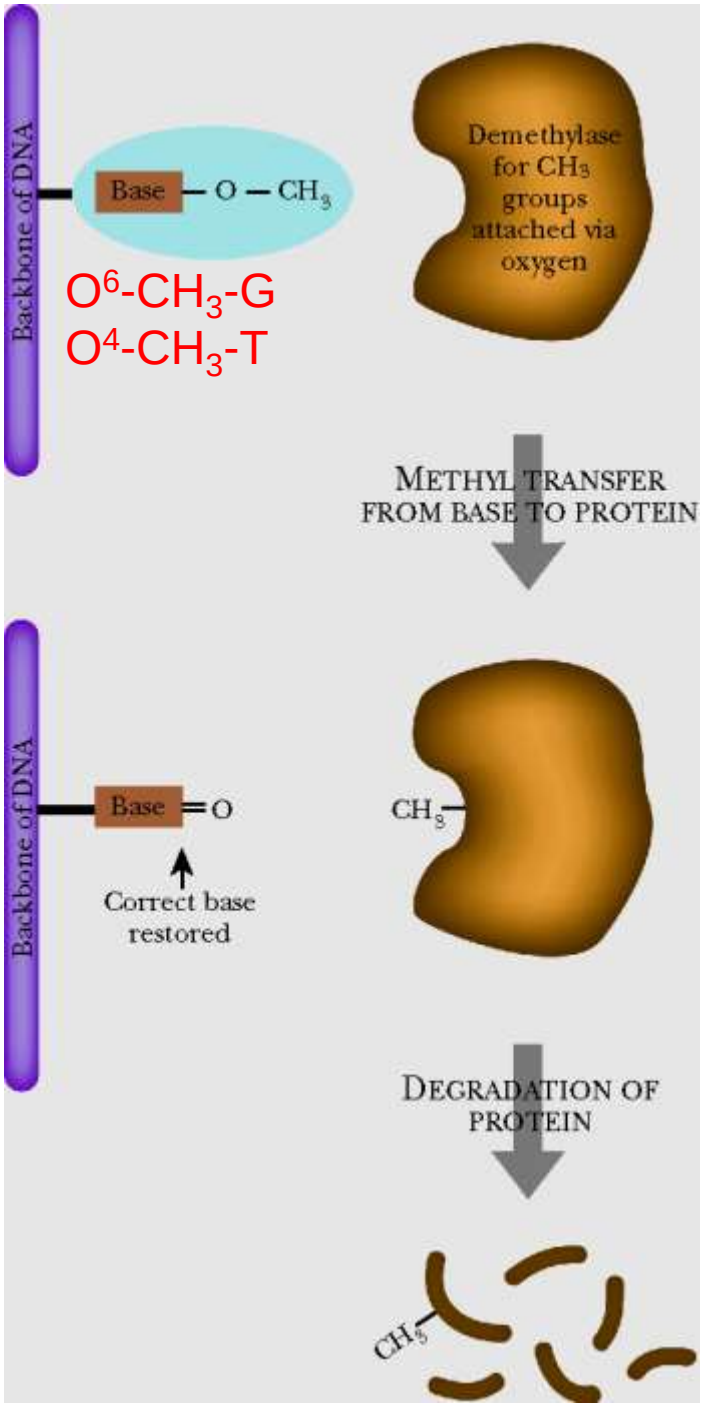
Лаборатория «Репарации ДНК»
Research Director, заведующий лабораторией
САПАРБАЕВ Мурат Калиевич

Suicide demethylase for O-methyl bases.



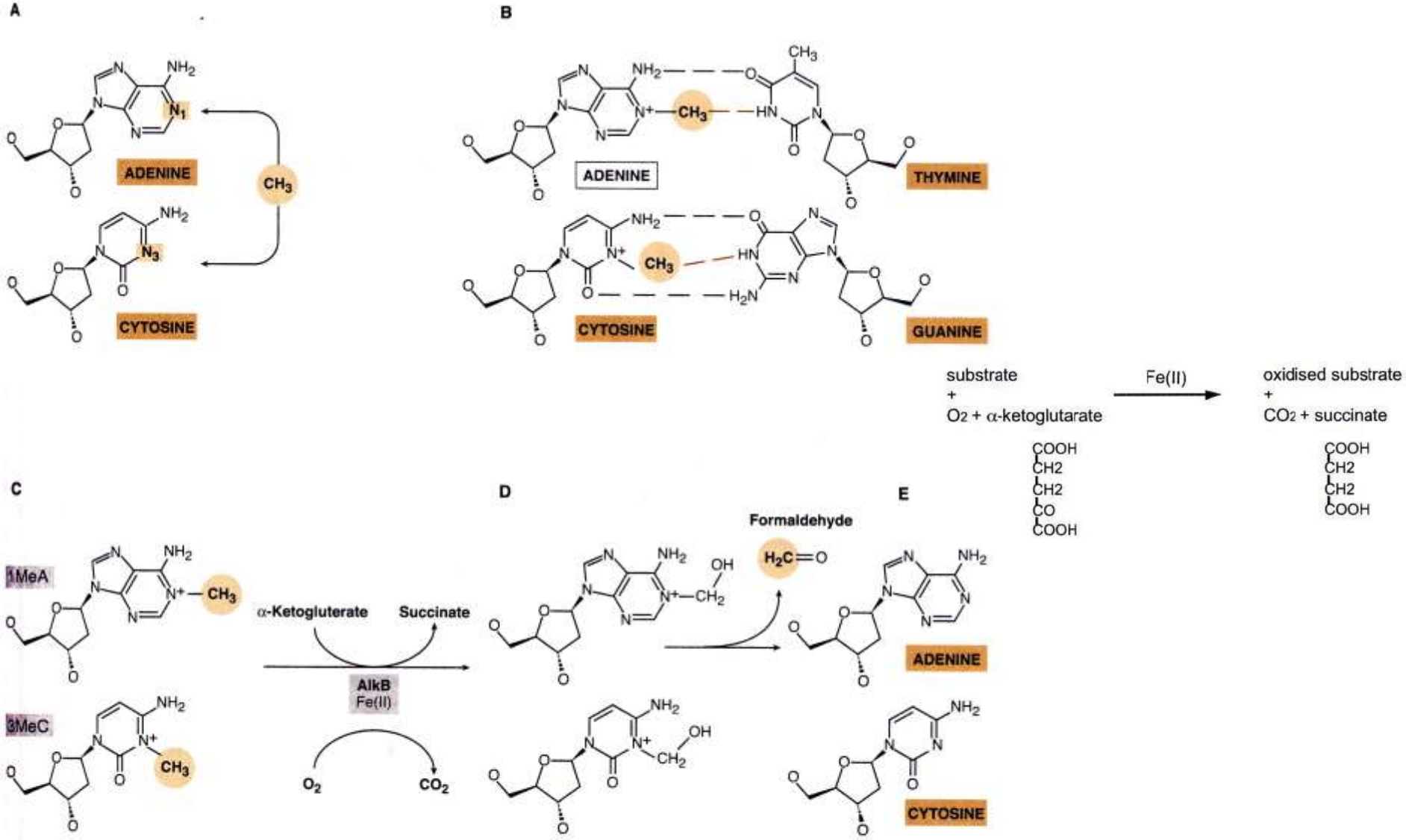
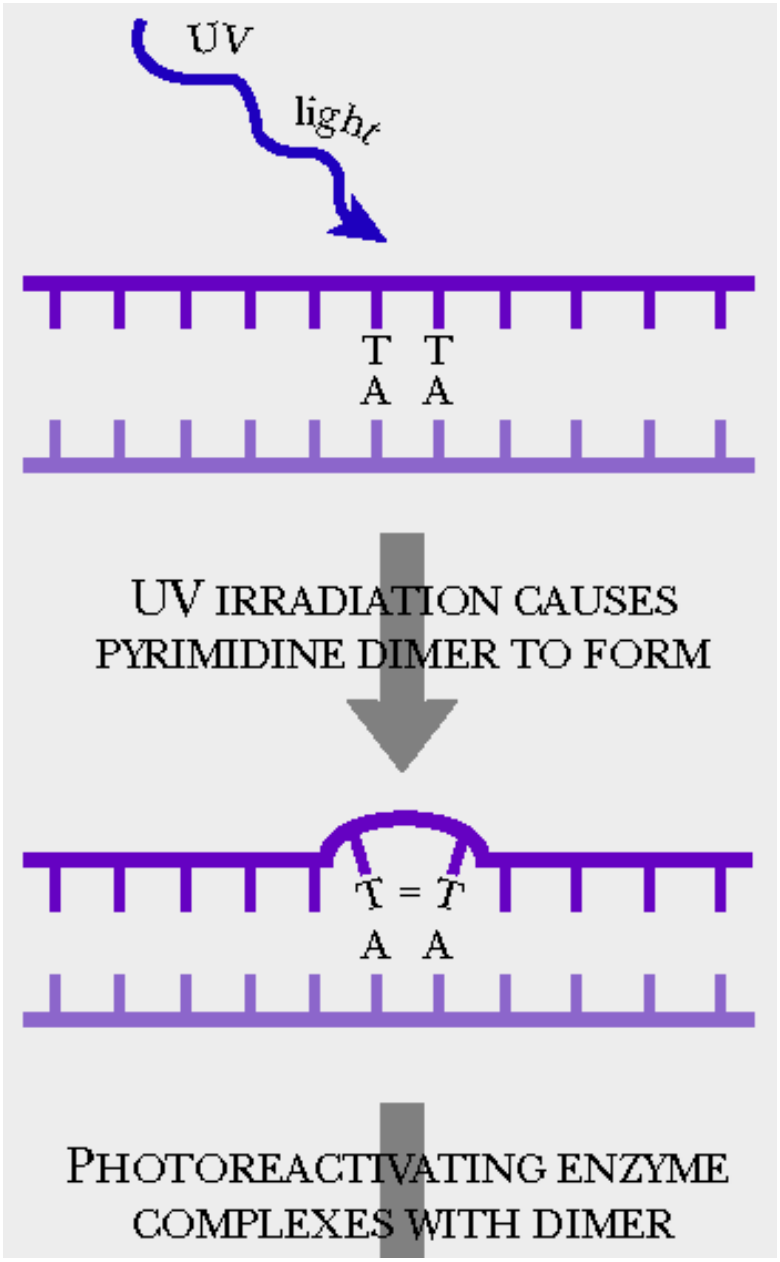
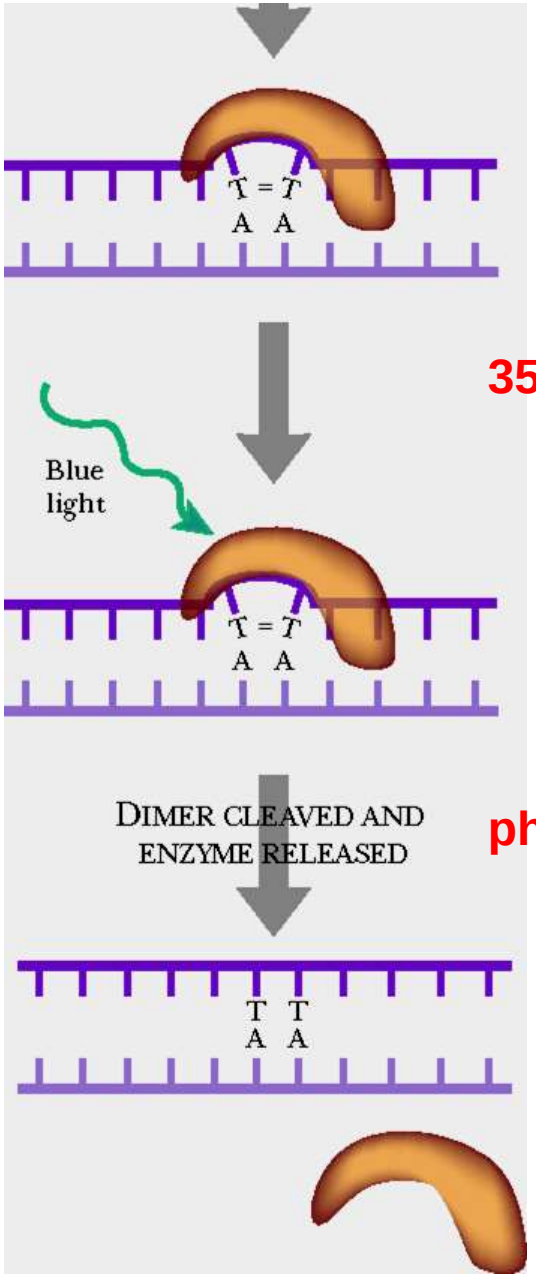


Figure The repair of N^1-methyladenine (1-MeA) and N^3-methylcytosine (3-MeC) by AlkB protein (A and B). The N-1 and N-3 positions of adenine and cytosine are equivalent in the sense that in single-stranded DNA they are both susceptible to attack by methylating agents (A) whereas in double-stranded DNA they are shielded from such attack (B). Both 1MeA and 3MeC lesions can be generated in regions of single-stranded DNA and on reannealing of the double helix these lesions persist. The lesions are buried within the double helix of DNA but are expected to disrupt hydrogen bonding with the complementary strand (broken gold lines) (B). (C and D) Both 1MeA and 3MeC in DNA are repaired by AlkB-catalyzed oxidative demethylation. The reaction requires α-ketoglutarate, O_2 and Fe^{2+} and generates succinate and CO_2. (E) The oxidized methyl groups are removed as formaldehyde, regenerating normal DNA bases. (Adapted from reference 10.)

Photoreactivation cleaves pyrimidine dimers.





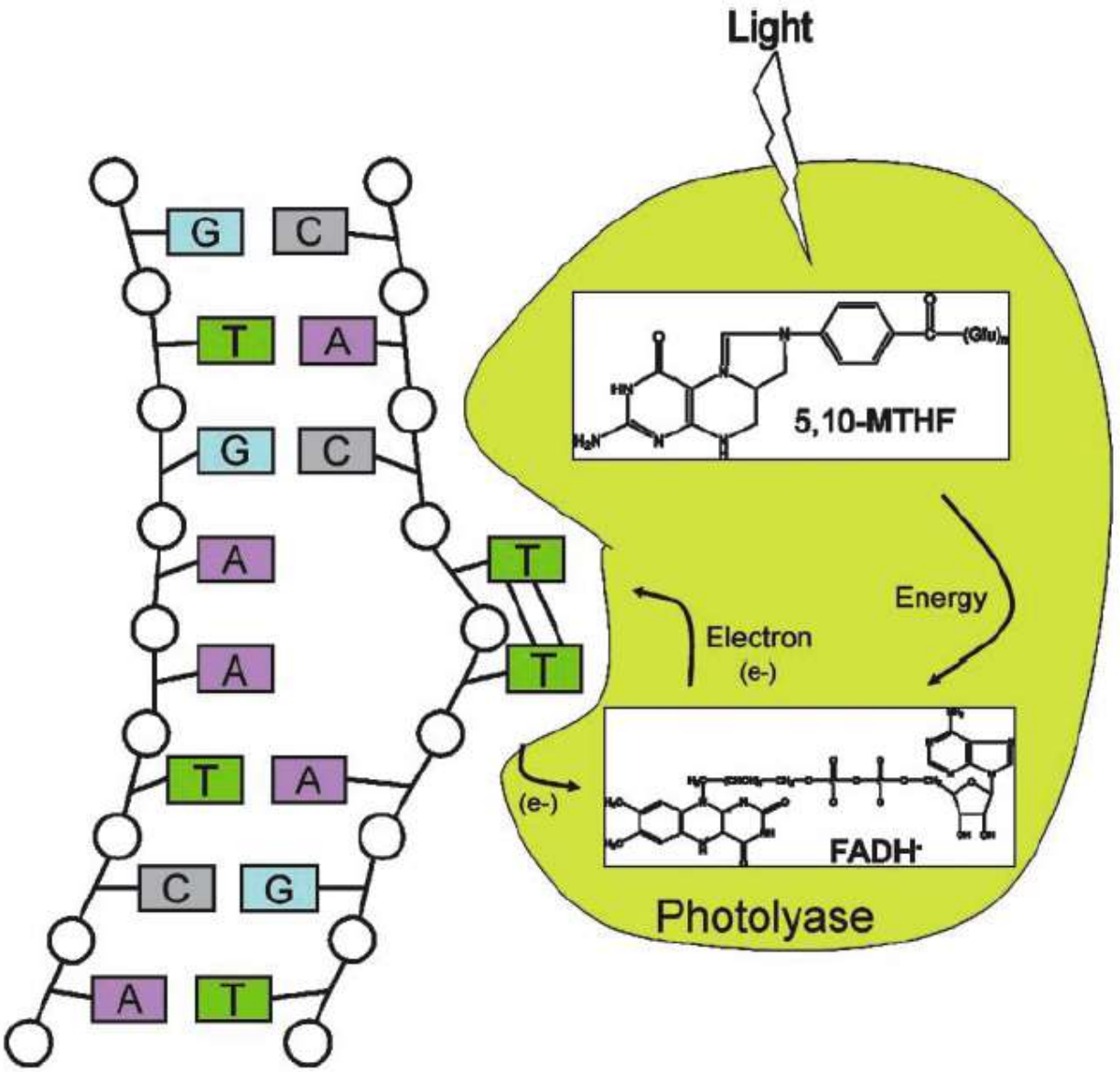
350-500nm

photolyase

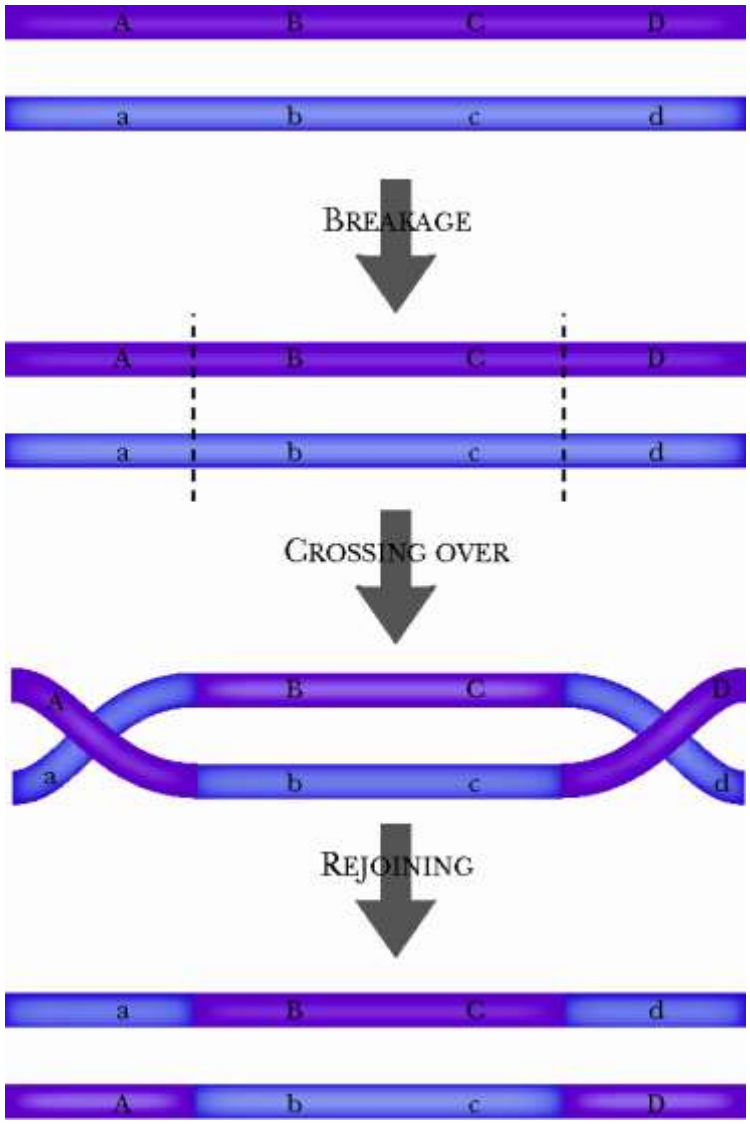
Bind to dimer in dark but lack energy to remove crosslink

No DNA synthesis

Alternative to nucleotide excision repair: direct repair by ***photolyase*** (not found in placental mammals; we have structural homologues with no repair abilities called cryptochromes that act as photoreceptors to set circadian clock)



Overview of Homologous Recombination

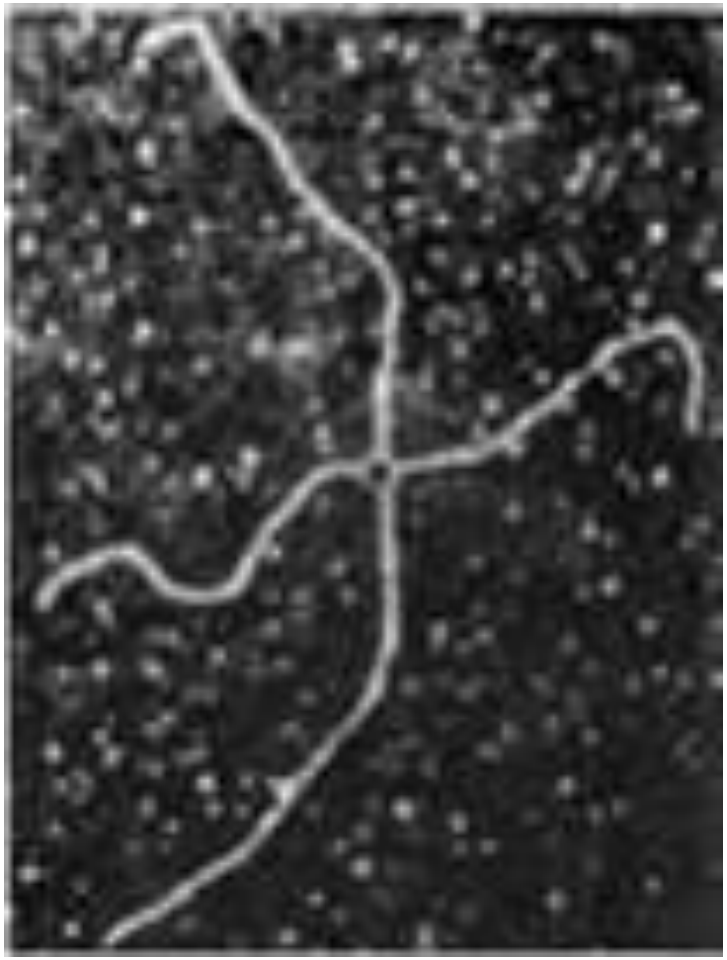


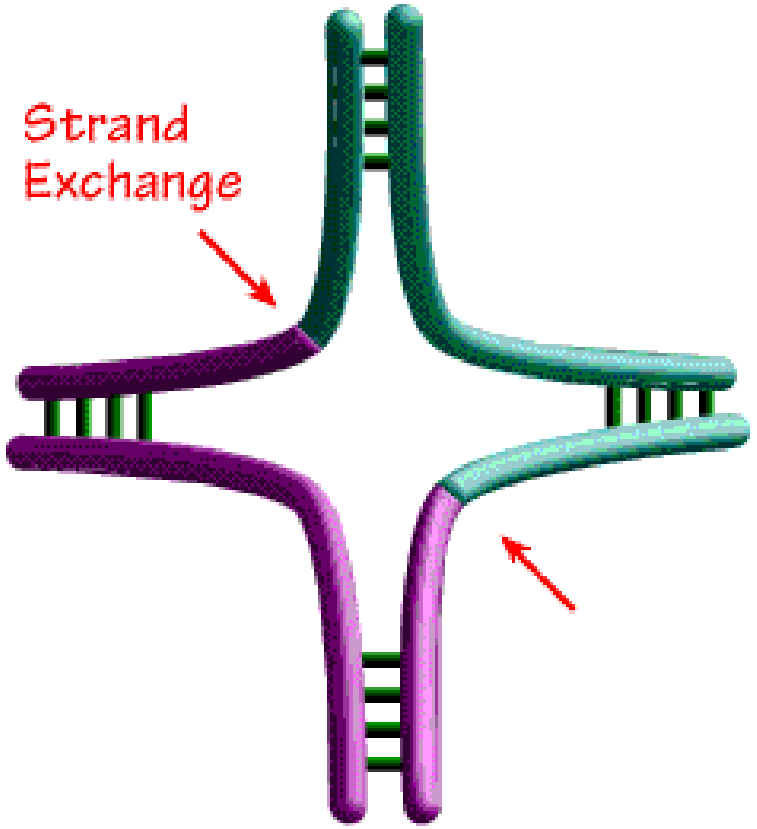
In all cases of recombination, two DNA molecules are broken and rejoined to each other forming a **crossover**.

Single crossover usually forms short-lived hybrid DNA molecules.
→promoter recombination of linear chromosomes.
→cannot cause recombination between two circular DNA molecules.

Double crossovers forms recombination.

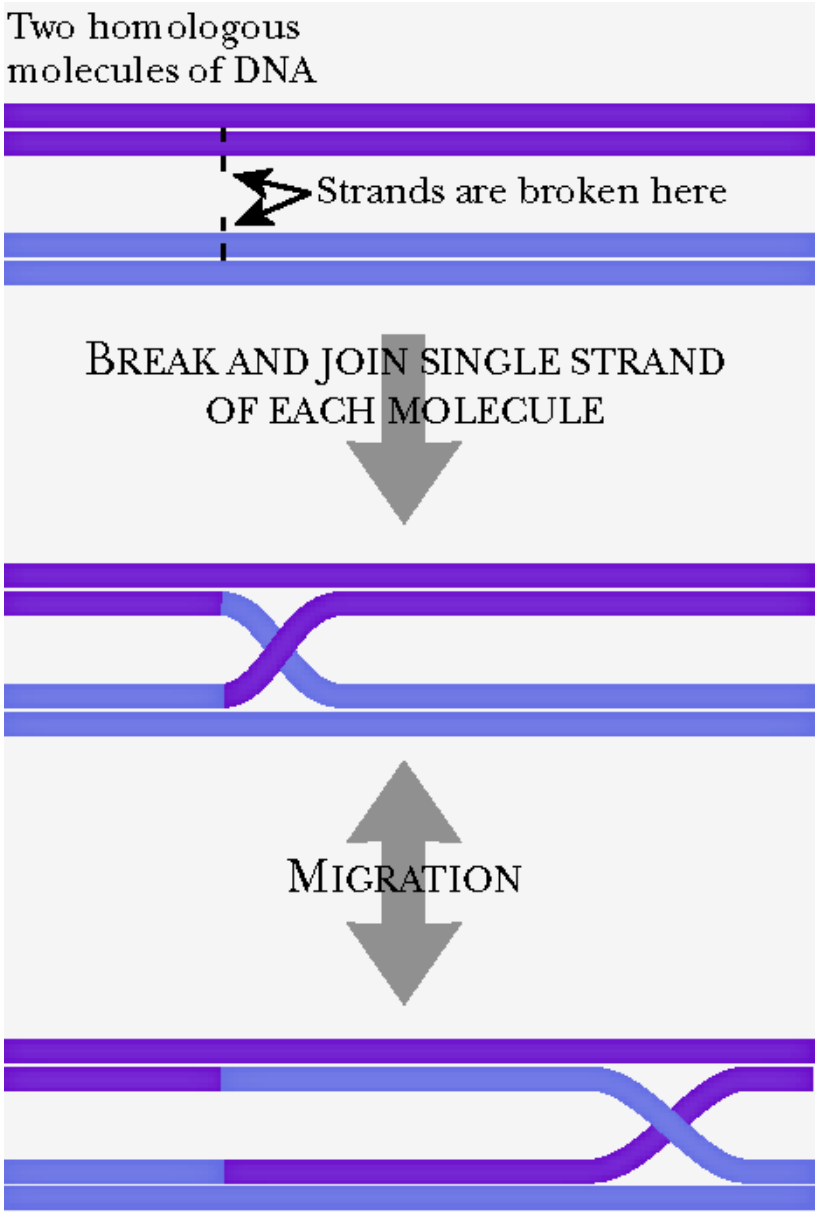
Fig14.1 Two crossovers result in **recombination**.



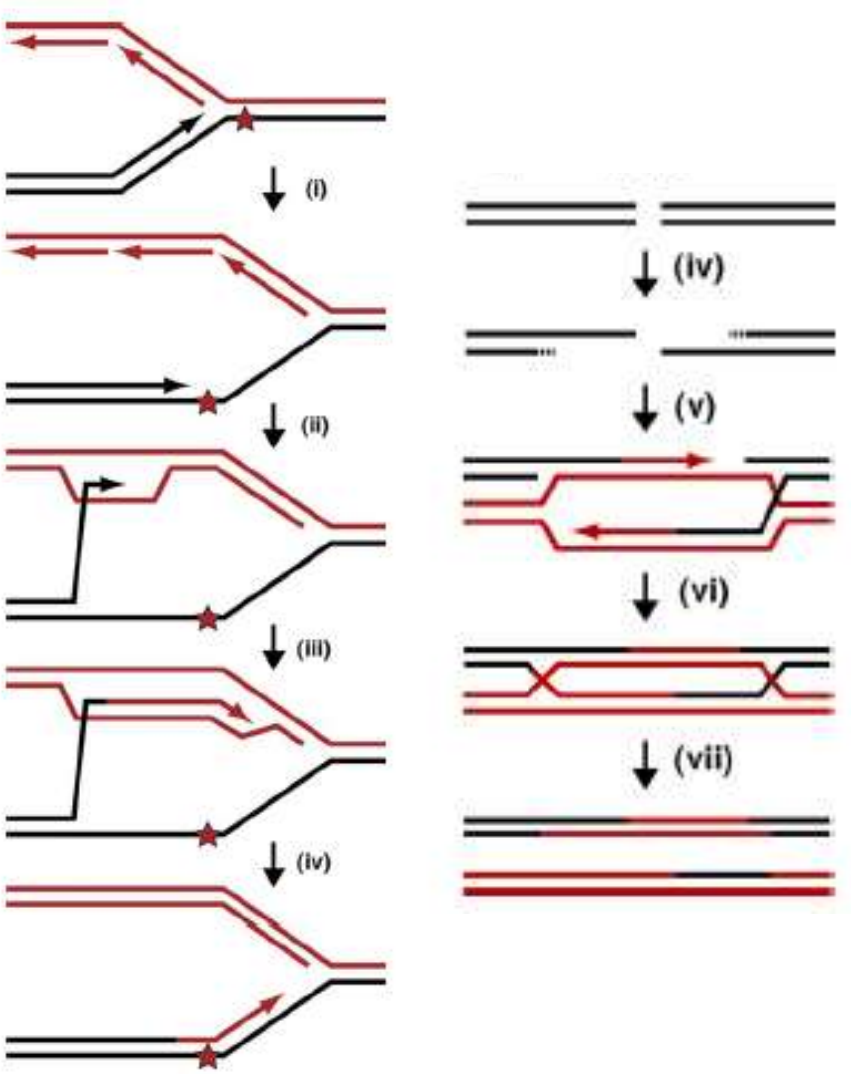


http://engels.genetics.wisc.edu/Holliday/holliday3D.html

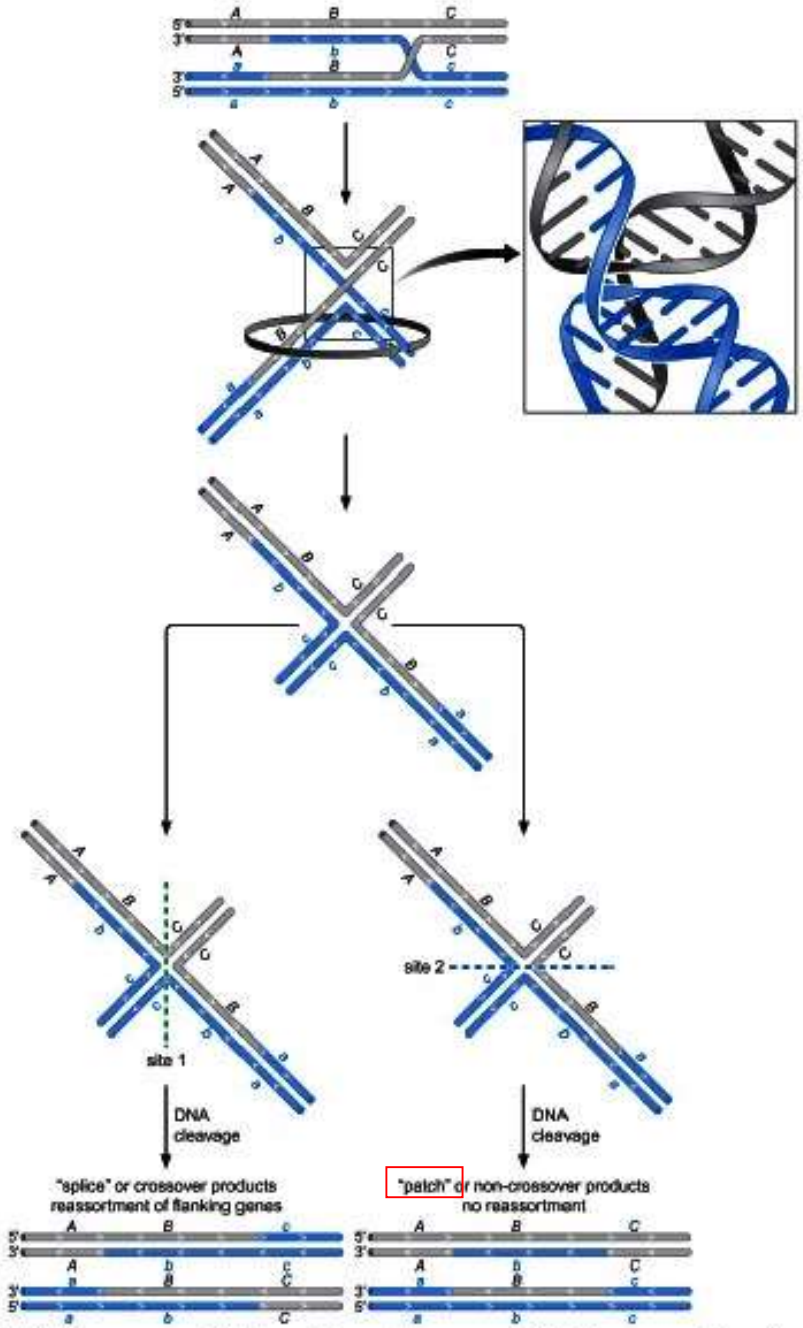
Molecular Basis of Homologous Recombination



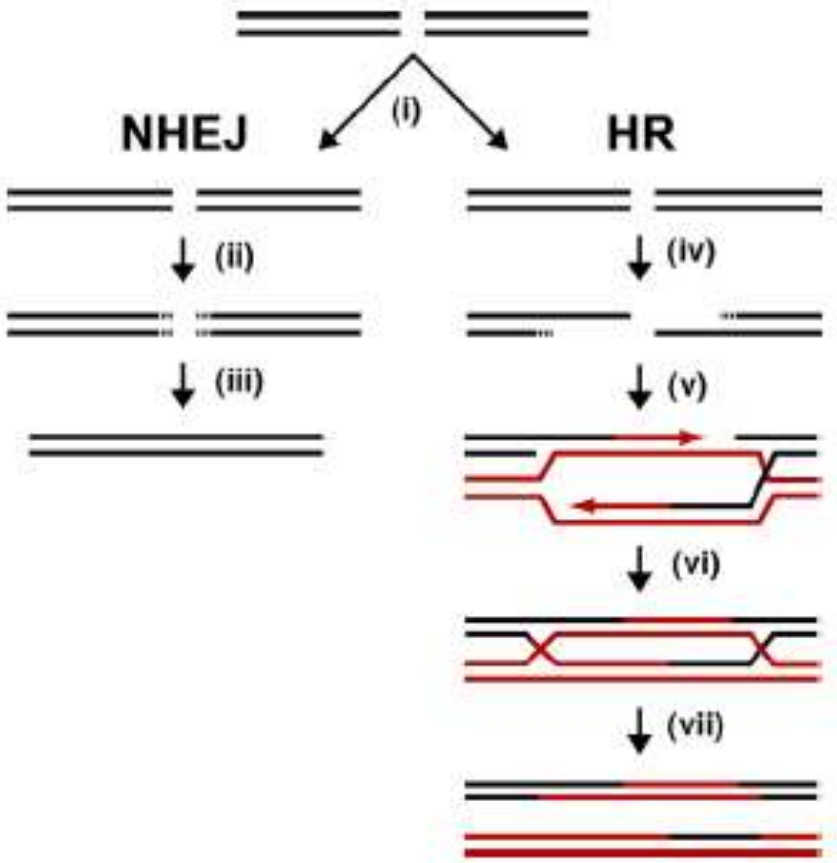
Formation of a crossover.



Recombinational restart of a collapsed replication fork. Upon replication fork blockage. And DSB repair.



DSB repair pathways in eukaryotes.



Left, non-homologous endjoining pathway (NHEJ). Right, homologous recombination pathway (HR). Depending on whether the NHEJ or HR pathway is used, DNA DSB repair proceeds through a number of distinct step: (i) damage detection, (ii) endprocessing, (iii) end-ligation, (iv) 52-resection, (v) strand-invasion (two end invasion shown), (vi) Holliday-junction formation, and (vii) Hollidayjunction resolution. Damaged DNA in black and intact homologous sequences as well as newly synthesized DNA in red.

TABLE 10-1 Prokaryotic and Eukaryotic Factors that Catalyze Recombination Steps

Recombination Step	*E. coli* Protein Catalyst	Eukaryotic Protein Catalyst
Pairing homologous DNAs and strand invasion	RecA protein	Rad51 Dcm1 (in meiosis)
Introduction of DSB	None	Spo11 (in meiosis) HO (for mating-type switching)
Processing DNA breaks to generate single strands for invasion	RecBCD helicase/nuclease	MRX protein (also called Rad50/58/60 nuclease)
Assembly of strand exchange proteins	RecBCD and RecFOR	Rad52 and Rad59
Holliday junction recognition and branch migration	RuvAB complex	Unknown
Resolution of Holliday junctions	RuvC	Perhaps Mus81 and others

Rad51

- Eukaryotic homolog of *E. coli* RecA
- Binds single-stranded DNA and double-stranded DNA
- Searches for regions of homology
- Exchanges homologous strands

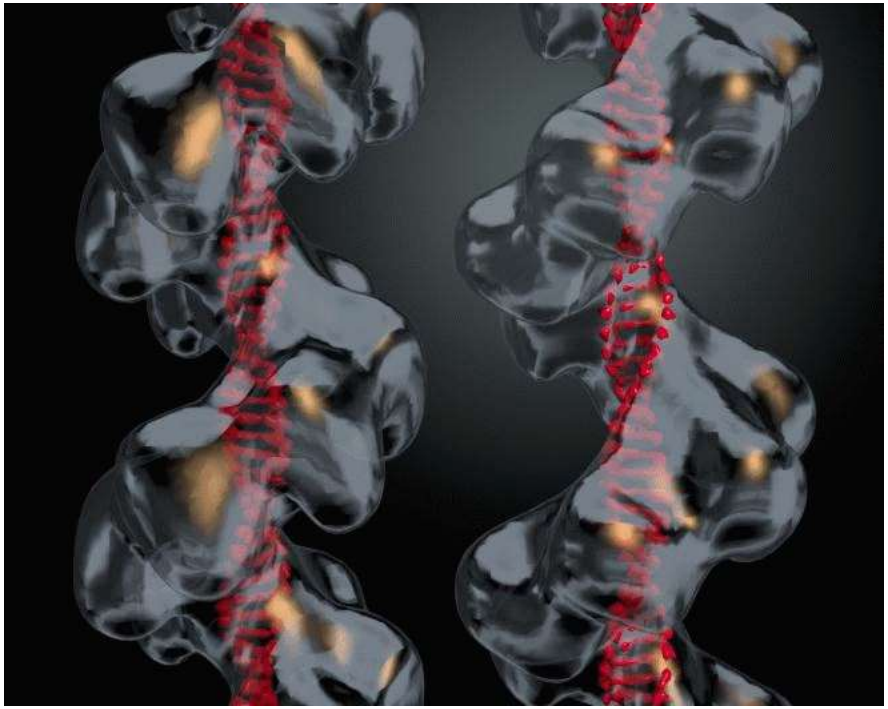
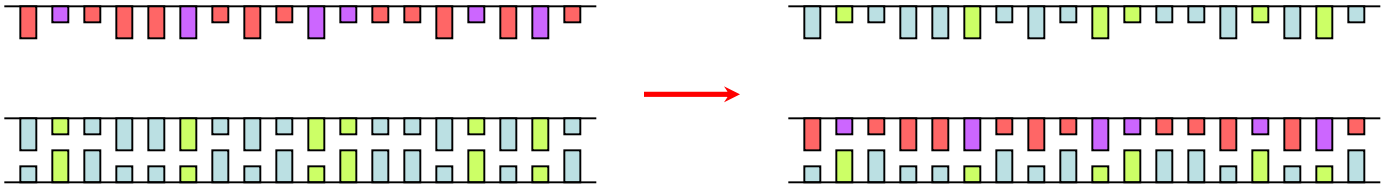


Image is from the cover of the March 26, 1993 issue of *Science*

Recombination: bringing DNA strands together in new ways

Strand annealing

Spontaneous or
Rad52 mediated



Strand invasion

RecA or
Rad51 mediated

Components of DNA double-strand repair pathways in human cells

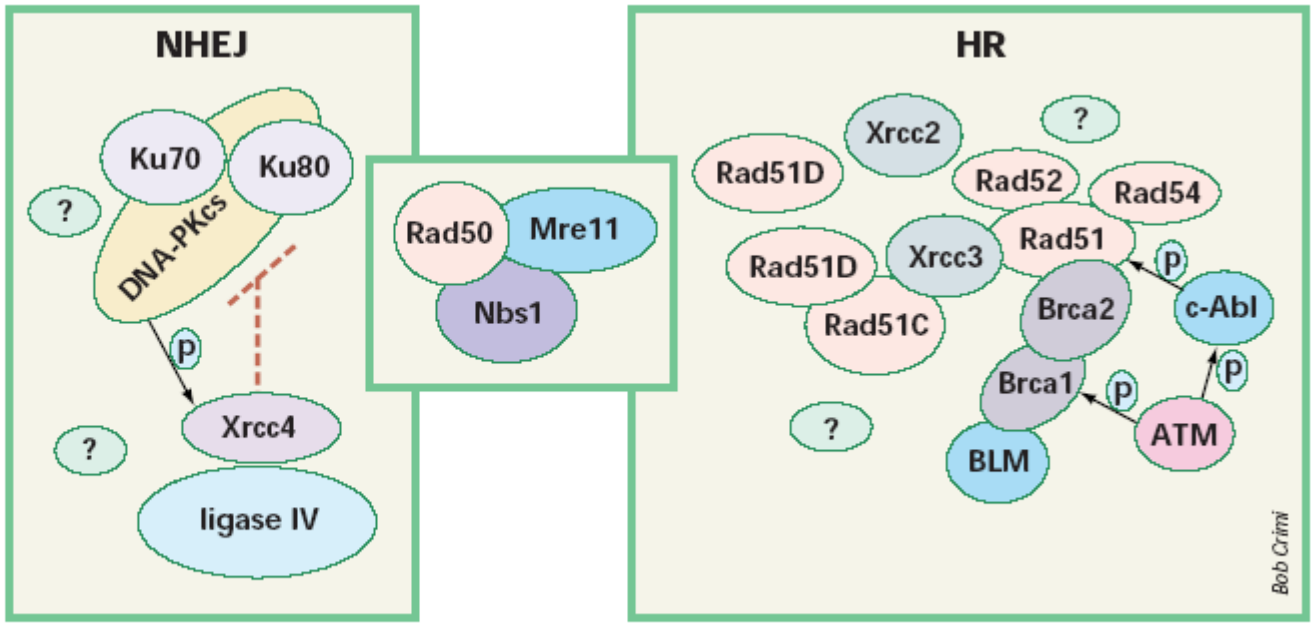
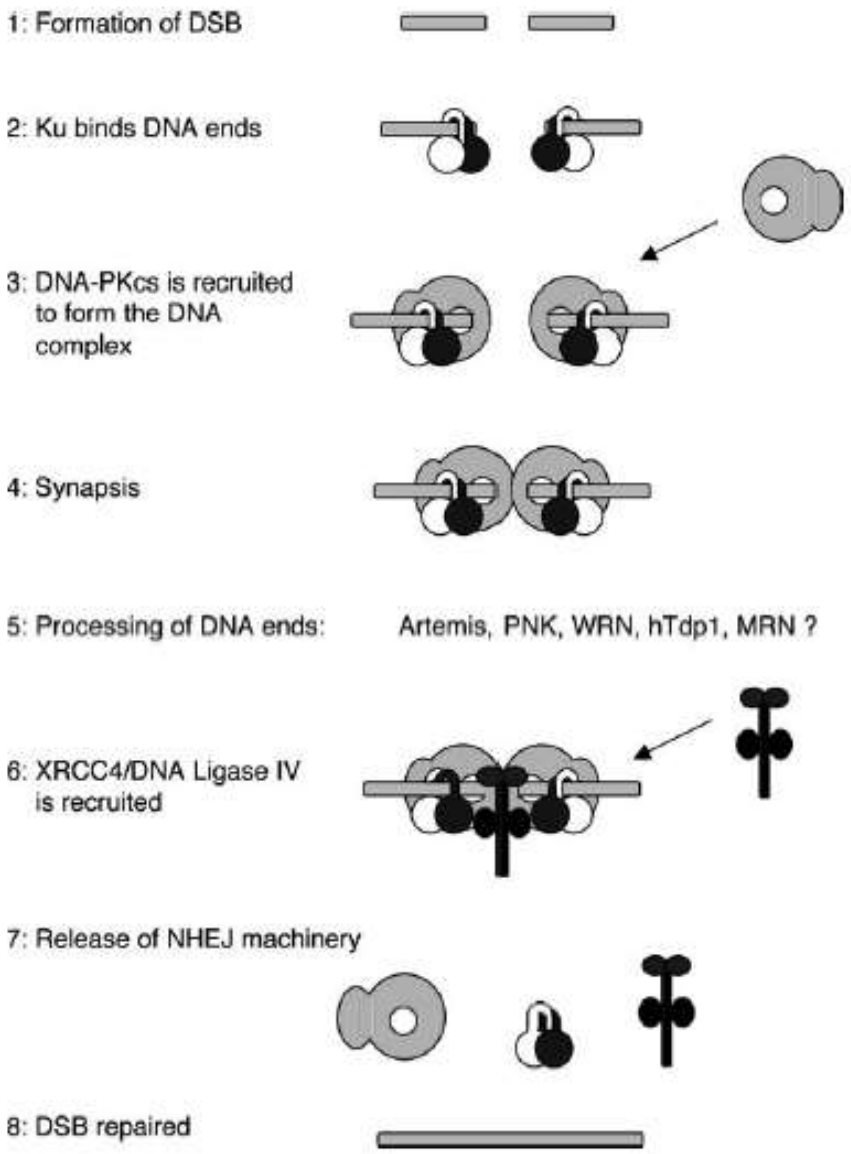


Fig. 2 Components of DNA DSB repair pathways. NHEJ: Ku binds a DBS, followed by recruitment and activation of DNA-PKcs. XRCC4 and ligase IV are recruited directly or indirectly by the DNA-PK holoenzyme and/or are activated by DNA-PK–mediated phosphorylation. HR: proteins involved in mammals are indicated. The strand-exchange reaction catalyzed by Rad51 is facilitated by Rad52 through direct interaction. Rad54, a DNA-dependent ATPase, also interacts directly with Rad51 and stimulates its activity. Rad51-related proteins (Rad51B-D, Xrcc2 and Xrcc3) are also involved in HR. There is a direct interaction between Xrcc3 and Rad51, and Rad51B and Xrcc3 interact with Rad51C. Rad51 also interacts with Brca2 and indirectly with Brca1 through Brca2. The c-Abl tyrosine kinase modulates Rad51 strand exchange activity through phosphorylation. Brca1 and c-Abl are phosphorylated by ATM. The Mre11/Rad50/Nbs1 complex, which participates in both NHEJ and HR, is also indicated.

Double-Strand Break Repair: Nonhomologous End Joining (NHEJ)

Mammalian pathway



Ku: dimer of Ku70 and Ku80

DNA-PKcs: DNA-dependent protein kinase catalytic subunit. Member of protein kinase family that includes ATM and ATR.

Synapsis is achived through microhomologies.

Factors involved in processing of ends not well understood. MRN is Mre11/Rad50/Nbs1 complex.

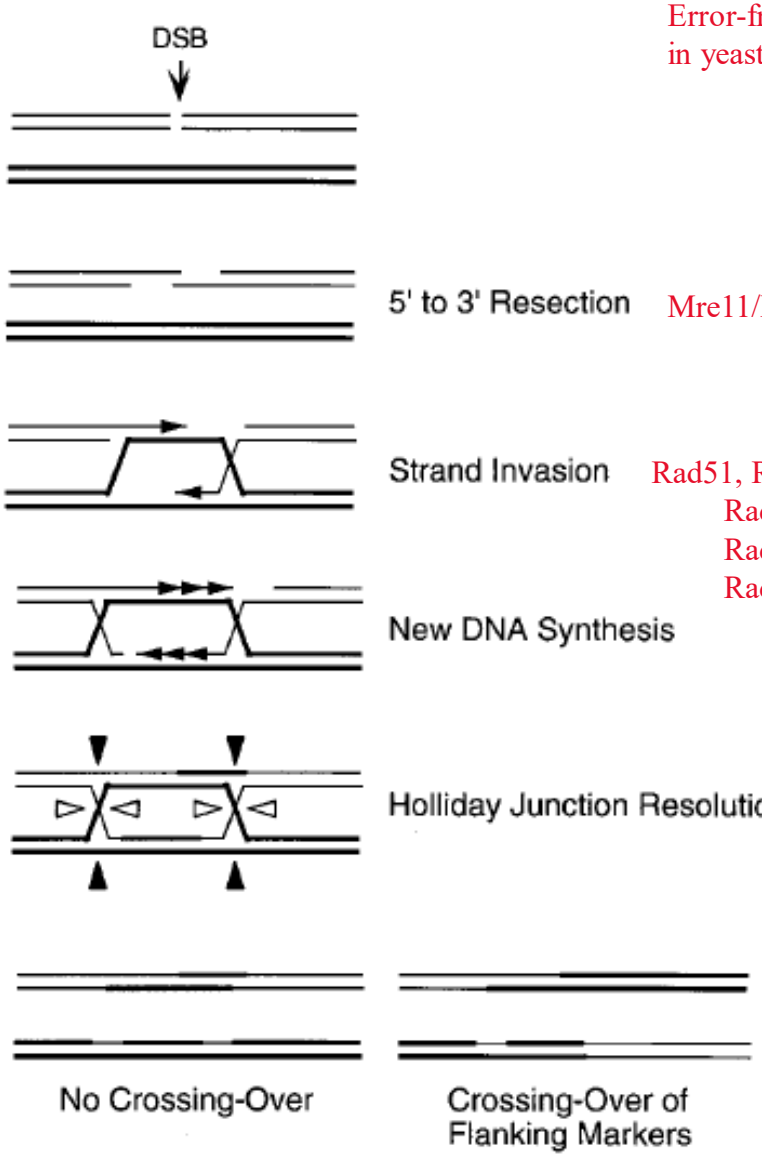
Xrcc4/DNA ligase IV are required for the final ligation step.

Error-prone; small insertions or deletions. Major pathway of DSB repair in mammals, minor pathway in yeast.

Further reading: Lees-Miller & Meek, Biochimie 85, 1161 (2003)

Double Strand Break Repair 1. Gene Conversion

Szostak model



Error-free pathway; a major pathway in yeast and mammals

Mre11/Rad50/Xrs2 (has 3' to 5' resection in vitro)

Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, RPA

Rad51, Rad55, Rad57 → Rec A homologs

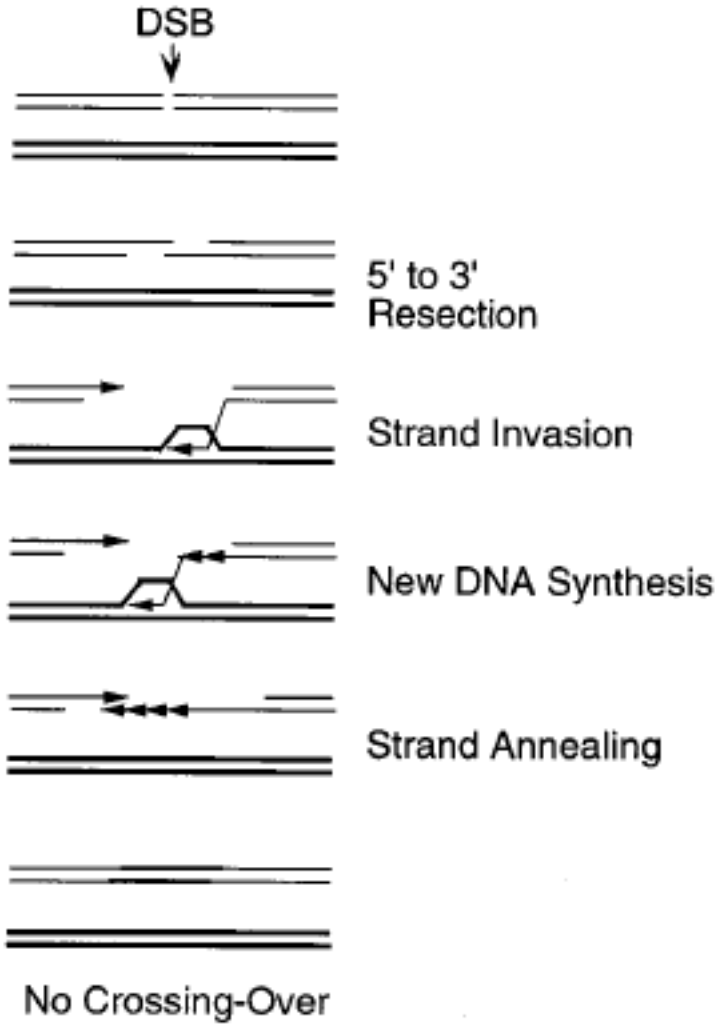
Rad54 → DNA helicase

Rad52 → DNA end-binding protein

Resolvases

Bloom's Syndrome protein (BLM) prevents crossovers

Double Strand Break Repair 1a. Synthesis-Dependent Strand Annealing (SDSA)



Error-free pathway; a major pathway in yeast and mammals

Mre11/Rad50/Xrs2 (has 3' to 5' resection in vitro)

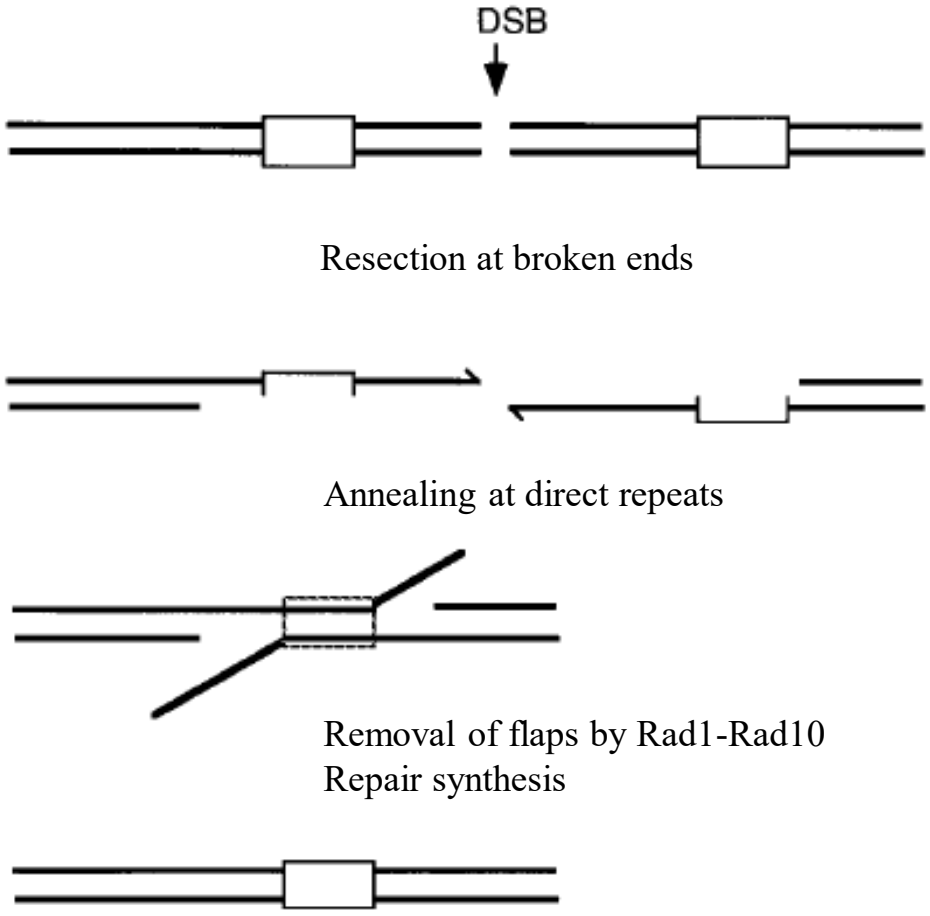
Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, RPA

Rad51, Rad55, Rad57 → Rec A homologs

Rad54 → DNA helicase

Rad52 → DNA end-binding protein

Double-Strand Break Repair 2: Single-Strand Annealing

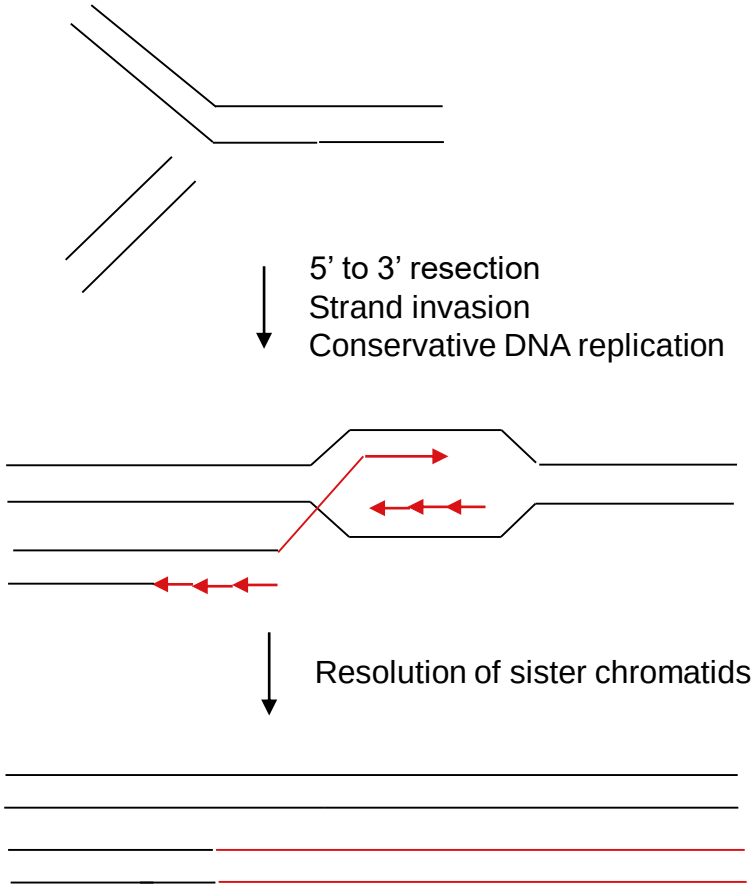


Requires Rad52, Rad1-Rad10, and replicative factors

Does not require Rad51

Highly error-prone as intervening sequences are deleted.

Double Strand Break Repair 3:Break-Induced Replication



Major pathway for S phase repair

Rad51, Rad52, Rad54, Rad55, Rad57

Requires replicative machinery

In principle BIR is error-free, but could give rise to translocations.

Not well studied in mammalian cells.

Davis and Symington MCB 24, 2344 (2004)

Nuclear foci formation upon DNA damage

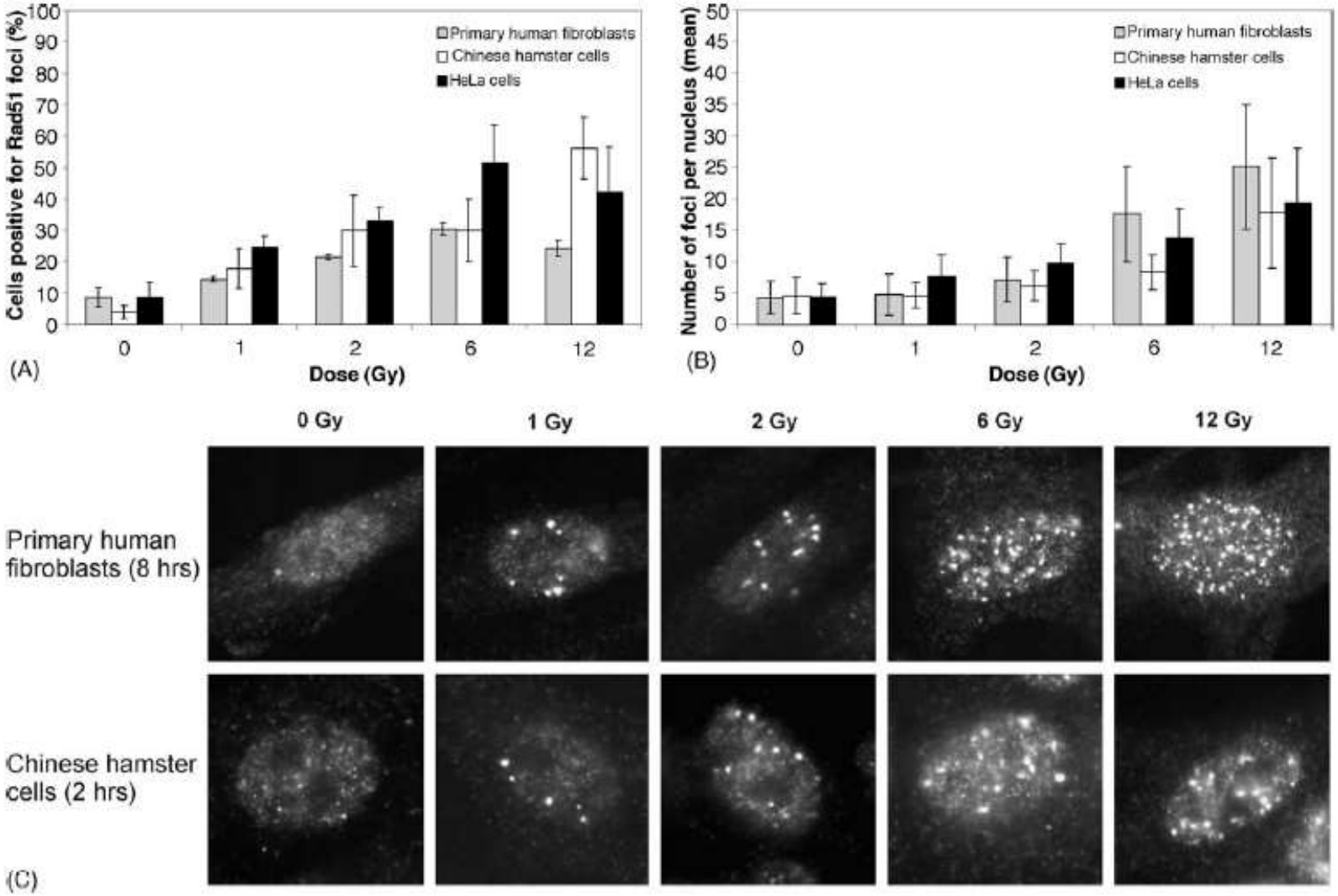


Fig. 2. Dose dependence of Rad51 IRIF. Primary human fibroblasts, Chinese hamster (V79) and HeLa cells were irradiated with 0, 1, 2, 6 and 12 Gy, fixed after 2 h (Chinese hamster cells) or 8 h (primary fibroblasts and HeLa cells) after which immuno-staining with antibodies against Rad51 was performed. Cells with ≥1 focus per nucleus were considered positive for foci formation. The error bars represent the 95% confidence interval. (A) The percentage of foci positive cells was determined by counting at least 200 cells per experiment. The experiment was performed 2–4 times for all cell lines. (B) The number of foci per foci-positive cell was determined by counting at least 50 cells with ≥1 focus per nucleus per experiment. (C) Representative pictures of primary fibroblasts and Chinese hamster cells at indicated dose points, 8 h (primary fibroblasts) or 2 h (Chinese hamster cells) after irradiation.

Co-localisation of nuclear foci upon DNA damage

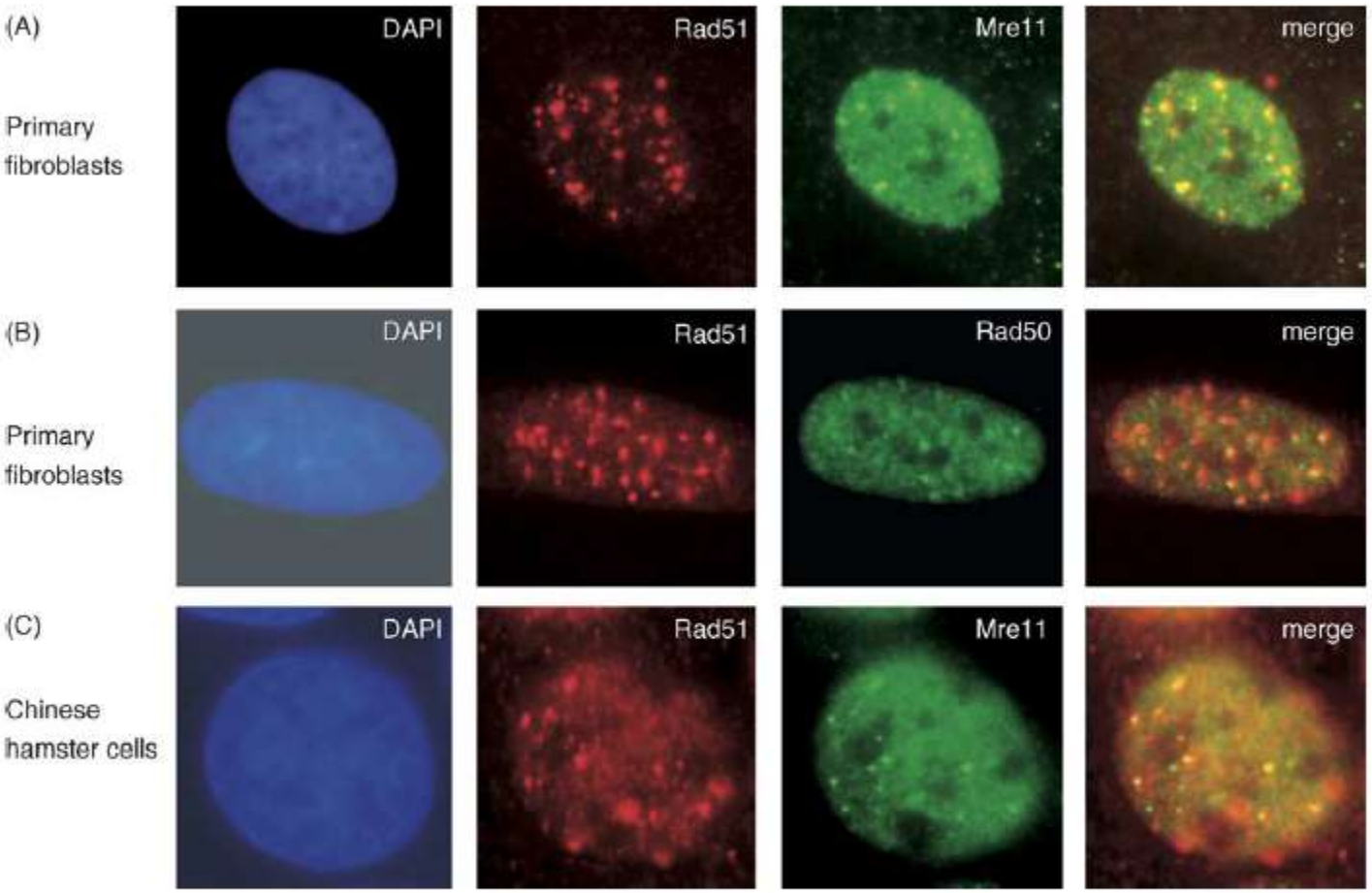
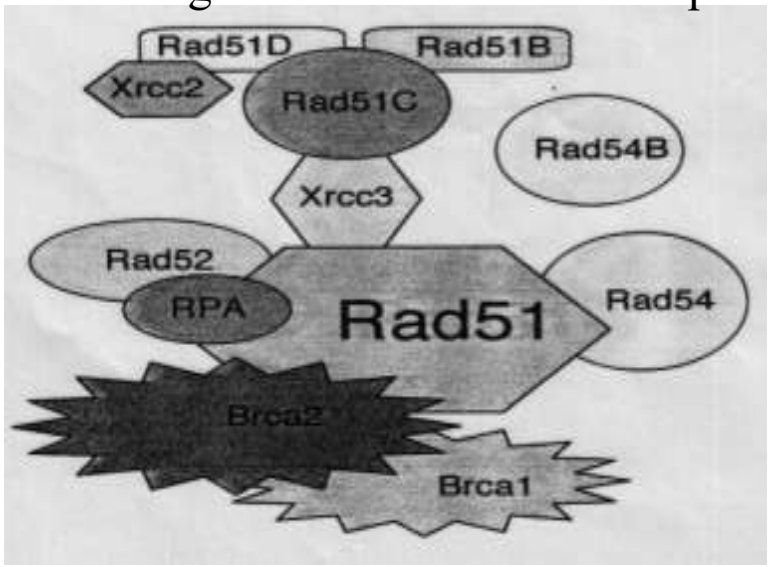


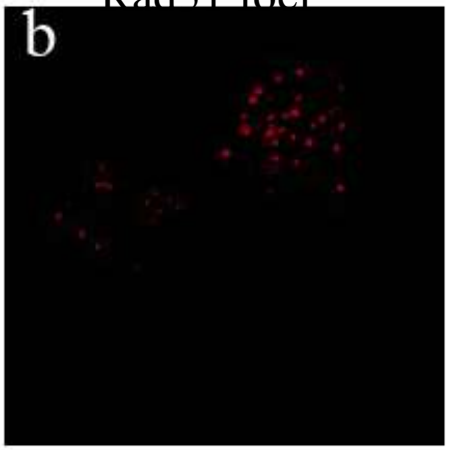
Fig. 5. Co-localization of Rad51 with Mre11 and Rad50 IRIF. (A) Primary human fibroblasts were irradiated with 12 Gy and fixed after 8 h. Double immuno-staining was performed using antibodies against Rad51 and Mre11. Some cells with Rad51 IRIF were observed which showed a partial co-localization with small Mre11 foci. (B) Primary human fibroblasts were irradiated with 12 Gy and fixed after 8 h. Double immuno-staining was performed using antibodies against Rad51 and Rad50. Some cells with Rad51 IRIF showed a partial co-localization with Rad50 foci. (C) Chinese hamster cells (CHO9) expressing Mre11-GFP were irradiated with 12 Gy and fixed after 2 h. Immuno-staining was performed using antibodies against Rad51. Cells with both Rad51 and Mre11 IRIF could be observed, in which the Rad51 foci partially co-localized with Mre11 foci.

Mammalian Recombination Complexes

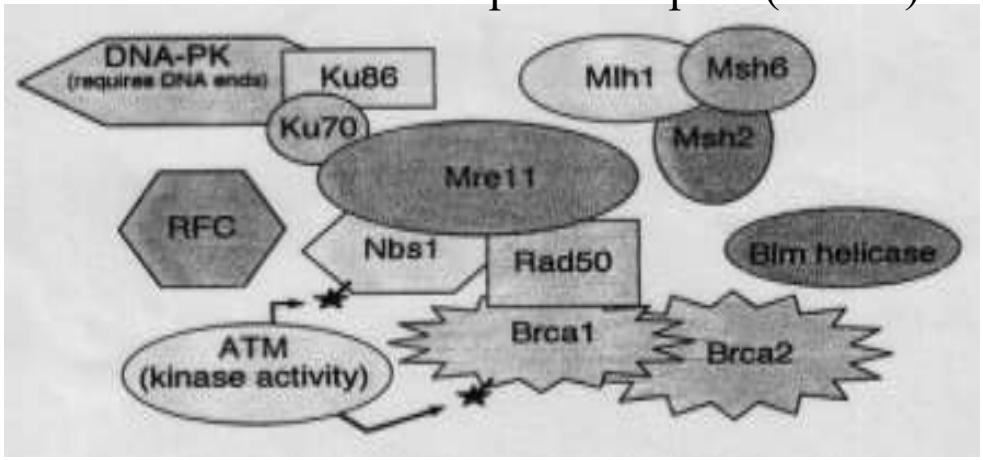
Homologous Recombination Complex



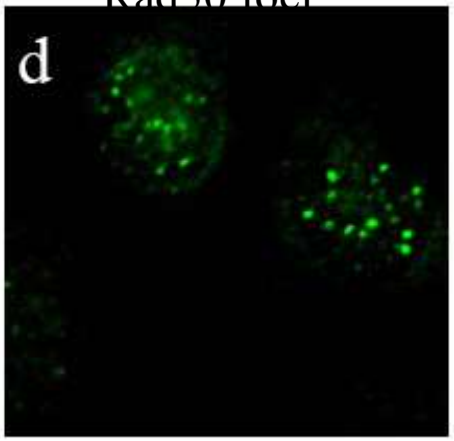
Rad51 foci



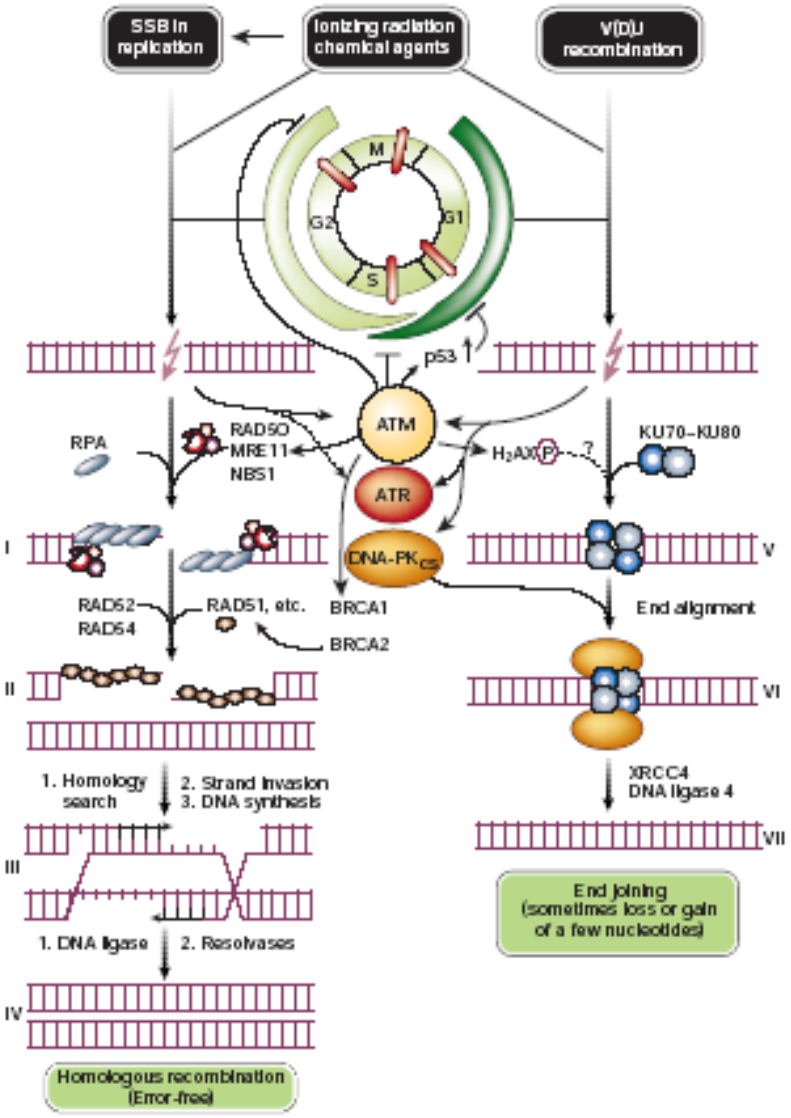
Surveillance and Repair Complex (BASC)



Rad50 foci



Mechanism of homologous recombination and end joining



A tentative scenario for the homologous-recombination reaction is depicted in the left panel of the figure. To promote strand invasion into homologous sequences, the 5′–3′ exonuclease activity of the RAD50/MRE11/NBS1 complex (also a substrate for ATM phosphorylation) exposes both 3′ ends[30] (I). RPA facilitates assembly of a RAD51 nucleoprotein filament that probably includes RAD51-related proteins XRCC2, XRCC3, RAD51B, C and D. RAD52 stimulates filament assembly (II). RAD51 has, like its *Escherichia coli* RecA counterpart, the ability to exchange the single strand with the same sequence from a double-stranded DNA molecule. Correct positioning of the sister chromatids by cohesins probably facilitates the identification of a homologous sequence. A candidate for the complex chromatin transactions associated with these DNA gymnastics is RAD54, a member of the SWI/SNF family of DNA-dependent ATPases. After identification of the identical sister chromatid sequence, the intact double-stranded copy is used as a template to properly heal the broken ends by DNA synthesis (III). Finally, the so-called Holliday-junctions are resolved by resolvases[27,33,60] (IV). Homologous recombination involves the simultaneous action of large numbers of the same molecules, which are found to be concentrated in radiation-induced nuclear foci. These depend on, and also include, the BRCA1 and BRCA2 proteins[35]. Recent evidence implicates BRCA2 directly or indirectly in nuclear translocation of RAD51 (ref. 61).

Cells in G1 have only the homologous chromosome for recombination repair. However, this may be difficult to find in the complex genome. Moreover, it is potentially dangerous as a template for repair as it may lead to homozygosity for recessive mutations. As an alternative, the end-joining reaction simply links ends of a DSB together, without any template, using the end-binding KU70/80 complex and DNA-PK$_{cs}$, followed by ligation by XRCC4–ligase4 (reviewed by 27,33; see the right panel of the figure, stages V–VII). The function of KU70/80 might involve end protection and approximating the ends, in addition to a signalling function by DNA-PK$_{cs}$. End joining may be further facilitated when the ends are still held together through nucleosomes or other structures. End joining is sometimes associated with gain or loss of a few nucleotides if internal microhomologies are used for annealing before sealing. This implies the involvement of DNA polymerases and/or nucleases. Note that the KU complex is also involved in telomere metabolism[27,62].

DNA Mismatch Repair or post-replication repair pathway

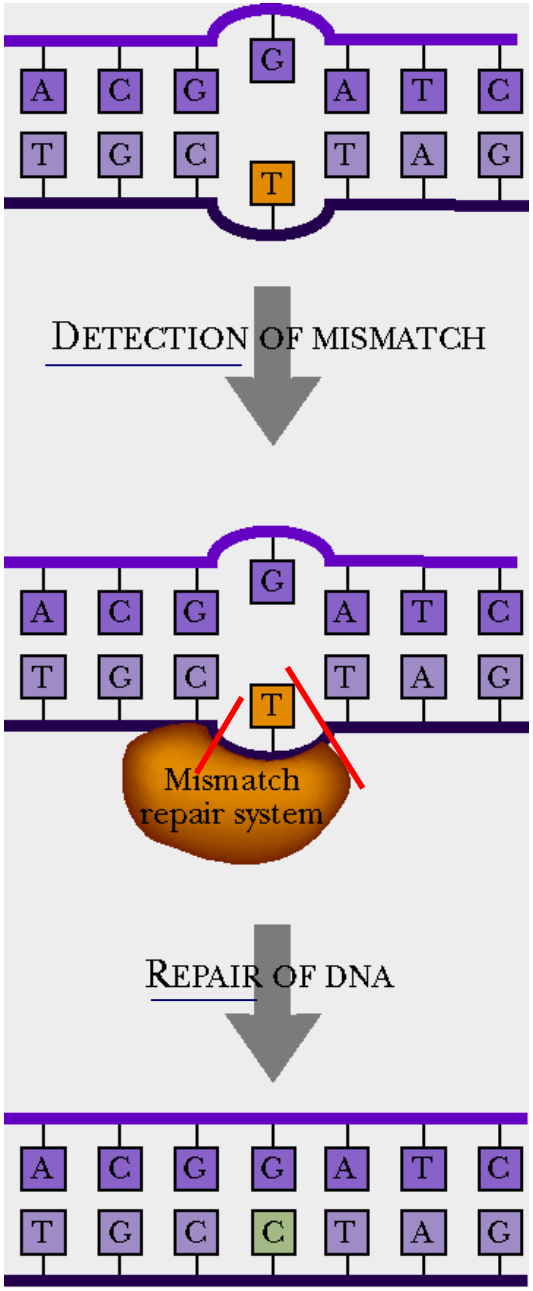
Repair of Replication Errors

Mechanisms for Insuring Replicative Fidelity

1. Base pairing	10^{-1} to 10^{-2}
2. DNA polymerases	10^{-5} to 10^{-6}
- base selection	
- proofreading	
3. Accessory proteins	10^{-7}
- single strand binding protein	
4. Mismatch correction	10^{-10}

Further reading: A. Bellacosa, Cell Death and Differentiation 8, 1076 (2001)
M. J. Schofield & P. Hsieh, Ann. Rev. Microbiol. 57, 579 (2003)

Principle of Mismatch Repair



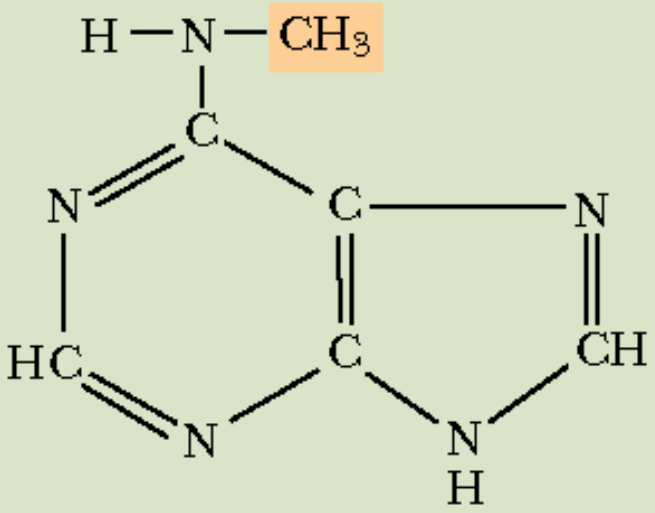
Cut out part of DNA strand containing wrong base.

Mismatch Repair Gap filled by DNA Pol III.

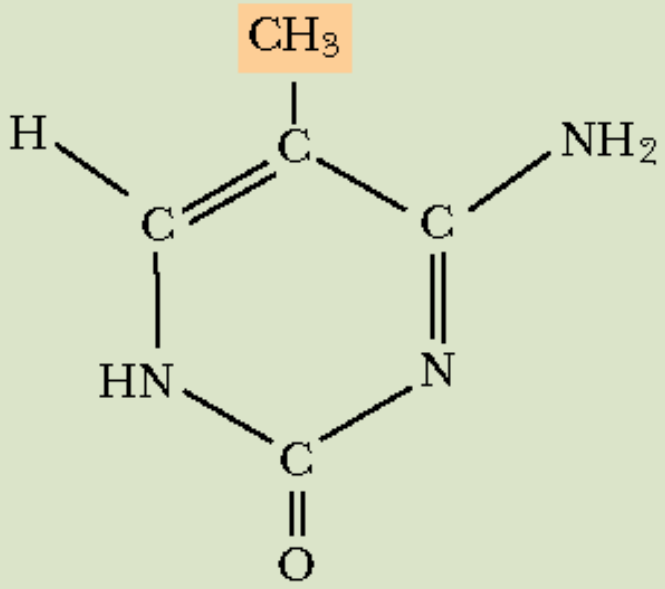
Note! most repair system using Pol I to replace short damaged region of DNA.

Methylated Bases in *E. coli* -Chemical Structure.

Not perturb base pairing



N6-methyl-adenine



C5-methyl-cytosine

Dam Protein (product of dam gene)
→ DNA adenine methylase

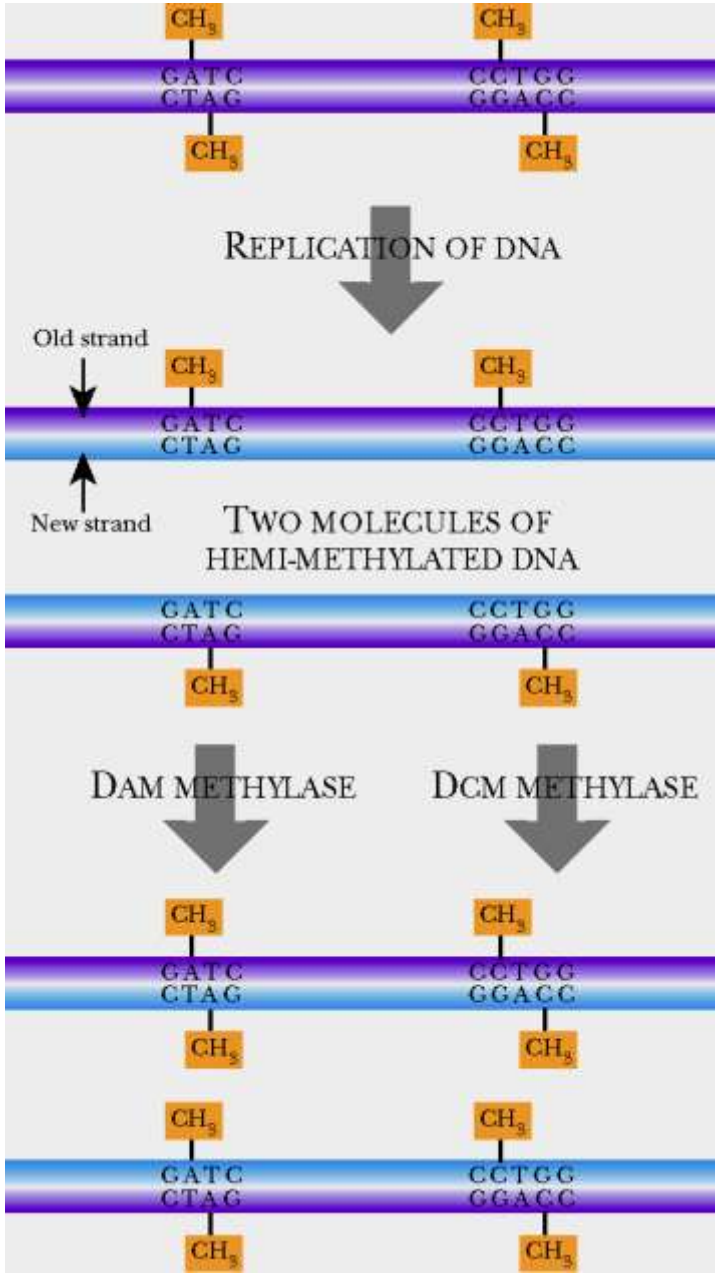
Dcm Protein (product of dcm gene)
→ DNA cytosine methylase

Recognition site is "Sequence-specific" & "Palindromic"

GATC

CCTGG

Sequence unique for E.Coli



Palindrome make the DNA methylated equally on both strands.

Not perturb base pairing

[delay in fully methylation]

1. During this period, many repair systems check DNA.
2. Control the initiation of new round of bacterial DNA replication

Function of methylation

→ Tell which is old, correct strand.

Fig14-14. Hemimethylated DNA

The major mismatch repair system of *E. coli* is MutSHL.

→ Consist of MutS, MutH, MutL (proteins)

→ Note! Genes are *mut*S, *mut*H, *mut*L

→mut = mutator,

def in mut → high mutation rate

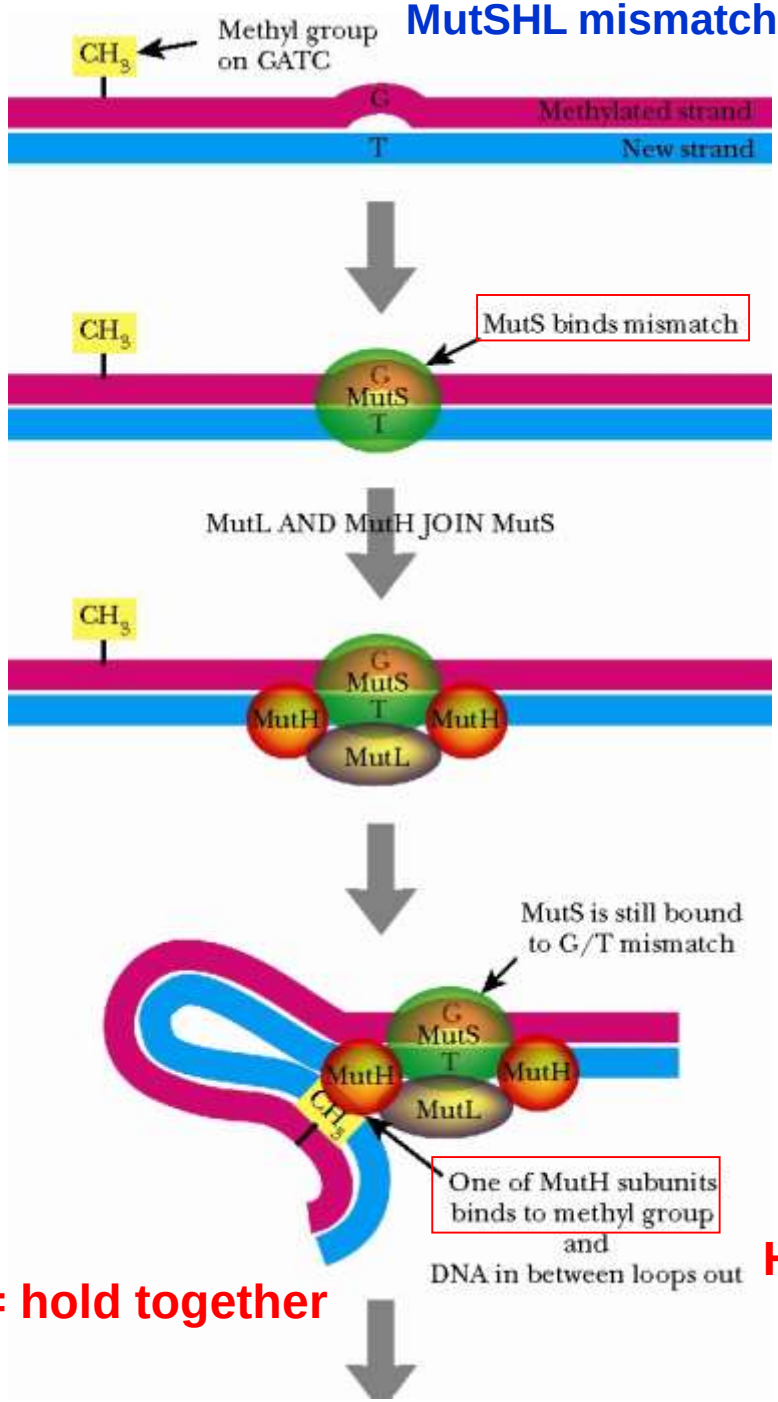
Damage Recognized:

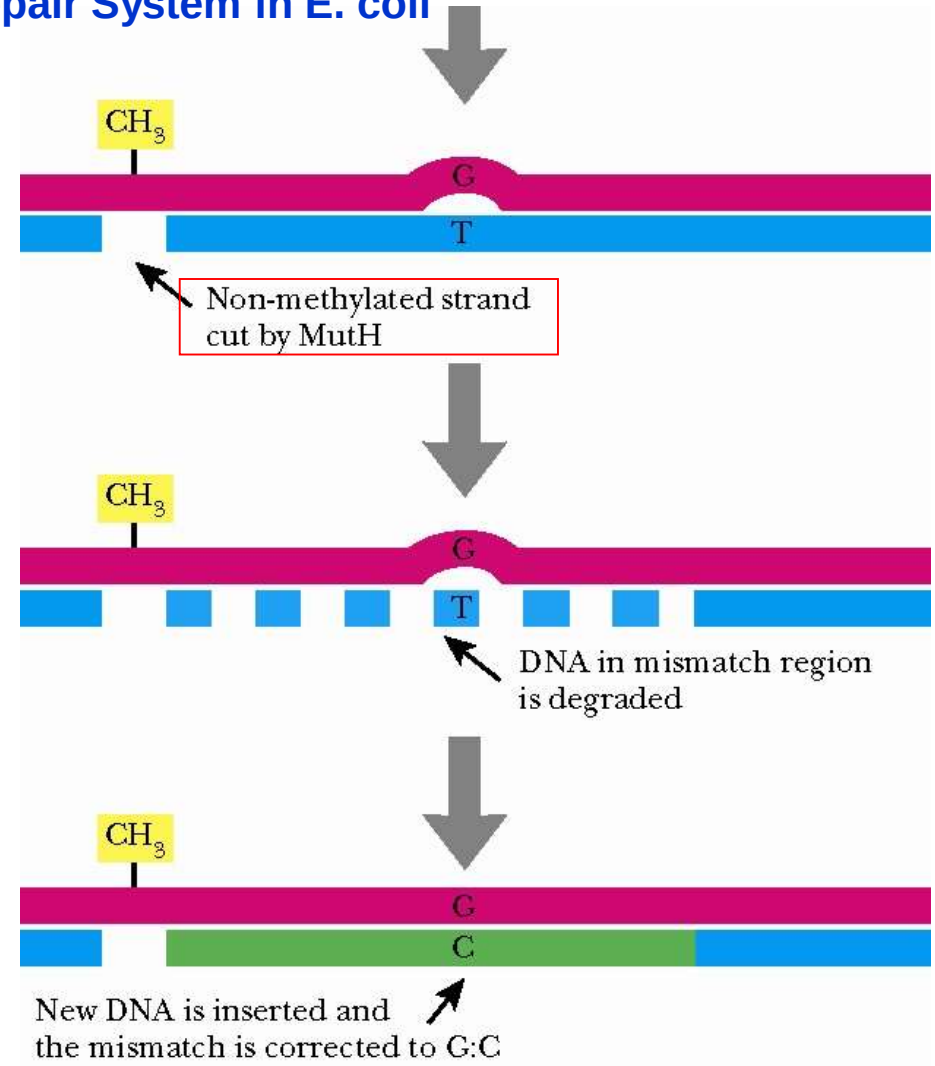
Base-base mismatch
Small insertion/deletion loops (IDLs)

Gene Products Required (11):

MutS (damage recognition)
MutL
MutH (endonuclease)
MutU (DNA helicase)
Exonucleases (ExoI, ExoVII, ExoX, RecJ)
DNA polymerase III
Single strand binding protein (SSB)
DNA Ligase

MutSHL mismatch Repair System in E. coli





Pol III attach & repair the gap created by MutSHL system.

L = hold together

H = find the nearest GATC site & nick the non-CH3 strand

Eucaryotic homologs of MMR genes

				Mutations in human cancer	
E. coli	*S. cerevisiae*	*H. sapiens*	Chromosome location	Hereditary (germline)	Sporadic (somatic)
mutS	*Msh2*	*MSH2*	2p22-p21	✓	✓
	Msh3	*MSH3*	5q11-q12		✓
	Msh6	*MSH6*	2p16	✓	✓
	Msh4	*MSH4*	1p31		
	Msh5	*MSH5*	6p21.3		
	Msh1	[a]	–		
mutL	*Mlh1*	*MLH1*	3p21.3	✓	✓[b]
	Pms1	*PMS2*	7p22	✓	✓
	Mlh2	*PMS1*	2q31-q33	✓	
	Mlh3	*MLH3*	14q24.3		✓
mutH	[a]	[a]	–		
mutU (uvrD)	[a]	[a]	–		

[a]Not identified. [b]Usually, loss of expression by promoter hypermethylation

Germline mutations occur in the syndrome: Hereditary nonpolyposis colon cancer - HNPCC
Approx. 90% of MMR mutations occur in Msh2 and Mlh1
HNPCC accounts for approx. 3% of all colon cancers

Mismatch Repair Mutations in Hereditary Nonpolyposis Colon Cancer (HNPCC)

- MMR mutations in 70% of families
- MLH1 (50%), MSH2 (40%)
- Minor role for MSH6, PMS1, PMS2

- Population prevalence 1:2851 (15-74 years)
- 18% of colorectal cancers under 45 years
- 28% of colorectal cancers under 30 years

Functions of MMR Proteins

- Repair of mismatches and insertion/deletion loops
  - Msh2, Msh3, Msh6, Mlh1, Pms2, (Pms1, Mlh3)

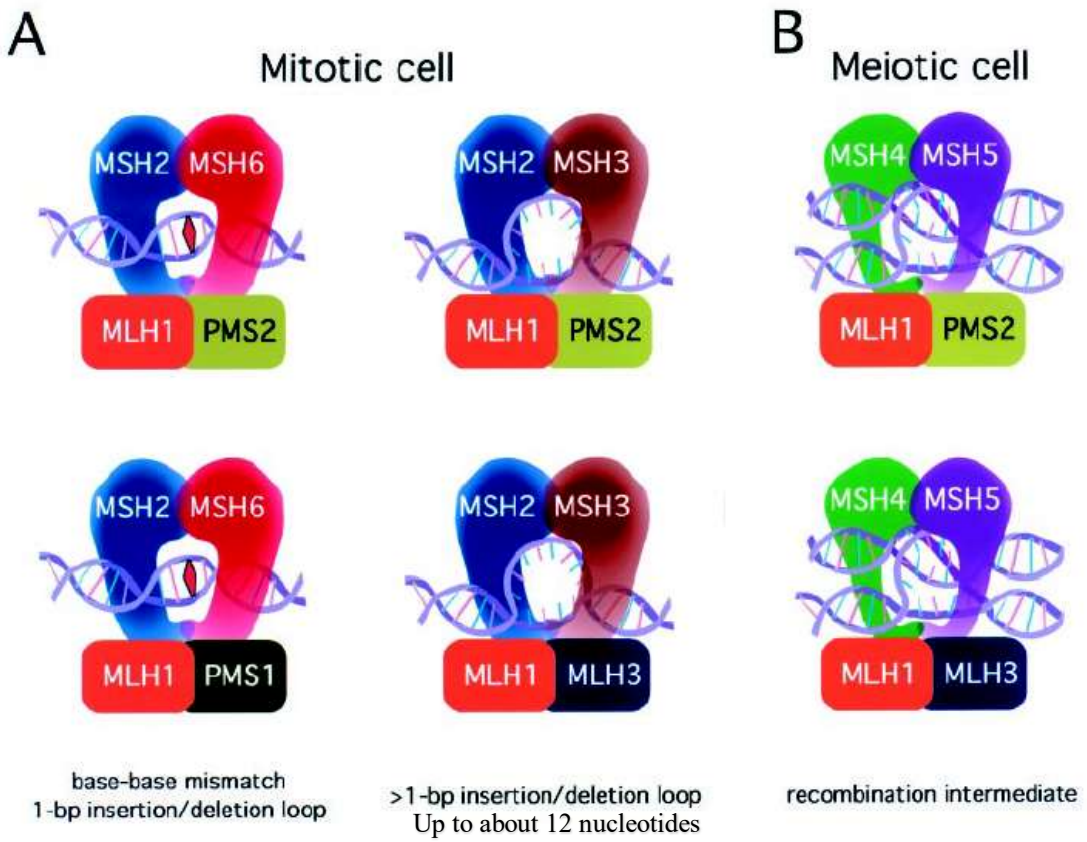
- Meiotic recombination
  - Msh4, Msh5, Mlh1, Pms2, Mlh3

- Mitotic recombination
  - Msh2, Msh3

- DNA damage signaling in apoptosis (alkylation damage)
  - Msh2, Msh6, Mlh1, Pms2

- Repair of DNA Interstrand Cross-links
  - Msh2, Msh3, Mlh1?, Pms2?

Interactions in Mammalian MMR



Msh2/Msh6 → MutSα (recognizes base-base mismatch and 1bp IDL)

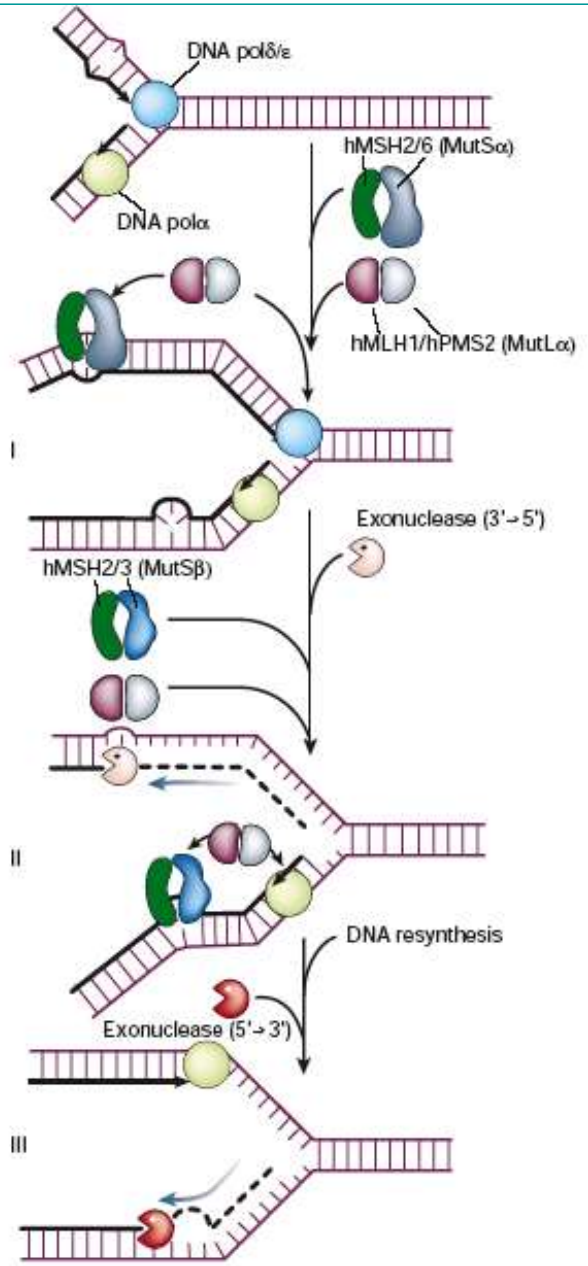
Msh2/Msh3 → MutSβ (recognizes 2 to approx. 12 bp IDLs)

Mlh1/Pms2 → MutLα

Mlh1/Pms1 → MutLβ

Mlh1/Mlh3

Nick-Directed Mismatch Repair in Mammalian Cells



MutSα (Msh2/Msh6) - recognizes mismatch or 1 bp IDL
MutSβ (Msh2/Msh3) - recognizes 2-12 bp IDL

MutLα (Mlh1/Pms2) - exact role unknown

Discrimination between parent and daughter strand is accomplished by presence of nick in daughter strands

PCNA is required and may couple replicative machinery to MMR
- PCNA interacts with Msh3 and Msh6

RPA protects single-stranded DNA and prevents extensive resection by exonucleases

Exonuclease I - only identified eucaryotic exonuclease; has 5'-3' polarity

Pols δ/ε perform resynthesis

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Genetics

The role of mismatch repair in the prevention of base pair mutations in *Saccharomyces cerevisiae*

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Table 3. Comparison of aerobic reversion rate versus anaerobic reversion rate

Strain	Reversion rate, $\times 10^{-6}$		Anaerobic suppression	Mutation
	Aerobic	Anaerobic		
YMH57/*msh2*	0.31	0.025	12	T → C
YMH57/*msh6*	0.33	0.0099	33	
YMH52/*msh2*	1.7	0.038	45	C → T
YMH52/*msh6*	1.5	0.051	29	
YMH54/*msh2*	5.6	0.12	47	G → T
YMH54/*msh6*	6.3	0.10	63	

Anaerobic suppression is the ratio of aerobic to anaerobic reversion rates. The mutation shown is the one most likely to occur due to oxidative damage (e.g., G → T rather than C → A).

Authors suggest that the high reversion rates observed in these MMR-deficient strains are caused by misincorporations opposite oxidatively damaged bases and that MMR normally prevents these mutations. Authors further suggest that recognition of mispairs opposite damaged bases may be a more important role for MMR in yeast than correction of errors opposite normal bases.