DNA Damage and Repair

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DNA Damage and Repair Contents

- **DNA damage**
- **DNA damage response**
- **DNA repair**
  - Direct reversal
  - Base excision repair
  - Nucleotide excision repair
  - Translesion synthesis
  - Mismatch repair
  - Double strand break repair
    - Recombination repair
    - Non-homologous end joining
  - Inter strand cross-link repair

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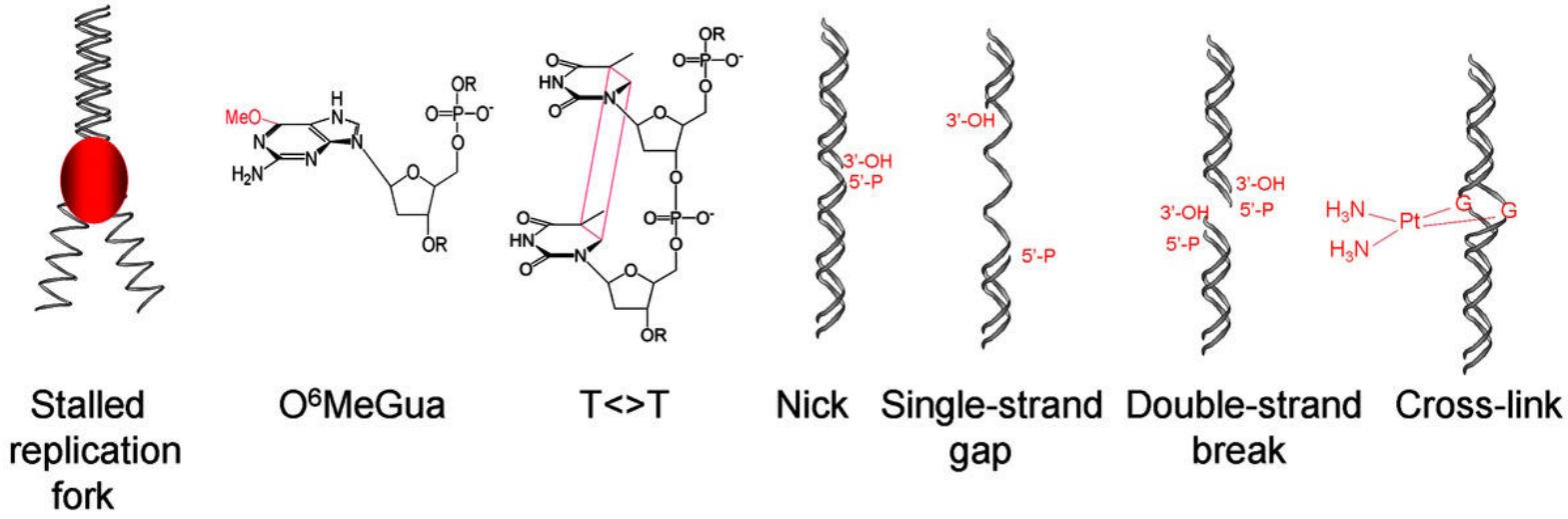
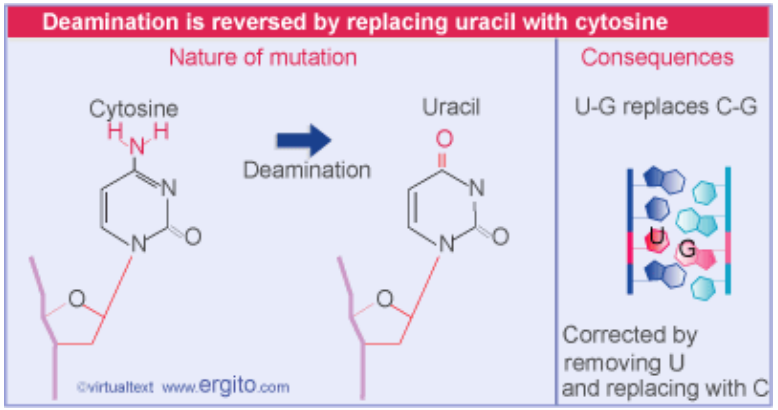
Major DNA Lesions

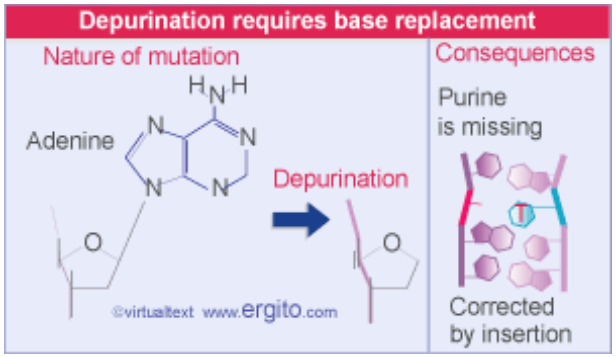


DNA lesions and structures that elicit DNA response reactions. Some of the base backbone lesions and noncanonical DNA structures that elicit DNA response reactions are shown. O^6MeGua indicates O^6-methyldeoxyguanosine, T<>T indicates a cyclobutane thymine dimer, and the cross-link shown is cisplatin G-G interstrand cross-link.

Endogenous DNA Damage



Deamination of cytosine to uracil can spontaneously occurs to creates a U·G mismatch.



Depurination removes a base from DNA, blocking replication and transcription.

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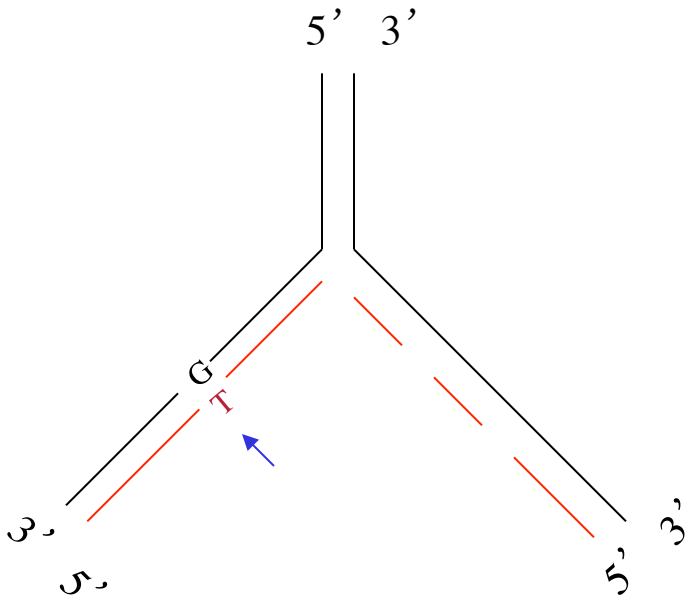
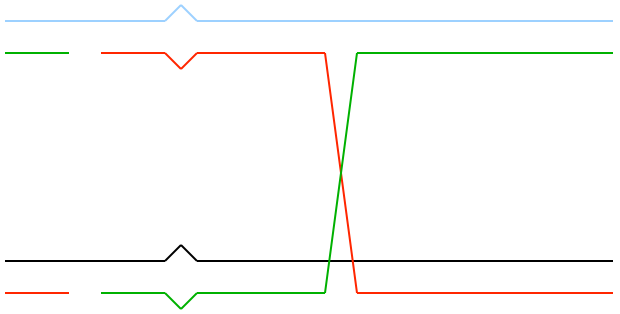
Mismatch Derived from Normal DNA Metabolism



DNA replication errors



DNA recombination

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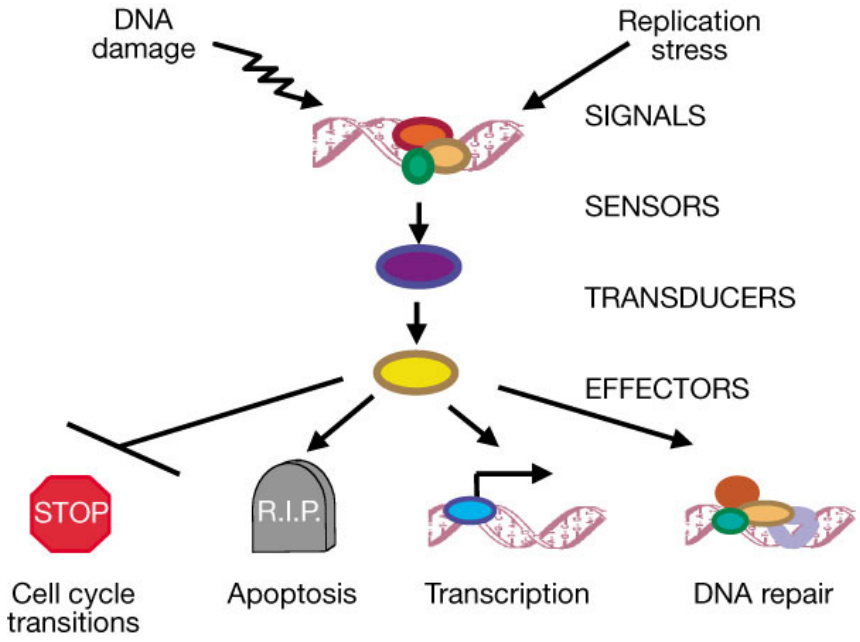
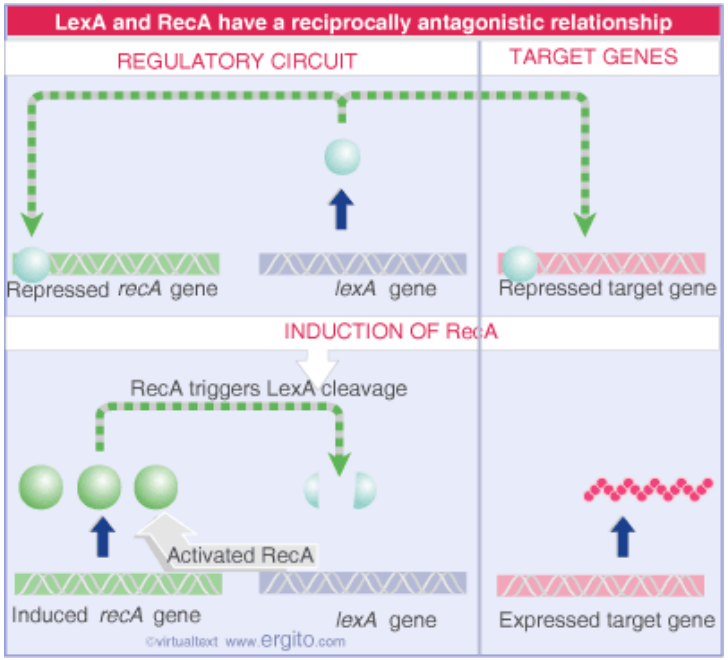
Cellular Response to DNA Damage



A contemporary view of the general outline of the DNA damage response signal-transduction pathway. Arrowheads represent activating events and perpendicular ends represent inhibitory events. Cell-cycle arrest is depicted with a stop sign, apoptosis with a tombstone. The DNA helix with an arrow represents damage-induced transcription, while the DNA helix with several oval-shaped subunits represents damage-induced repair. For the purpose of simplicity, the network of interacting pathways are depicted as a linear pathway consisting of signals, sensors, transducers and effectors.

SOS Response in E. coli



Key Terms

•An **SOS response** (discovered by Miroslav Radman in 1974) in *E. coli* describes the coordinate induction of many enzymes (more than 20), including repair activities, in response to damage to DNA; results from activation of protease activity by **RecA** to cleave **LexA** repressor.

•The **SOS box** is the DNA sequence (operator) of ~20 bp recognized by **LexA** repressor protein.

Key Concepts

•Damage to DNA causes **RecA** to trigger the SOS response, consisting of genes coding for many repair enzymes.
•**RecA** activates the autocleavage activity of **LexA**.
•**LexA** represses the SOS system; its autocleavage activates target gene expression.

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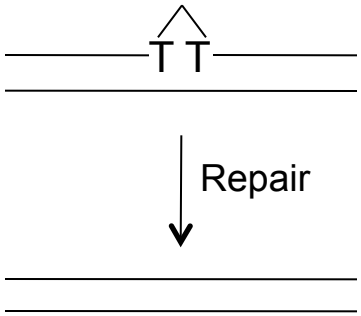
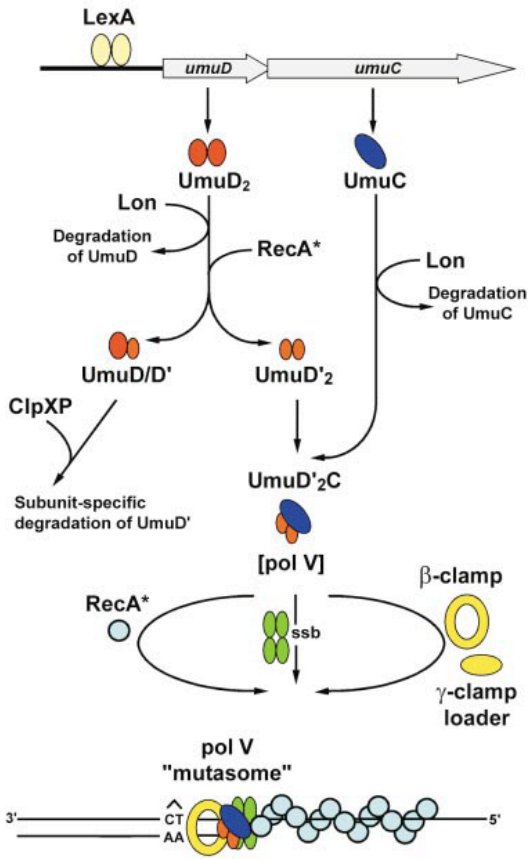
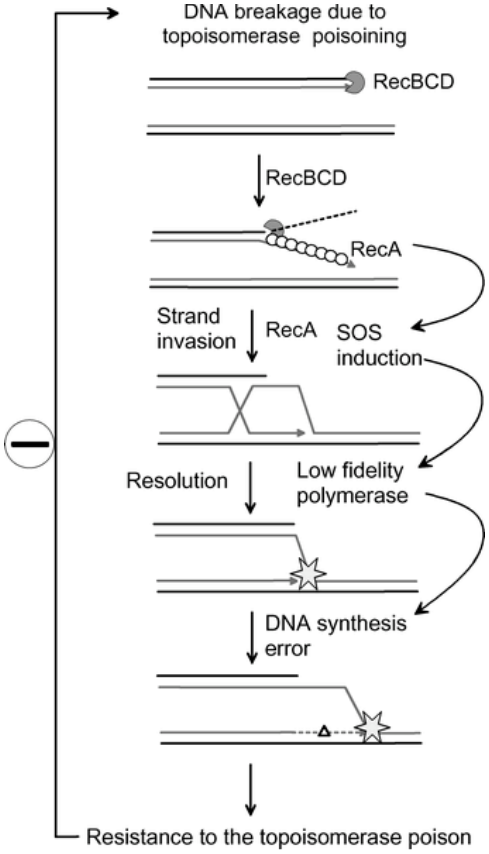
Consequences of SOS Response

- **DNA repair:** Induce expression of many DNA repair genes, including recA, uvrA, uvrB, and uvrD.

- **Mutagenesis**: Activate low fidelity translesion DNA polymerases to bypass the DNA lesions.







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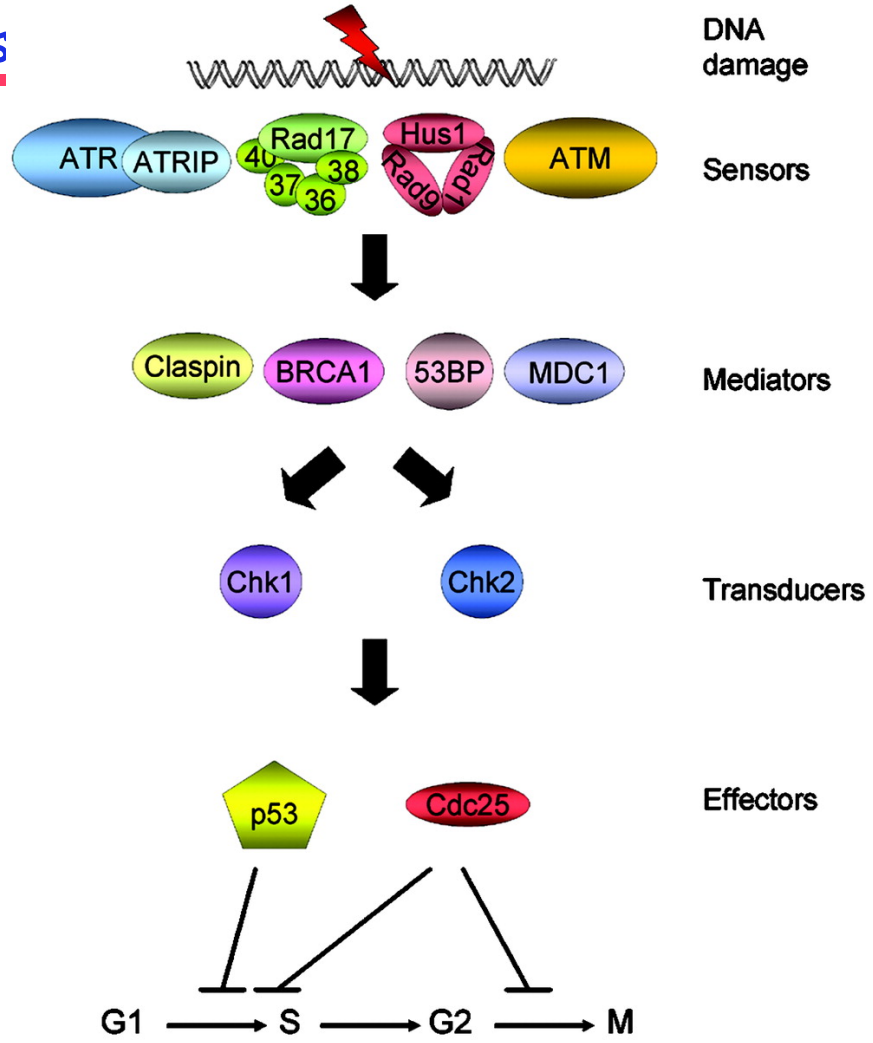
DNA Damage Checkpoint Proteins

DNA damage **Checkpoints** are biochemical pathways that delay or arrest cell cycle progression in response to DNA damage. Like other signal transduction pathways, the DNA damage checkpoint has three types of components: sensors, signal transducers, and effectors. The damage is detected by **sensors** that, with the aid of **mediators**, transduce the signal to **transducers**. The transducers, in turn, activate or inactivate other proteins (**effectors**) that directly participate in inhibiting the G1/S transition, S-phase progression, or the G2/M transition.



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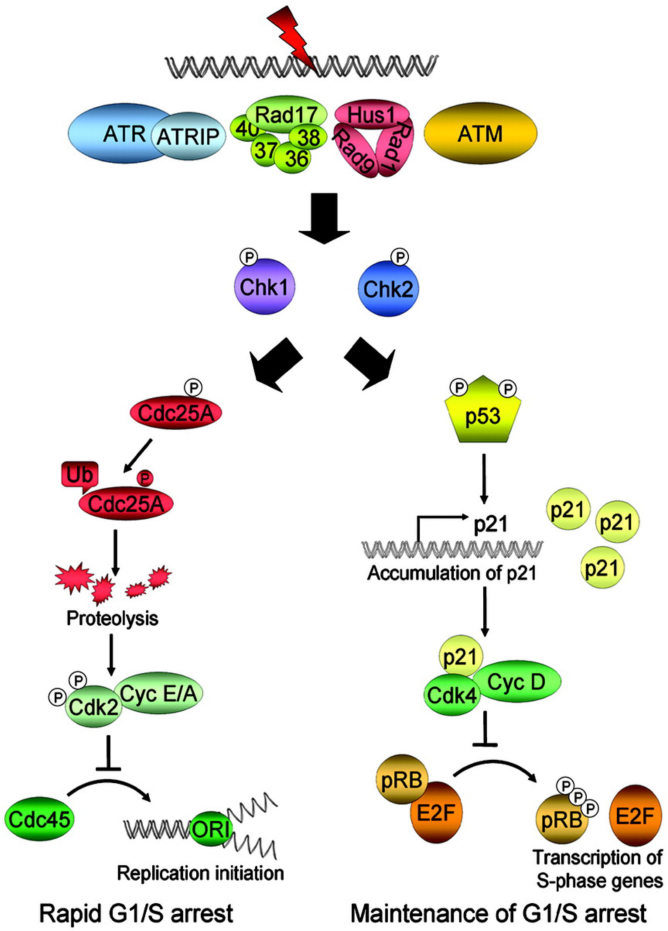
Cellular Response to DNA Damage



The G1/S checkpoint. DNA damage is sensed by ATM after double-strand breaks or by ATR, Rad17-RFC, and the 9-1-1 complex after UV-damage. ATM/ATR phosphorylates Rad17, Rad9, p53, and Chk1/Chk2 that in turn phosphorylates Cdc25A, causing its inactivation by nuclear exclusion and ubiquitin-mediated degradation. Phosphorylated and inactivated Cdk2 accumulates and cannot phosphorylate Cdc45 to initiate replication. Maintenance of the G1/S arrest is achieved by p53, which is phosphorylated on Ser15 by ATM/ATR and on Ser20 by Chk1/Chk2. Phosphorylated p53 induces p21WAF-1/Cip1 transcription, and p21WAF-1/Cip1 binds to the Cdk4/CycD complex, thus preventing it from phosphorylating Rb, which is necessary for the release of the E2F transcription factor and subsequent transcription of S-phase genes. p21WAF-1/Cip1 also binds to and inactivates the Cdk2/CycE complex, thus securing the maintenance of the G1/S checkpoint.

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DNA Damage Checkpoint proteins

Protein function	Mammals	*S. pombe*	*S. cerevisiae*
Sensors			
RFC-like	Rad17	Rad17	Rad24
PCNA-like	Rad9	Rad9	Ddc1
	Rad1	Rad1	Rad17
	Hus1	Hus1	Mec3
PI3-Kinases (PIKK)	ATM	Tel1	Tel1
	ATR	Rad3	Mec1
PIKK binding partner	ATRIP	Rad26	Ddc2/Lcd1/Pie1
Mediators			
	MDC1		
	53BP1		
	TopBP1	Cut5	Dpb11
	Claspin	Mrc1	Mrc1
	BRCA1	Crb2/Rph9	Rad9
Transducers			
Kinase	Chk1	Chk1	Chk1
	Chk2	Cds1	Rad53

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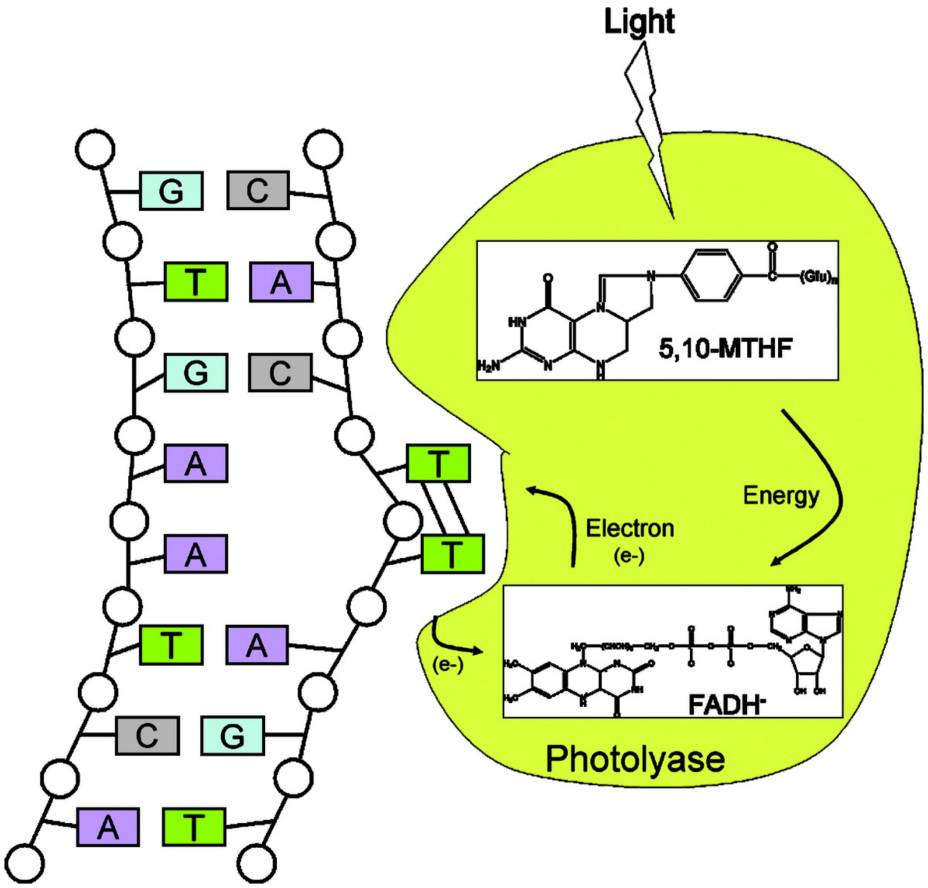
Direct Reversal



Direct repair by photoreactivation. Photolyase binds to DNA containing a pyrimidine dimer in a light-independent reaction and flips the dimer out into the active site pocket. Catalysis is initiated by light. The photoantenna cofactor, methenyltetrahydrofolate (5,10-MTHF), absorbs a photon and transfers the excitation energy to the catalytic cofactor, $FADH^-$. Then, the excited state FADH transfers an electron to the pyrimidine dimer, splitting the dimer into two pyrimidines. The electron returns to the flavin radical to regenerate $FADH^-$, and the enzyme dissociates from the repaired DNA.

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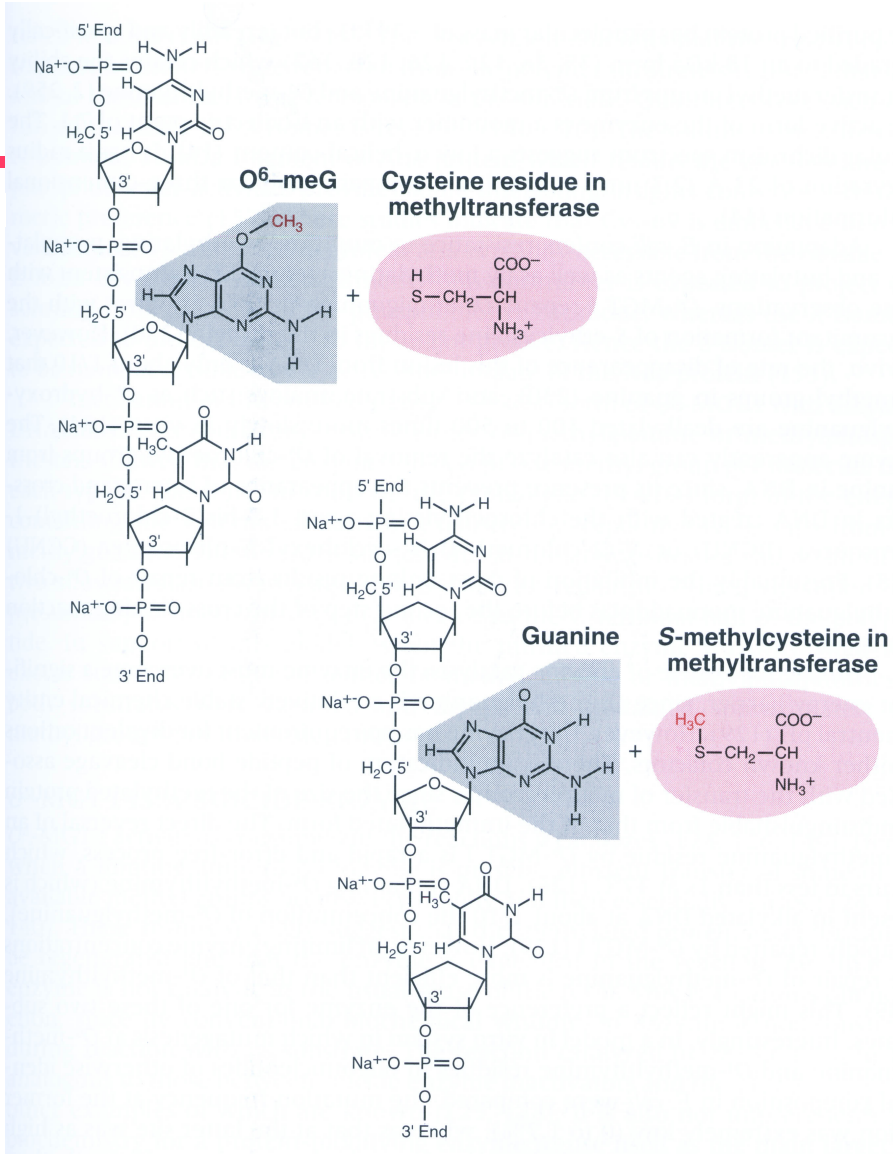
Direct Reversal

Repair of O6-me-G by O6-methylguanine methyltransferase



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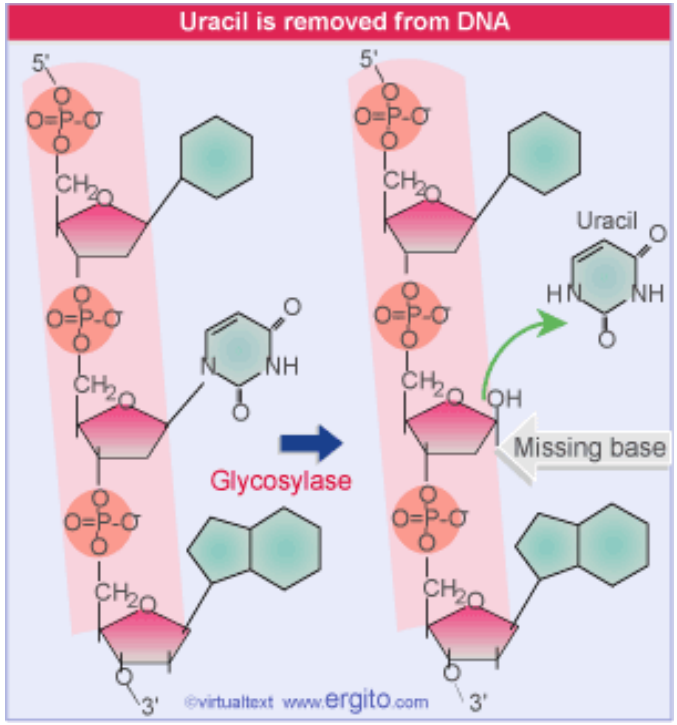
Base Excision Repair



•Excision repair that is initiated by DNA glycosylase is called **base excision repair (BER)**, because the damaged moieties are excised as free bases.

•DNA **glycosylases** are key enzymes required in BER, and they remove the damaged or mismatched base from DNA by cleaving the bond between the base and the deoxyribose.

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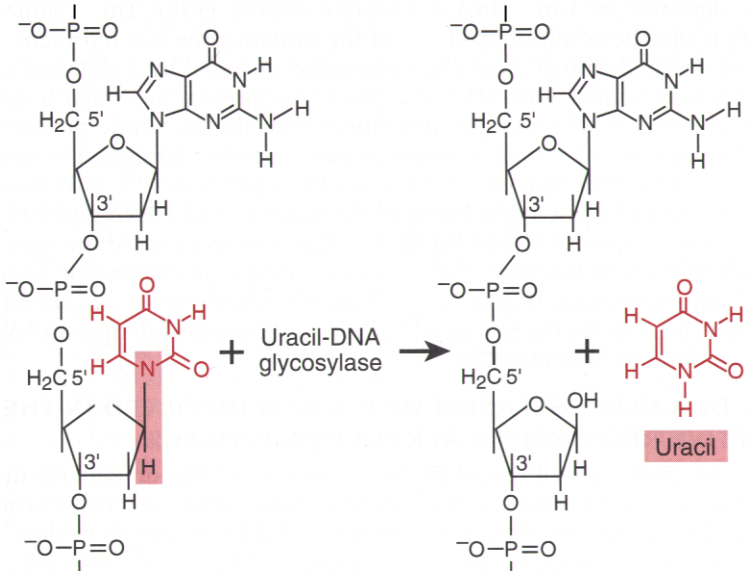
Uracil-DNA Gylycosylase

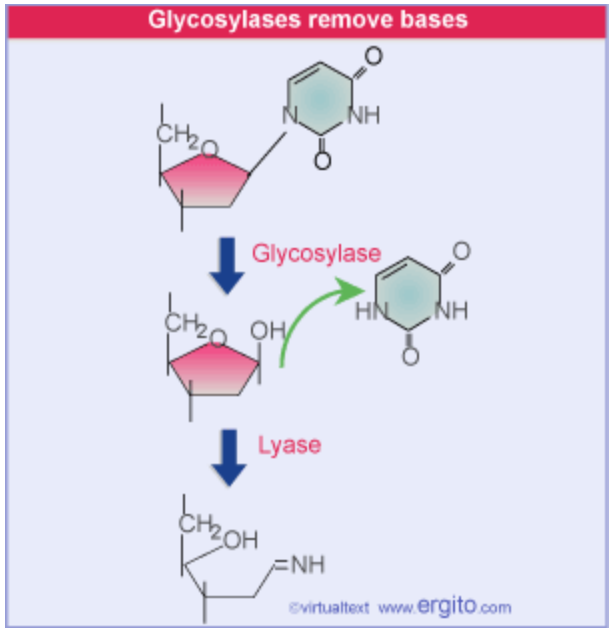


Uracil-DNA glycosylase specifically removes uracil from DNA by hydrolyzing the N-glycosylic bond linking the base to the sugar-phosphate backbone.

DNA Glycosylases

Enzyme	Substrate
Uracil-DNA glycosylase	Uracil
5’-mC-DNA glycosylase	5-methylcytocine
Thymine-DNA glycosylase	G-T mispair
MutY	G-A mispair
3-mA-DNA glycosylase	3-methyladenine
FaPy-DNA glycosylase	Formamidopyrimidine or 8-ox-G
PD-DNA glycosylase	Pyrimidine dimer

Glycosylase Associated with Lyase Activity



Some glycosylases also contain lyase activity. Glycosylase hydrolyzes the bond between base and deoxyribose (using H_20), but a lyase takes the reaction further by opening the sugar ring using an amino (NH_2) group to attack the deoxyribose ring.

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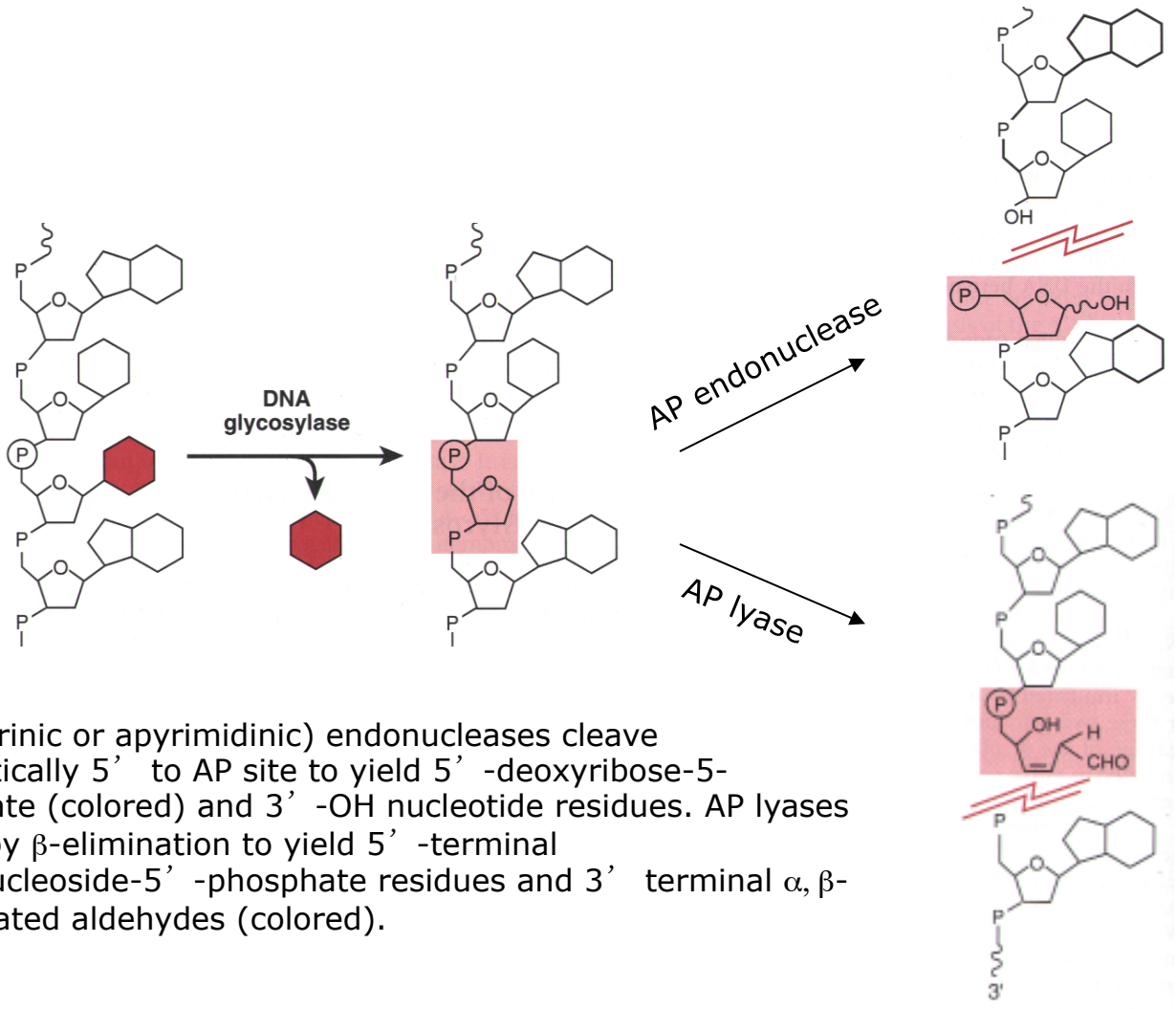
AP Endonuclease and Lyase



AP (apurinic or apyrimidinic) endonucleases cleave hydrolytically 5' to AP site to yield 5' -deoxyribose-5-phosphate (colored) and 3' -OH nucleotide residues. AP lyases cleave by β-elimination to yield 5' -terminal deoxynucleoside-5' -phosphate residues and 3' terminal α, β-unsaturated aldehydes (colored).

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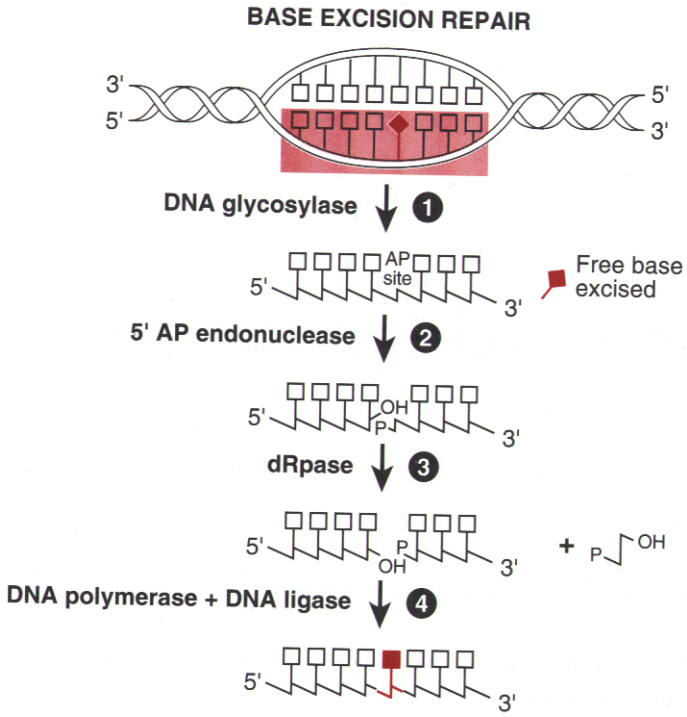
Base Excision Repair



DNA glycosylase removes the damaged base as a free base, generating another DNA damage called **apurinic** or **apyrimidinic** (**AP**) site. The removal of an AP site is initiated by a second class of BER enzymes called AP endonucleases, which specifically recognize AP site and produce incisions or nicks in duplex DNA by hydrolyzing the phosphodiester bonds immediately 5' the AP site, resulting in a 5' terminal deoxyribose-phosphate. An exonuclease called DNA **deoxyribophosphodiesterase** (**dRpase**) removes the sugar moiety. The single nucleotide gap can be filled by DNA polymerase.

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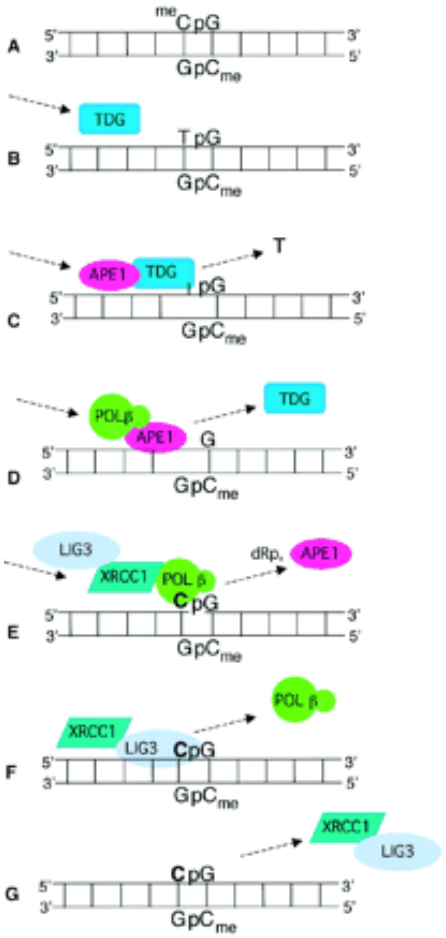
BER in Mammalian Cells



Single-nucleotide replacement pathway for BER. The example shown is for repair of a T residue arising when 5-methylcytosine (meC) in a CpG sequence (**A**) is deaminated (**B**). Thymine DNA glycosylase (TDG) removes the thymine and recruits the APE1 endonuclease (**C**). APE1 cleaves the chain on the 5' side of the abasic site and recruits POL β; TDG dissociates (**D**). POL β releases the remnant 5'-deoxyribosephosphate (dRp), inserts a C residue, and recruits the LIG3-XRCC1 complex (**E**). LIG3 seals the nick as POL β dissociates (**F**). The LIG3-XRCC1 complex is liberated (**G**). To restore the DNA to its original methylation state, a DNA methyltransferase would need to act on the newly synthesized C residue. Sequential binding of protein monomers would be expected to improve repair accuracy and specificity. Not shown here is an alternative longer patch BER pathway that can act after chain cleavage in step (D) and involves POL β, POL δ, or POL ε together with PCNA, FEN1, and LIG1

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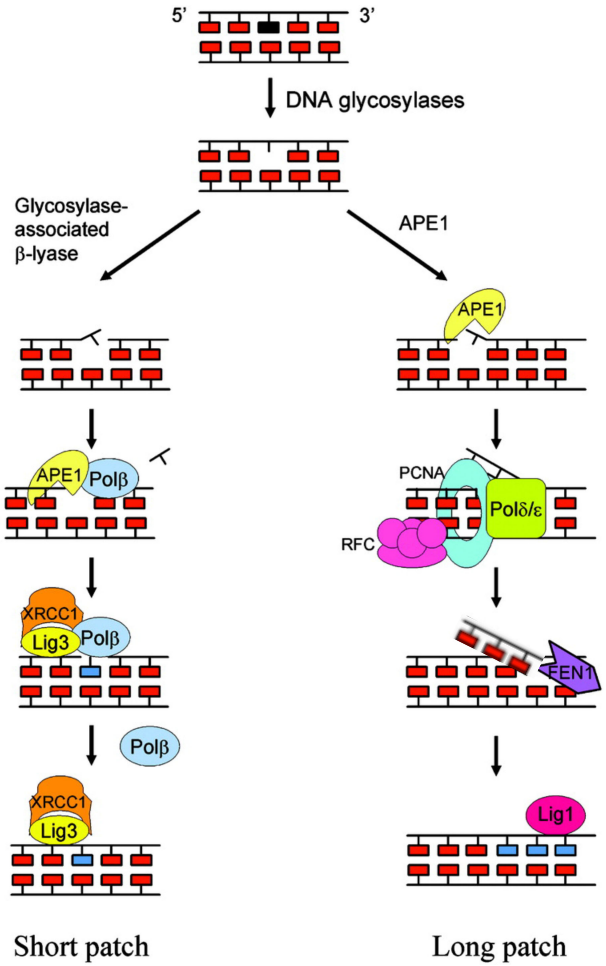
Short and long Patch BER



Base excision repair mechanisms in mammalian cells. A damaged base is removed by a DNA glycosylase to generate an AP site. Depending on the initial events in base removal, the repair patch may be a single nucleotide (short patch) or 2-10 nucleotides (long patch). When the base damage is removed by a glycosylase/AP lyase that cleaves the phosphodiester bond 3' to the AP site, APE1 endonuclease cleaves the 5' bond to the site and recruits Pol β to fill in a 1-nt gap that is ligated by Lig3/XRCC1 complex. When the AP site is generated by hydrolytic glycosylases or by spontaneous hydrolysis, repair usually proceeds through the long-patch pathway. APE1 cleaves the 5' phosphodiester bond, and the RFC/PCNA-Pol δ/ε complex carries out repair synthesis and nick translation, displacing several nucleotides. The flap structure is cleaved off by FEN1 endonuclease and the long-repair patch is ligated by Ligase 1.

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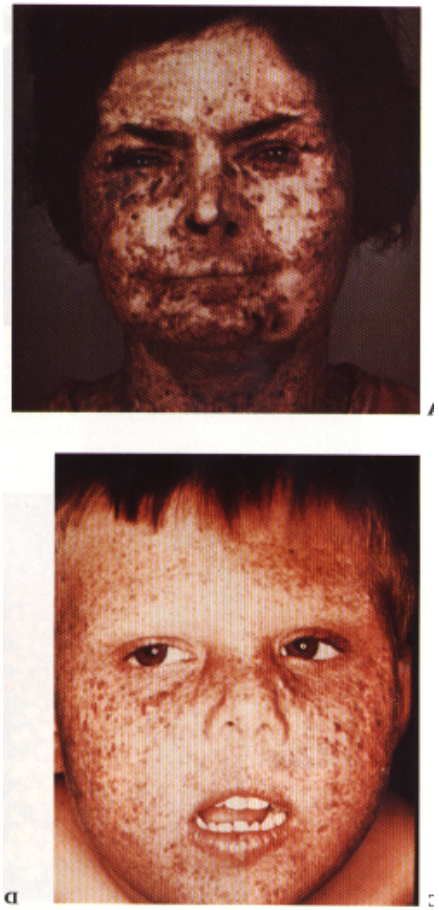
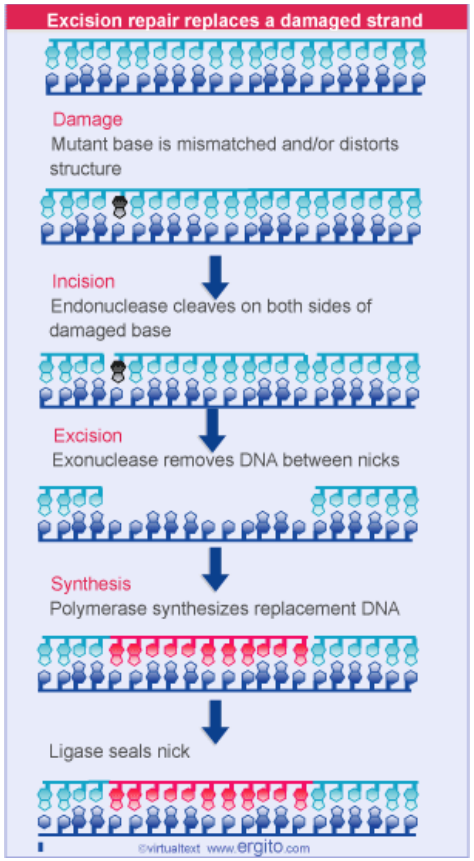
Defects in Nucleotide excision Repair Predispose to Cancer



Clinical features of xeroderma pigmentosum (**XP**). Upper, The disease typically presents with severe pigmentary disturbances on the sun-exposed areas of the skin. Lower, These clinical features can occur at a very early age.

Nucleotide Excision Repair in *E. coli*



Nucleotide excision repair (NER) removes and replaces a stretch of DNA that includes the damaged base(s).

In *E. coli*, the UvrABC system is responsible for NER.

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UvrABC Endonuclease

UvrA:

- 103 kDa
- DNA-independent ATPase
- DNA-damage binding activity
- Forms a dimer with UvrB

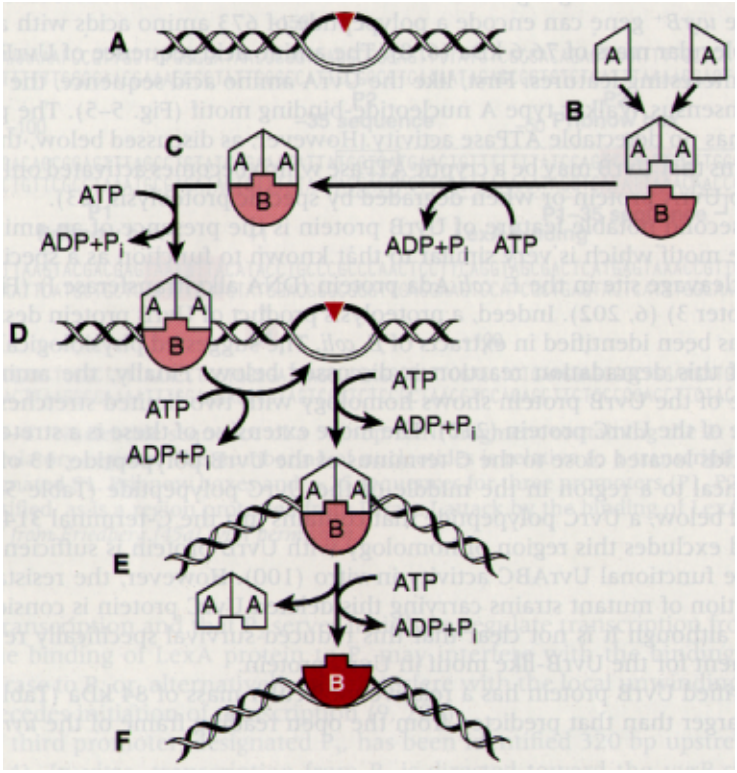
UvrB:

- 76 kDa
- Endonuclease activity
- Forms a dimer with UvrA

UvrC:

- 68 kDa
- Endonuclease activity
- Interacts with UvrB

Damage Recognition by UvrAB



A (**UvrA**)$_2$(**UvrB**)$_1$ complex forms (B and C) and initially binds to DNA at a site removed from the damage (D). The protein complex tracks along the DNA using a DNA helicase activity until it encounter the damage. **UvrA** protein dissociates from the complex, leaving a stable UvrB-DNA complex (F). This is associated with bending and kinking of the DNA.

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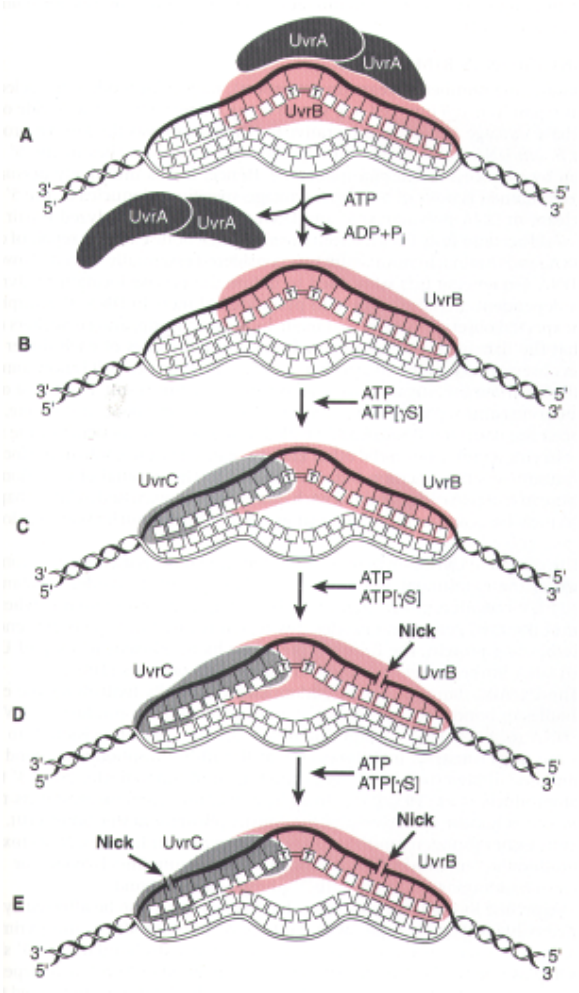
Incision of the Damaged Nucleotides by UvrBC



Following the formation of a stable **UvrB**-damaged DNA complex (A and B), **UvrC** protein binds at the site (C) and induces a conformational change which enables bound **UvrB** protein to nick the DNA 4 nucleotides 3' to the site of damage (D) (shown as a pyrimidine dimer). This reaction requires the binding of ATP (or ATP[γS]) by **UvrB** protein, but no ATP hydrolysis occurs at this step. Following the 3' incision, **UvrC** protein catalyzes nicking of the DNA 7 nucleotides 5' to the dimer(E).

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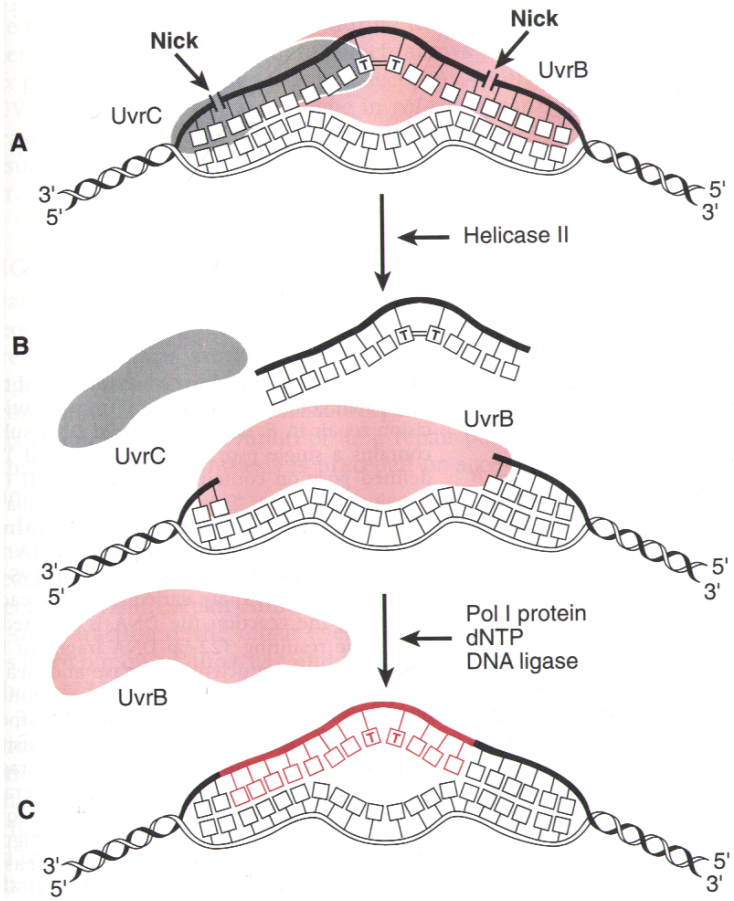
Repair Synthesis



DNA helicase II (**UvrD**) is required for the release of an oligonucleotide fragment following bimodal incision and for the replacement of **UvrC** protein (A and B). **UvrB** protein remains bound to the gapped DNA during the excision reaction and is released during the repair synthesis reaction catalyzed by **Pol I** (C). DNA ligation completes the nucleotide excision repair reaction.

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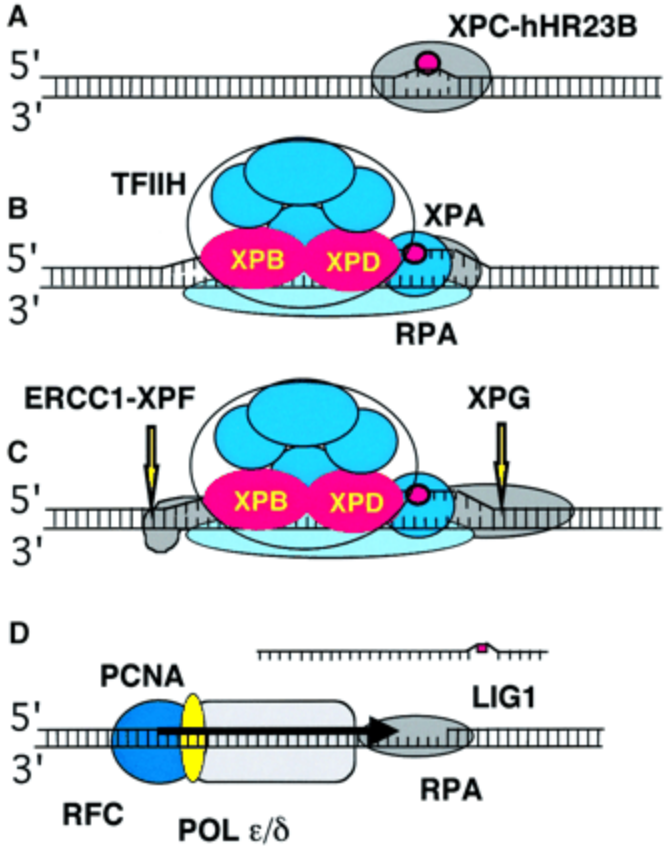
Nucleotide Excision Repair in Human Cells



There are 8 xeroderma pigmentosum (**XP**) genes, called XP complementation groups (from **XPA** to **XPG**, and **XPV**) DNA damage is recognized by the **XPC-hHR23B** protein (A). An open bubble structure is then formed around a lesion in a reaction that uses the ATP-dependent helicase activities of **XPB** and **XPD** (two of the subunits of **TFIIH**) and also involves **XPA** and **RPA** (B). Formation of this open complex creates specific sites for cutting on the 3' side by the **XPG** nuclease and then on the 5' side by the **ERCC1-XPF** nuclease(C). After a 24- to 32-residue oligonucleotide is released, the gap is filled in by **PCNA**-dependent **POL** ε or δ and sealed by a DNA ligase, presumably **LIG1**(D).

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Xeroderma Pigmentosum Complementation Group

XPA
XPB
XPC
XPD
XPE
XPF
XPG

Nucleotide excision repair genes

XPV (variant) — A DNA polymerase (*Pol*η) involved in **Translesion DNA Synthesis (TLS)**

Translesion DNA Synthesis: Replicative DNA synthesis is a faithful process that employs high-fidelity DNA polymerases that cannot deal with damage in the DNA template. Most DNA lesions can block the progress of the replication fork. To overcome such blocks, the cell uses specialized low-fidelity DNA polymerases, which synthesize DNA past lesions.

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Factors Involved in Human NER

Factor	Proteins (yeast homolog)	Activity	Role in repair
XPA	XPA/p31 (Rad14)	DNA binding	Damage recognition
RPA	p70	DNA binding	Damage recognition
	p32	Replication factor	
	p11		
XPC	XPC/p106 (Rad4)	DNA binding	Damage recognition
	HR23B/p58 (Rad23)		Molecular matchmaker
TFIIH	XPB/ERCC3/p89 (Rad25)	DNA-dependent ATPase	Unwinding the duplex Kinetic proofreading
	XPD/ERCC2/p80 (Rad3)	Helicase	
	p62 (Tfb1)	General transcription factor	
	p52 (Tfb2)		
	p44 (Ssl1)		
	p34 (Tfb4)		
XPG	XPG/ERCC5/p135 (Rad2)	Nuclease	3'-incision
XPF-ERCC1	XPF/ERCC4/p112 (Rad1)	Nuclease	5'-incision
	ERCC1/p33 (Rad10)		

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Translesion DNA Polymerases

Bacterial	Yeast (S. cerevisiae)	Humans	Polymerase Family
Pol II			B
Pol IV			Y
Pol V			Y
	Rev 1	Rev 1	Y
	Pol ζ	Pol ζ	B
	Pol η	Pol η	Y
		Pol ϰ	Y
		Pol ι	Y
		Pol λ	X
		Pol μ	X
		Pol β	X
		Pol θ	A
		Pol ν	A

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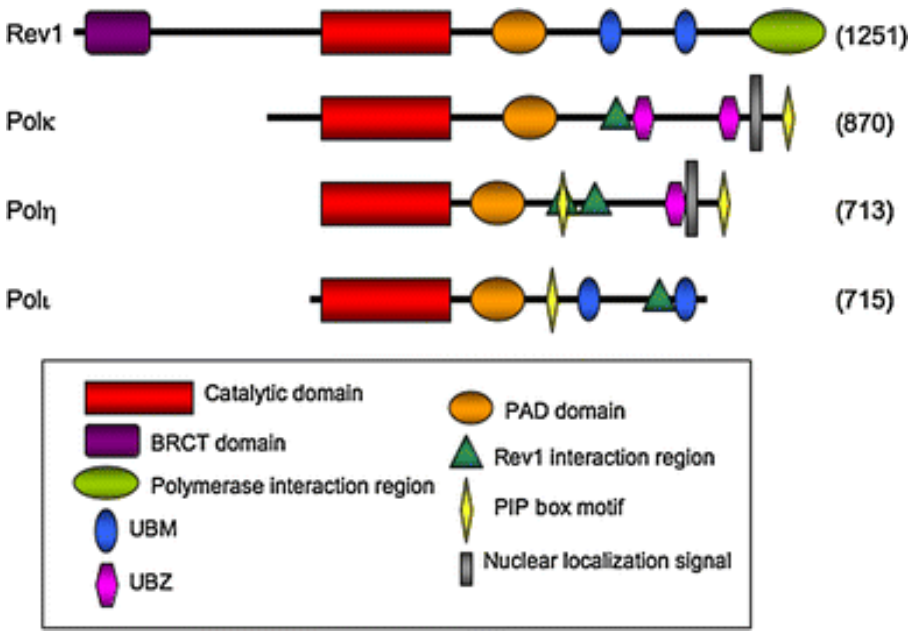
Biochemical Properties of Transletion DNA Polymerases

1. **Lacking 3’-5’ proofreading exonuclease activity**
2. **Synthesizing DNA in a distributive manner**
3. **Capable of both error-free and error-prone translesion synthesis, depending on the lesion**
4. **Synthesizing DNA from undamaged templates with extraordinarily low fidelity**

The structural domains of the Y-family polymerases



BRCT, BRCA1 C terminus-like domain.
UBM, ubiquitin binding motif.
UBZ ubiquitin binding zinc finger motif.
PAD polymerase associated domain.
NLS nuclear localization signal.
PIP PCNA interaction peptide.

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Model for Translesion Synthesis

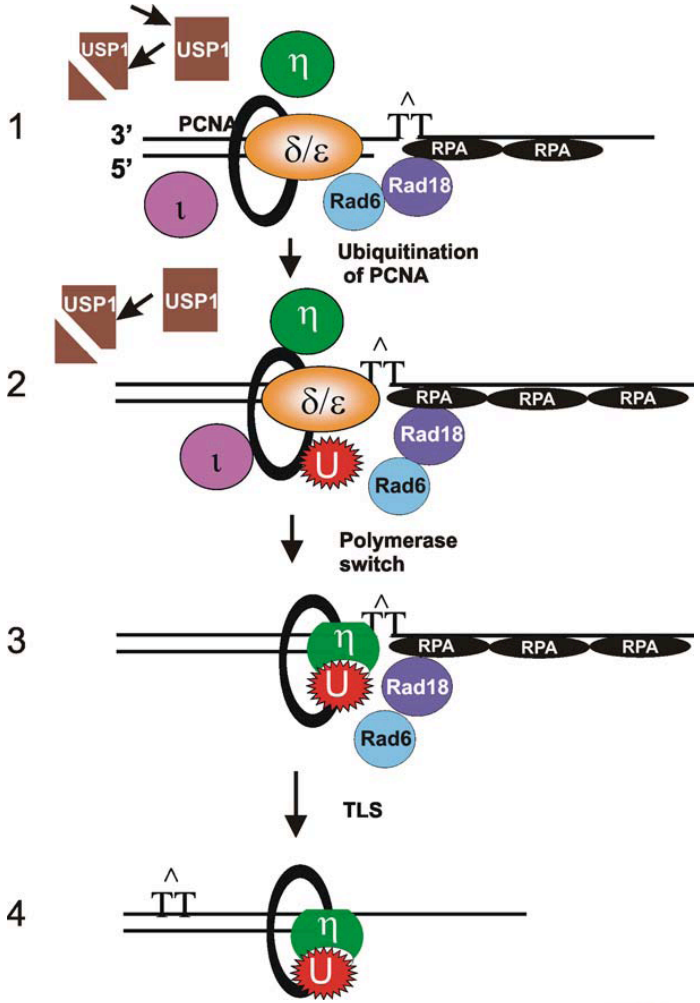
Model for translesion synthesis (TLS). (1) The replication machinery including PCNA and polδ or ε is stalled at a CPD.

(2) Exposure of single-stranded DNA coated with RPA recruits Rad18-Rad6, USP1 is cleaved and PCNA is mono-ubiquitinated. For clarity only one PCNA monomer is shown to be ubiquitinated, although in reality, all three monomers of one trimer are probably ubiquitinated.

(3) PCNA ubiquitination increases the affinity for Y-family polymerases, so polη is recruited.

(4) polη carries out TLS past the CPD.

Alan R. Lehmann



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DNA Damage and Repair Contents

- **DNA damage**
- **The DNA damage response**
- **DNA repair**
  - Direct reversal
  - Base excision repair
  - Nucleotide excision repair
  - Translesion synthesis
  - Mismatch repair
  - Double strand break repair
    - Recombination repair
    - Non-homologous end joining
  - Inter strand cross-link repair

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What is DNA Mismatch?

What is mismatch?

Non-Watson-Crick base pairs

Two classes of mismatches

Base-base mismatch

G-T, A-C, A-A, A-G, G-G, C-C, C-T, T-T

Insertion-deletion mismatch

```
                          CA
                          | |
——————— CACACACACACACA CACACACACACACACA ———————
——————— GTGTGTGTGTGTGT-GTGTGTGTGTGTGTCA ———————
```

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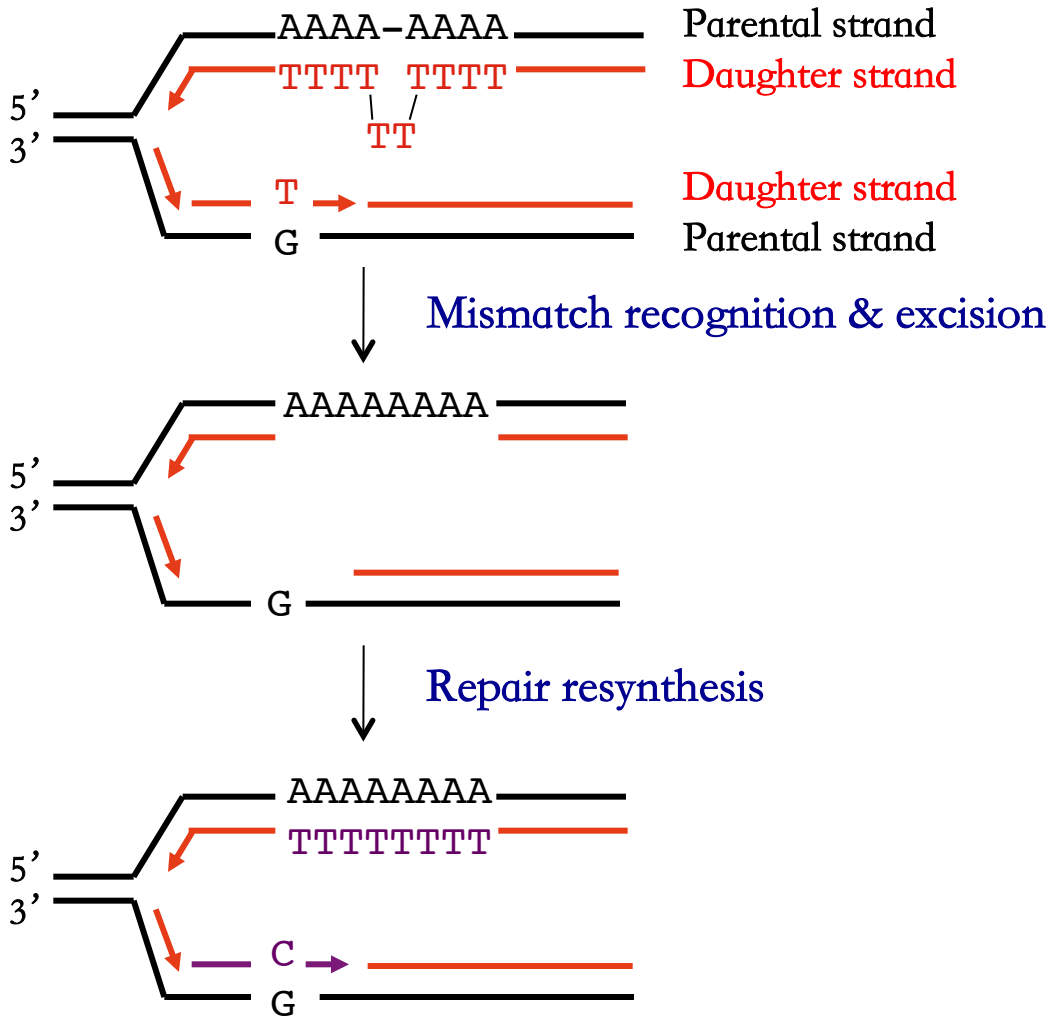
Mismatch Repair Ensures Replication Fidelity



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Key MMR Components

MutS:

97 kDa
Mismatch recognition protein
ATPase activity

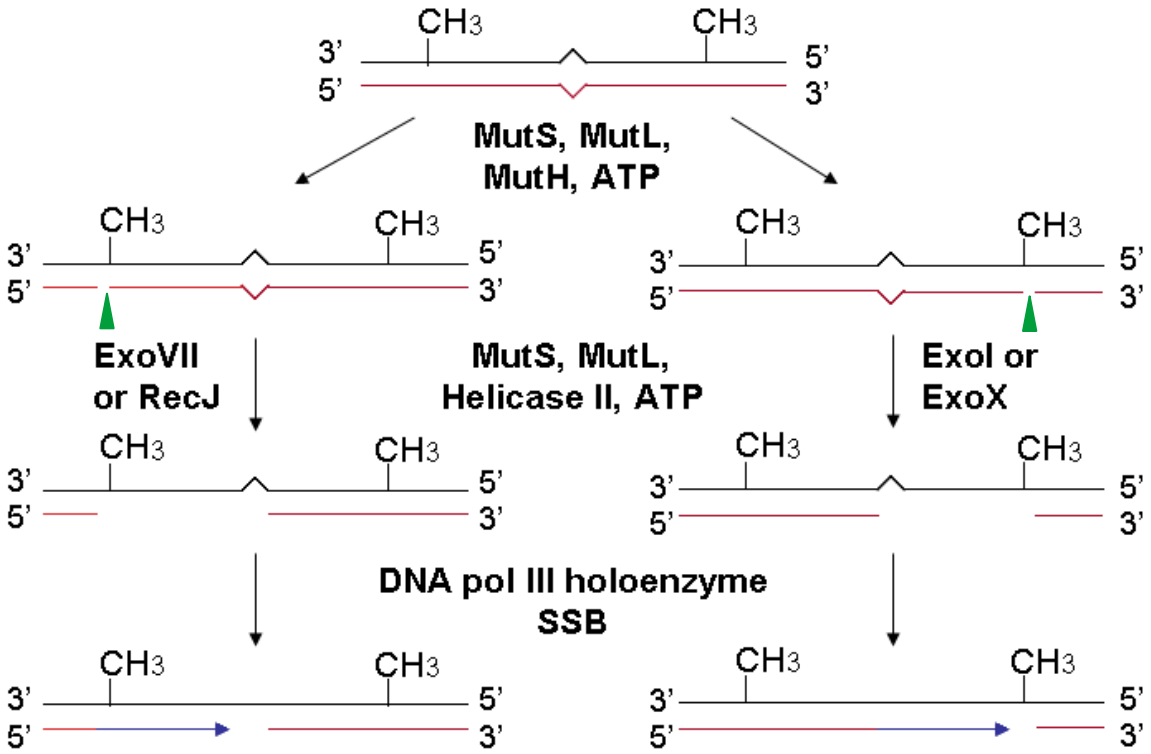
MutL:

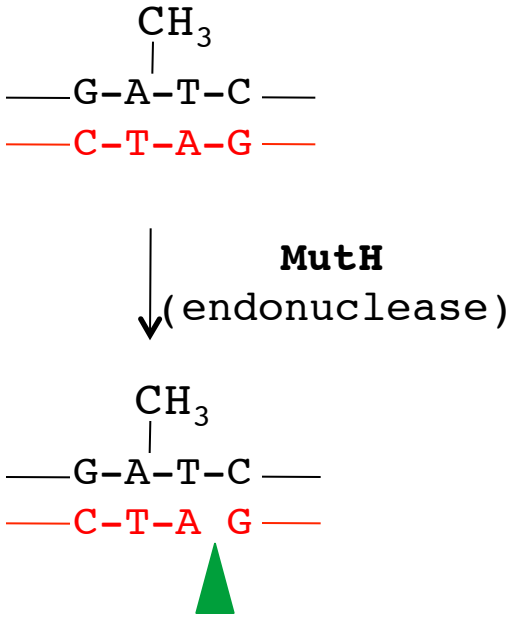
70 kDa
ATPase activity

MutH:

23 kDa
Endonuclease activity

Mismatch Repair In *E. coli*





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Characteristic of MMR

- MutSHL-dependent
- Bi-directional process
- Strand specificity (repair occurs only on the newly synthesized strand)

Mismatch Repair Components

E. coli	Human
MutS	MutSα, MutSβ
MutL	MutLα
MutH	?
UvrD	?
ExoI, ExoVII, ExoX, RecJ	ExoI
SSB	RPA
Pol III holoenzyme	Pol δ
DNA ligase	DNA ligase I
	PCNA RFC HMGB1

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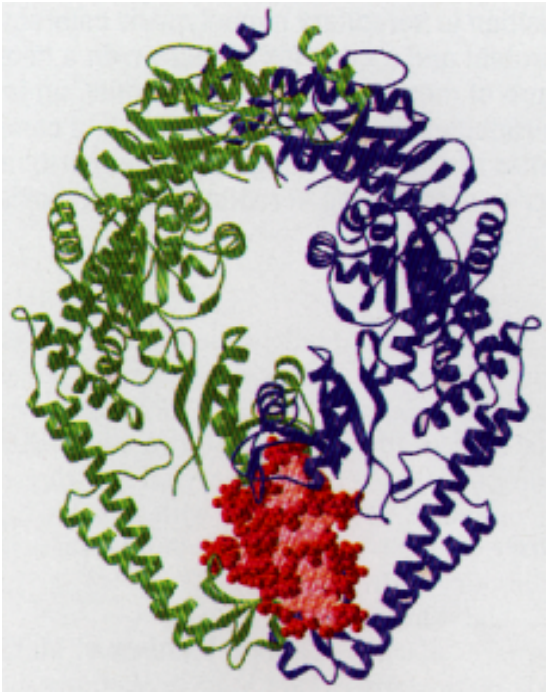
Human Mut Homologs

E. coli	Human
$(MutS)_2$	MutSα: MSH2, MSH3, MSH6; MutSβ: MSH2, MSH3
$(MutL)_2$	MutLα: MLH1, PMS1; MutLβ: MLH1, PMS1; MutLγ: MLH1, PMS2, MLH3
MutH	?

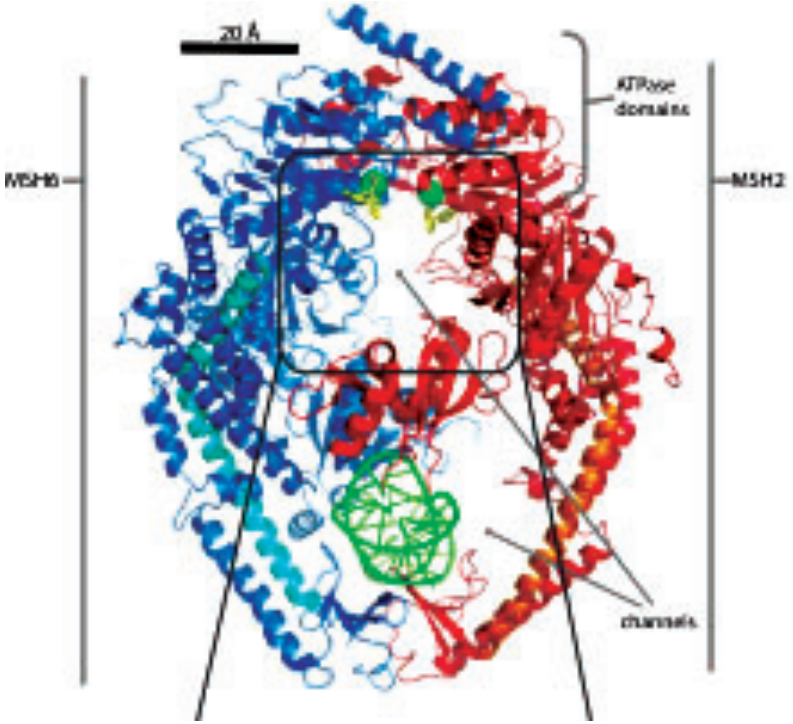
Crystal Structure of MutS

Bacteria



Nature 407: 703-710, 2000
Nature 407: 711-717, 2000

Human



Mol Cell 26: 579-92, 2007

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Microsatellite and Its Instability

Key Terms

Microsatellite:

Simple repetitive DNA sequences are called microsatellite.

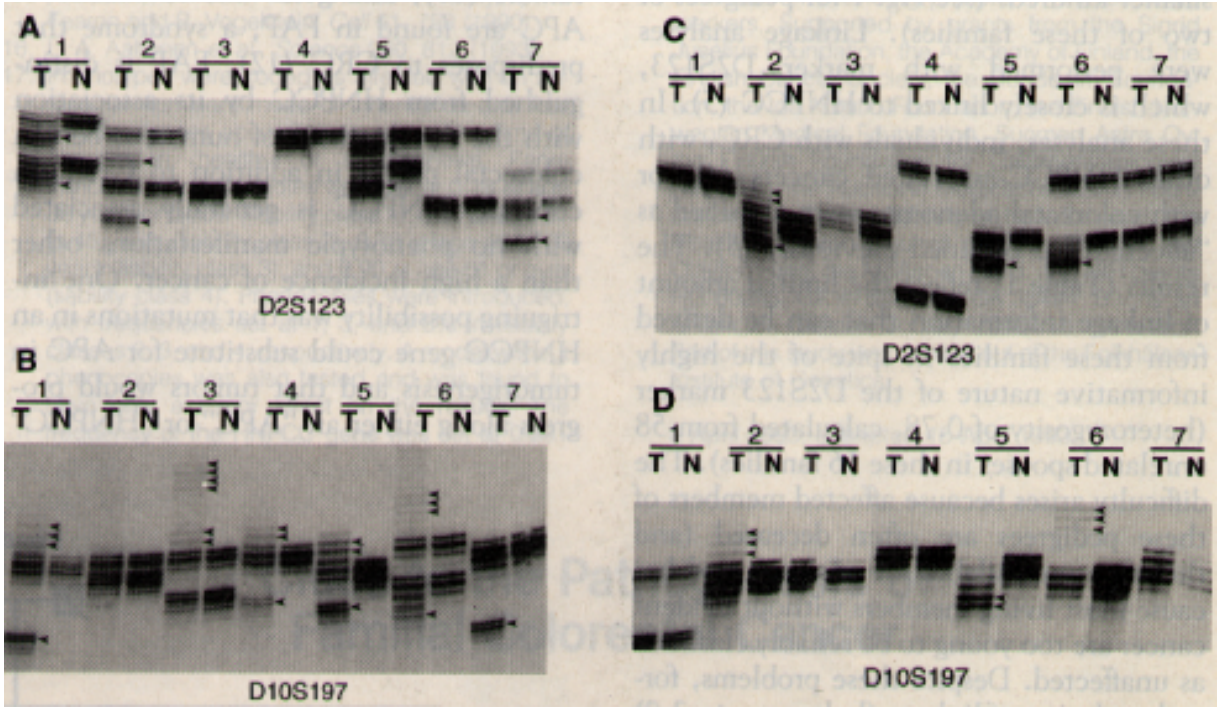
For example: $(A)_n$
$(CA)_n$
$(CAG)_n$
$(CACA)_n$

There are hundreds and thousands of microsatellite sequences in our DNA, and the are located mostly in non-coding regions.

Microsatellite instability (MSI):

Alterations in repeat numbers of microsatellite sequences from one generation to another.

Microsatellite Instability in Cancer



Dinucleotide repeat polymorphisms in normal (N) and tumor (T) tissue from patients with hereditary non-polyposis colorectal cancer (HNPCC) (A and B) and patients with sporadic colorectal cancer (C and D). Genomic DNAs were amplified by PCR using microsatellite markers D2S123 abd D10S197, and the products were separated in 6% polyacrylamide gels.

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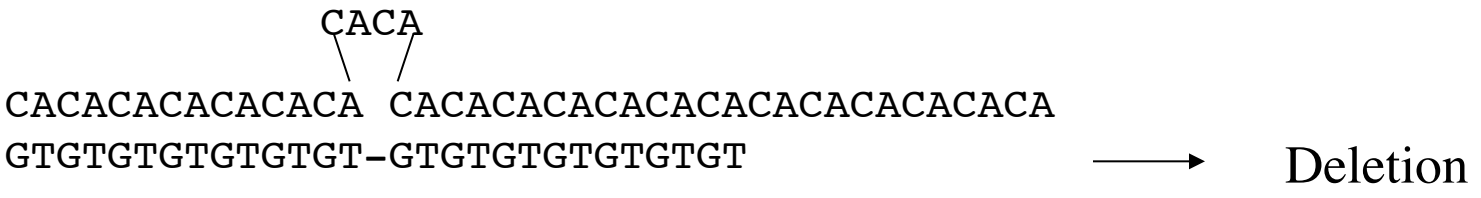
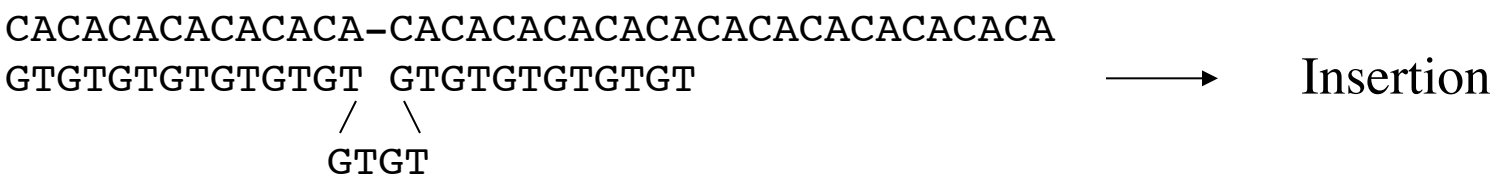
Mechanism of MSI

Loop out in the template strand



Loop out in the primer strand



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Mismatch Repair Defects Cause Cancer

		HNPCC[a]	Sporadic
Population incidence		~1 in 500	1 in 20
Micro**S**atellite **I**nstability		>90%	13-20%
MMR gene mutations		80%?	~65% of CRC with **MSI**
MutS homologs	MSH2	~40%	30%
	MSH3	0%	
	MSH6	~0.5%	
MutL homologs	MLH1[b]	~50%	70%
	PMS1	<5%	
	PMS2		
	MLH3		

[a] Hereditary Nonpolyposis Colorectal Cancer, also called Lynch Syndrome

[b] Hypermethylation of the *MLH1* promoter silences MLH1 expression.

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MutSα Binds to Damaged DNA

O^6-methylguanine (MNNG, procarbazine, temozolomide)

Cisplatin adducts

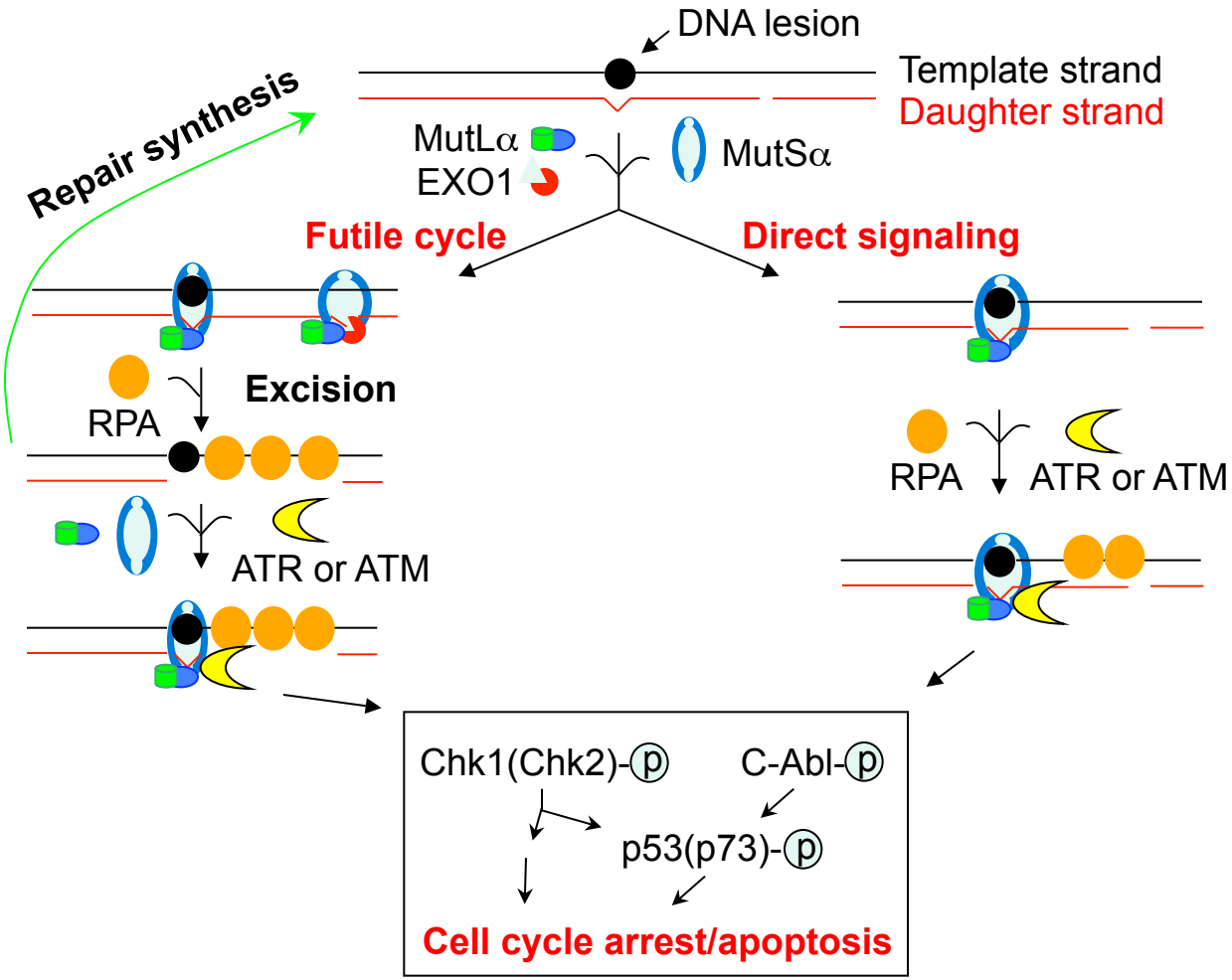
8-Hydroxyguanine (oxidative damage)

Aminofluorene adducts

Adducts of polycyclic aromatic hydrocarbon (B[a]P, B[c]Phe)

UV dimer

Mechanism of MMR-Mediated Apoptosis



The futile DNA repair cycle model (left) suggests that DNA adducts (solid black circle) induce misincorporation, which triggers the MMR reaction. Since MMR only targets the newly synthesized strand for repair, the offending adduct in the template strand cannot be removed, and will provoke a new cycle of MMR upon repair resynthesis. Such a futile repair cycle persists and activates the ATR and/or ATM signaling network to promote cell cycle arrest and/or apoptosis. The direct signaling model proposes that recognition of DNA adducts by MSH-MLH complexes allows the proteins to recruit ATR and/or ATM to the site, activating the downstream damage signaling.

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Functions of MMR

- Repair function

  Correcting heteroduplexes

- Apoptosis function

  Eliminating damaged cells from body by promoting apoptosis

Tumor Suppression of MMR

	Wild-type cells	MMR mutant cells
Biosynthetic errors	Correction	Hypermutable
DNA damage	Apoptosis	Survival with mutation
	⇩	⇩
Consequence	Mutation-free genome	Tumorigenesis

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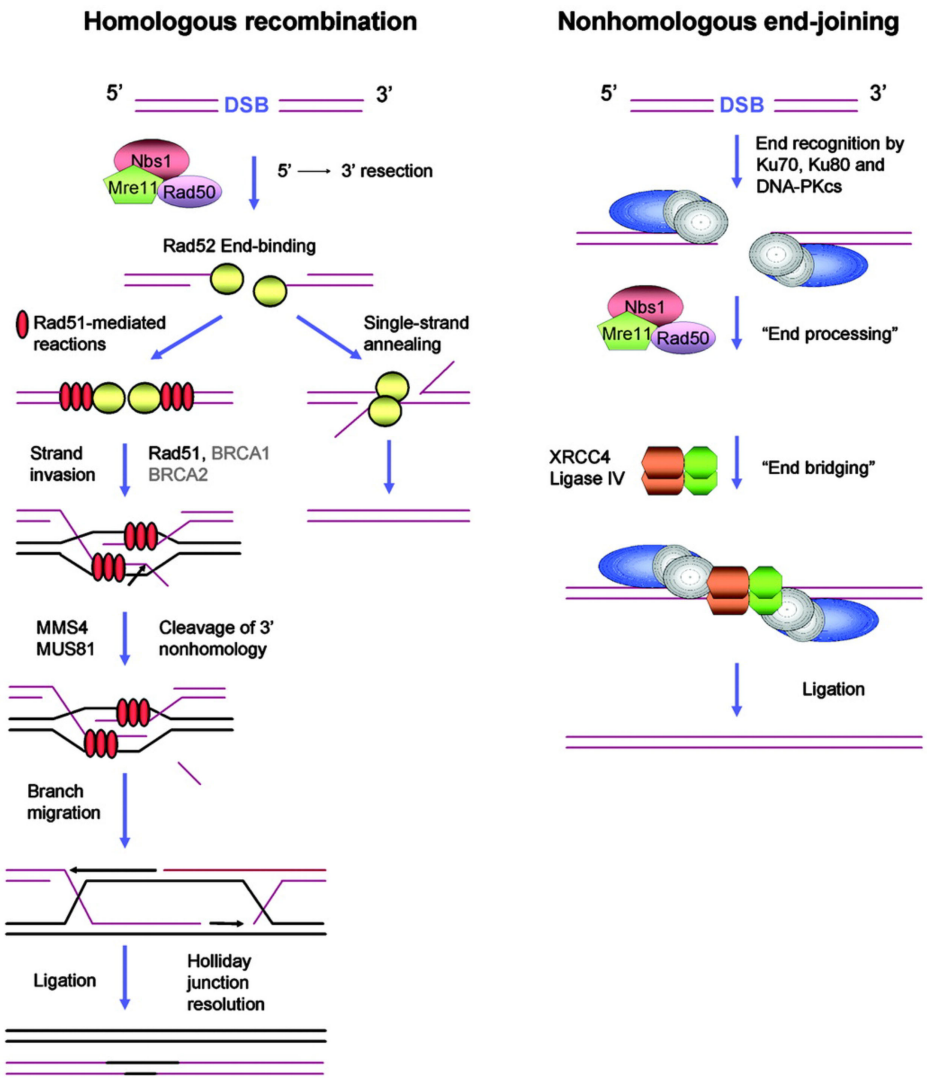
Double Strand Break Repair



Double-strand break/recombinational repair. Double-strand breaks are repaired by either homologous recombination, which in eukaryotes depends on the Rad51-family proteins-orthologs of the bacterial RecA recombinase, or by nonhomologous end-joining mediated by the DNA-PK complex. A key intermediate in homologous recombination is the Holliday intermediate, in which the two recombining duplexes are joined covalently by single-strand crossovers. Resolvases such as MUS81·MMS4 cleave the Holliday junctions to separate the two duplexes. In the single-strand annealing (SSA) mechanism, the duplex is digested by a 5' to 3' exonuclease to uncover microhomology regions that promote pairing, trimming, and ligation. BRCA1 and BRCA2 are also involved in homologous recombination, but their precise roles are unclear.

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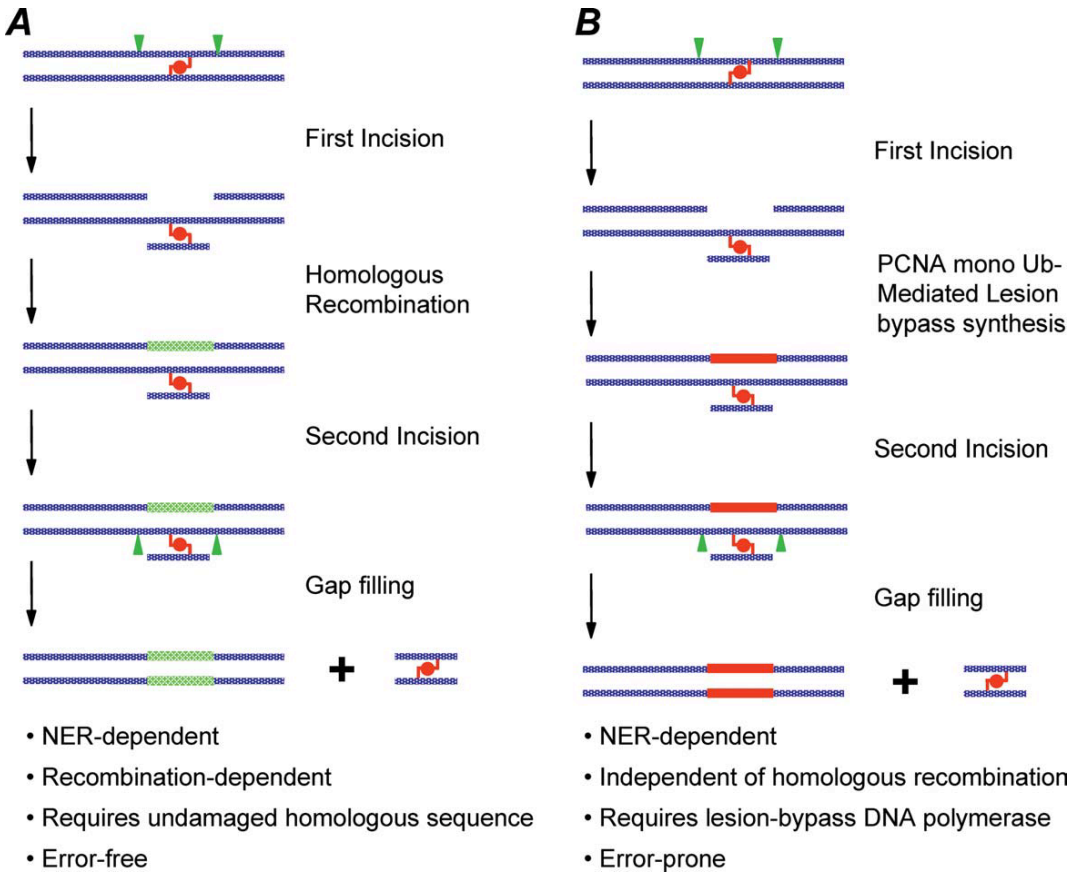
Models for Inter-Strand Cross-link Repair



Models and their characteristics of ICL repair in Escherichia coli and eukaryotes. A, The Cole's model depicting homology-dependent error-free repair of ICLs. B, Recombination-independent ICL repair. ' ' ' ' indicates crosslinking moiety. Green arrows indicate positions of NER incision. DNA patches in green depicting error-free repair synthesis. DNA patches in red depicting error-prone repair synthesis.

Shen and Li, *Environ Mol Mutagen*. 51(6):493-9, 2010.

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