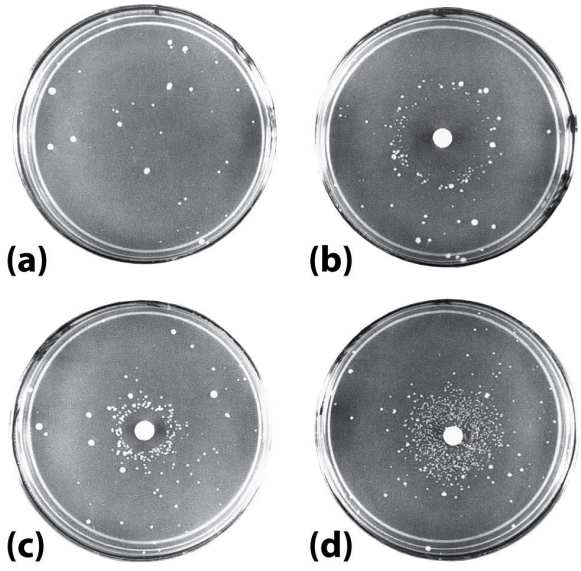
Chapter 25.2: Outline

1. Sources and types of DNA damage
   - cellular consequences

2. Types of DNA repair pathways
   - Mismatch repair
   - Base-excision repair
   - Nucleotide-excision repair
   - Direct repair
   - Error-prone repair

3. DNA repair and cancer

Ames Test for mutagenicity



Salmonella strain:
- his$^-$
- membrane defect
- liver homogenate

Figure 25-21
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Sources of DNA damage

Ionizing radiation- γ-rays and X-rays

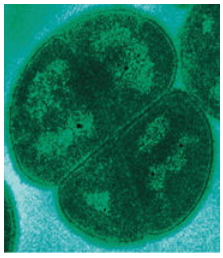
UV radiation- UVC very potent, UVB (blocked by O_3), UVA indirectly through ROS

Oxygen radicals (ROS)- generated by UV, leaked from mitochondria, other cellular processes

Chemicals- environmental
hydrocarbons- particularly cigarette smoke
aflatoxin- peanut mold

Chemotherapy- by design cause DNA damage

"Superbug"…



Deinococcus radiodurans

Gram-positive bacterium

So-called polyextremophile
survives: dehydration, radiation, acid, vacuum

Withstand 5000 Gy dose with no loss in viability
10 Gy is lethal to humans!

How?
Not really known but some unusual DNA repair features
- multiple copies of genome
- DNA repair different, more rapid (RecA)

Types of DNA damage that are observed

Mismatch

Mostly replication errors

Base covalent modification

Spontaneous: C to U, A to I, G to X
Chemical induced: oxidation, alkylation

Breaks

single or double strand (radiation, chemicals)

Crosslinks

intrastrand vs interstrand (radiation, chemicals, chemotherapy)

DNA lesions can be mutagenic

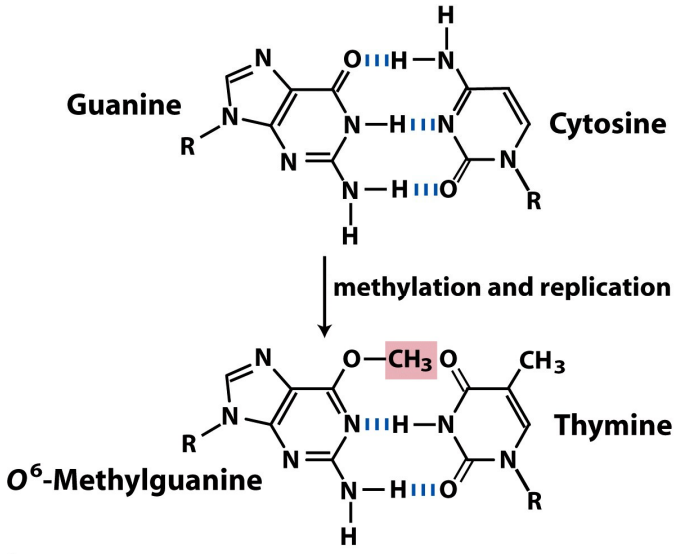


Figure 25-28a
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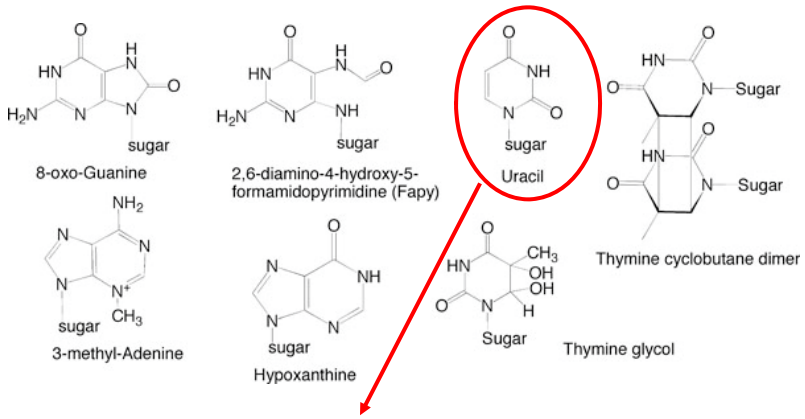
others just block replication, transcription

Multiple types of DNA lesions



Why do cells go to so much trouble to avoid U in DNA??

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DNA repair systems

Strategy:

Recognize
Remove
Repair

} Question: Energy conservation vs. Fidelity?

Most take advantage of presence of a <u>complementary strand</u> (use undamaged DNA to correctly repair)

If no complementary strand (ds breaks, breaks in replication fork)- recombination (Ch 25.3)

Types of DNA repair systems

1. Mismatch repair (replication errors)

<u>Recognize-</u>
Problem: how does cell know correct nucleotide?

1st evidence: mix DNA strands from dam+/dam- strains

System in *E. coli*: recognizes methyl strand, corrects the other one

E. coli mismatch repair system

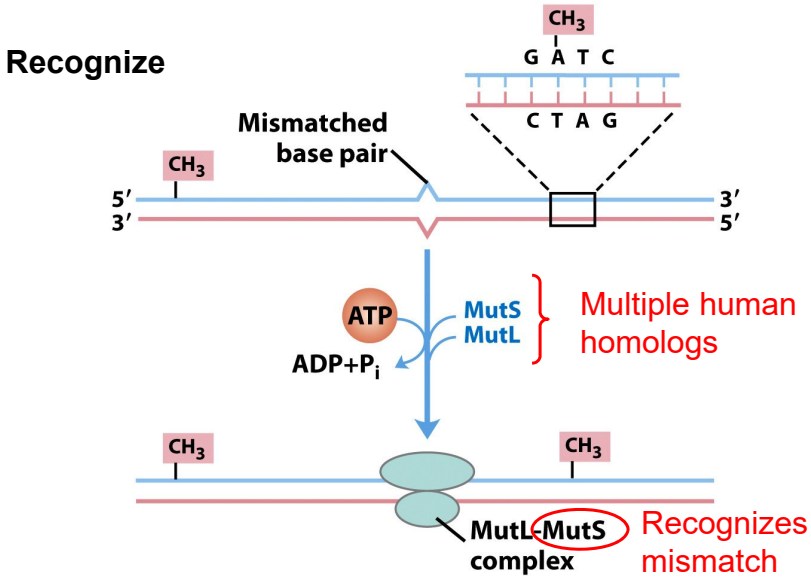

Figure 25-23 part 1
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E. coli mismatch repair system

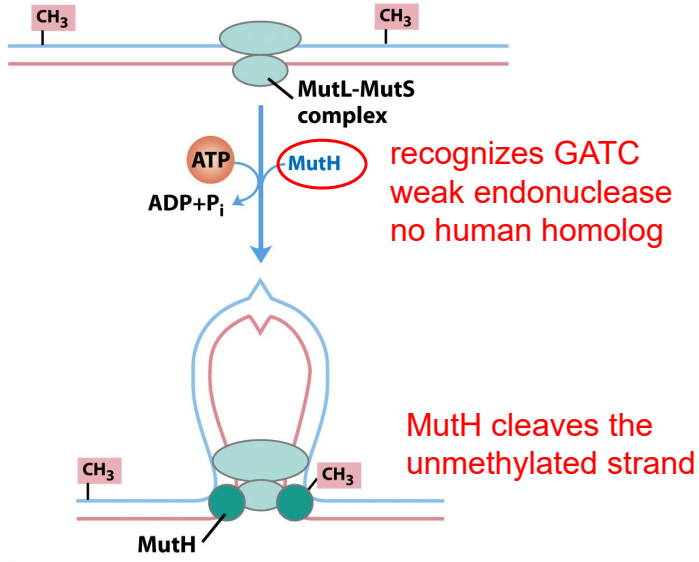

Figure 25-23 part 2
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E. coli mismatch repair system

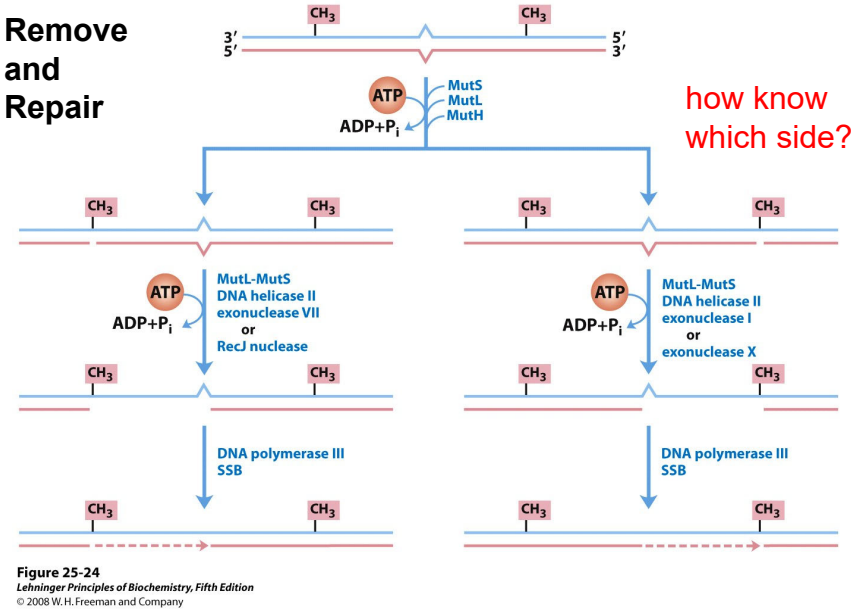

Figure 25-24
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Types of DNA repair systems

2. Base-excision repair

Recognize specific lesions

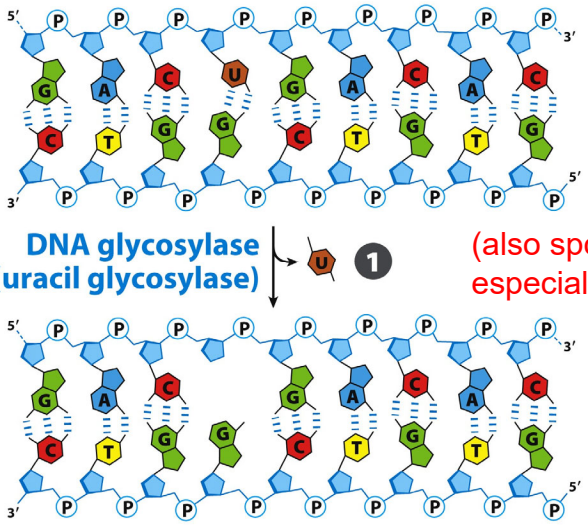
- Spontaneous deamination (C to U)
- Damaged bases (8-oxoG, HX, alkylation)

Important features:

- DNA *N*-glycosylases: recognize and remove
  - Uracil N-glycosylase (Ung)
- Create AP site (Apurinic, apyrimidinic)
- AP endonuclease: cut DNA at site
- Repair: DNAPol I in *E. coli*, euks other pols

DNA glycosylase causes formation of AP site

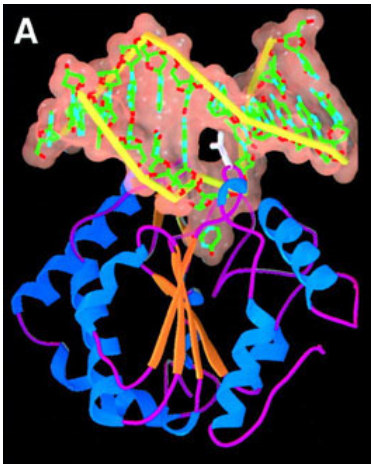
Recognize



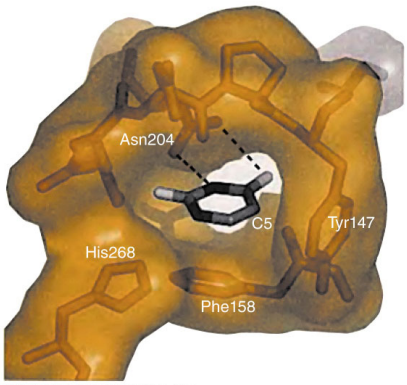
(also spontaneous, especially purines)

DNA glycosylases recognize specific lesions

Human UNG and DNA duplex with U-G mismatch



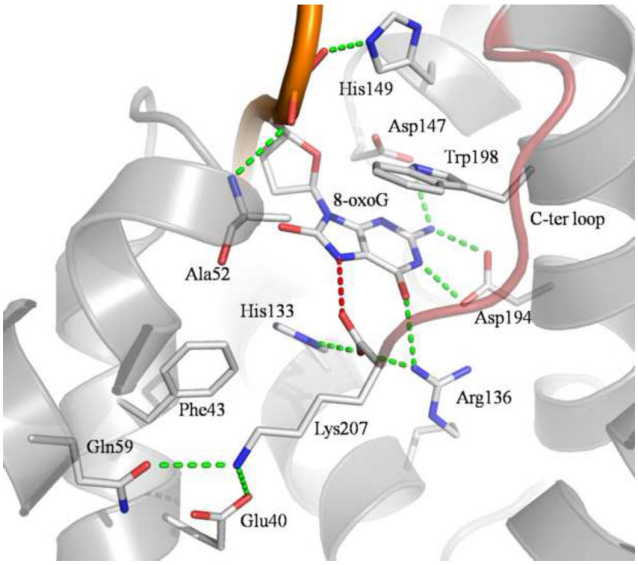
Parikh et al, EMBO J, 1998



Appling Biochemistry, Fig 23.10

DNA glycosylases recognize specific lesions

MjaOgg2 with DNA duplex containing 8-oxoG lesion



Faucher, Wallace and Doublié JMB 2010

Base excision repair in *E. coli*

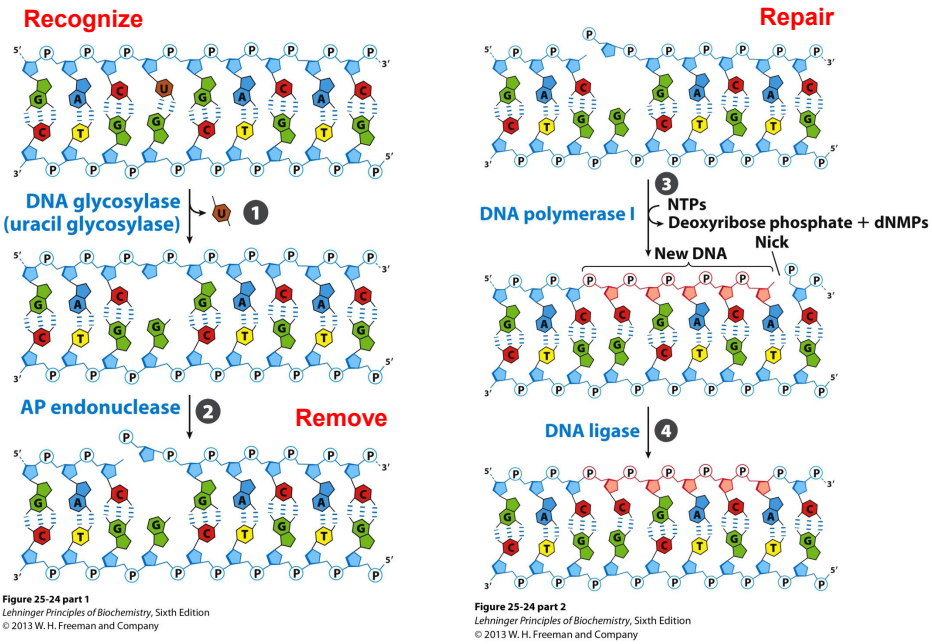


Figure 25-24 part 1
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Figure 25-24 part 2
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Types of DNA repair systems

3. Nucleotide-excision repair

Generally removal of larger lesions (distort structure)- cyclopyrimidine dimers, base adducts

Important features:

Dual cleavage sites (on either side of damage)

Recognize: Excinuclease in all organisms

E.coli: UvrA,B,C

human: 16 polypeptides

Remove (helicase) and repair (polymerase)

Nucleotide-excision repair in *E. coli*

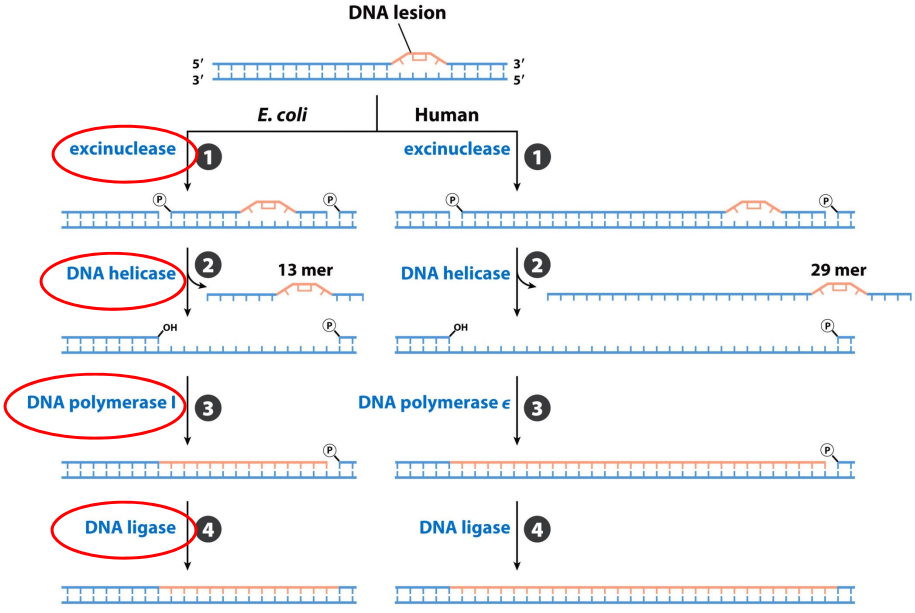

Figure 25-25
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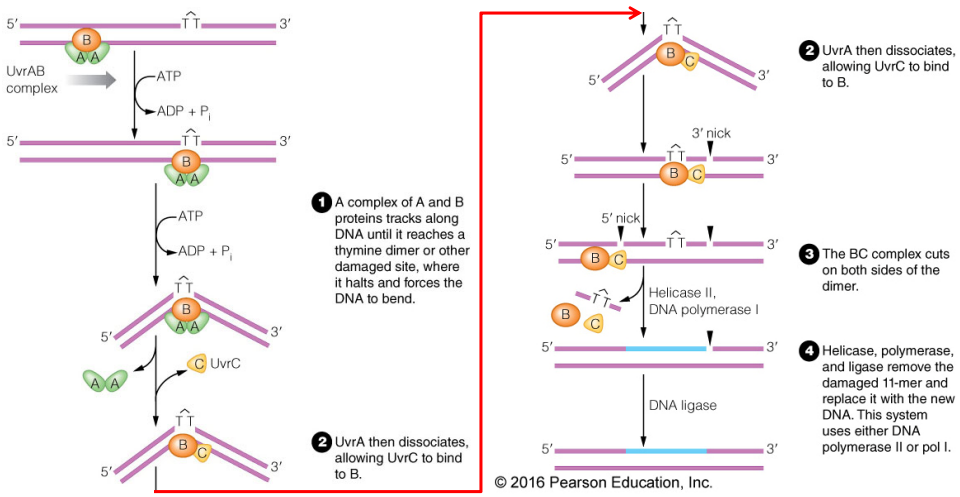
UvrABC excinuclease in *E. coli*



Appling Biochemistry, Fig 23.7

Types of DNA repair systems

4. Direct repair

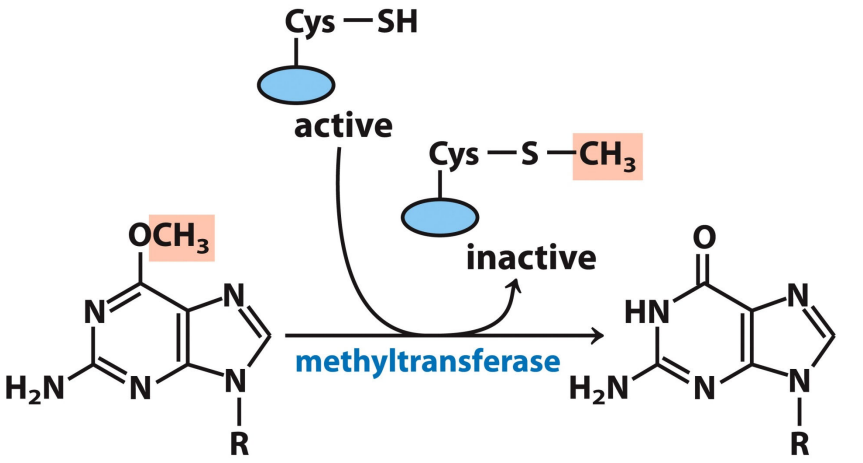
Mechanism to repair without removal (and new DNA synthesis)

<u>Important features:</u>

specific enzymes for each type of damage

- DNA photolyases (not in humans)
  - UV induced pyrimidine dimers
  - energy from hv→ THF → radical
- Methyltransferases (AlkA, AlkB)
  - m^1A, m^3C by AlkB
  - O^6-meG by MGMT

MGMT is a "suicide enyzme"



O^6-Methylguanine nucleotide → **Guanine nucleotide**

Don't need to know exact names, but know types of proteins involved

TABLE 25–5 Types of DNA Repair Systems in *E. coli*

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase MutH, MutL, MutS proteins DNA helicase II SSB DNA polymerase III Exonuclease I Exonuclease VII RecJ nuclease Exonuclease X DNA ligase	Mismatches
Base-excision repair	
DNA glycosylases AP endonucleases DNA polymerase I DNA ligase	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
Nucleotide-excision repair	
ABC excinuclease DNA polymerase I DNA ligase	DNA lesions that cause large structural changes (e.g., pyrimidine dimers)
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine
AlkB protein	1-Methylguanine, 3-methylcytosine

Table 25-5
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Types of DNA repair systems

5. Error-prone repair (translesion synthesis,TLS)

ds breaks, interstrand cross links: template damaged too- replication fork can't proceed

<u>Important features:</u>
Two methods to deal with this situation:
1) Recombination (Ch. 25.3)
2) TLS- less accurate synthesis

SOS response: LAST resort to avoid death
existing proteins (UvrABC) activated
new proteins (UmuC,D)→ Pol V
Mutations result! (fidelity ~ 1 error/10^3 bp)

The need for error prone TLS

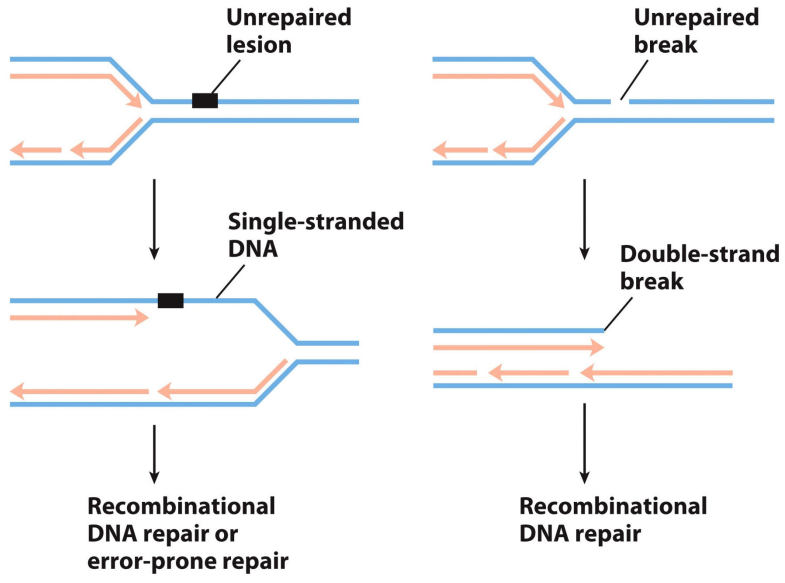

Figure 25-29
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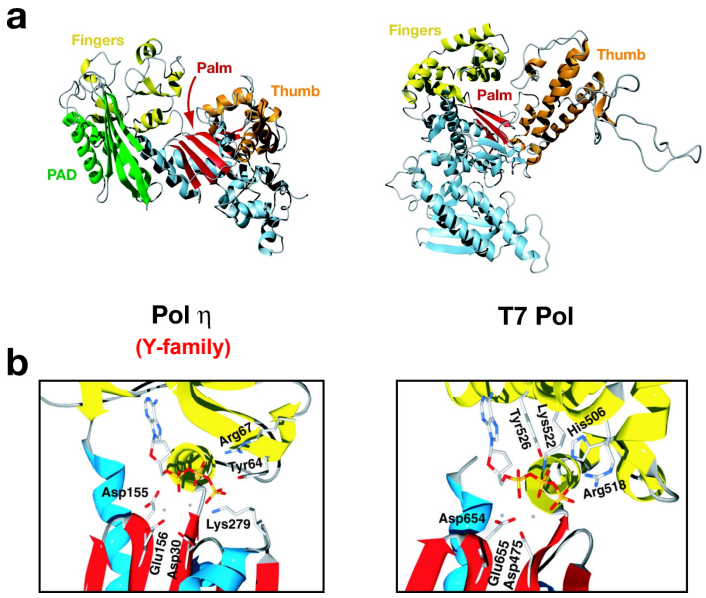
How do TLS polymerases bypass lesions?



Prakash, Johnson, Prakash, Ann Rev Biochem 2005

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DNA repair and cancer

Mutagens are often carcinogens

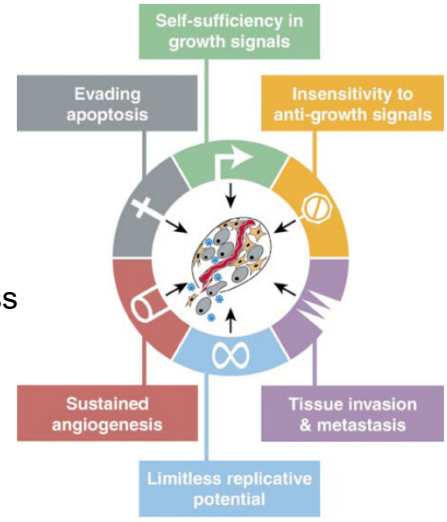
Hereditary predisposition to cancer:

- Loss/damage of one copy is hereditary
- Subsequent somatic loss of 2nd allele over lifetime is required
- Several documented hereditary predispositions

Still does NOT cause majority of cancers

Alterations to genes involved with normal cell physiology can lead to cancer

Conundrum: mutation is an inefficient process



Hanahan and Weinberg, Cell (2000), The Hallmarks of Cancer 100:57-70

DNA repair and cancer

HNPCC- most common known
- defects in hMLH and hMSH: mismatch repair (*h*uman *M*ut*L* *H*omolog, etc)
- tumor cells from patients impaired mismatch repair

BRCA1/BRCA2
- huge proteins: implicated in repair (homologous recombination), among MANY other functions

Xeroderma Pigmentosum genes (XP)
- nucleotide excision repair genes (pyr dimers especially)

DNA repair and cancer

Unwanted side effects of chemotherapy:
many chemotherapy drugs are potent mutagens

cyclophosphamide	Cytoxan	Crosslinks (inter/intra), alkylation
melphalan	Alkeran	
busulfan	Myleran	
chlorambucil	Leukeran	
mitomycin	Mutamycin	
cisplatin	Platinol	→ Crosslinks
bleomycin	Blenoxane	→ Strand breaks

Ch 25.2: Recap

DNA damage has important cellular consequences
- mutations of many types from many sources

DNA repair pathways exist in all organisms to correct damage
- a great deal of redundancy
- "efficiency" not necessarily the goal

Defects in DNA repair processes have important consequences for human disease (cancer)