DNA damage and repair summary

1. Defects in repair cause disease
2. Common types of DNA damage
3. DNA repair pathways
   - Direct enzymatic repair
   - Base excision repair
   - Nucleotide excision repair
   - Mismatch repair
   - Double-strand break repair
     - Non-homologous end joining
     - Homologous recombination

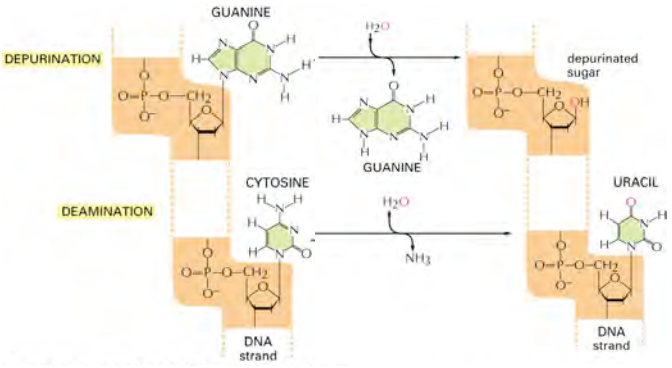
DNA repair defects cause disease

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

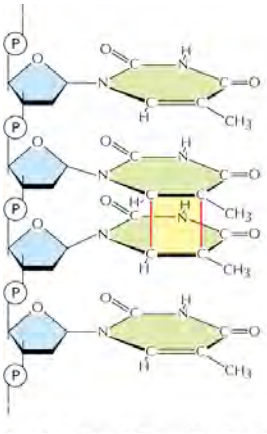
SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411**:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509**:49.

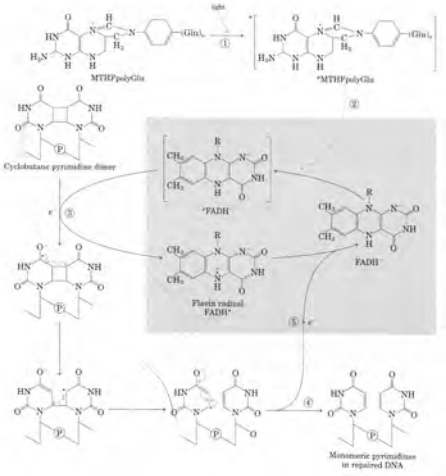
Common types of DNA damage -- 1



1. Depurination: A, G
2. Deamination: C --> U, A --> Hypoxanthine

Common types of DNA damage -- 2



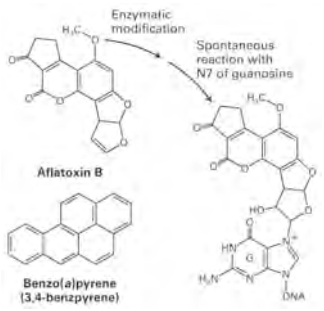


Pyrimidine dimers (UV induced).

Repair pathways

Common types of DNA damage -- 3

Two carcinogens that mutate (the P53 gene) by base alkylation



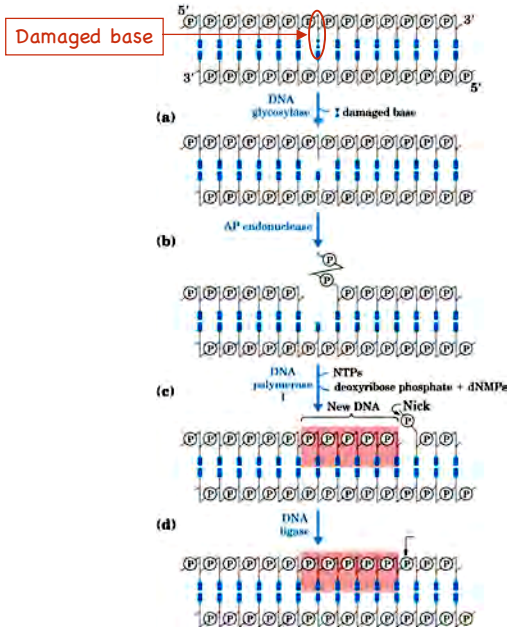
+ Mismatches (mistakes in DNA synthesis)
Interstrand cross-links,
Double-strand DNA breaks

Total damage from all mechanisms: 10^4 - 10^6 lesions/day!

Diverse DNA repair systems

- Augment DNA polymerase proofreading
- Mostly characterized in bacteria
- General mechanisms shared in eukaryotes
    1. Direct repair, e.g. pyrimidine dimers
    2. Base excision repair
    3. Nucleotide excision repair
    4. Mismatch excision repair
    5. Double-strand break repair and recombination

Base excision repair



Base excision repair pathway (BER).
(a) A DNA glycosylase recognizes a damaged base and cleaves between the base and deoxyribose in the backbone.

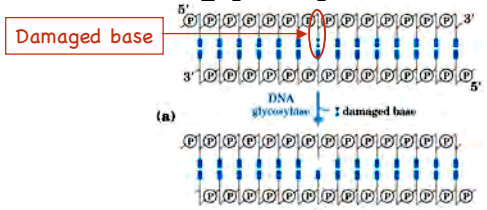
(b) An AP endonuclease cleaves the phosphodiester backbone near the AP site.

(c) DNA polymerase I initiates repair synthesis from the free 3′ OH at the nick, removing a portion of the damaged strand (with its 5′→3′ exonuclease activity) and replacing it with undamaged DNA.

(d) The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.

AP= apurinic or apyrimidinic
(a=without)

A DNA glycosylase initiates base excision repair



Examples of bases cleaved by DNA glycosylases:

Uracil (deamination of C)

8-oxoG paired with C (oxidation of G)

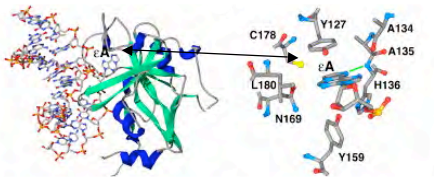
Adenine across from 8-oxoG (misincorporation)

Thymine across from G (5-meC deamination)

Alkyl-adenine (3-meA, 7-meG, hypoxanthine)

Human alkyl-adenine DNA glycosylase

DNA bent & modified base flipped out of duplex -- "Non-Watson-Crick" structure



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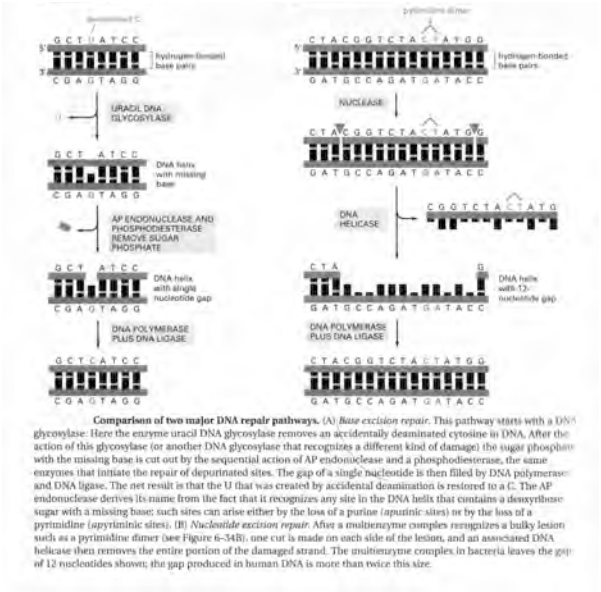
Two pathways of increasing complexity

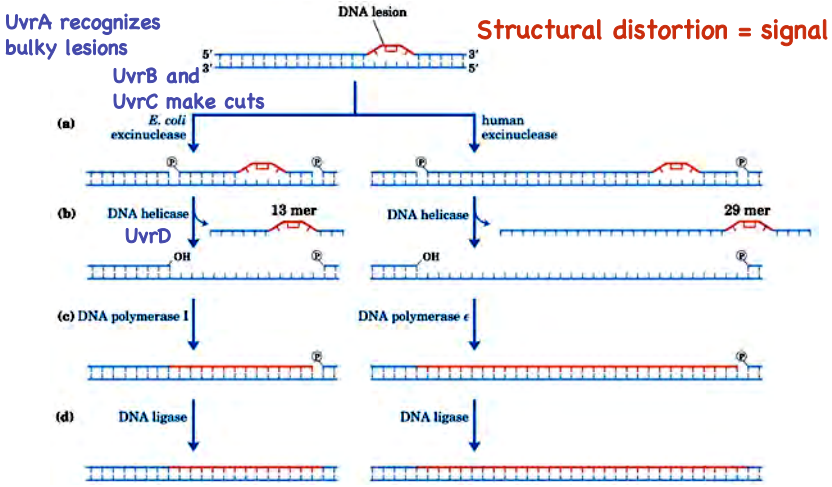
Base Excision repair



Comparison of two major DNA repair pathways. (A) *Base excision repair.* This pathway starts with a DNA glycosylase. Here the enzyme uracil DNA glycosylase removes an accidentally deaminated cytosine in DNA. After the action of this glycosylase (or another DNA glycosylase that recognizes a different kind of damage) the sugar phosphate with the missing base is cut out by the sequential action of AP endonuclease and a phosphodiesterase, the same enzymes that initiate the repair of depurinated sites. The gap of a single nucleotide is then filled by DNA polymerase and DNA ligase. The net result is that the U that was created by accidental deamination is restored to a C. The AP endonuclease derives its name from the fact that it recognizes any site in the DNA helix that contains a deoxyribose sugar with a missing base; such sites can arise either by the loss of a purine (apurinic sites) or by the loss of a pyrimidine (apyrimidinic sites). (B) *Nucleotide excision repair.* After a multienzyme complex recognizes a bulky lesion such as a pyrimidine dimer (see Figure 6-34B), one cut is made on each side of the lesion, and an associated DNA helicase then removes the entire portion of the damaged strand. The multienzyme complex in bacteria leaves the gap of 12 nucleotides shown; the gap produced in human DNA is more than twice this size.

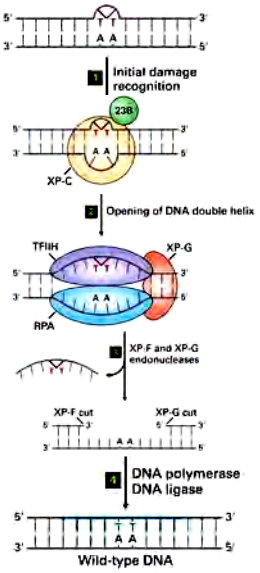
Nucleotide Excision repair

Nucleotide excision repair



(a) Two excinucleases (excision endonucleases) bind DNA at the site of bulky lesion. (b) One cleaves the 5′ side and the other cleaves the 3′ side of the lesion, and the DNA segment is removed by a helicase. (c) DNA polymerase fills in the gap and (d) DNA ligase seals the nick.

Nucleotide excision repair -- eukaryotes



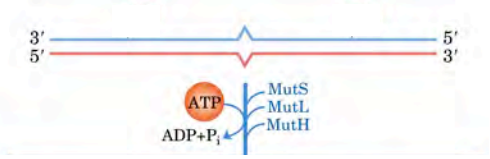
Mutations in any of at least seven genes, *XP-A* through *XP-G*, cause an inherited sensitivity to UV-induced skin cancer called xeroderma pigmentosum. The XP proteins are among >30 required for nucleotide excision repair.

Diverse DNA repair systems

- Augment DNA polymerase proofreading
- Mostly characterized in bacteria
- General mechanisms shared in eukaryotes
    1. Direct repair, e.g. pyrimidine dimers
    2. Base excision repair
    3. Nucleotide excision repair
    4. Mismatch excision repair -- replication errors
    5. Double-strand break repair and recombination

Mismatch repair

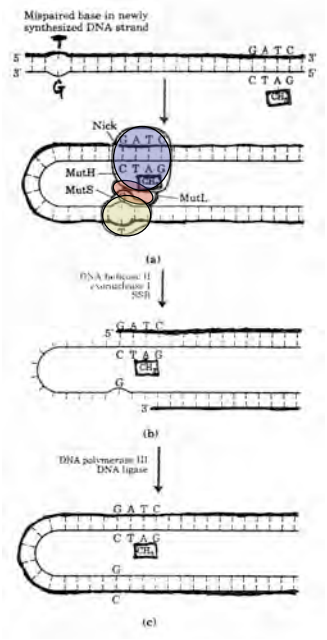
Which strand is new and which is the parent?



Mut S binds mismatch
Mut L links S to H
Mut H recognizes the parental strand

Mismatch repair

Which strand is new and which is the parent?
The mutation is in the new strand!
-CH3 marks the parental strand!



A model for methyl-directed mismatch repair. The proteins involved in this process in *E. coli* have been purified (see Table 24–5). Recognition of the sequence GATC and of the mismatch are specialized functions of the MutH and MutS proteins, respectively. **(a)** The MutL protein links the MutH and MutS proteins together in a complex. The MutH protein cleaves the unmethylated strand on the 5′ side of the G in the GATC sequence. **(b)** The combined action of DNA helicase II, exonuclease I, and SSB then removes a segment of the new strand between the cleavage site and a point just beyond the mismatch. **(c)** The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase.

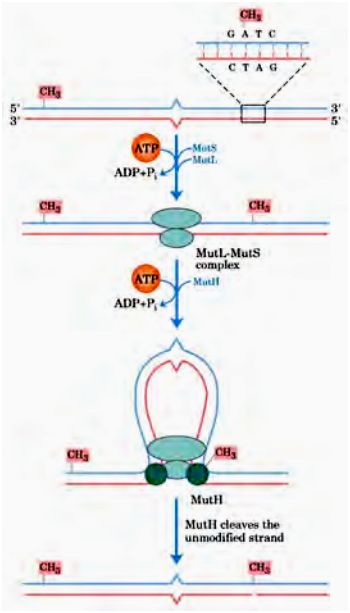
MutH - Binds 7-meGATC
MutS - Binds mismatch
MutL - links MutH and MutS

Mismatch repair -- Recognition

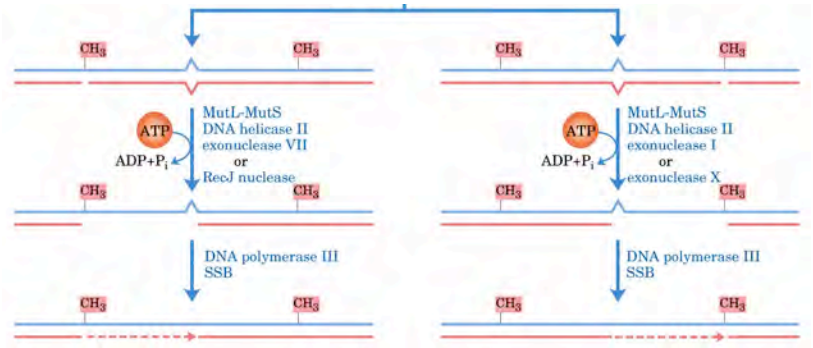
Which strand is new and which is the parent?
The mutation is in the new strand!
A-CH3 marks the parental strand!

MutS - Binds mismatch
MutL - links MutH and MutS
MutH - Binds G^{me}ATC

DNA is threaded through the MutS/MutL complex. The complex moves simultaneously in both directions along the DNA until it encounters a MutH protein bound at a hemimethylated GATC sequence. MutH cleaves the unmethylated strand on the 5′ side of the G in the GATC sequence.



Mismatch repair -- Resolution



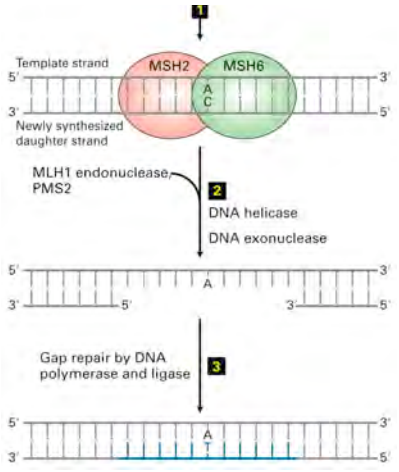
1. The combined action of DNA helicase II, SSB, and one of many different exonucleases (only two are labeled) removes a segment of the new strand between the MutH cleavage site and a point just beyond the mismatch.
2. The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase.

Mismatch repair -- Hereditary Non-Polyposis Colon Cancer (HNPCC) gene (Humans)

HNPCC results from mutations in genes involved in DNA mismatch repair, including:

- several different MutS homologs
- Mut L homolog
- other proteins: perhaps they play the role of MutH, but not by recognizing hemi-methylated DNA (no 6meA GATC methylation in humans, no dam methylase)

Mismatch repair -- MSH proteins -- eukaryotes



Defects in mismatch excision repair lead to colon and other cancers.

1. MSH2:MSH6 complex binds the mismatch and identifies newly synthesized strand.
2. MLH1 endonuclease and other factors such as PMS2 bind, recruiting a helicase and exonuclease, which together remove several nucleotides including the lesion.
3. The gap is filled by Pol δ and sealed by DNA ligase.

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- Augment DNA polymerase proofreading
- Mostly characterized in bacteria
- General mechanisms shared in eukaryotes
    1. Direct repair, e.g. pyrimidine dimers
    2. Base excision repair
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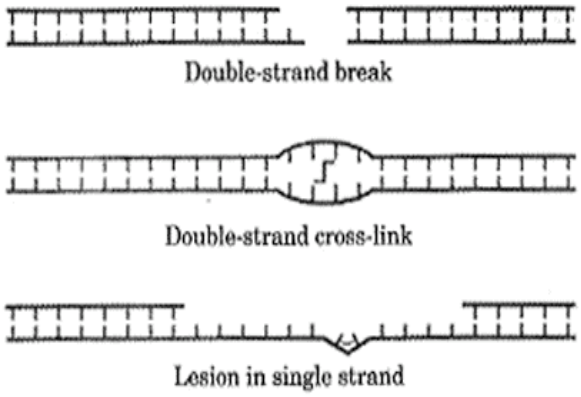
    4. Mismatch excision repair -- replication errors
    5. Double-strand break repair and recombination

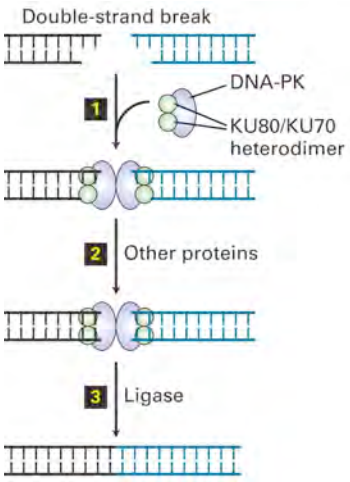
Double-strand break repair



NO TEMPLATE FOR REPAIR!!

Double-strand break repair

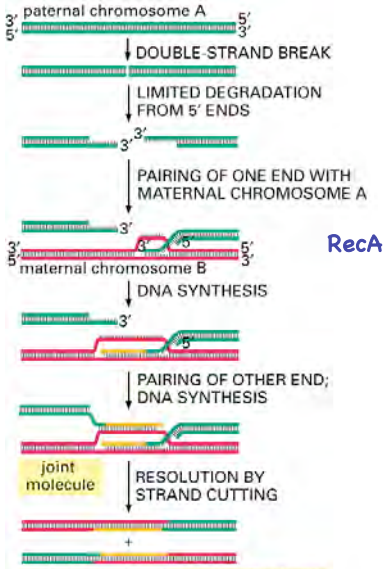
Two basic mechanisms: End-joining and Recombination



The end-joining pathway of ds break repair is mutagenic, because it removes several base pairs at the break site.

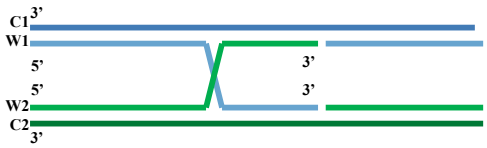
Mediated by Ku proteins.

Double-strand break repair -- Homologous recombination pathways



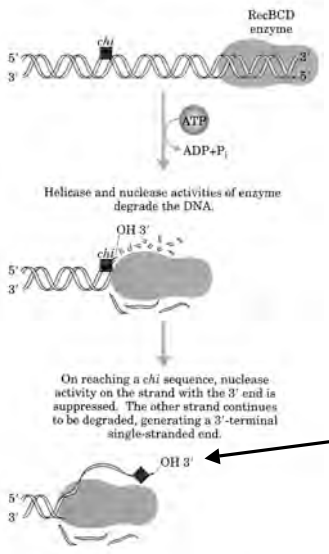
RecA

RecBCD



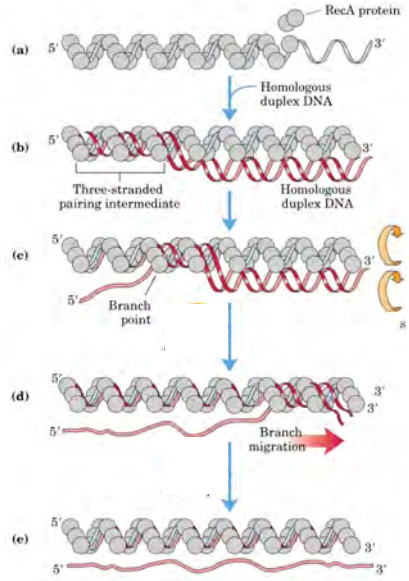
Strand exchange with nicks

RecBCD helicase/nuclease in bacteria



RecBCD recognizes ends and unwinds and degrades DNA until it encounters a *chi* site. Nuclease activity is suppressed on that strand, generating a ssDNA 3′ overhanging end that initiates recombination.

RecA mediates strand exchange



Model for 3-strand strand exchange reaction.

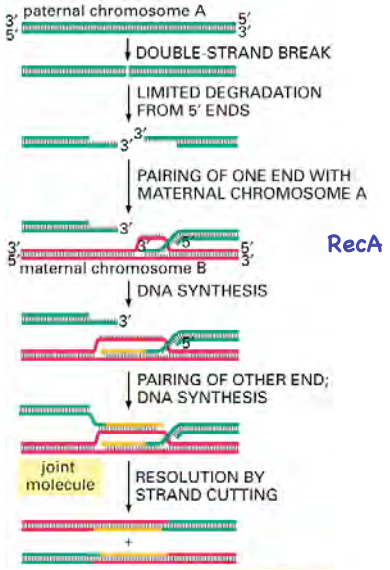
(a) RecA protein forms a filament on the single-stranded DNA.

(b) A homologous duplex incorporates into this complex.

(c) One of the strands in the duplex is transferred to the single strand originally bound in the filament.

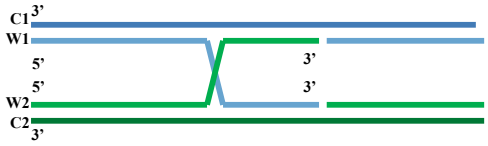
(d) The other strand of the duplex is displaced.

Double-strand break repair -- Homologous recombination pathways



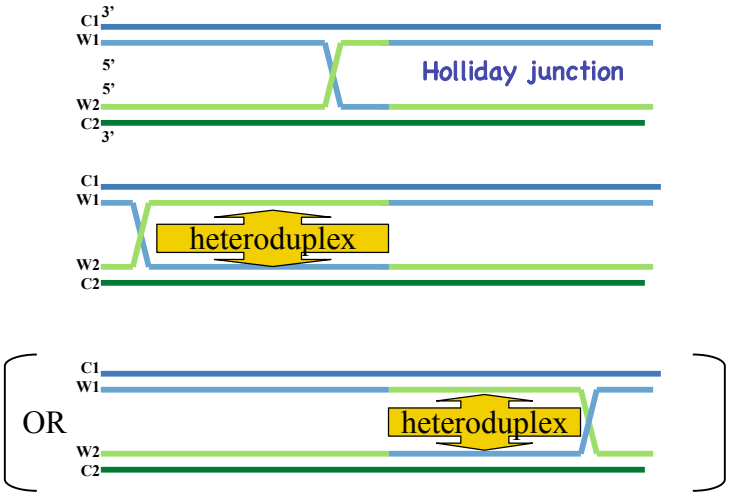
RecA

RecBCD

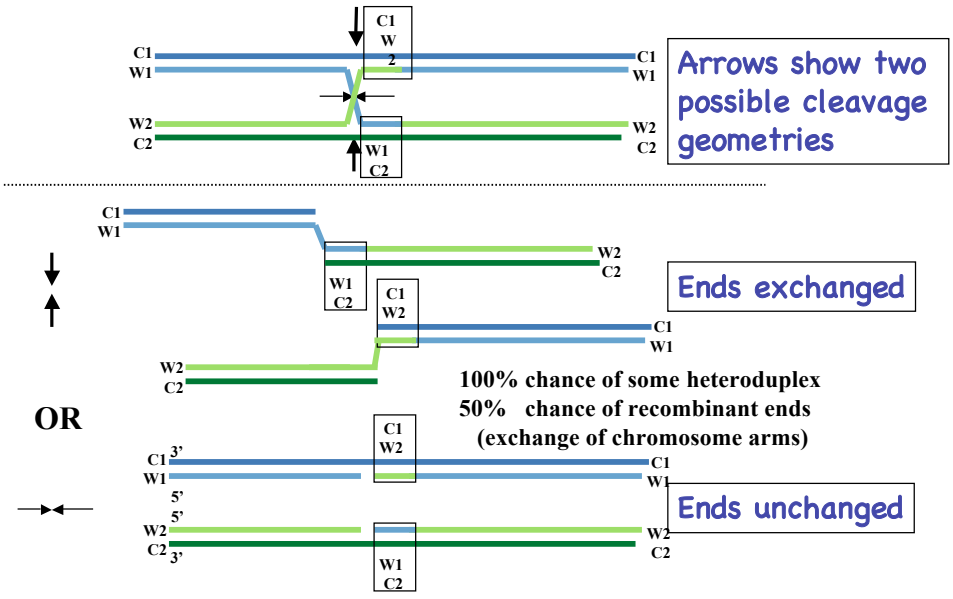


Strand exchange with nicks

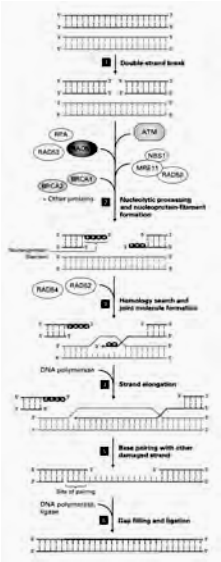
Branch migration extends heteroduplex



Resolution --cleavage separates chromosomes



Double-strand break repair -- Homologous recombination in eukaryotes



1. Ds break

2. dsDNA activates ATM kinase, which activates exo-nucleases that create ss 3′ ends. In a reaction that depends on BRCA 1 & 2, Rad51 coats the ss 3′ ends.

3. Rad51 and friends pair the 3′ end with the sister chromatid.

4. DNA polymerase elongates.

5. Pairing of the new DNA bridges the gap.

6. The gap is filled and ligated.

Crossing over (recombination) is common

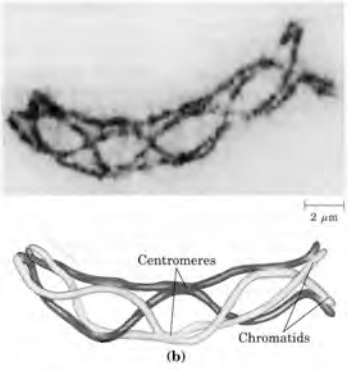
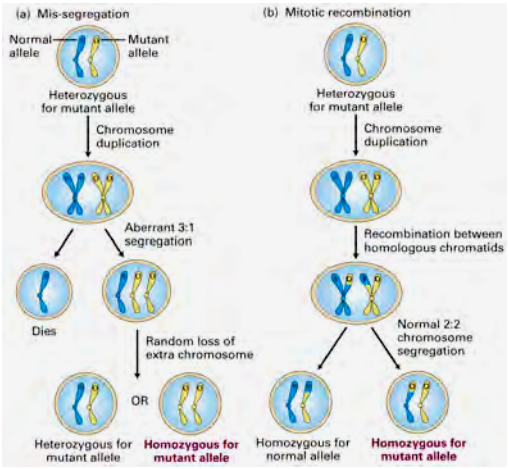


figure 25–28
Crossing over. (a) Crossing over often produces an exchange of genetic material. **(b)** The homologous chromosomes of a grasshopper are shown during prophase I of meiosis. Multiple points of joining (chiasmata) are evident between the two homologous pairs of chromatids. These chiasmata are the physical manifestation of prior homologous recombination (crossing over) events.

5 crossovers in a pair of grasshopper meiotic chromosomes

Three mechanisms of Loss of Heterozygosity



(2) (3)

1. Spontaneous second mutation (not shown),
2. Mis-segregation and
3. Mitotic recombination.

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