

## Intragenic (gene) mutations

Base substitutions

Transversions  $\text{Pu} \leftrightarrow \text{Py}$

Transitions  $\text{Pu} \rightarrow \text{Pu}$  or  $\text{Py} \rightarrow \text{Py}$

Insertions (small)

Deletions (small)

*Effects: Change or loss of function of single genes,*

*Mutation types: silent, missense, nonsense, frameshift*

## Intergenic (chromosome) mutations

Deletions Insertions Inversions

Translocations Amplifications

*Effects:: Change or loss of function of larger units*

## Ploidy mutations

Euploidy

Aneuploidie

Haploidy

Hypoploidy (e.g. Monosomy)

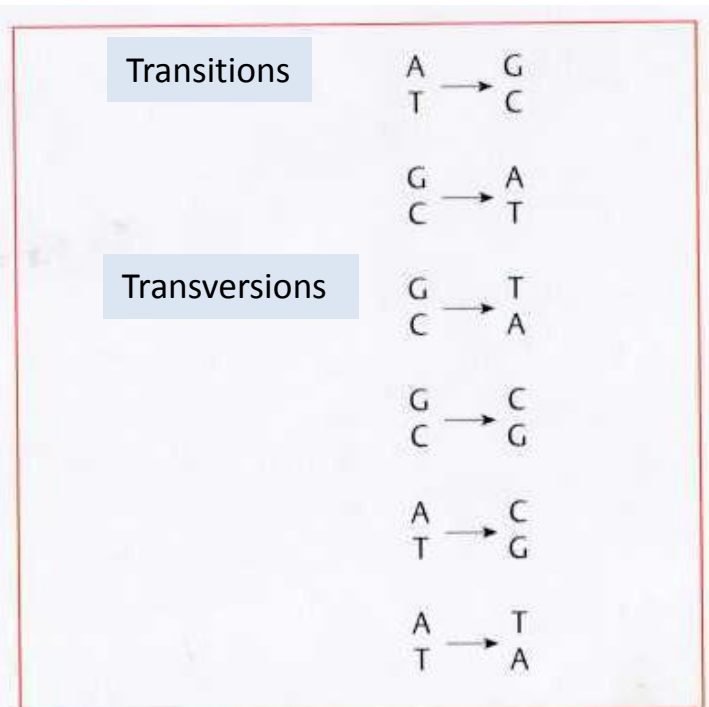
Polyploidy

Hyperploidy (e.g. Trisomy)

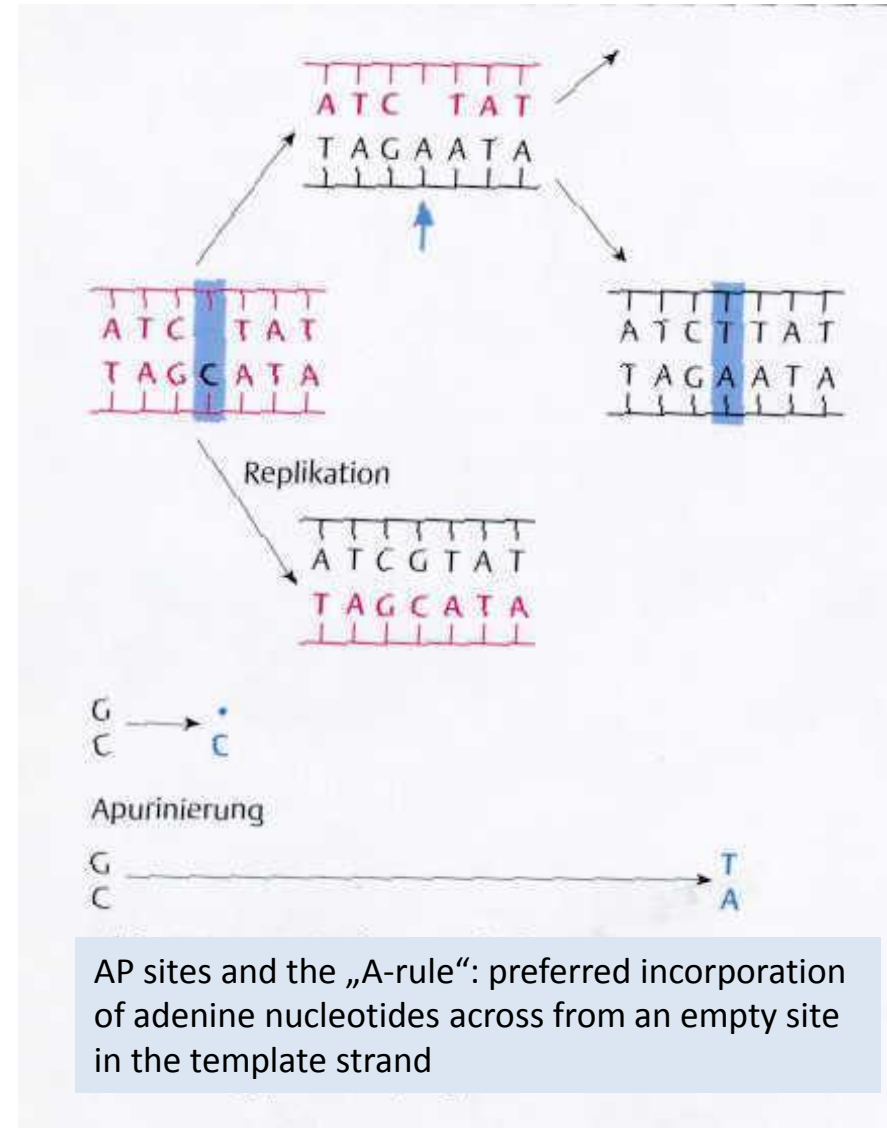
*Effects: mostly pleiotropic or loss of function*

Wild type	C T T A G T G A C T A C G G T A A A G A A T C A C T G A T G C C A T T T C U U A G U G A C U A C G G U A A A Leu · Ser · Asp · Tyr · Gly · Lys Protein	DNA mRNA Protein
Neutral mutations	C T T A G <b>C</b> G A C T A C G G T A A <b>G</b> G A A T <b>C</b> <b>G</b> C T G A T G C C A T T <b>C</b> C U U A G <b>C</b> G A C U A C G G U A A <b>G</b> Leu · Ser · Asp · Tyr · Gly · Lys Protein	DNA mRNA Protein
Missense mutations	C <b>C</b> T A G T G A <b>A</b> T A C G G T A A A G <b>G</b> A T C A C T <b>T</b> A T G C C A T T T C <b>C</b> U A G U G A <b>A</b> U A C G G U A A A Pro · Ser · Glu · Tyr · Gly · Lys Protein	DNA mRNA Protein
Nonsense mutations	C T T A G T G A C T A <b>G</b> G G T A A A G A A T C A C T G A T <b>C</b> C C A T T T C U U A G U G A C U A <b>G</b> Stop-Codon Leu · Ser · Asp Protein	DNA mRNA Protein

**Nucleotide exchange causing mutations.** Note: Mutations are very rare. An independent exchange of two closely adjacent nucleotides is highly unlikely, almost excluded.



Two kinds of nucleotide exchanges:  
transitions and transversions



AP sites and the „A-rule“: preferred incorporation of adenine nucleotides across from an empty site in the template strand

# Frameshift Mutations

4

Wild type

DNA  
 A C A A A A A G T C C A T C A C T T A A C G C C  
 T G T T T T T C A G G T A G T G A A T T G C G G

mRNA  
 A C A A A A A G U C C A U C A C U U A A C G C C

Protein  
Thr · Lys · Ser · Pro · Ser · Leu · Asn · Ala

Addition  
of an AT  
pair

DNA  
 A C A A A A A **A** G T C C A T C A C T T A A C G C C  
 T G T T T T T **T** C A G G T A G T G A A T T G C G G

mRNA  
 A C A A A A A **A** G U C C A U C A C U U A A C G C C

Protein  
 Thr · Lys · Lys · Ser · Ile · Thr · **Stop-Codon**

Deletion  
of an AT  
pair

DNA  
 A C A A A **A G** T C C A T C A C T T A A C G C C  
 T G T T T **T C** A G G T A G T G A A T T G C G G

mRNA  
 A C A A A **A G** U C C A U C A C U U A A C G C C

Protein  
Thr · Lys · Val · His · His · Leu · Thr · Pro

Deletion  
of an AT  
pair and  
addition  
of a GC  
pair

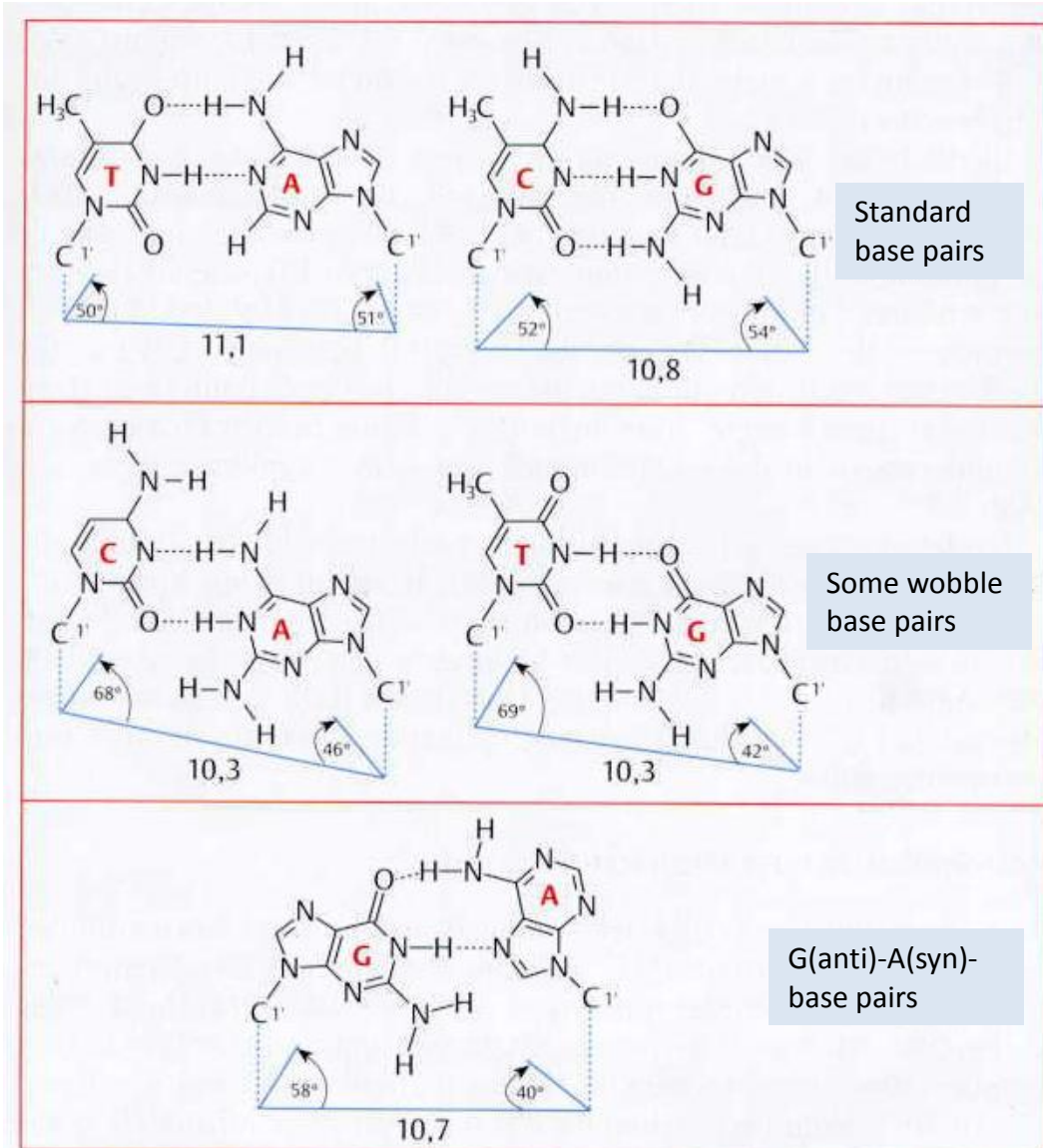
DNA  
 A C A A A **A G** T C C A T C A C T T A A C C **G** C C  
 T G T T T **T C** A G G T A G T G A A T T G G **C** G G

mRNA  
 A C A A **A A G** U C C A U C A C U U A A C C **G** C C

Protein  
Thr · Lys · Val · His · His · Leu · Thr · Ala

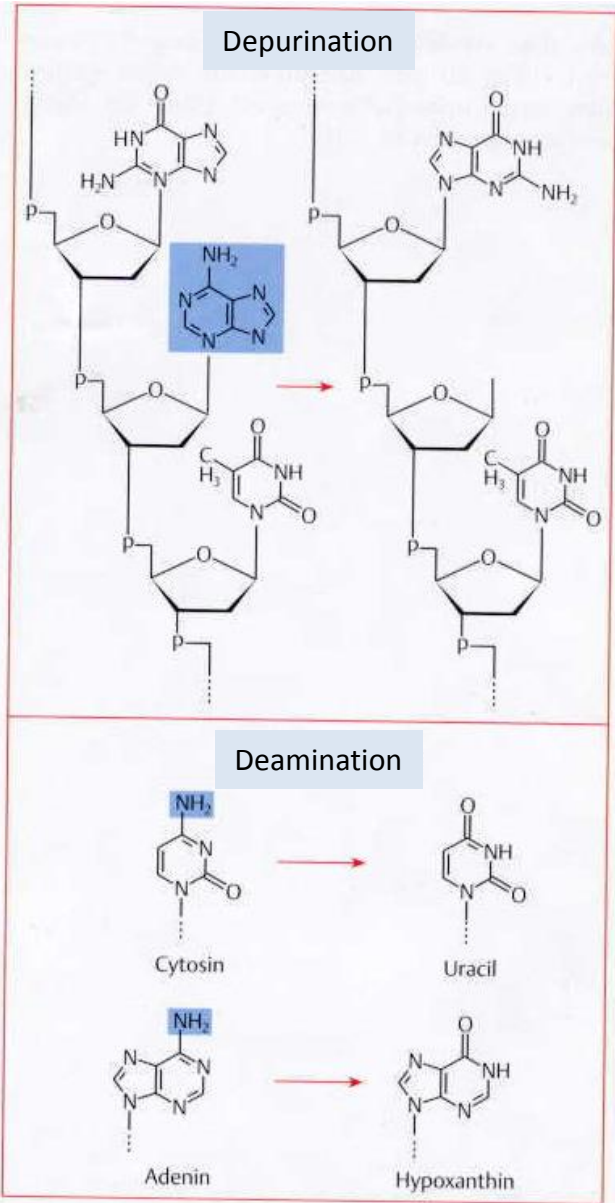
**Frameshift Mutations.** Underlined sequences represent the correct reading frame.

## Mutations by Replication errors

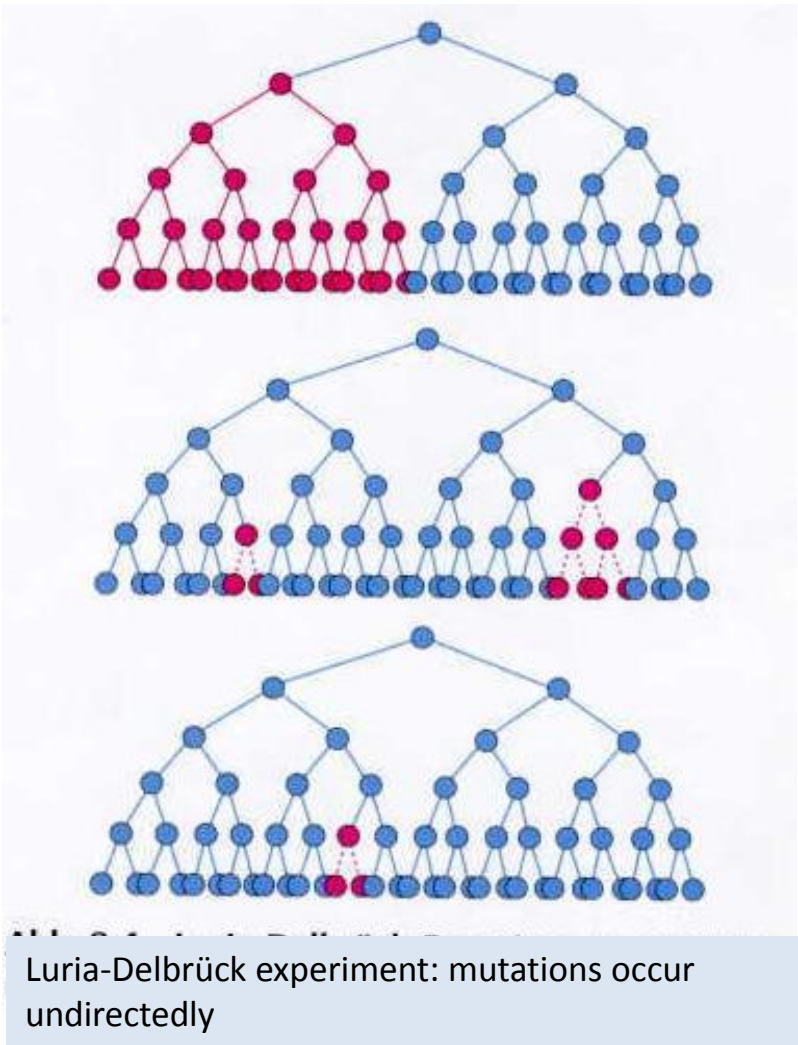


Unusual base pairs as reason for false incorporations at the replication fork





Spontaneous hydrolytic decomposition reactions: depurination and deamination



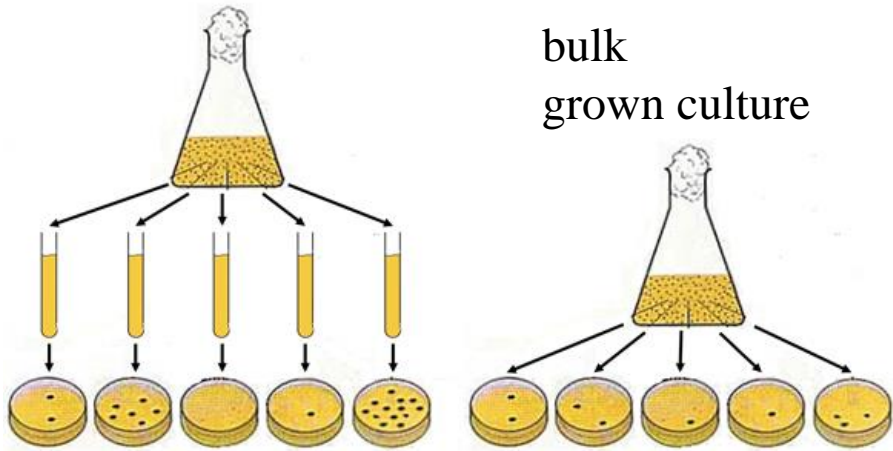
# Fluctuation test

# Spontaneous Mutations

*E.coli* – Mutation to Phage resistance

Question: is mutation occurring spontaneously or only induced by contact with phage?

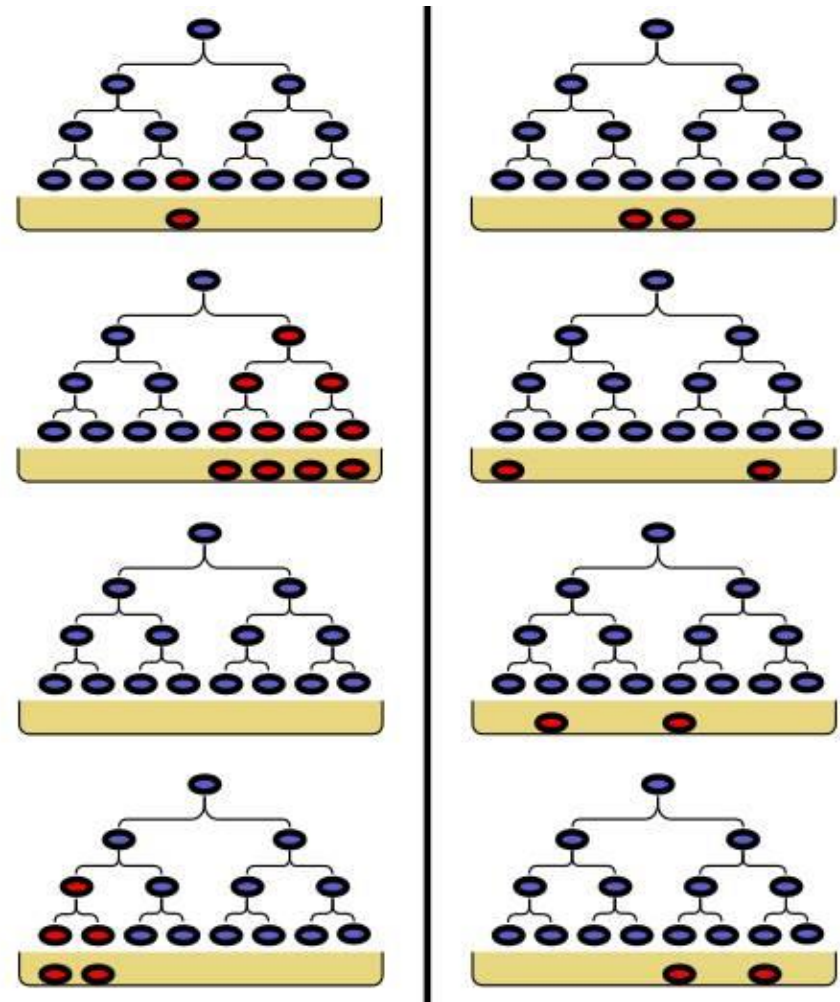
## Luria-Delbruck Fluctuation Test



**culture separately**

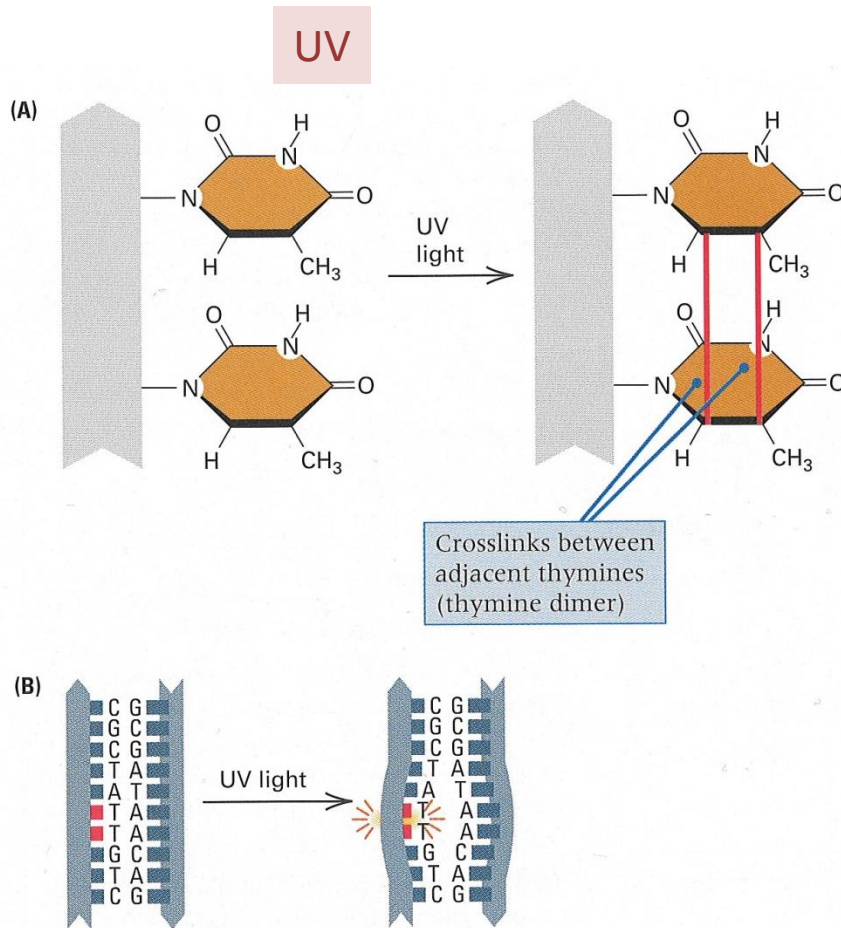
**sample repeatedly**

- Cultivation for same number of generations
- Plate on medium and spray with phage
- Count phage resistant colonies



Separately  
grown culture

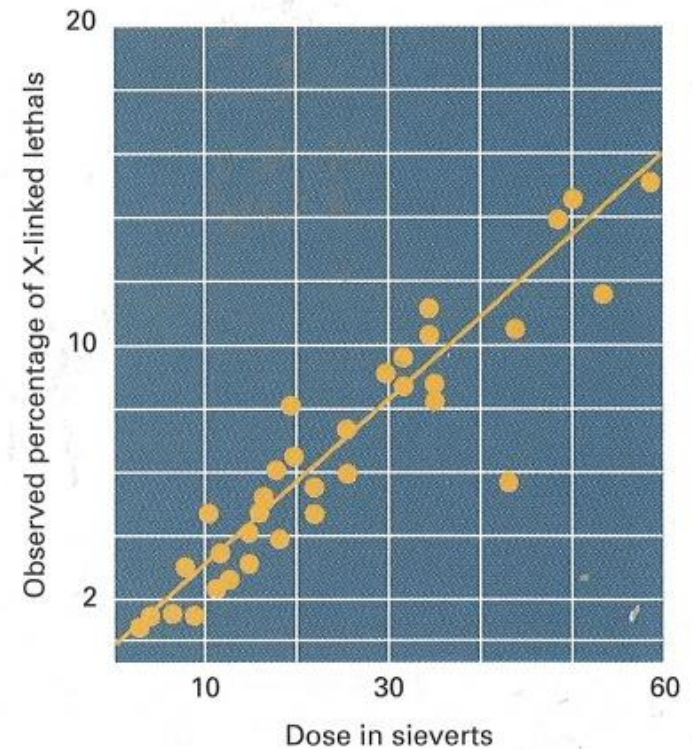
bulk  
grown culture



**Figure 14.27** (A) Structural view of the formation of a thymine dimer. Adjacent thymines in a DNA strand that have been subjected to ultraviolet (UV) irradiation are joined by formation of the bonds shown in red. Other types of bonds between the thymine rings also are possible. Although not drawn to scale, these bonds are considerably shorter than the spacing between the planes of adjacent thymines, so the double-stranded structure becomes distorted. The shape of each thymine ring also changes. (B) The distortion of the DNA helix caused by two thymines moving closer together when joined in a dimer.

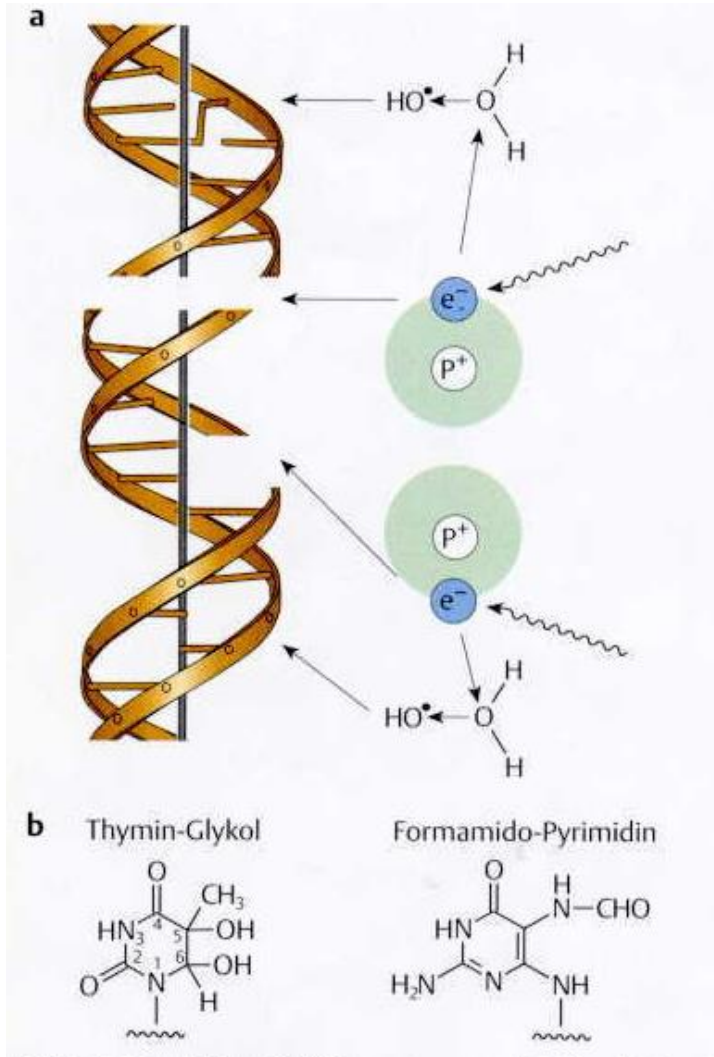
## Radiation mutagenesis

X-ray: Chromosome breaks  
Rearrangements



**Figure 14.28** The relationship between the percentage of X-linked recessive lethals in *D. melanogaster* and x-ray dose, obtained from several experiments. The frequency of spontaneous X-linked lethal mutations is 0.15 percent per X chromosome per generation.

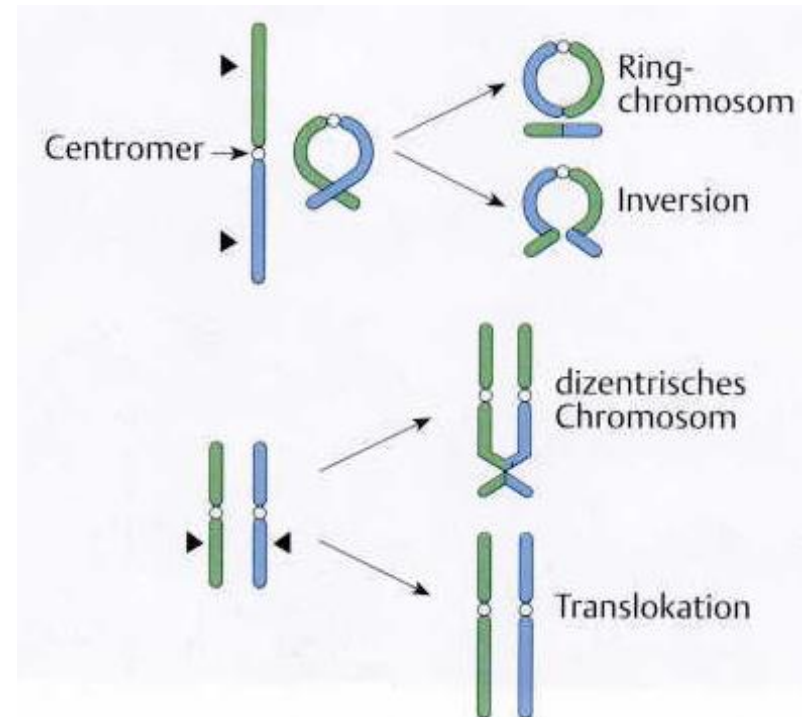




**DNA damages caused by ionizing radiation.** **a** (at the double helix) cross links by covalent linking of opposite bases; double strand break; single strand break; destruction or modification of DNA bases. **b** Some examples for bases damaged by radiation.

## High energy irradiations

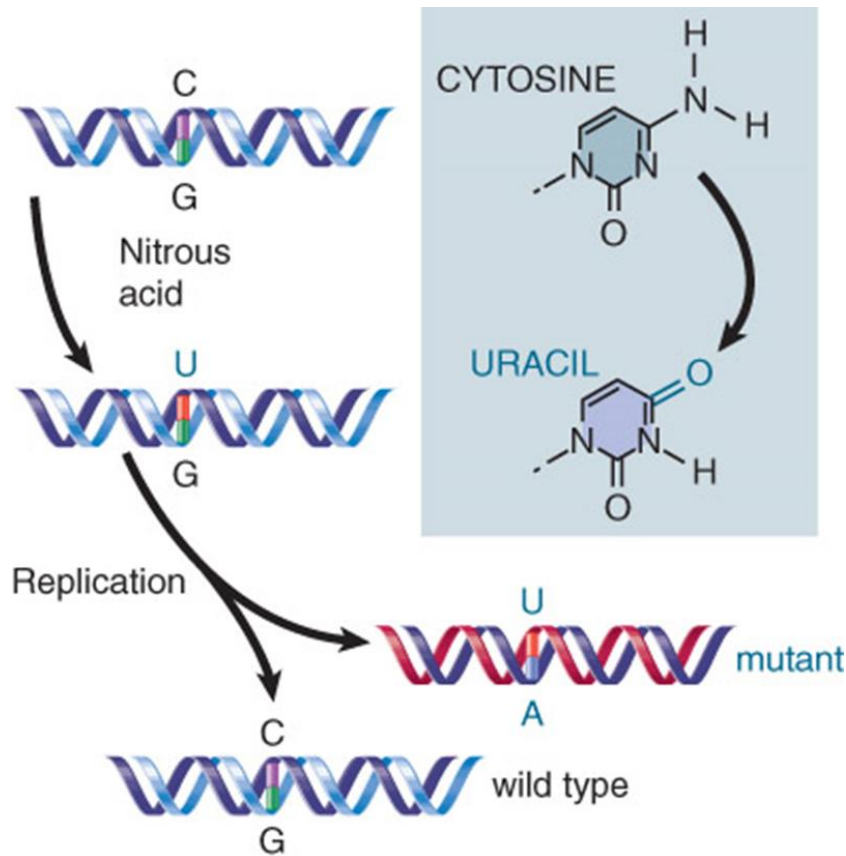
- Roentgen
- Alpha, Gamma, (Beta)



Chromosome breaks in irradiated cells

Mutations can be induced by chemical modification of a base.

## Chemical Mutagenesis



Simple Chemicals:  
 Bisulfite  
 Nitrous acid  
 Hydroxylamine

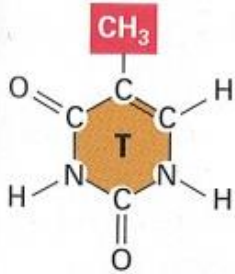
Desaminations: example: C → G

# Chemical Mutagenesis

## Base analogs

Base Analogs → Shift of tautomeric equilibrium

(A)

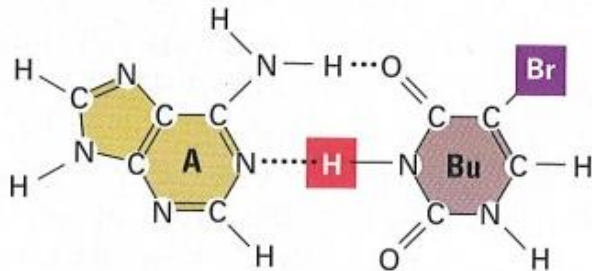


Thymine



5-Bromouracil (keto form)

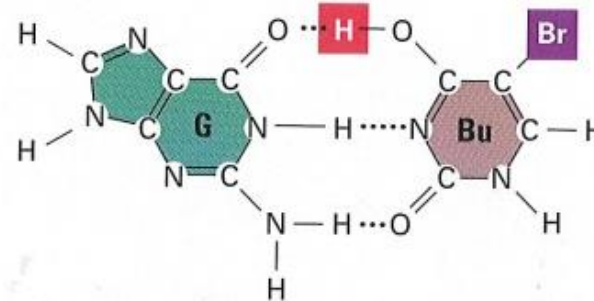
(B) A-Bu base pair



Adenine

5-Bromouracil (keto form)

(C) G-Bu base pair



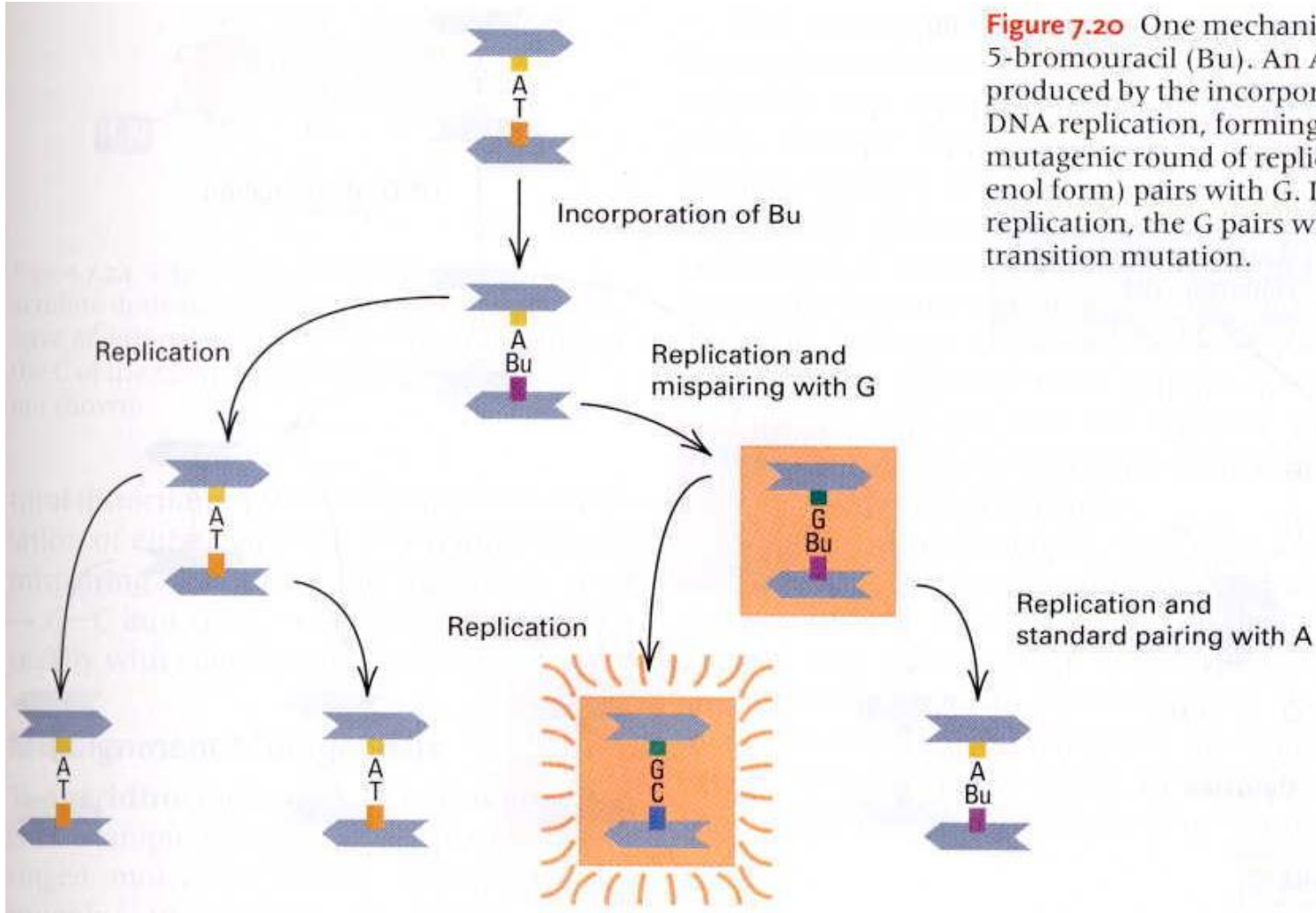
Guanine

5-Bromouracil (enol form)

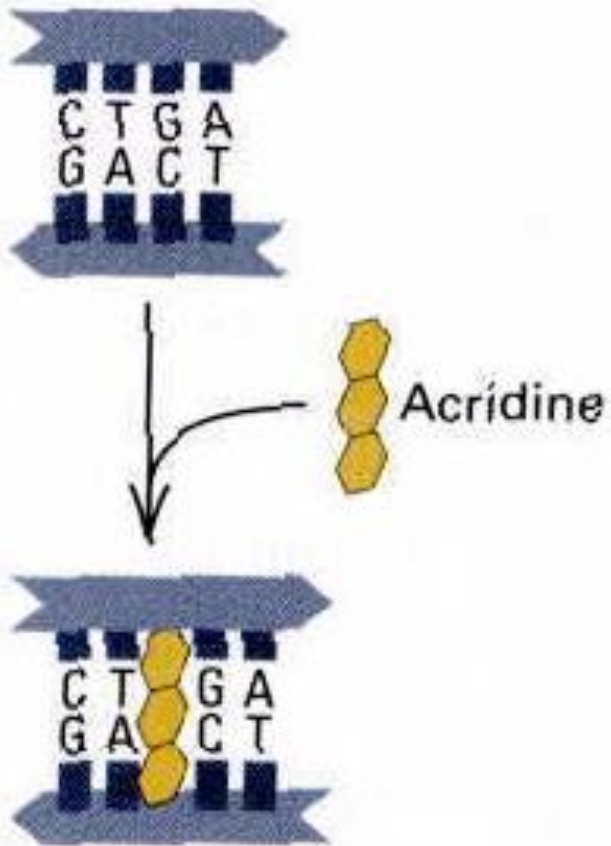
**Figure 14.23** Mismatching mutagenesis by 5-bromouracil. (A) Structures of thymine and 5-bromouracil. (B) A base pair between adenine and the keto form of 5-bromouracil. (C) A base pair between guanine and the rare enol form of 5-bromouracil. One of 5-bromouracil's hydrogen atoms changes position to create the keto form.

# Chemical Mutagenesis

**Figure 7.20** One mechanism for mutagenesis by 5-bromouracil (Bu). An AT → GC transition is produced by the incorporation of 5-bromouracil in DNA replication, forming an A-Bu pair. In the mutagenic round of replication, the Bu (in its rare enol form) pairs with G. In the next round of replication, the G pairs with C, completing the transition mutation.





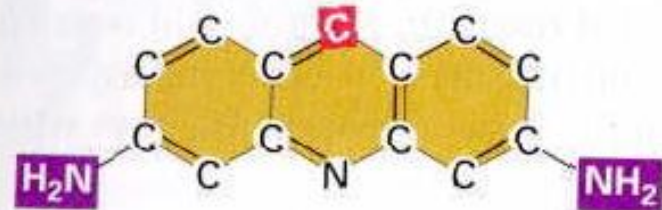


**Figure 7.25** Separation of two base pairs caused by intercalation of an acridine molecule.

## Chemical Mutagenesis

### Intercalating agents

Intercalation causes  
Frameshift mutations

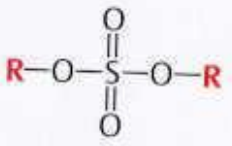


**Proflavin**

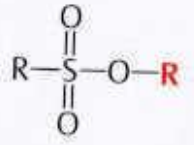
**Figure 7.24** The structure of proflavine, an acridine derivative. Other mutagenic acridines have additional atoms on the NH<sub>2</sub> group and on the C of the central ring. Hydrogen atoms are not shown.

# Alkylating agents

## Alkyl-Sulfate



Dialkylsulfat  
Beispiel:  
Dimethylsulfat

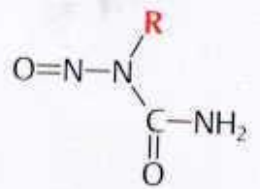


Alkyl-Alkan-Sulfonat  
Beispiele:  
Methylmethansulfonat, MMS;  
Ethylmethansulfonat, EMS

## N-Nitroso-Verbindungen



Dialkylnitrosamine  
Beispiel:  
Dimethylnitrosamin



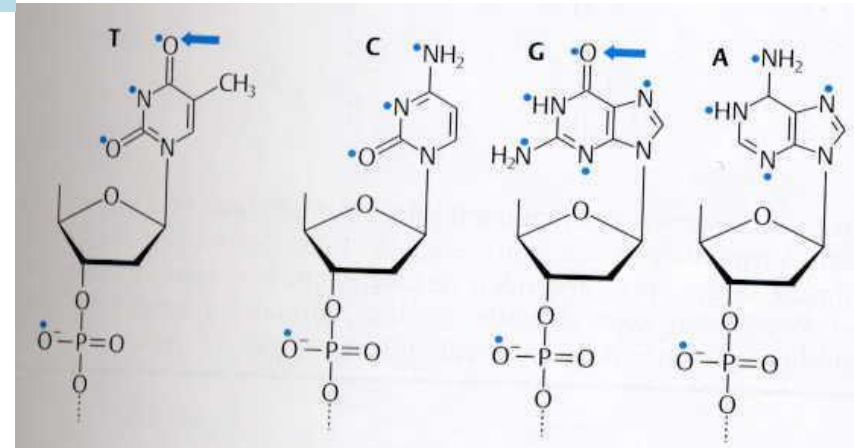
N-Nitrosoharnstoff-Derivate  
Beispiel:  
Methyl-Nitrosoharnstoff, MNN



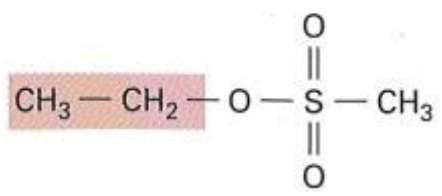
N-Alkyl-N'-Nitro-N-Nitroso-guanidin  
Beispiel:  
N-Methyl-N'-Nitro-N-Nitroso-guanidin (NNG)

Mutagenic chemicals: Some alkylating compounds.

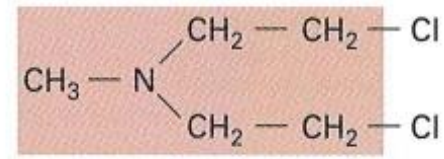
# Chemical Mutagenesis



**Alkylated nucleotides in the DNA.** Dots: putative attachment sites for methyl or ethyl groups; arrows: trigger of direct mutations (by false pairings).



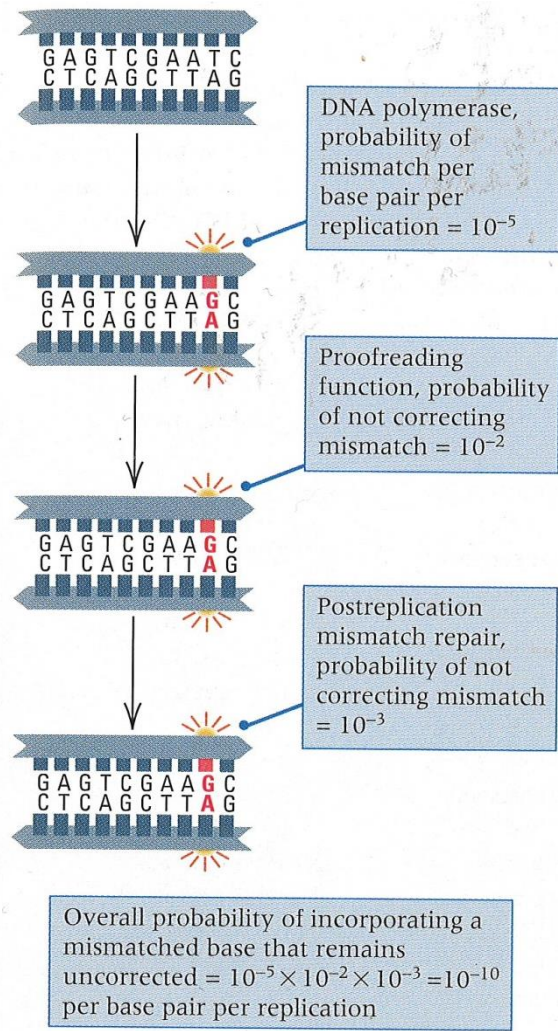
**Ethyl methanesulfonate**



**Nitrogen mustard**

**Figure 14.25** The chemical structures of two highly mutagenic alkylating agents; the alkyl groups are shown in red.





# DNA-Repair Overview

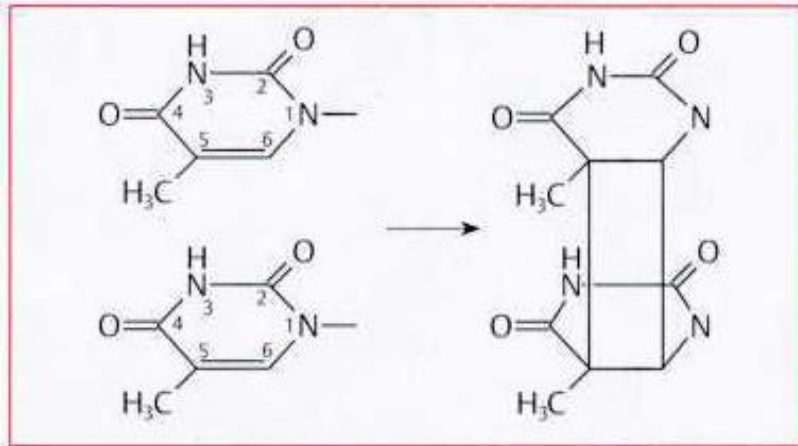
**Figure 14.31** Summary of rates of error in DNA polymerization, proofreading, and postreplication mismatch repair. The initial rate of nucleotide misincorporation is  $10^{-5}$  per base pair per replication. The proofreading function of DNA polymerase corrects 99 percent of these errors, and of those that remain, postreplication mismatch repair corrects 99.9 percent. The overall rate of misincorporated nucleotides that are not repaired is  $10^{-10}$  per base pair per replication.

## The human genome has many repair genes

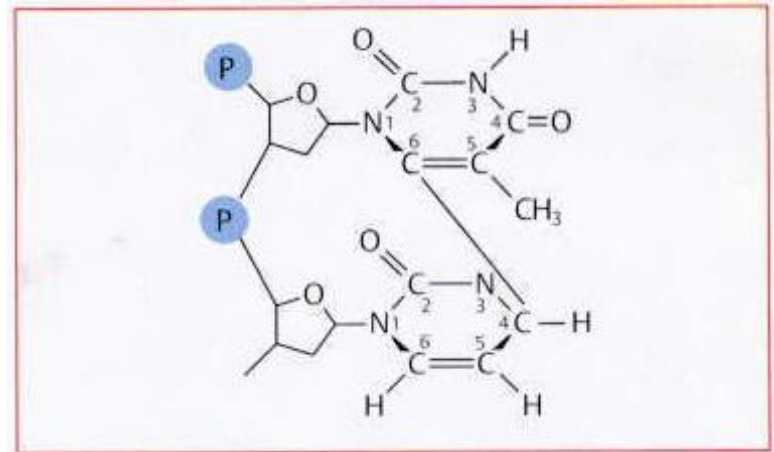
- Direct reversal of damage: 1 gene \*
- Base excision repair: 15 genes
- Nucleotide excision repair: 28 genes
- Mismatch excision repair: 11 genes
- Recombination repair: 14 genes
- Nonhomologous end-joining: 5 genes
- DNA polymerase catalytic subunits: 16 genes

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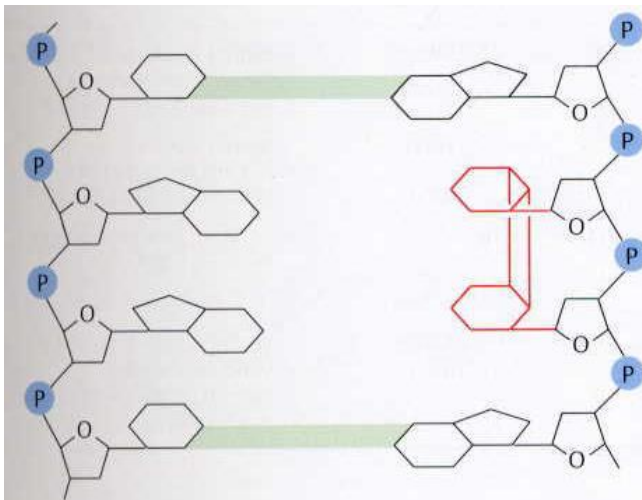
## Radiation Damage of DNA → UV



**Abb. 8.23** Thymin-Dimer, der häufigste DNA-Schaden nach UV-Bestrahlung.



**Abb. 8.24** Das TC(6-4)-Produkt, ein Photoprodukt nach UV-Bestrahlung von DNA.





**Abb. 8.25** Ein Pyrimidin-Dimer in der DNA. Benachbarte Thymin-Nucleotide sind *Hot Spots* der UV-Mutagenese.

Photo-reactivation:  
 Enzyme activated by light → 340-400 nm  
 Direct restoration of original base pairing



## Repair by alkyltransferases

**Methylation can distort the structure of DNA**

Nature of mutation	Consequences
<p>Guanine</p> <p>Methyl-guanine</p> <p>Alkylation</p>  <p>©virtualtext www.ergito.com</p>	<p>Methyl group distorts double helix</p>  <p>Corrected by dealkylation</p>

# Nucleotide Mismatch Repair

**Replication errors introduce mismatched base pairs**

**Nature of mutation**

Cytosine

Nc1nc(=O)nc(C1=O)n1

Adenine

Nc1ncnc2n(cnc12)N

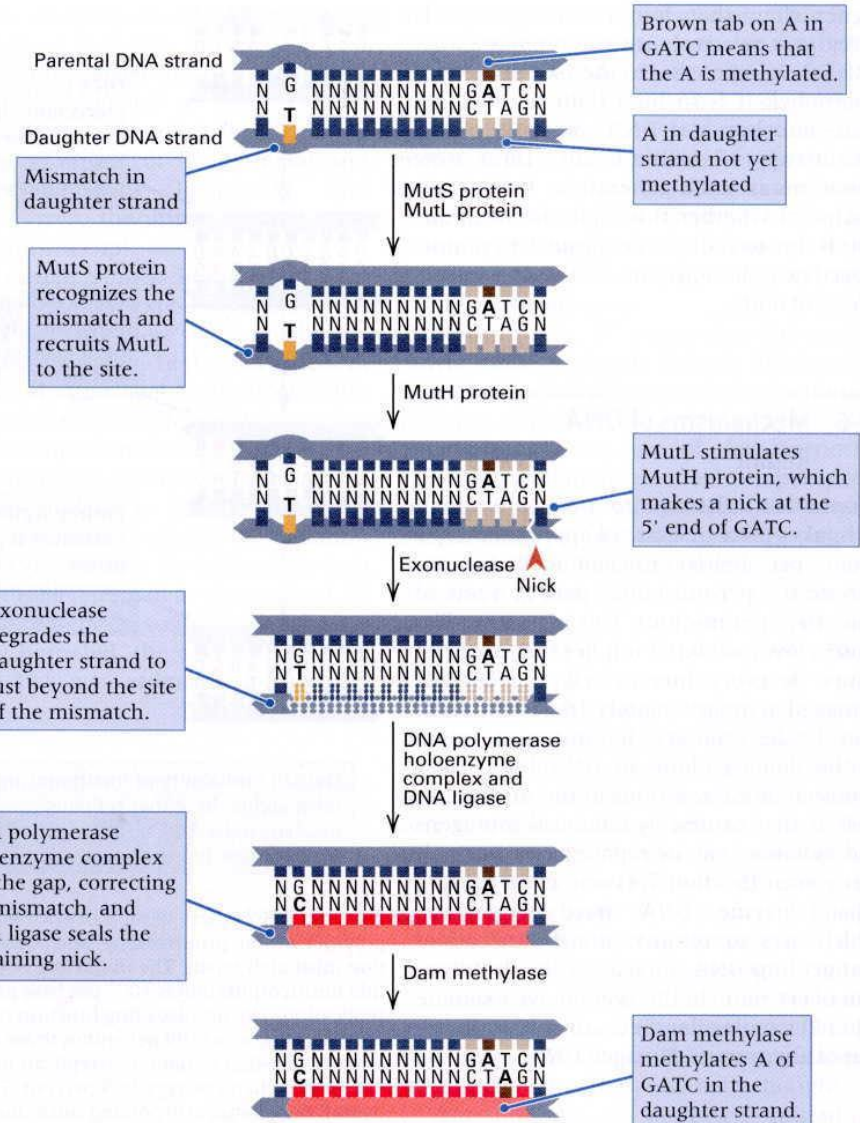
Replication errors

**Consequences**

Purine pair distorts duplex

Corrected by removing A or G in newly synthesized strand

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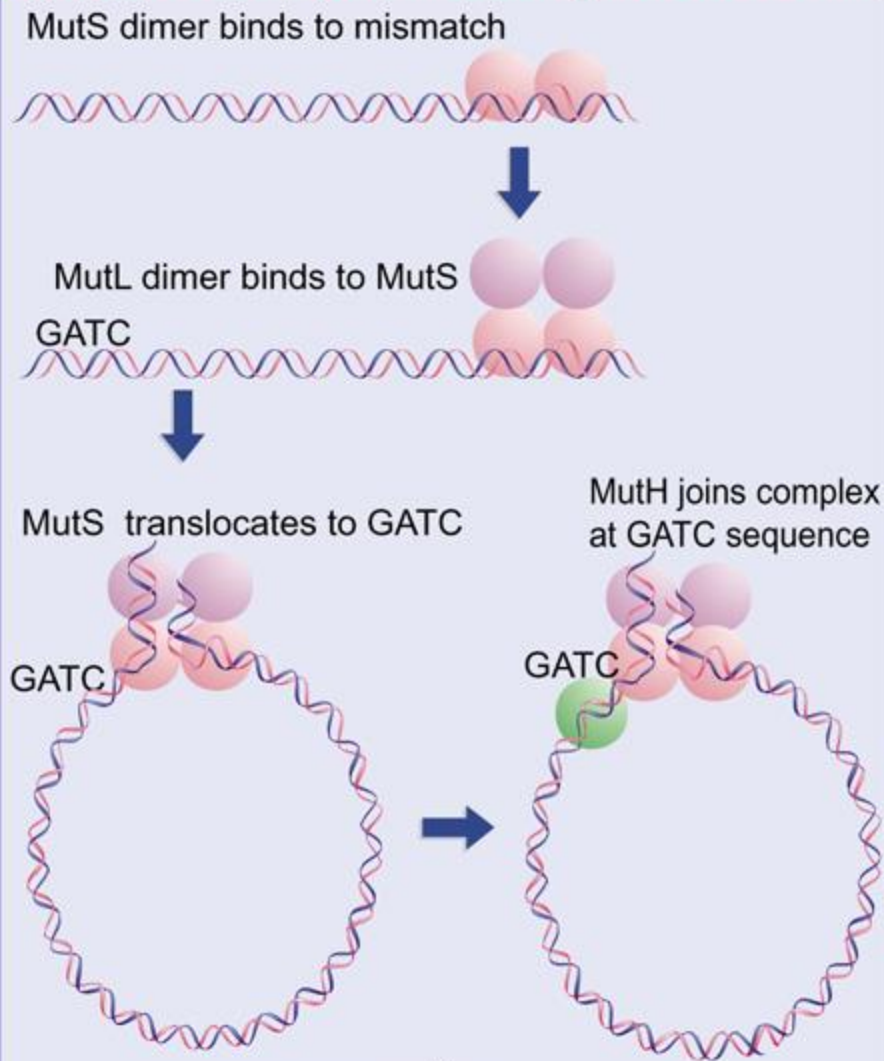


**Figure 7.30** Mismatch repair consists of the excision of a segment of a DNA strand that contains a base mismatch, followed by repair synthesis. In *E. coli*, cleavage takes place at the nearest methylated GATC sequence in the unmethylated strand. An exonuclease removes successive nucleotides until just past the mismatch, and the resulting gap is repaired. Either strand can be excised and corrected, but in newly synthesized DNA, methylated bases in the template strand often direct the excision mechanism to the newly synthesized strand that contains the incorrect nucleotide.

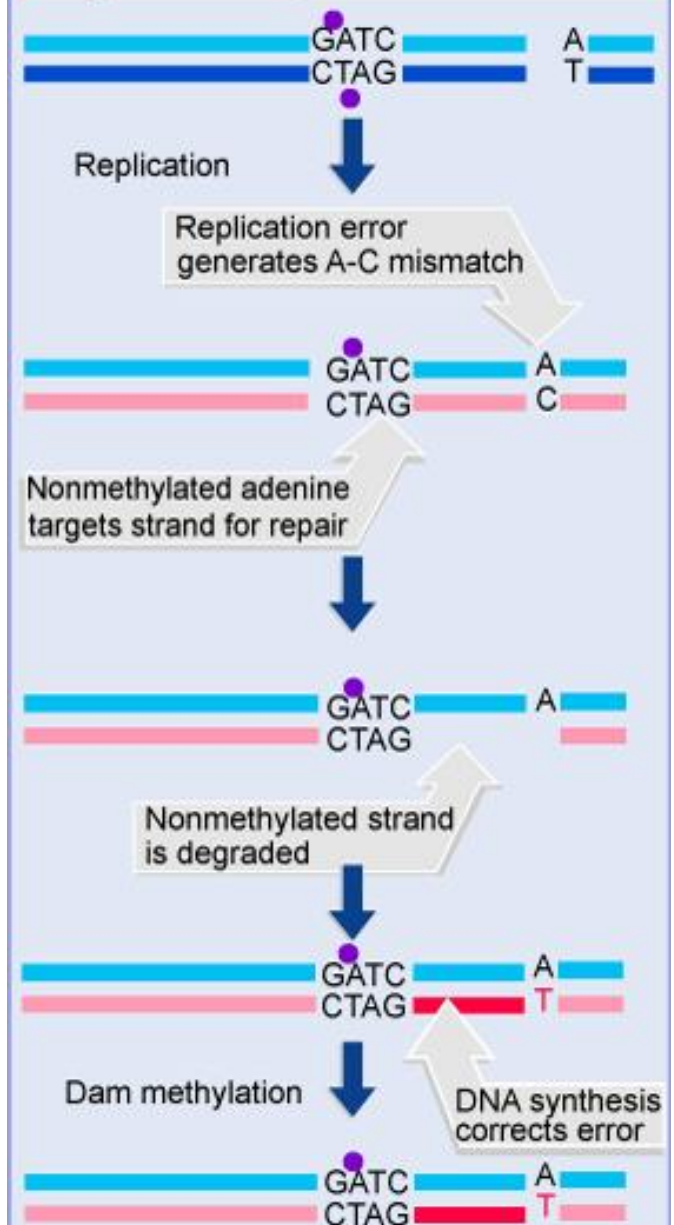
Methylation status defines mutated strand

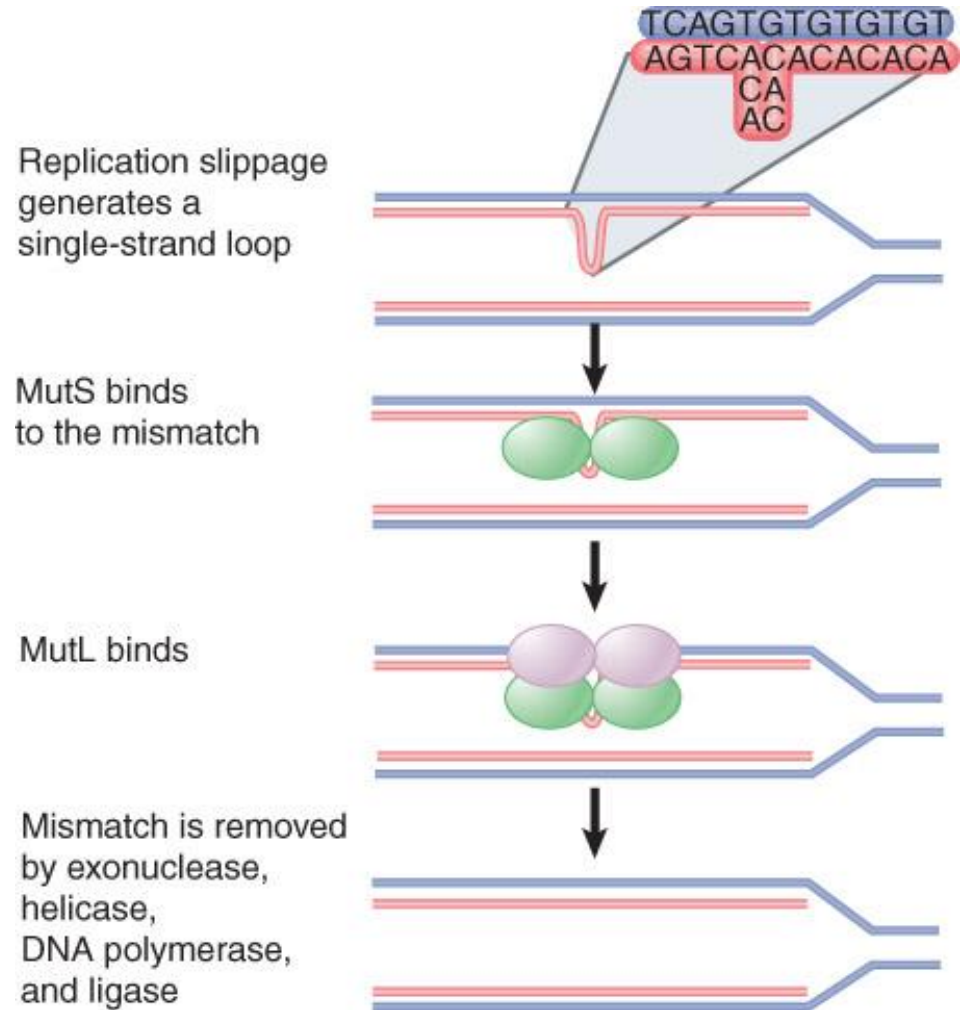
# Nucleotide Mismatch Repair

## MutSL binds mismatches on unmethylated DNA strands



## Methylation distinguishes the DNA strands

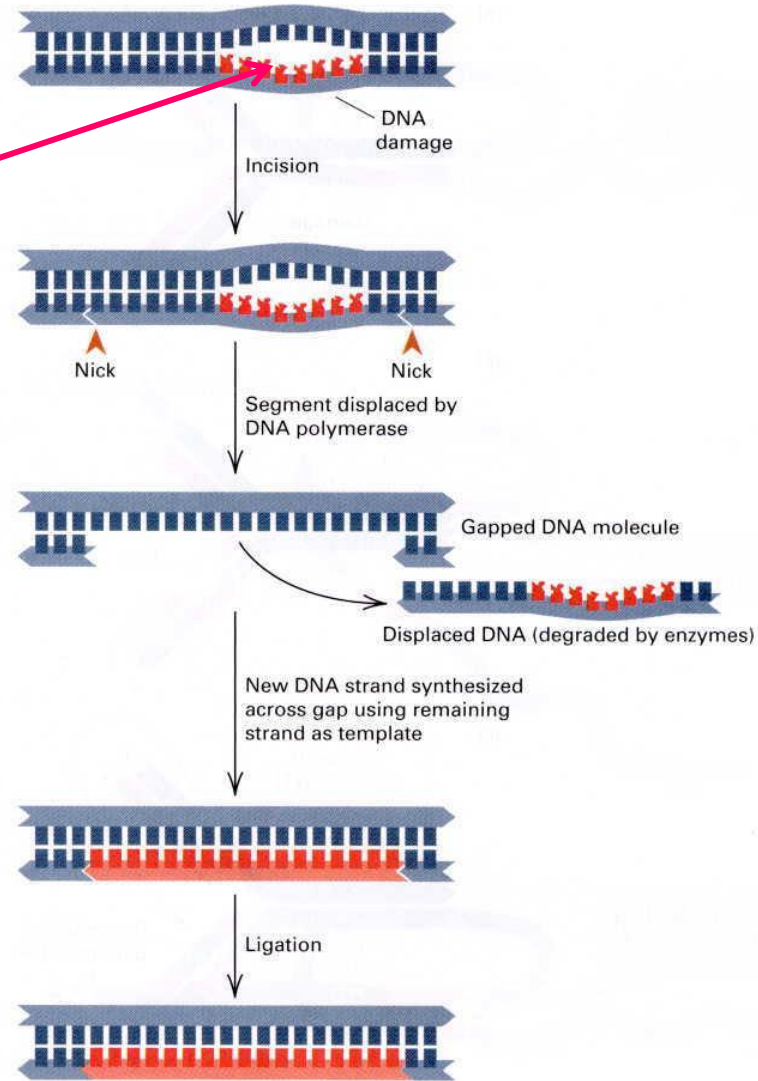




The MutS/MutL system initiates repair of mismatches produced by replication slippage.

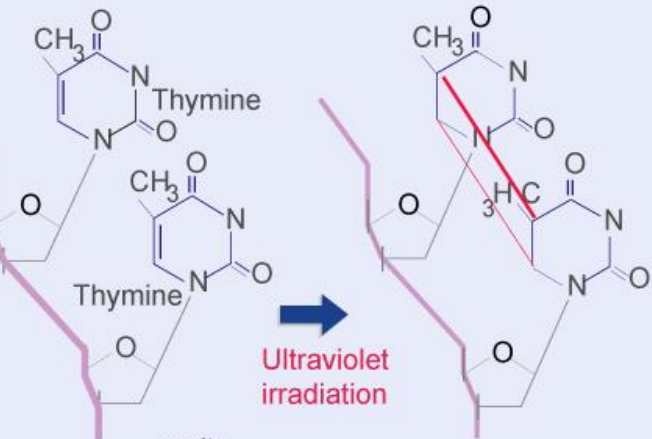


# Excision Repair



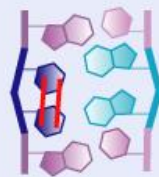
## Thymine dimers must be removed by excision

### Nature of mutation



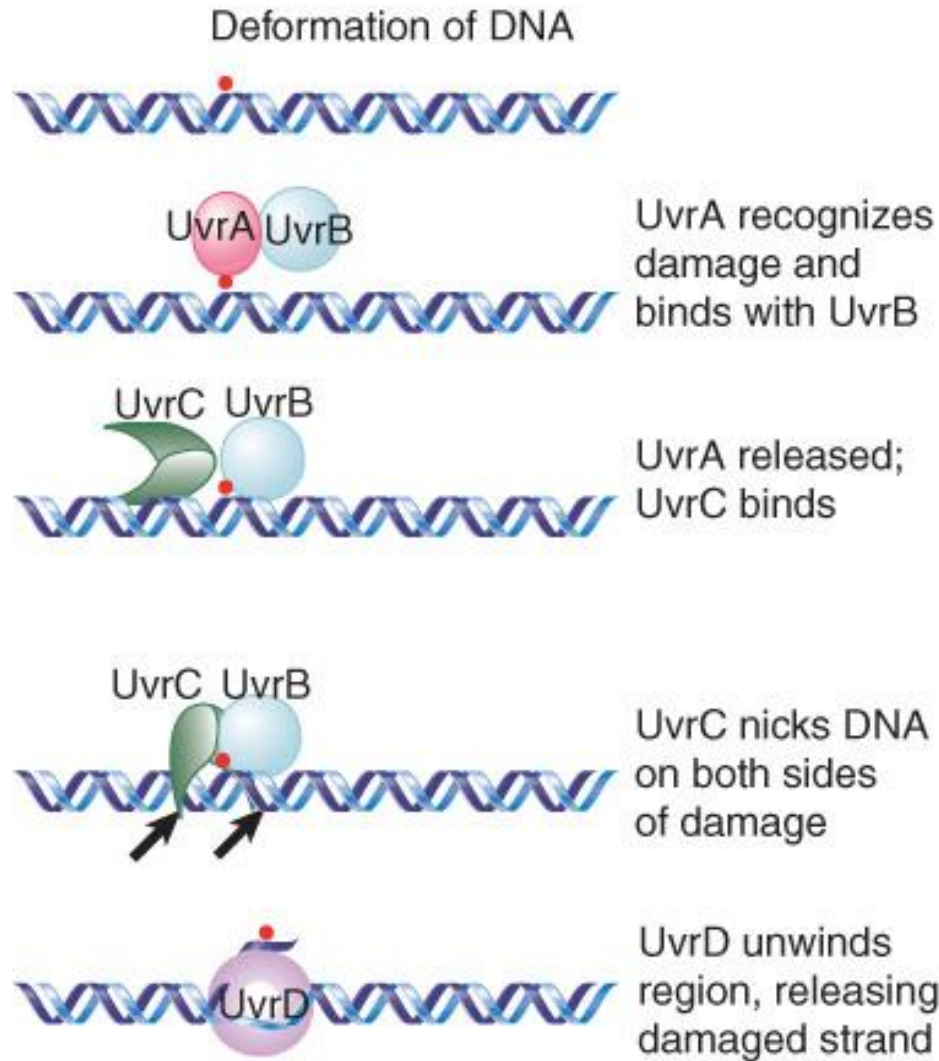
### Consequences

Thymine dimer distorts duplex



Corrected by excision

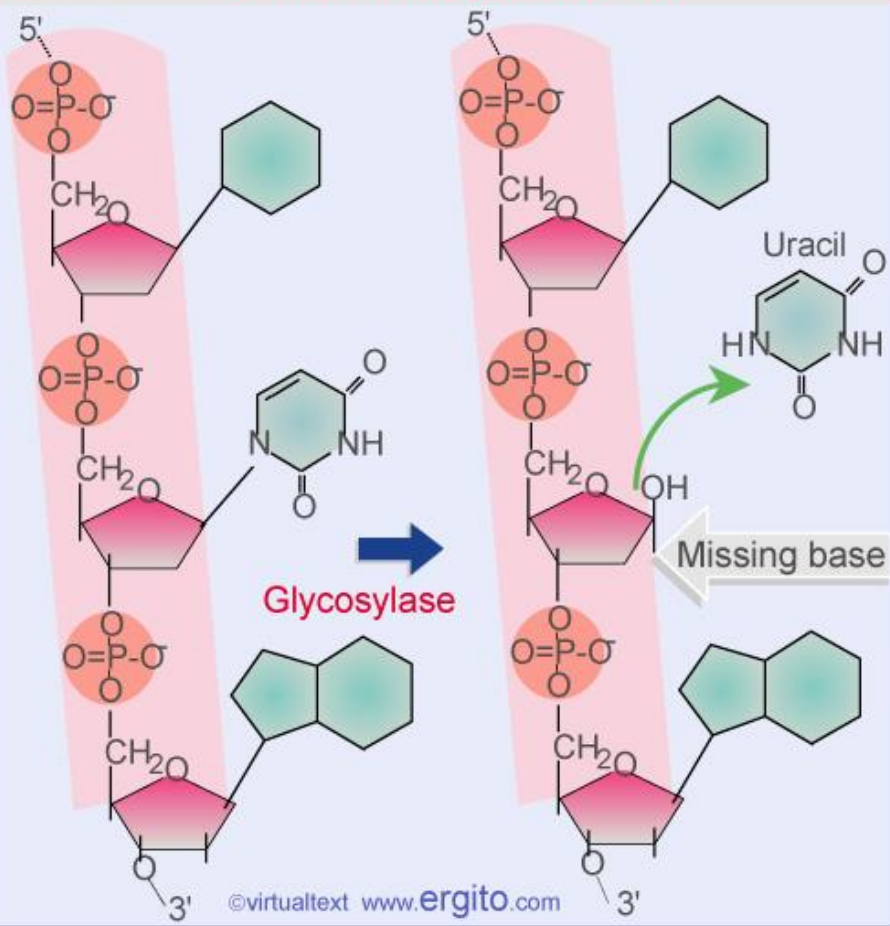
Figure 7.33 Mechanism of excision repair of DNA damage.



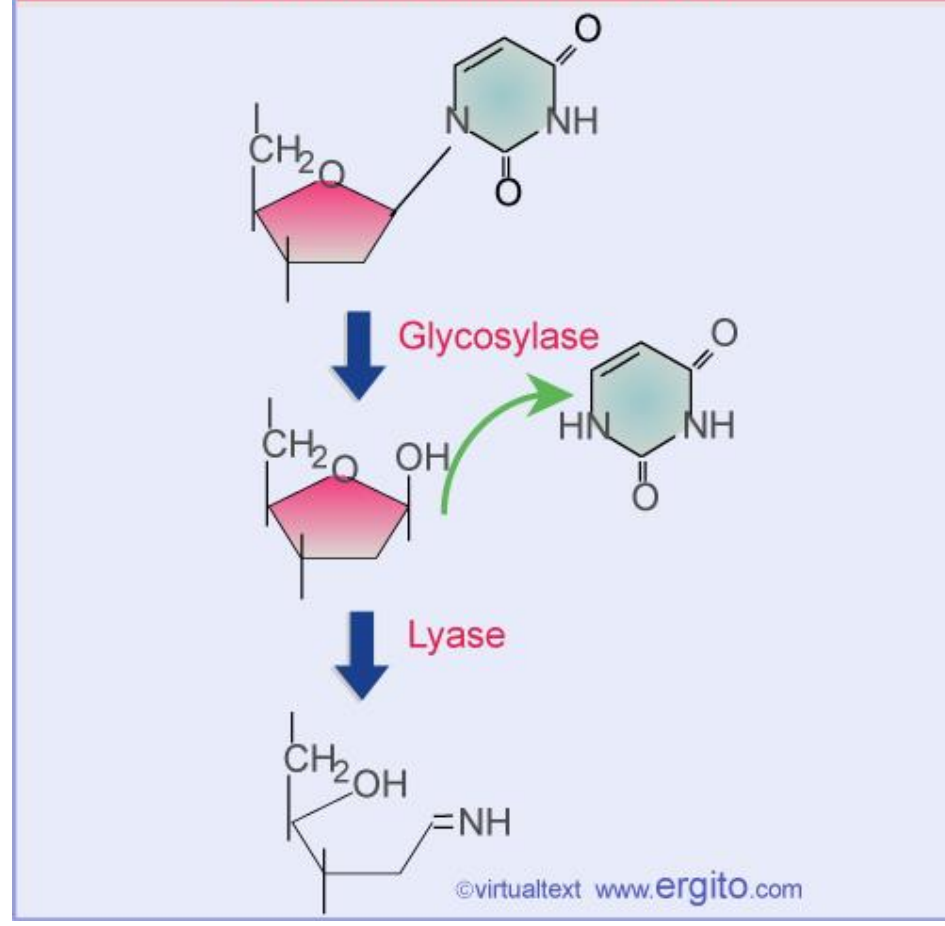
The Uvr system operates in stages in which UvrAB recognizes damage, UvrBC nicks the DNA, and UvrD unwinds the marked region.

# Removal of non-natural (modified) bases by glycosylases

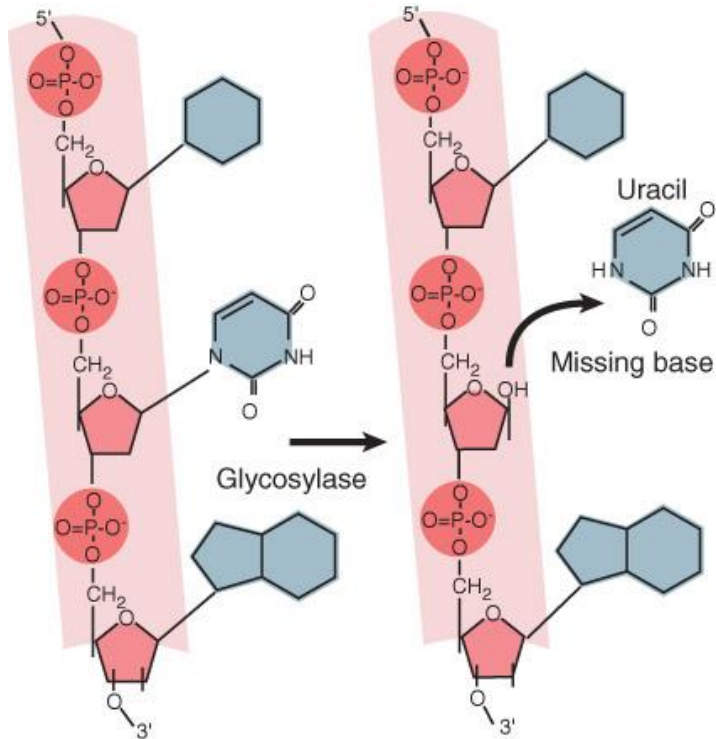
Uracil is removed from DNA



Glycosylases remove bases

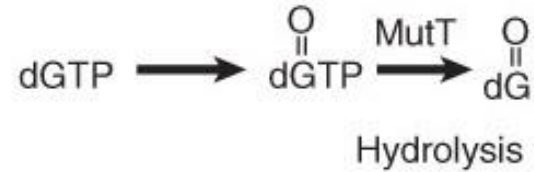


## Removal of bases by glycosylases

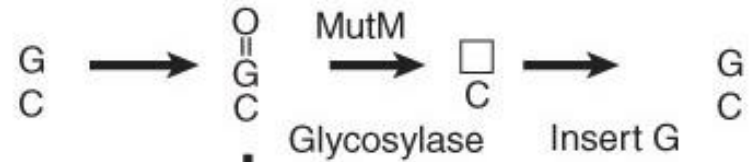


A glycosylase removes a base from DNA by cleaving the bond to the deoxyribose.

MutT hydrolyzes 8-oxo-dGTP

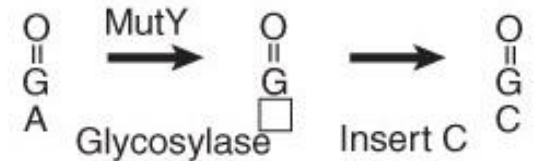


MutM removes G=O that is paired with C



Replication

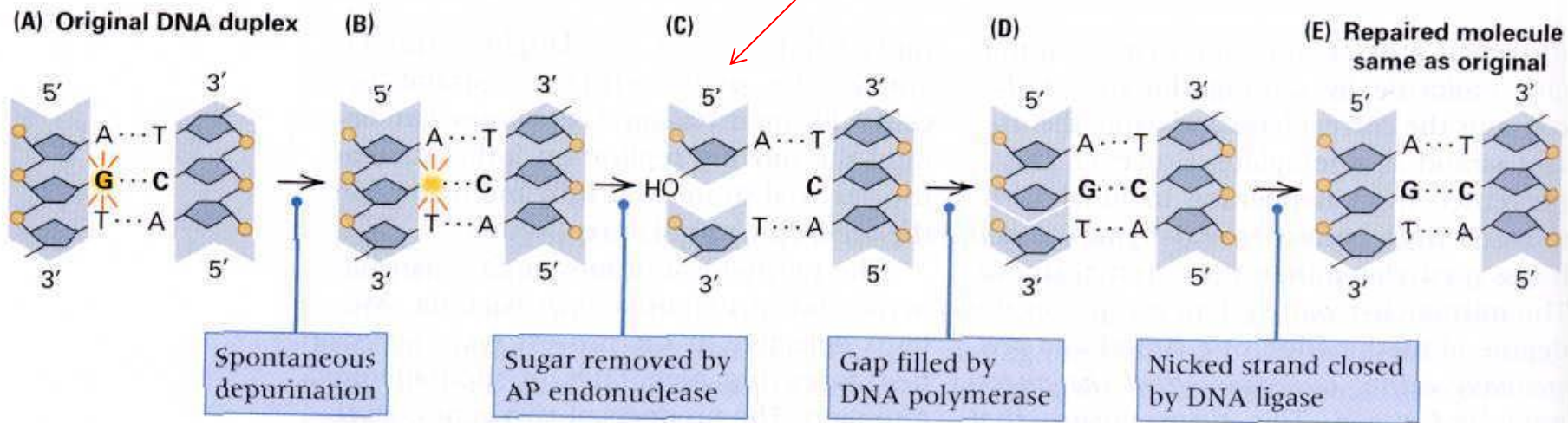
MutY removes A that is paired with G=O



Preferential removal of bases in pairs that have oxidized guanine is designed to minimize mutations.



Strand break, removal of nucleotides by exonuclease

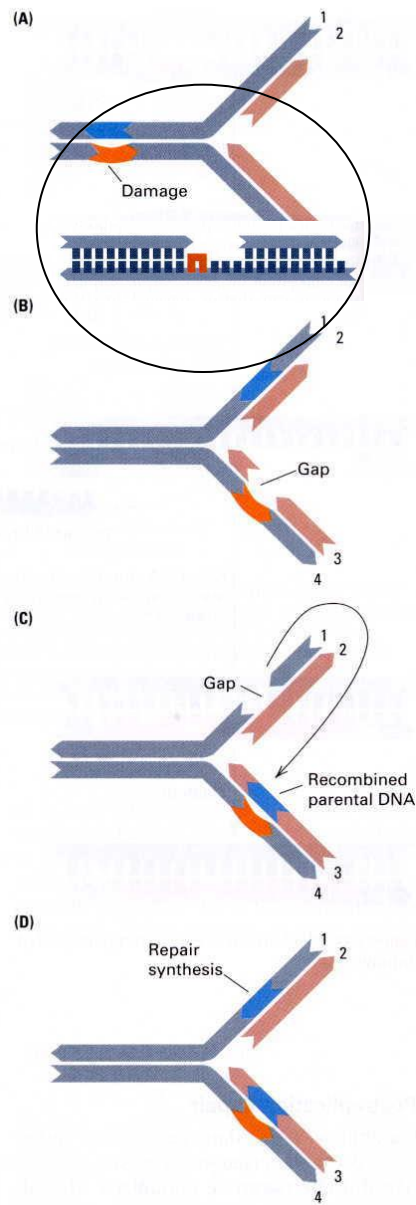


**Figure 7.32** Action of AP endonuclease. (A) Original DNA duplex. (B) Spontaneous hydrolysis of guanine results in loss of the base. (C) AP endonuclease excises the empty deoxyri-

bose from the DNA strand. (D) DNA polymerase fills the gap using the continuous strand as a template. (E) The remaining nick is closed by DNA ligase, restoring the original sequence.



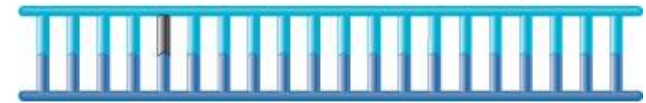
# Post-replication repair



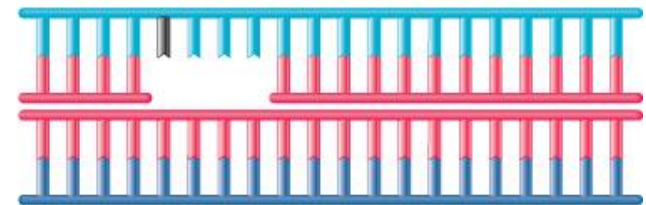
**Figure 7-34** Postreplication repair. (A) A molecule with DNA damage in strand 4 is being replicated. (B) By reinitiation of synthesis beyond the damage, a gap is formed in strand 3. (C) A segment of parental strand 1 is excised and inserted in strand 3. (D) The gap in strand 1 is next filled in by repair synthesis.

## Damage

Bases on one strand of DNA are damaged

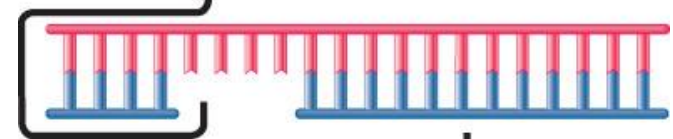
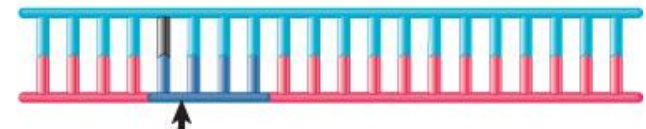


Replication generates a copy with gap opposite damage and a normal copy



## Retrieval

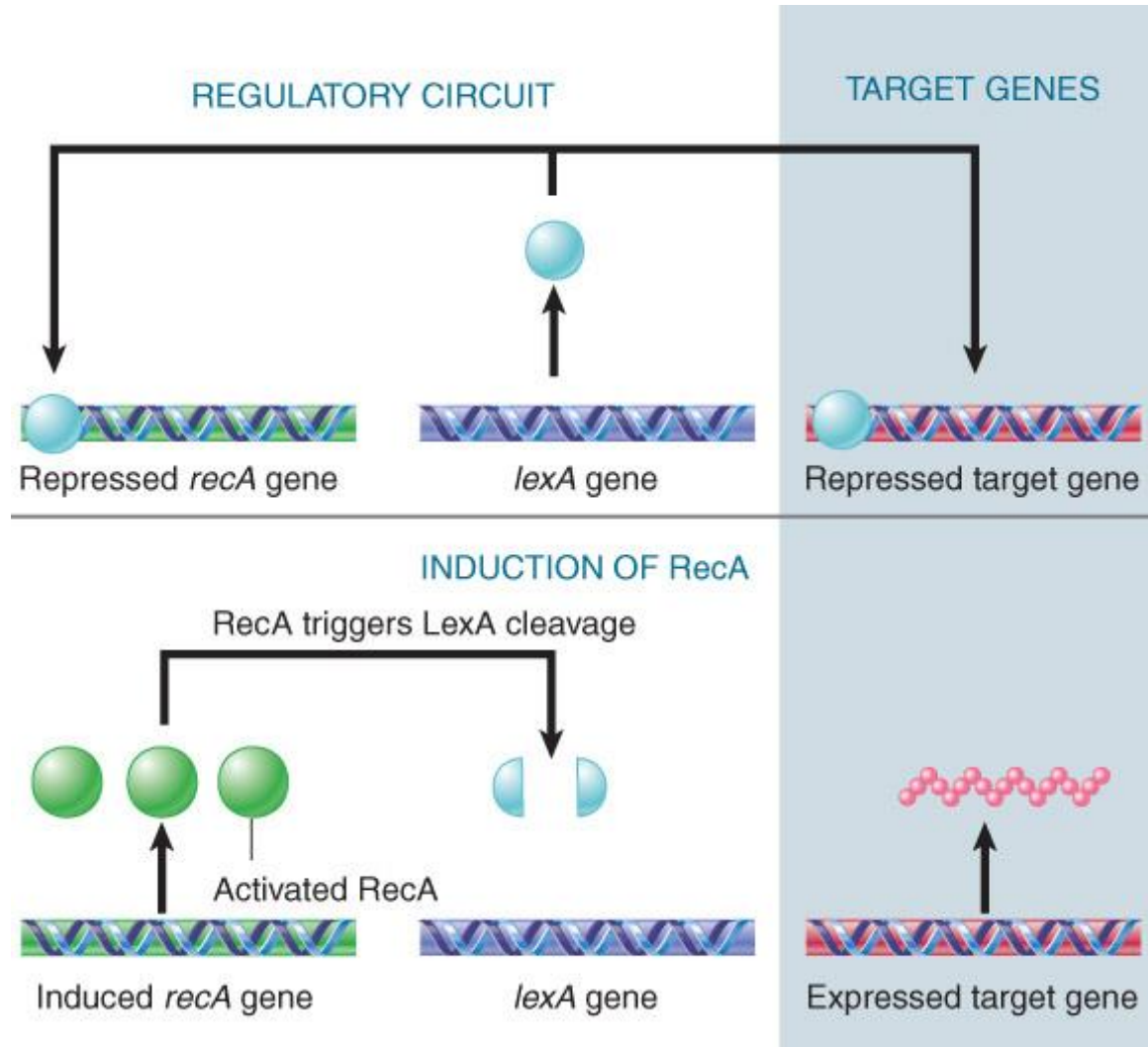
Gap is repaired by retrieving sequence from normal copy



Gap in normal copy is repaired

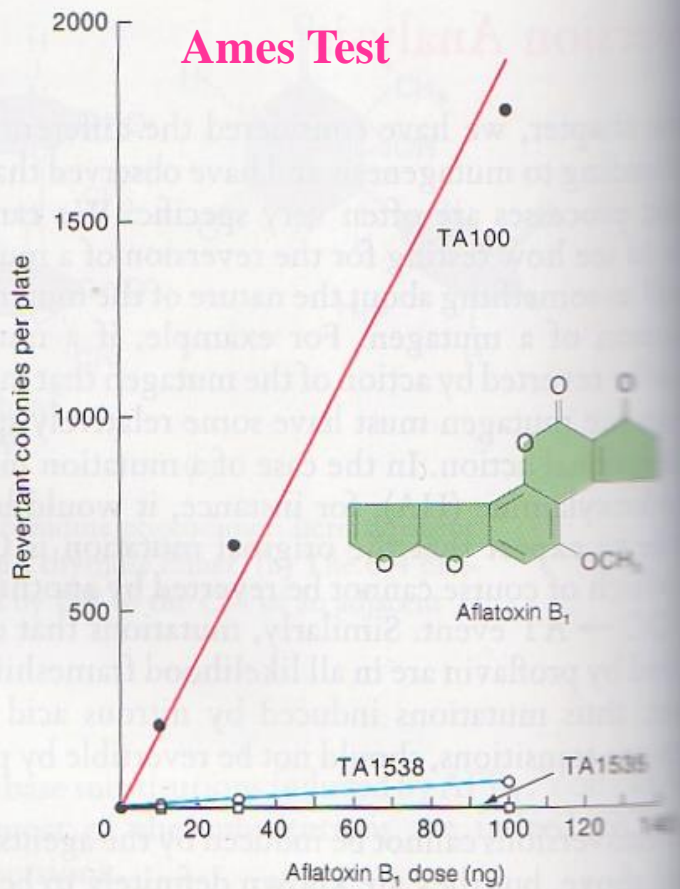


An *E. coli* retrieval system uses a normal strand of DNA to replace the gap left in a newly synthesized strand.

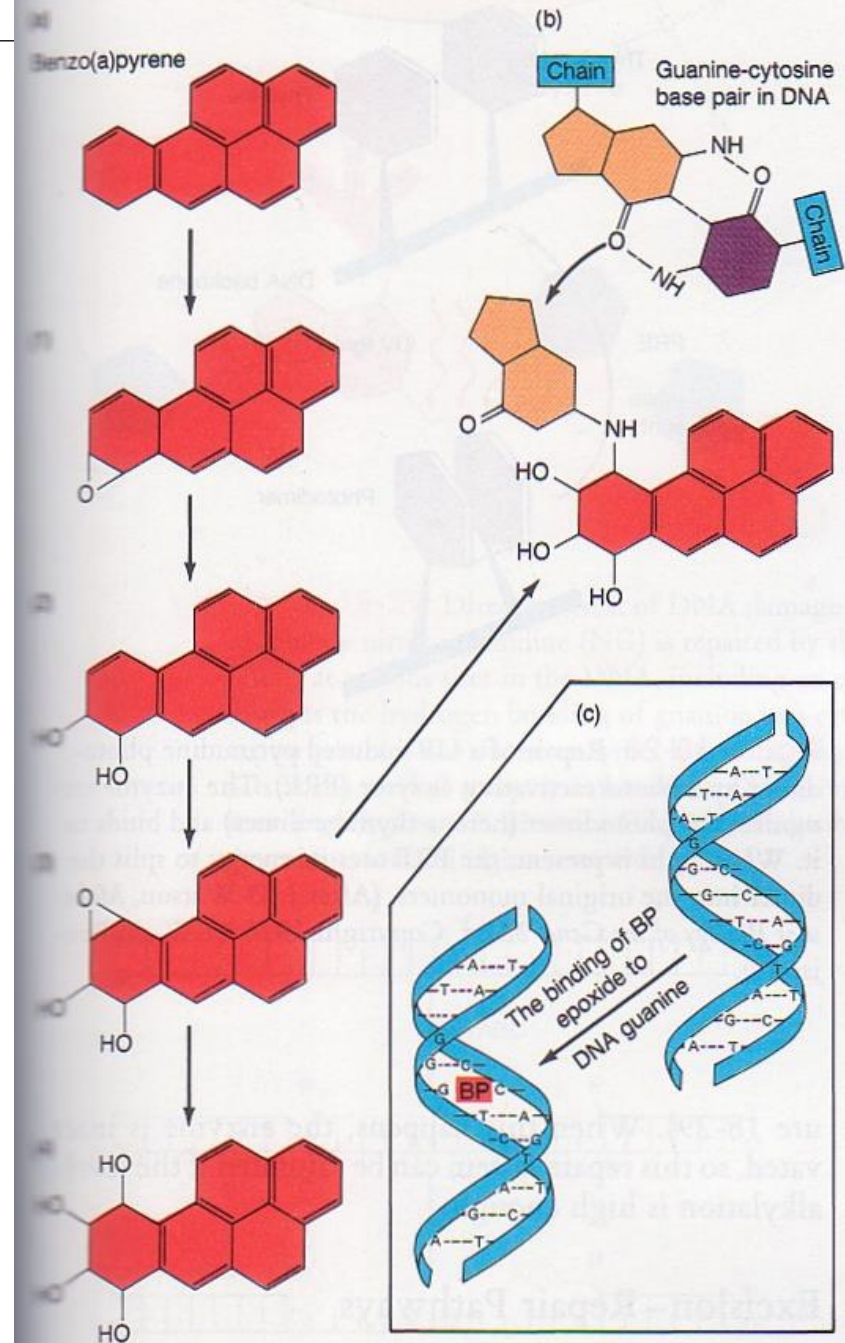


The LexA protein represses many genes, including repair genes, *recA* and *lexA*.

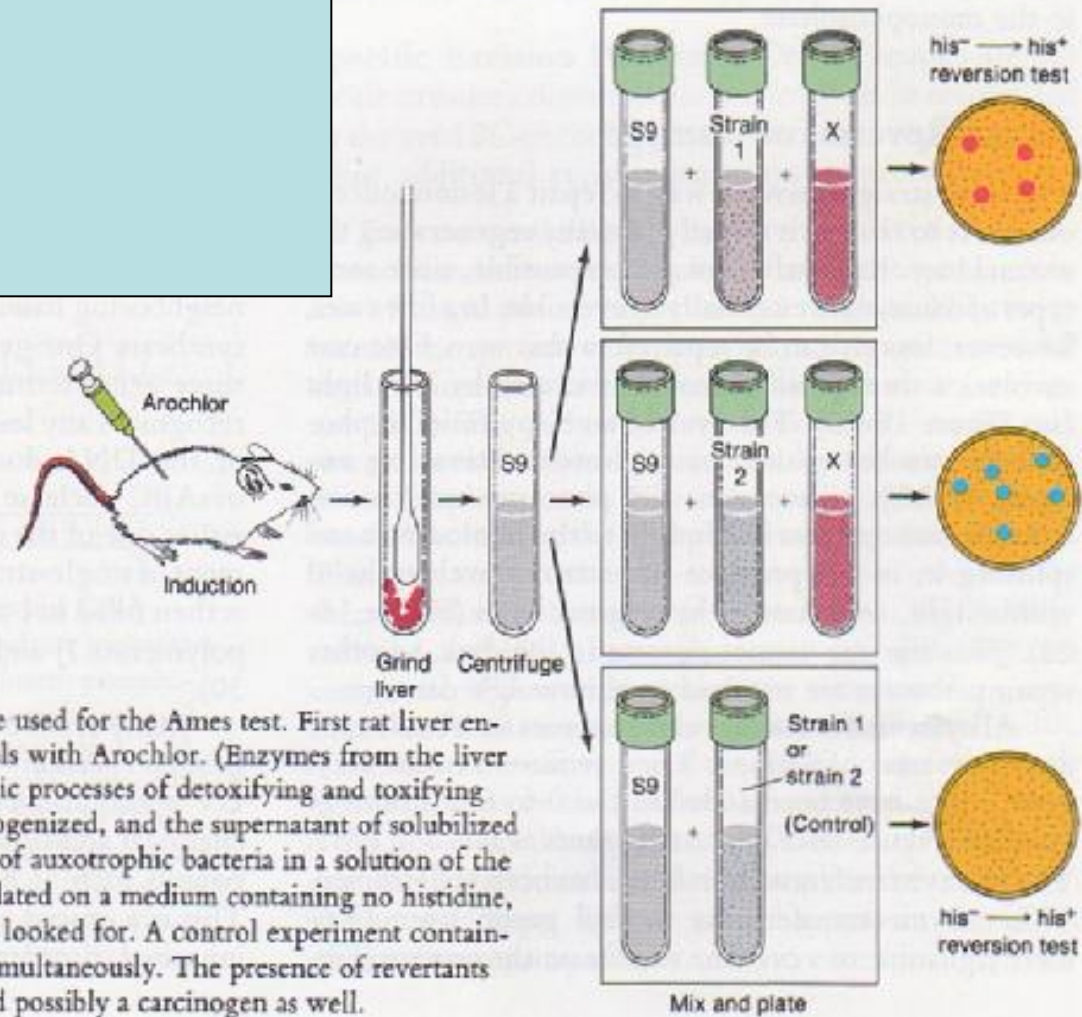




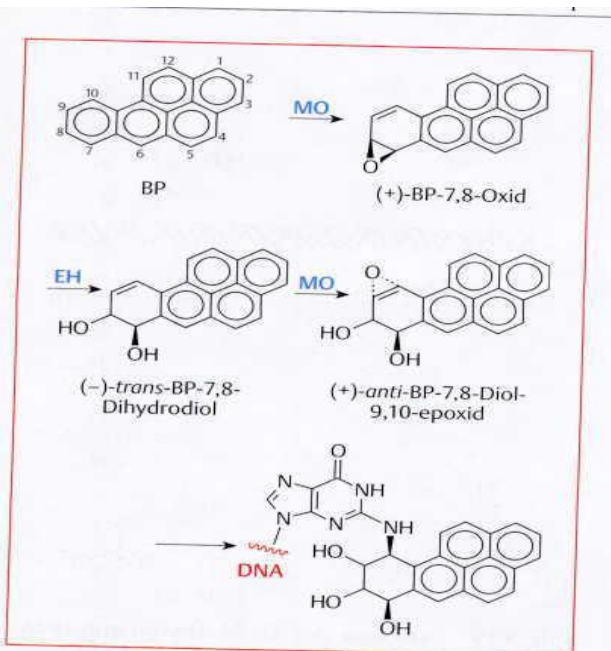
**Figure 18-25** Ames test results showing the mutagenesis of aflatoxin B<sub>1</sub>, which is also a potent carcinogen. TA100, TA1538, and TA1535 are strains of *Salmonella* bearing different *his* auxotrophic mutations. The TA100 strain is highly sensitive to reversion through base-pair substitution. The TA1535 and TA1538 strains are sensitive to reversion through frameshift mutation. The test results show that aflatoxin B<sub>1</sub> is a potent mutagen that causes base-pair substitution but not frameshifts. (From J. McCann and B. N. Ames, *Advances in Modern Toxicology*, Vol. 5. Edited by W. G. Flamm and M. A. Mehlman. Copyright by Hemisphere Publishing Corp., Washington, DC.)



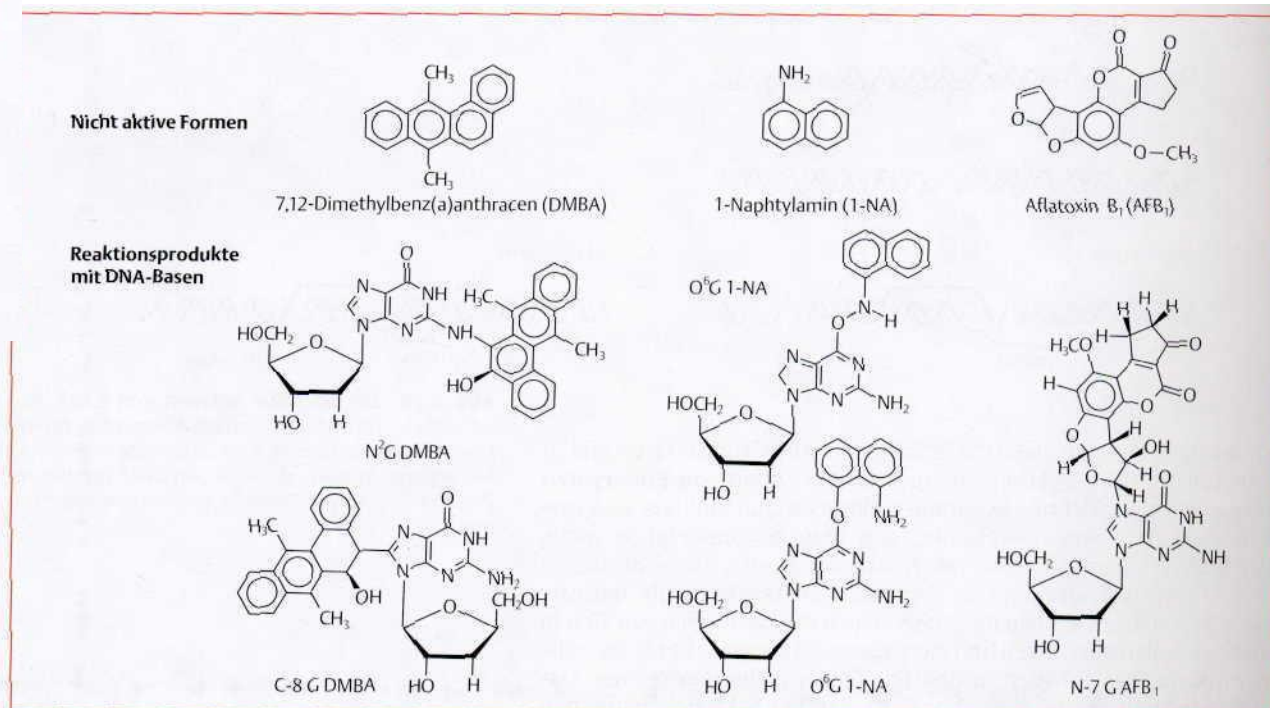




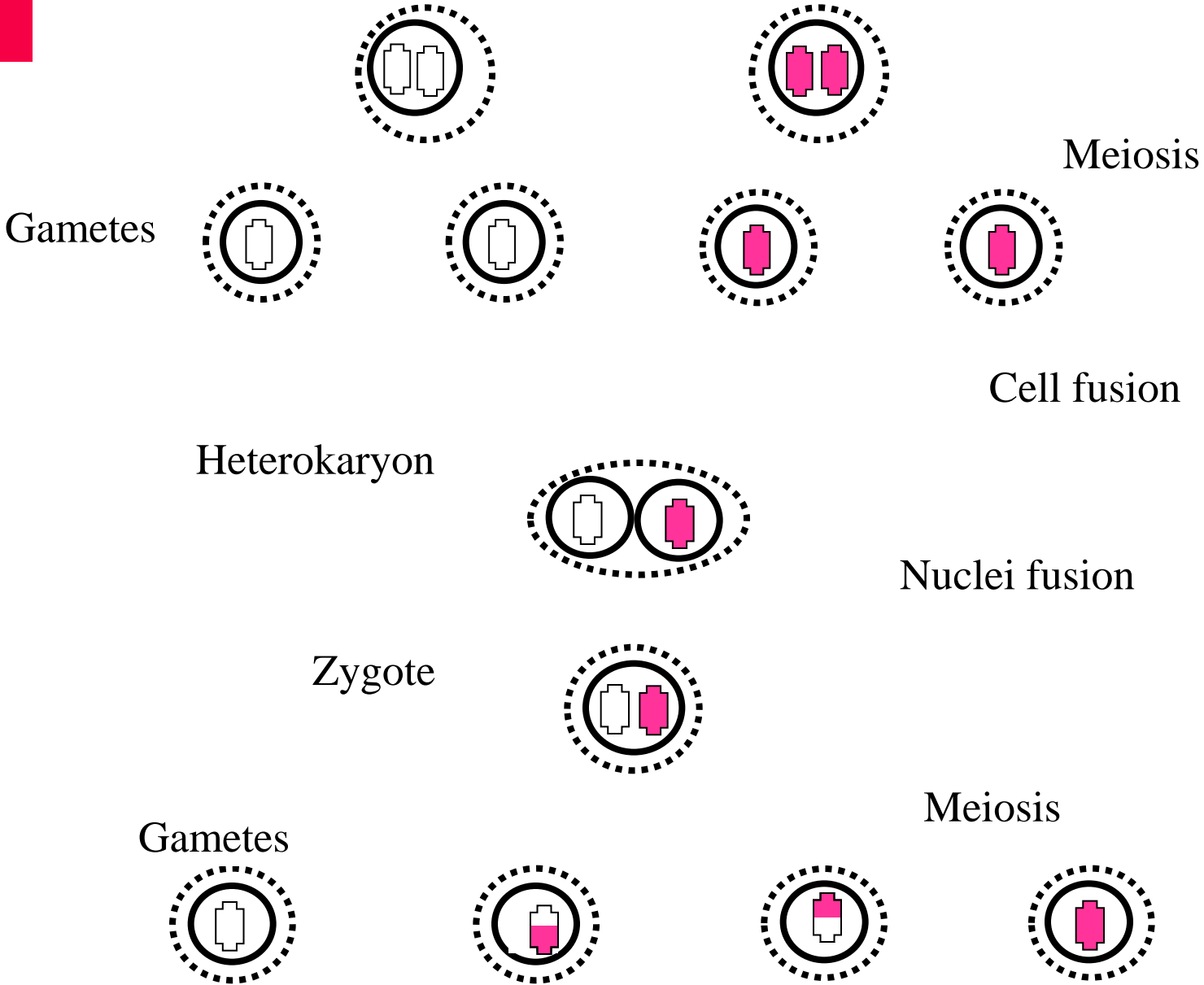
**Figure 18-27** Summary of the procedure used for the Ames test. First rat liver enzymes are mobilized by injecting the animals with Arochlor. (Enzymes from the liver are used because they carry out the metabolic processes of detoxifying and toxifying body chemicals.) The rat liver is then homogenized, and the supernatant of solubilized liver enzymes (S9) is added to a suspension of auxotrophic bacteria in a solution of the potential carcinogen (X). This mixture is plated on a medium containing no histidine, and revertants of mutant strains 1 and 2 are looked for. A control experiment containing no potential carcinogen is always run simultaneously. The presence of revertants indicates that the chemical is a mutagen and possibly a carcinogen as well.



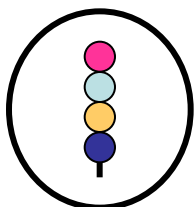
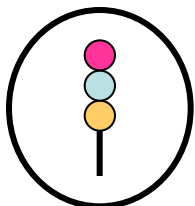
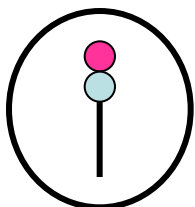
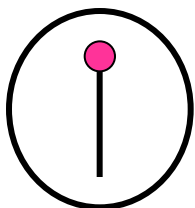
**Abb. 8.21 Aktivierung von Benz(a)pyren.** BP, Benz(a)pyren; MO, Monooxygenase; EH, Epoxidhydrolase. Die aktivierte Verbindung reagiert bevorzugt mit Guanin [nach 20].



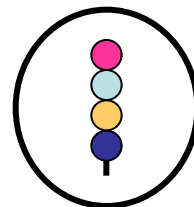
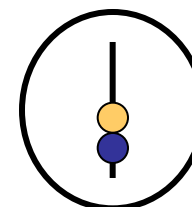
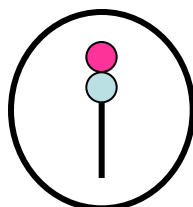
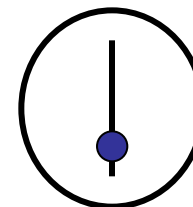
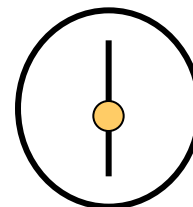
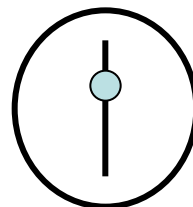
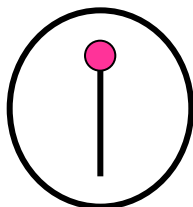
# Genetic Recombination



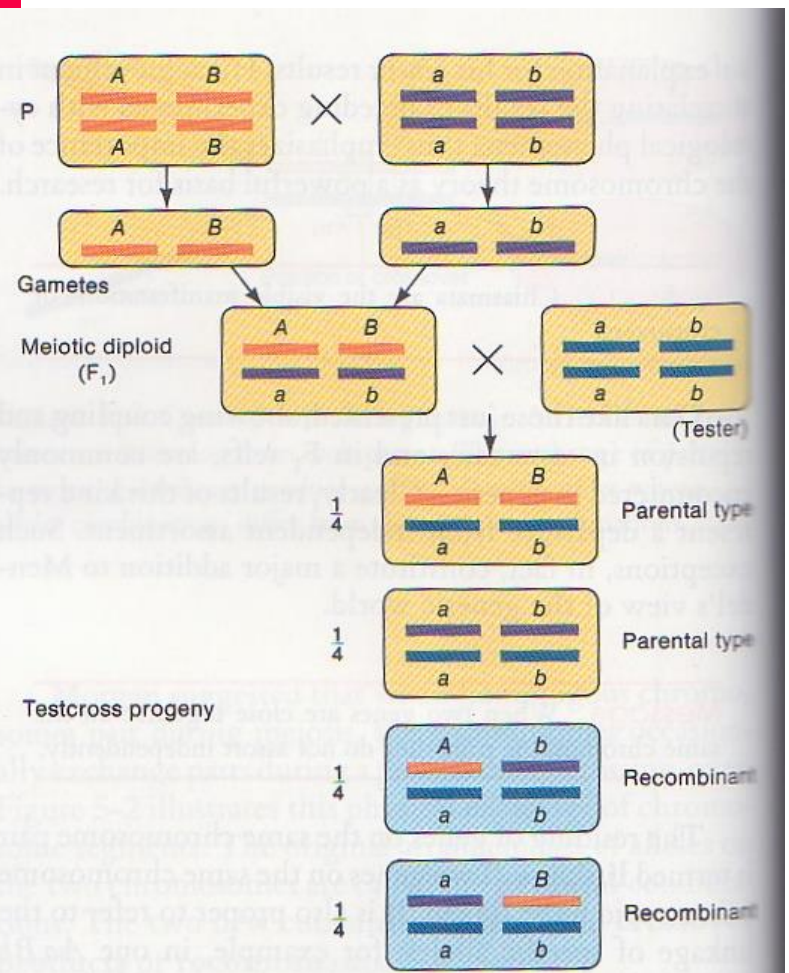
# Mutation



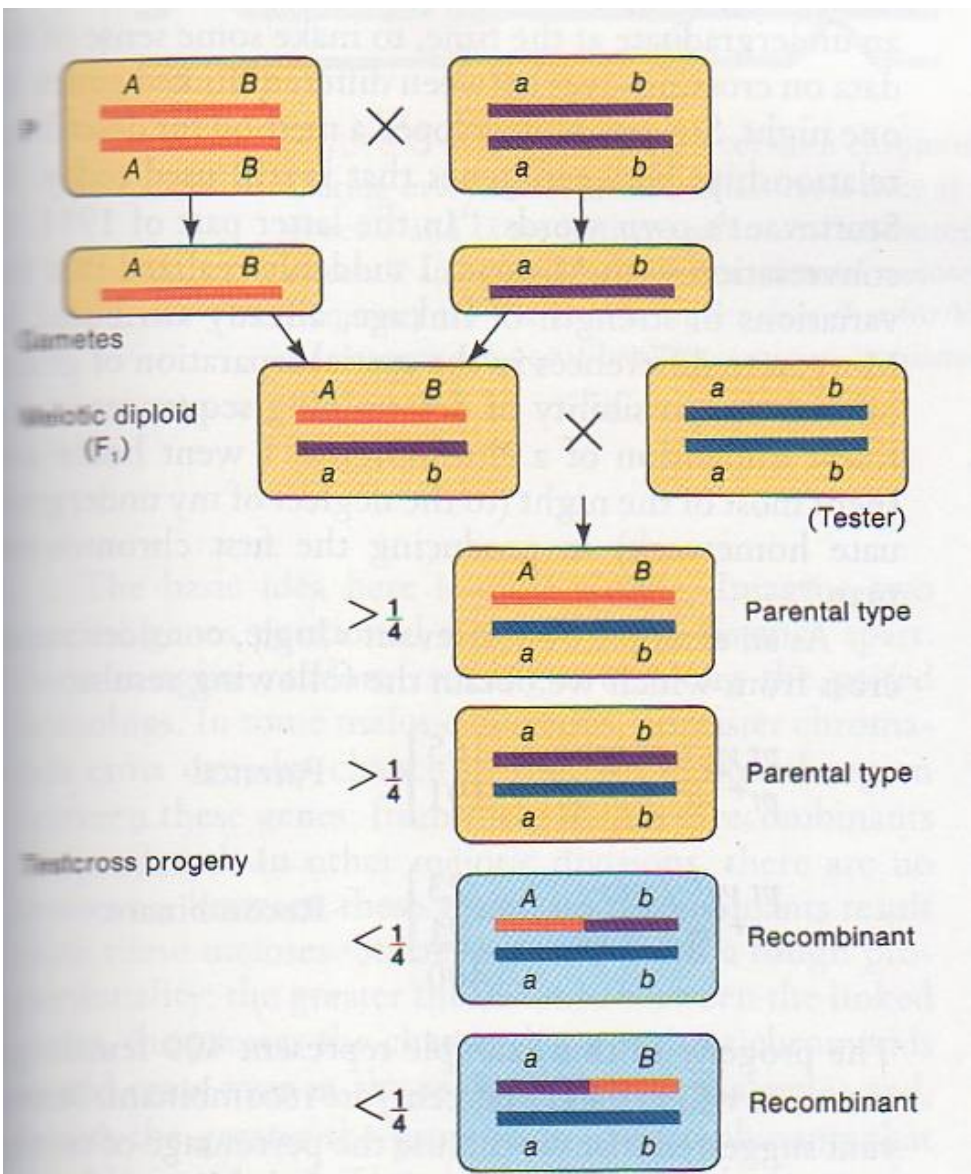
# Recombination





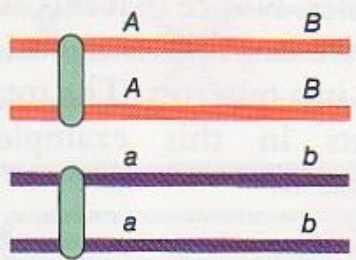
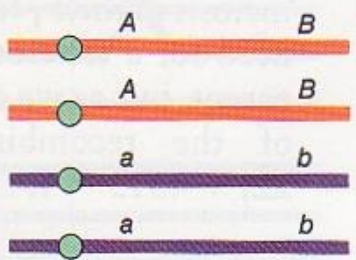



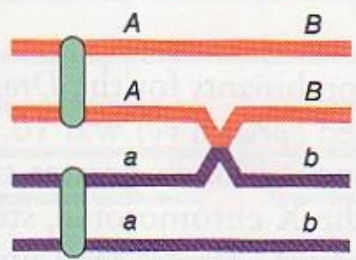
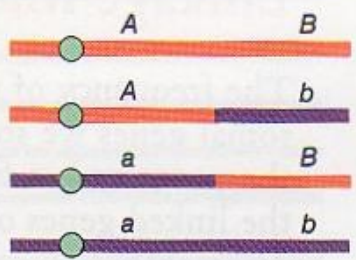





**Figure 5-6** Interchromosomal recombination, which always produces a recombinant frequency of 50 percent. This diagram shows two chromosome pairs of a diploid organism with *A* and *a* on one pair and *B* and *b* on the other. Note that we could represent the haploid situation by removing the part marked P and the testcross.



**Figure 5-8** Intrachromosomal recombination. Notice that the frequencies of the recombinants add up to less than 50 percent.

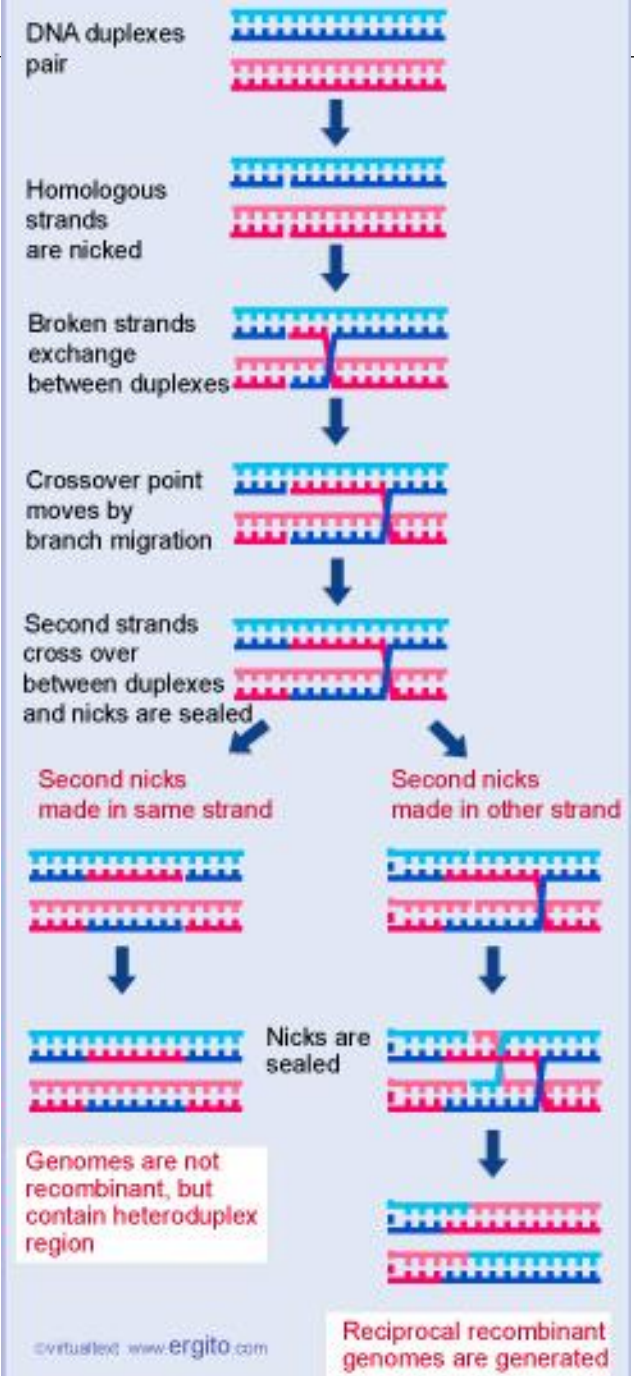
Linkage I: Basic Eukaryotic Chromosome Mapping

	Meiotic chromosomes	Meiotic products	
Meioses with no crossover between the genes			Parental
			Parental
			Parental
			Parental
Meioses with a crossover between the genes			Parental
			Recombinant
			Recombinant
			Parental

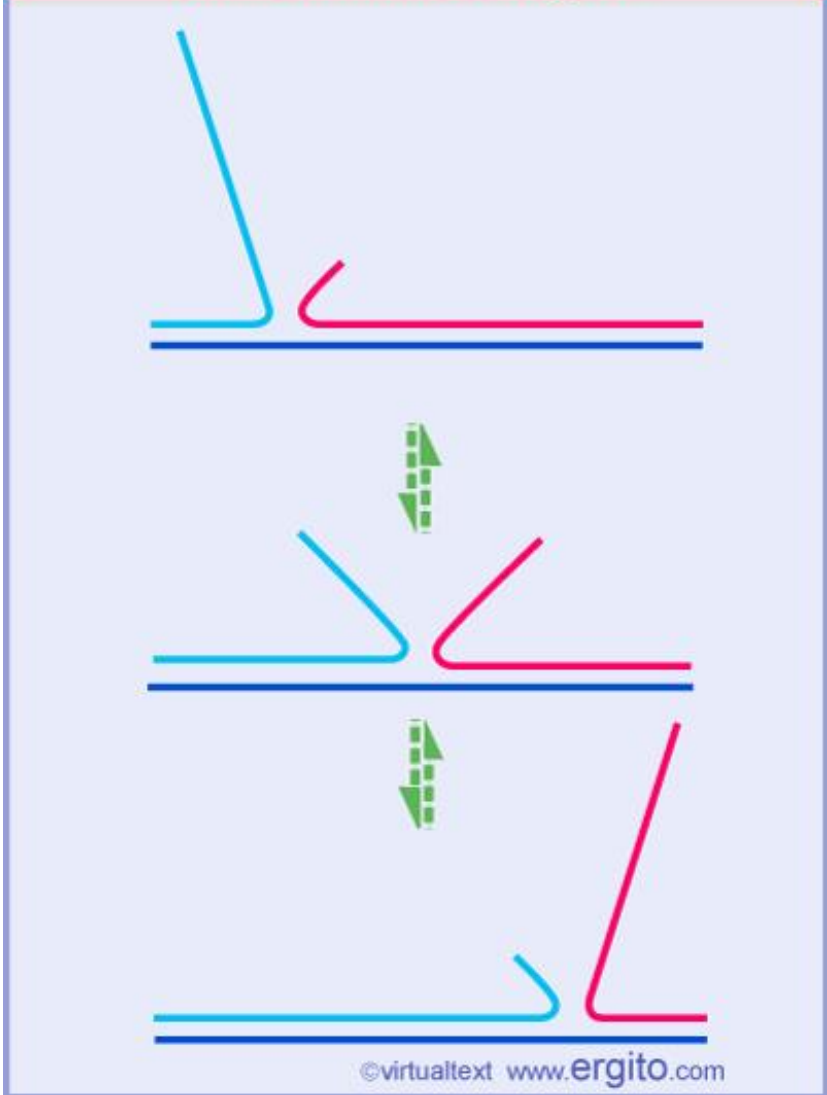
**Figure 5-7** Intrachromosomal recombinants arise from meioses in which nonsister chromatids cross over between the genes under study.



A recombination reaction has two possible outcomes



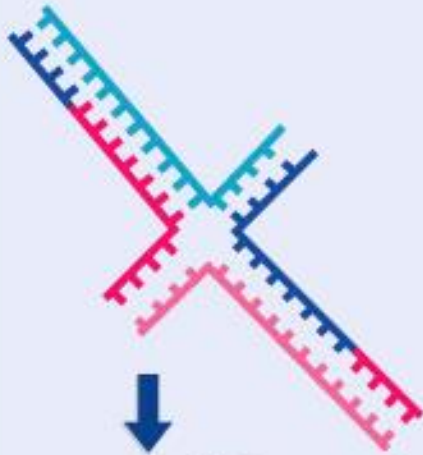
Branch sites can migrate



# Recombination has alternative resolutions



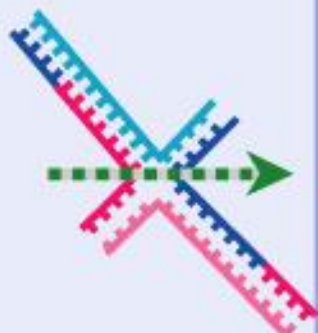
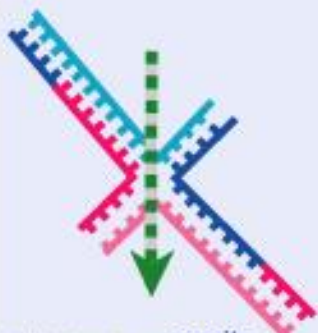
Rotation shows structure of Holliday junction



Nicking controls outcome

Nicks in other strands release splice recombinants (conventional)

Nicks in same strands release patch recombinants



# A double-strand break initiates recombination

Double-strand break made in recipient



Break is enlarged to gap with 3' ends



3' end migrates to other duplex



Synthesis from 3' end displaces one strand in gap



Displaced strand migrates to other duplex



DNA synthesis occurs from other 3' end



Gap replaced by donor sequence

Reciprocal migration generates double crossover



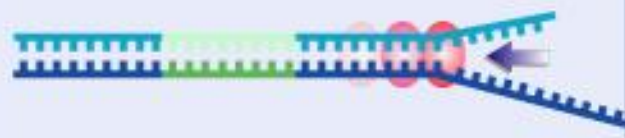


## RecBCD unwinds DNA and cleaves at Chi

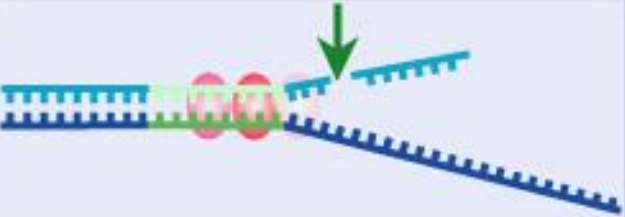
RecBCD binds a double-strand break



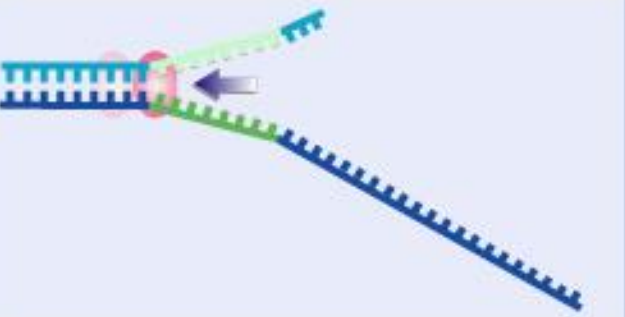
RecBCD unwinds & degrades DNA as exonuclease



RecBCD endonuclease cleaves single strand at chi



RecD dissociates at chi sequence



RecBC continues as helicase



# Homologous Recombination in E.coli

*RecBCD* complex generates strand break at preferred specific sites (chi).

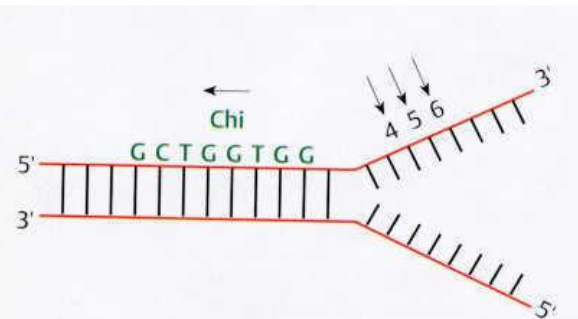
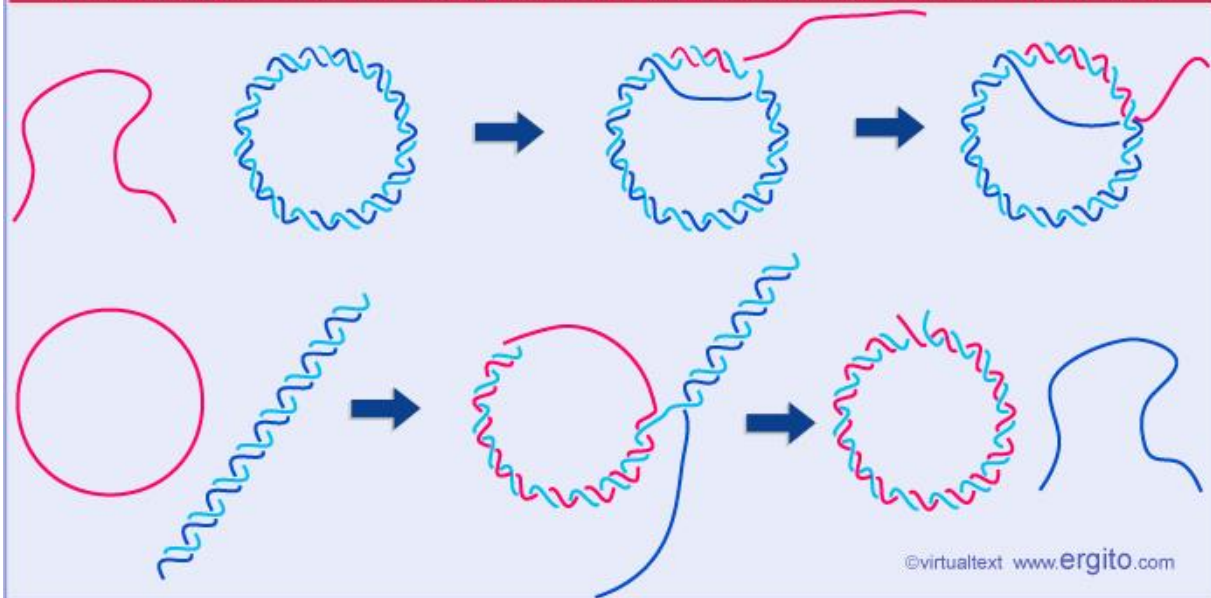
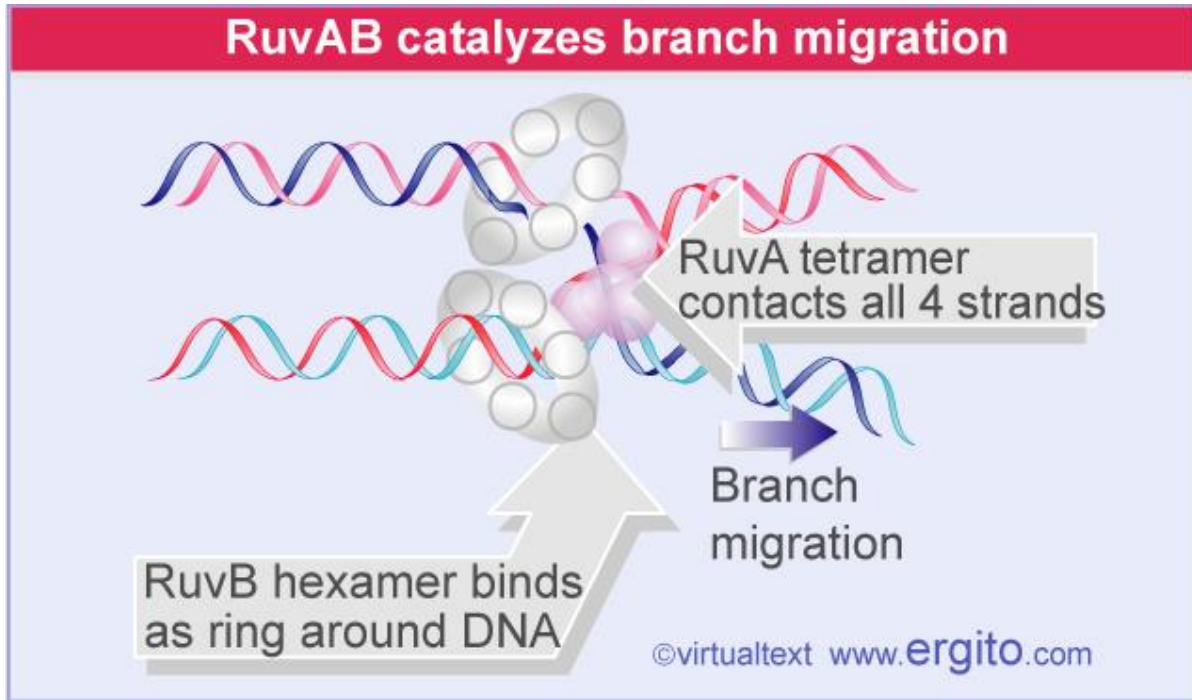
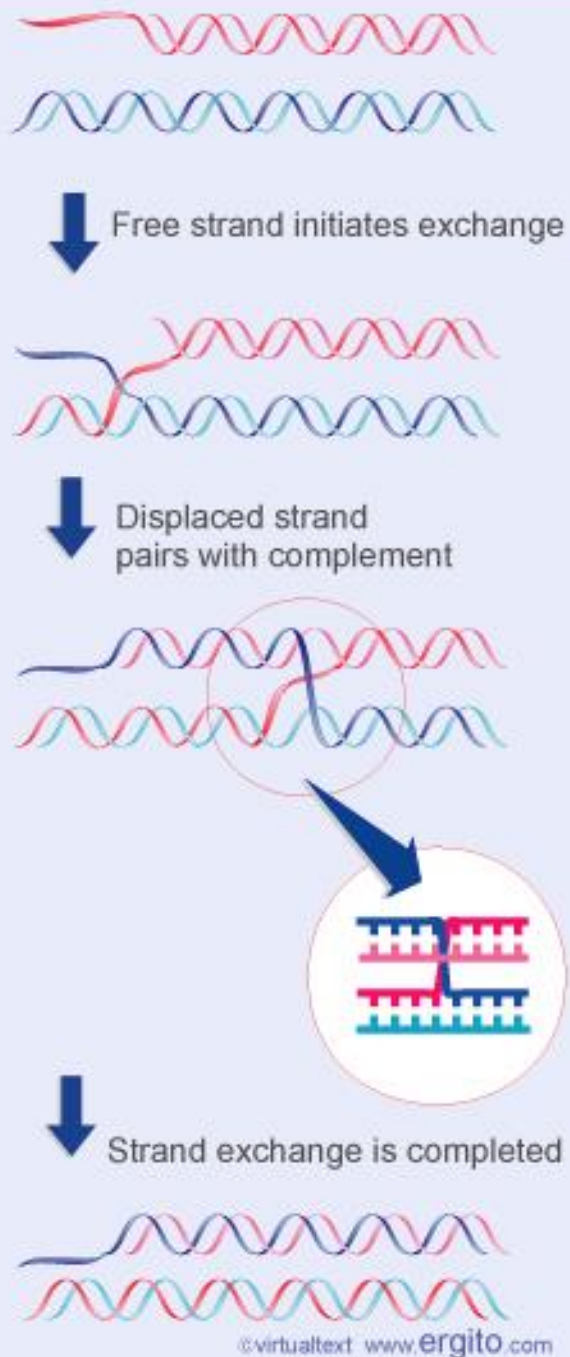


Abb. 7.15 Das Rec BCD-Protein schneidet kurz vor der Chi-Sequenz [nach 11].

RecA, supported by SSB binds to ssDNA and is involved in initiation of strand exchange

## RecA catalyzes strand exchange between duplex and single-stranded molecules





RuvC: Nuclease cleaving Holliday junction

# Gene transfer - parasexual mechanisms with Micro-organisms

## Transformation

Uptake of free DNA from environment

Natural Competence – Induced Competence

Forced Transfer

## Transduction

Bacteriophage mediated gene transfer

## Conjugation

In vivo plasmid transfer

Direct cell-cell contact

## Mitotic/Somatic Cell Fusion

Parasexual fusion

Induced Fusion

# Transduction

## Generalized Transduction

Prototype: Phage P1

Phage reproduces by autonomous replication.

Random pieces of bacterial DNA (generated due to degradation of bacterial genome at late stages of phage infection) are incorporated upon phage assembly

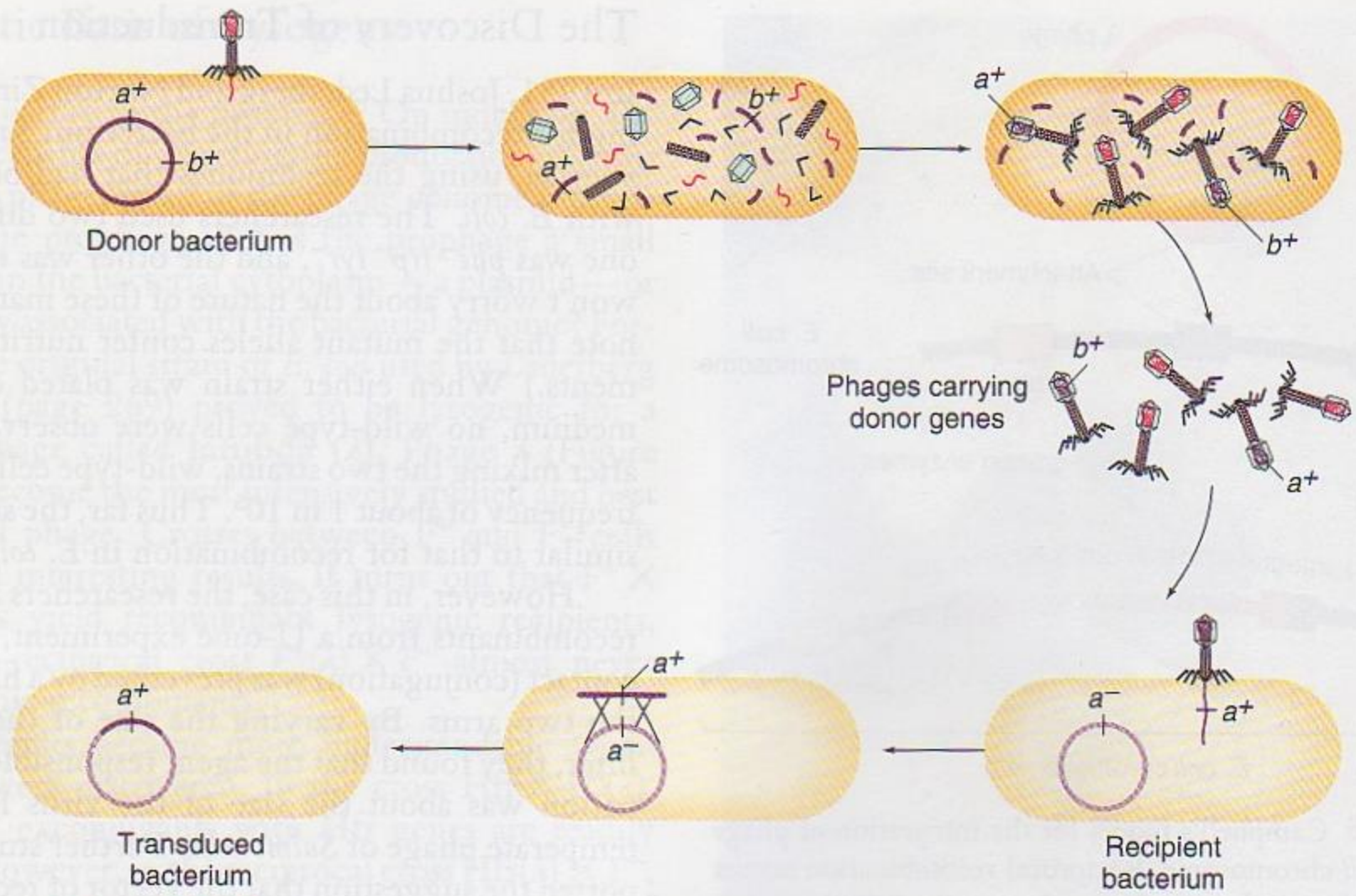
## Specialized Transduction

Prototype: Phage Lambda

Phage integrates into bacterial genome at specific sites

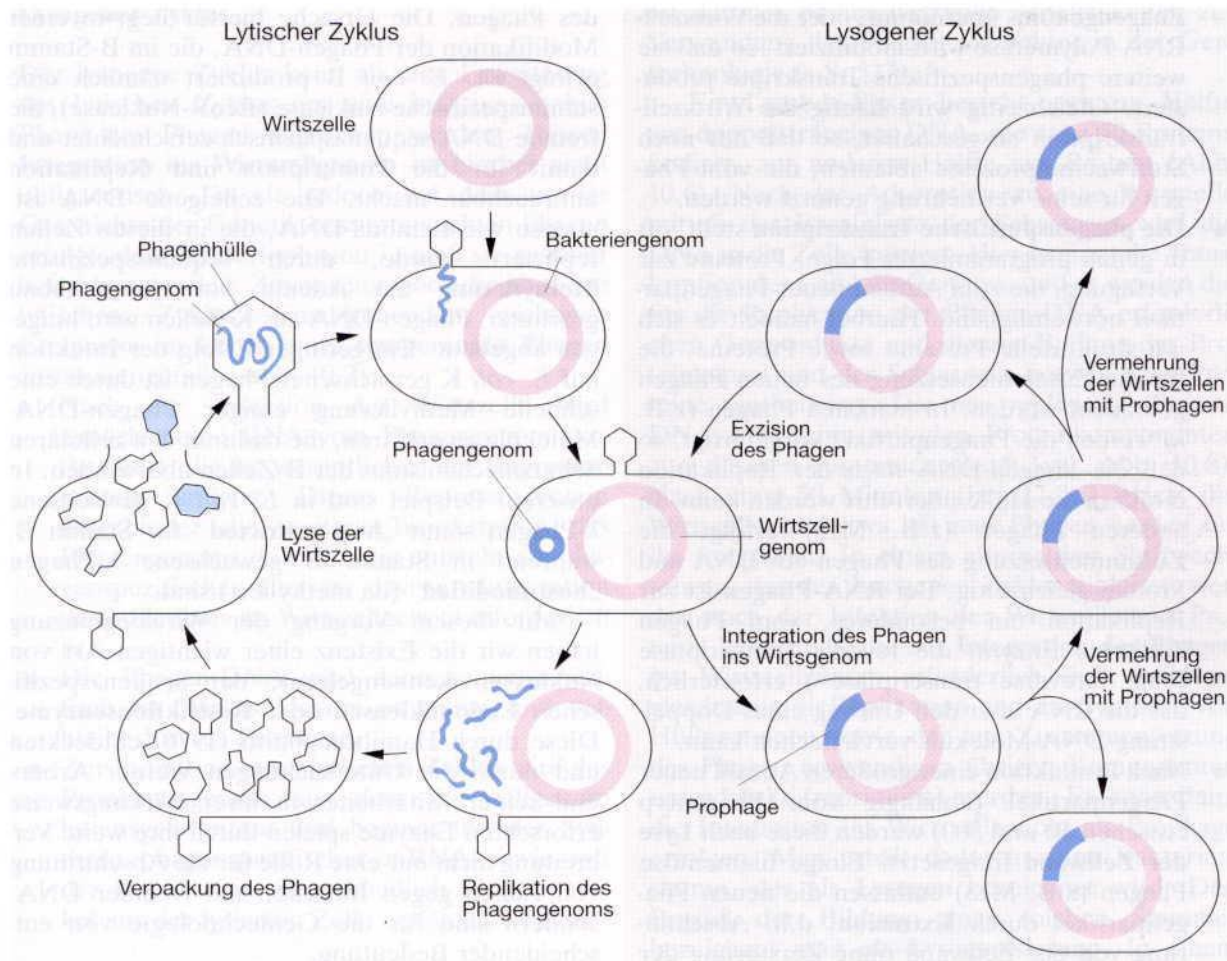
Only sequences adjacent to the integration site can be transduced





**Figure 10-29** The mechanism of generalized transduction. In reality, only a very small minority of phage progeny (1 in 10,000) carries donor genes.

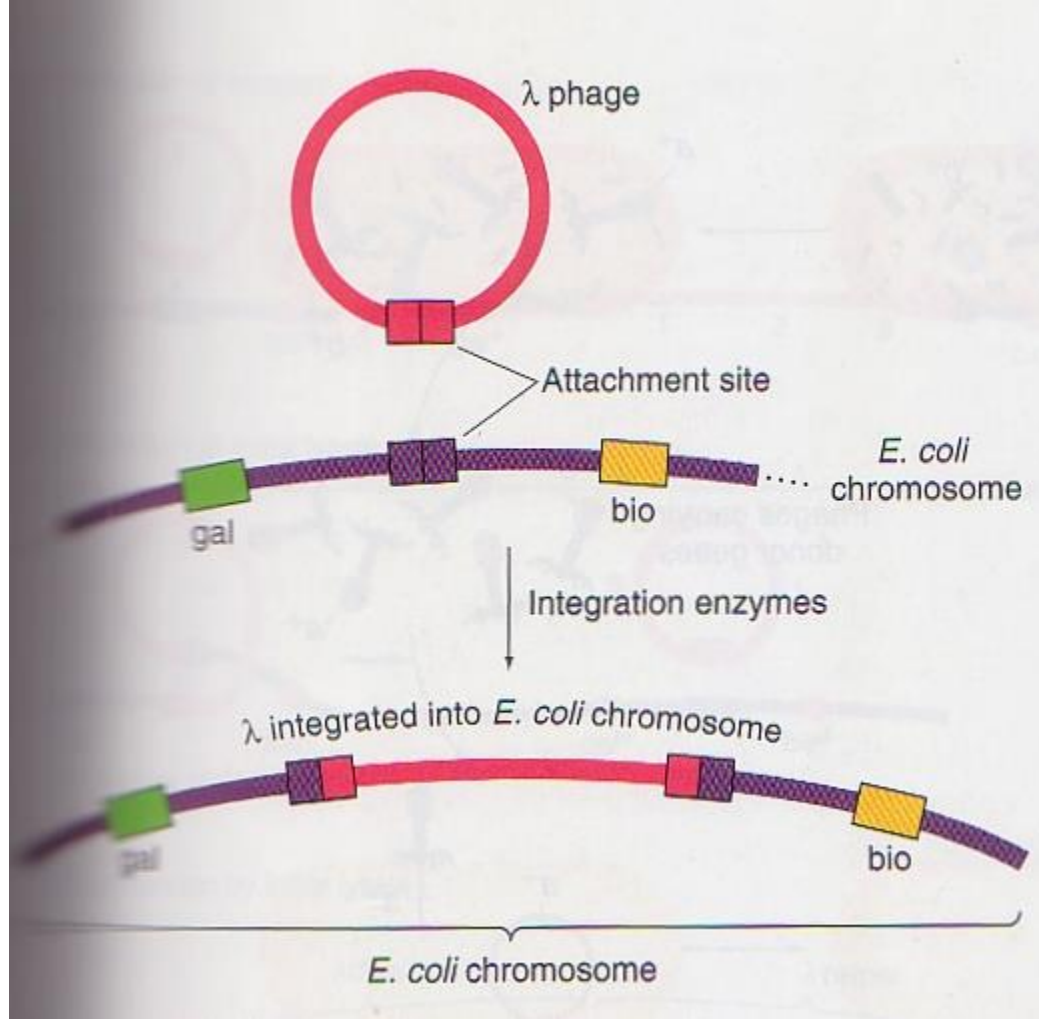
# Phage Lambda Life Cycle



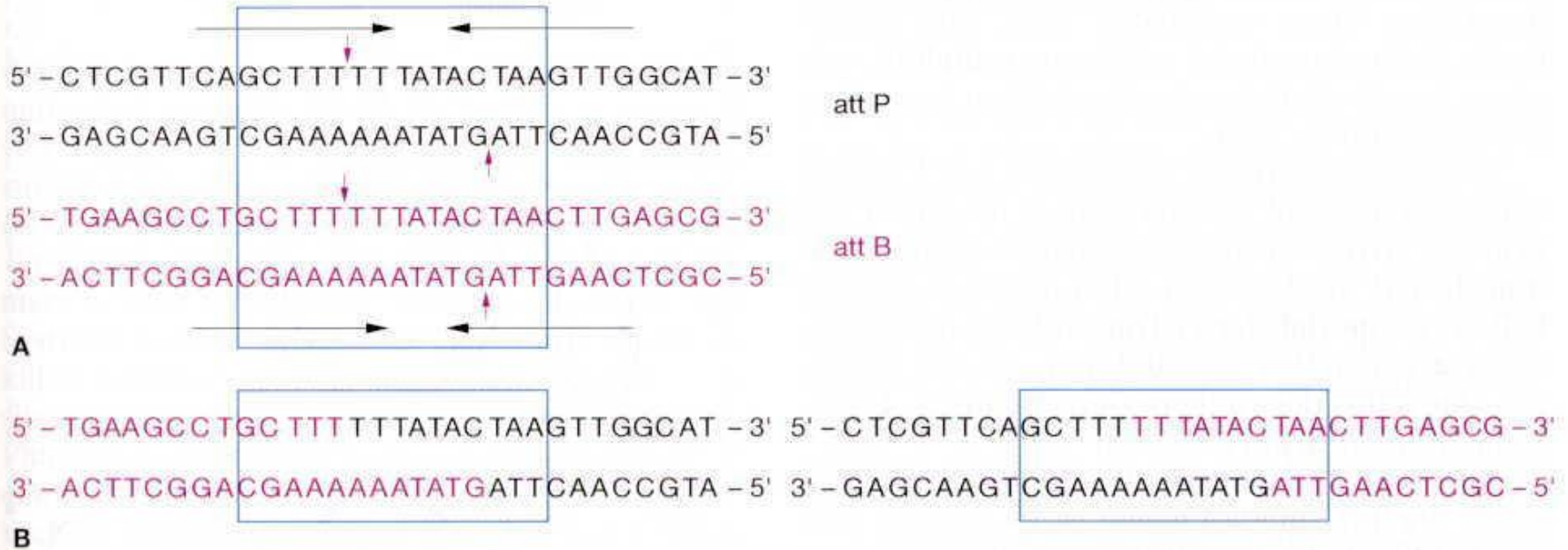
**Abb. 10.7.** Zyklus des Bakteriophagen Lambda. Nach der Infektion der Wirtszelle durch die Phagen-DNA wird diese zunächst zirkularisiert. Im lytischen Zyklus (*links*) werden an dieser DNA als Matrize nach dem Rolling-circle-Mechanismus (Abb. 10.10) neue lineare Phagen-DNA-Moleküle synthetisiert. Gleichzeitig werden die Hüllproteine hergestellt, so daß schließlich eine Verpackung der DNA in den vorbereiteten Phagenkopf und ein Anfügen des ebenfalls vorbereiteten Phagenschwanzes erfolgen kann. Die Zelle lysiert dann und entläßt infektiöse neue Phagenpartikel.

Im lysogenen Zyklus (*rechts*) erfolgt zunächst eine Integration des Lambda-Phagen als Prophage ins bakterielle Genom. In dieser Form kann der Prophage über viele Zellgenerationen im Bakteriengenom verbleiben, ohne daß seine Anwesenheit erkennbar wird oder Folgen für die Wirtszelle hat. Erst bei einer spontanen oder induzierten Exzision des Prophagen kann es zu einer intrazellulären Vermehrung seines Genoms kommen und die Zelle mündet in den lytischen Zyklus ein. (Nach Watson et al. 1987)

**Figure 10-28** Campbell's model for the integration of phage  $\lambda$  into the *E. coli* chromosome. Reciprocal recombination occurs between a specific attachment site on the circular  $\lambda$  DNA and a specific region on the bacterial chromosome between the *gal* and *bio* genes.





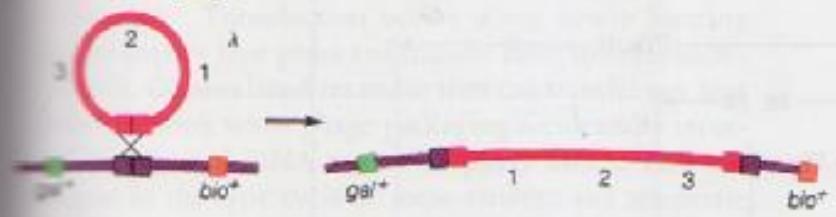


**Abb. 10.9 A, B.** Sequenzspezifische Integration des Phagen Lambda ins *E. coli*-Genom. Sequenzhomologien zwischen den *attP*- und *attB*-Regionen von Lambda und *E. coli* (*oben*) führen zu der Integration der Phagen in einer Position zwischen dem *gal*- und dem *bio*-Gen (*unten*). Die *hori-*

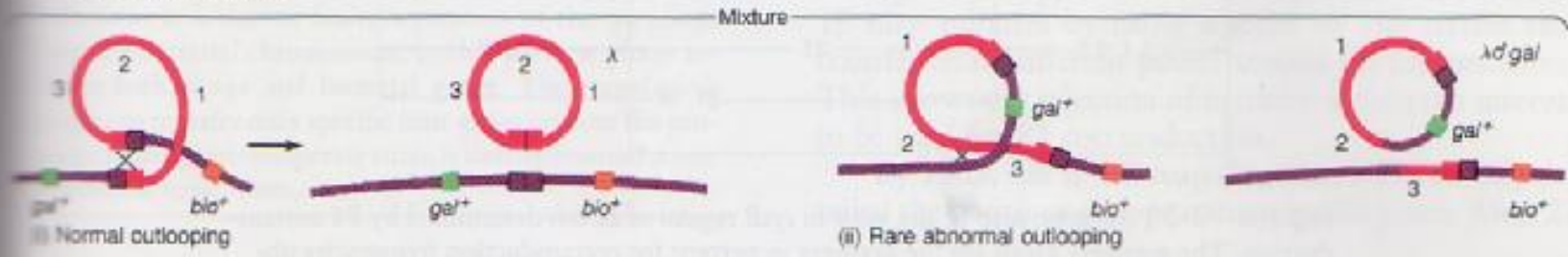
*zontalen Pfeile* zeigen die invertierten Repeats an, die *vertikalen kurzen Pfeile* die Schnittstellen, an denen die *att*-Regionen geöffnet werden. Die beiden Grenzbereiche *links* und *rechts* vom Phagengenom, innerhalb deren die Integration des Phagen erfolgt ist



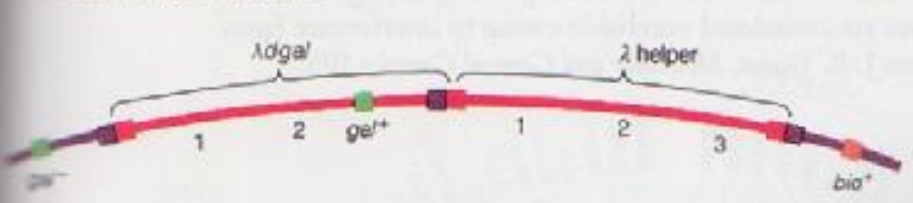
(a) Production of lysogen



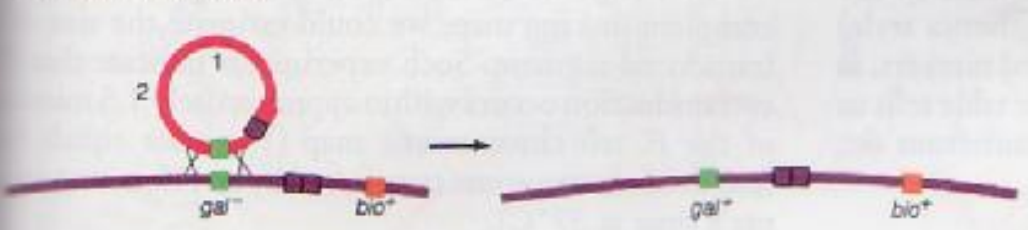
(b) Production of initial lysate



(c) Transduction by initial lysate



(d) Lysogenic transductants

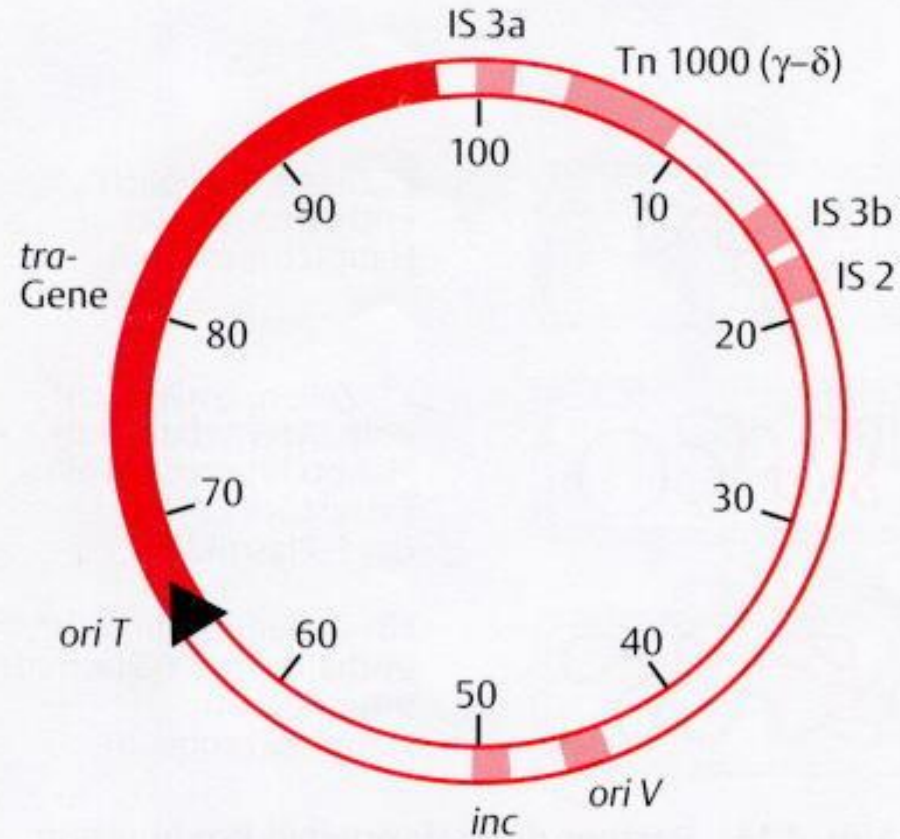


(e) Transductants produced by recombination

**Figure 10-30** Specialized transduction mechanisms in phage  $\lambda$ . (a) The production of a lysogenic bacterium takes place by crossing-over in a specialized region. (b) The lysogenic bacterial culture can produce normal  $\lambda$  or, rarely, an abnormal particle,  $\lambda dgal$ , which is the transducing particle. (c) Transduction by the mixed lysate can produce  $gal^+$  transductants by the coincorporation of  $\lambda dgal$  and a  $\lambda$  helper phage or, more rarely, by crossovers flanking the  $gal$  gene. The purple double squares are bacterial integration sites, the red double squares are  $\lambda$  integration sites, and the pairs of purple and red squares are hybrid integration sites, derived partly from *E. coli* and partly from  $\lambda$ .

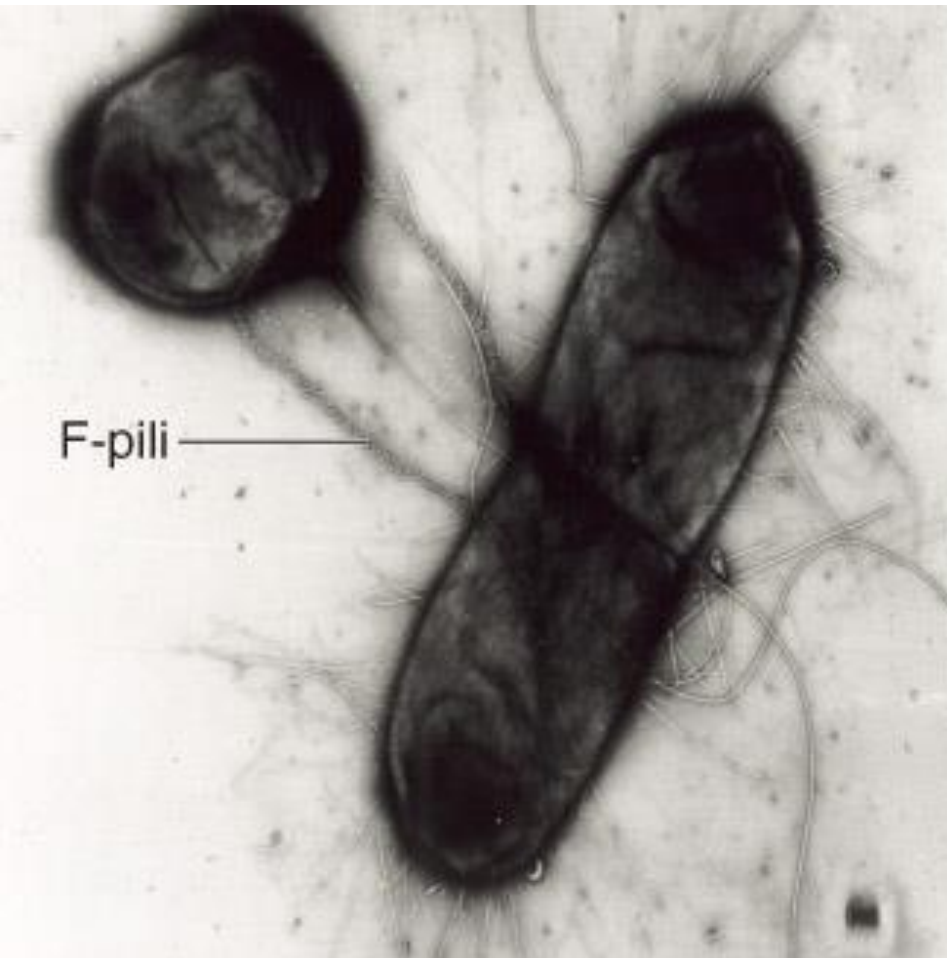
# 12.1.16

# Conjugation



**Abb. 4.16 Physikalische Karte des F-Plasmids.** Die DNA besteht aus 100 000 Basenpaaren. Das linke Ende des IS 3-Elementes gilt als Beginn der Karte. Nur ein kleiner Teil der ca. 60 Gene des F-Plasmids ist angegeben. Weitere Beschreibungen im Text [nach 25].

## Bacterial Conjugation



Mating bacteria are initially connected when donor F-pili contact the recipient bacterium.

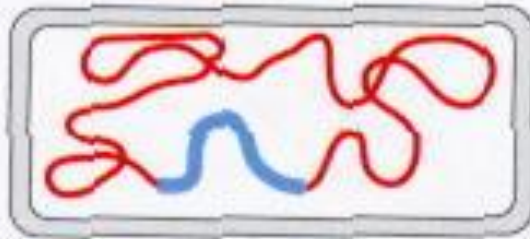




F<sup>-</sup> cells (female)  
No F-plasmid present



F<sup>+</sup> cells (male)  
F-plasmid present



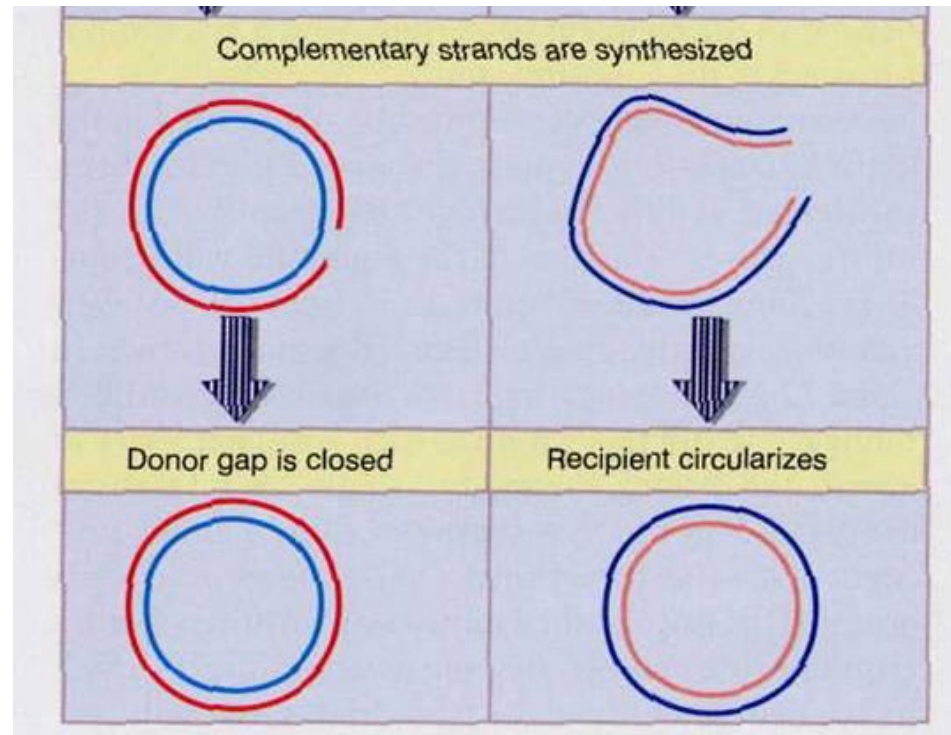
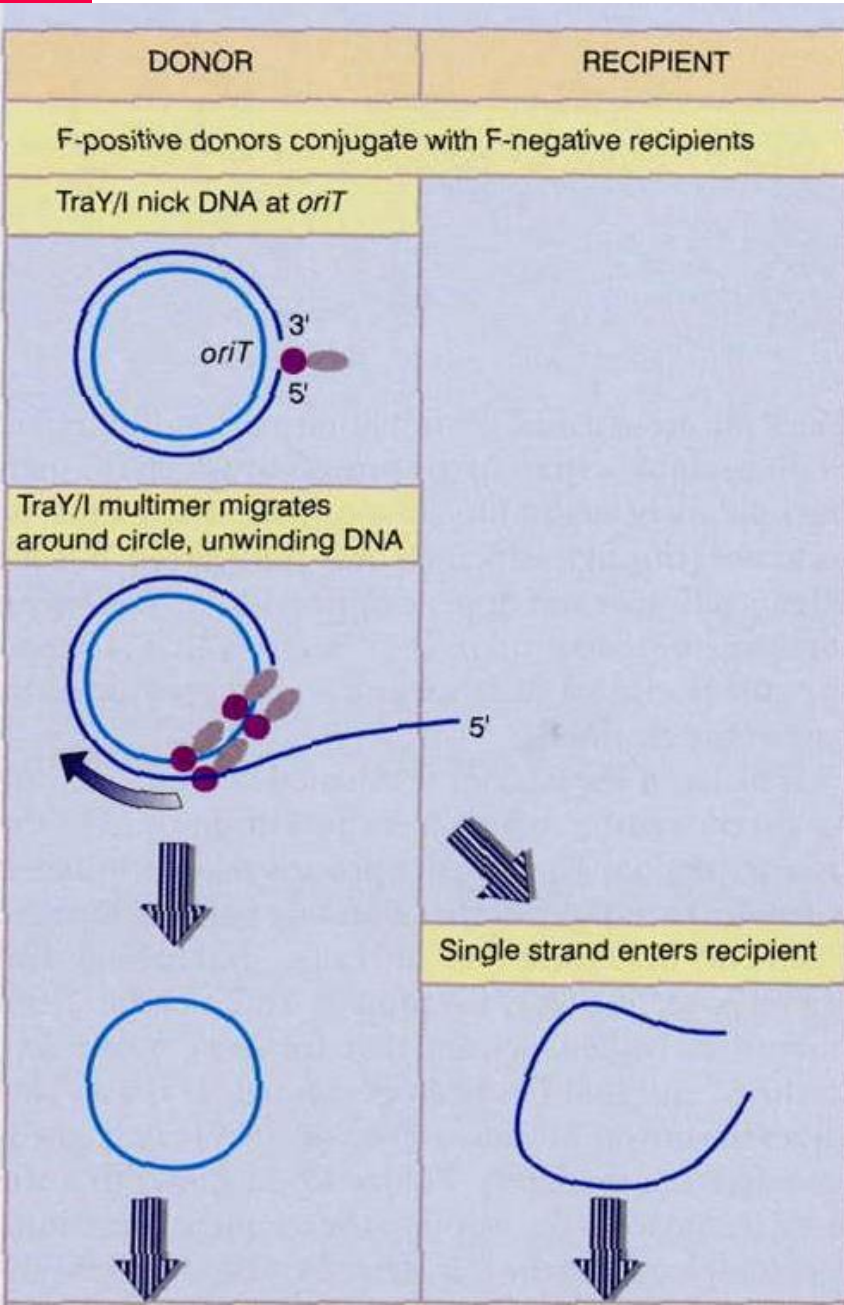
Hfr cells  
F-plasmid is integrated into  
Bacterial chromosome

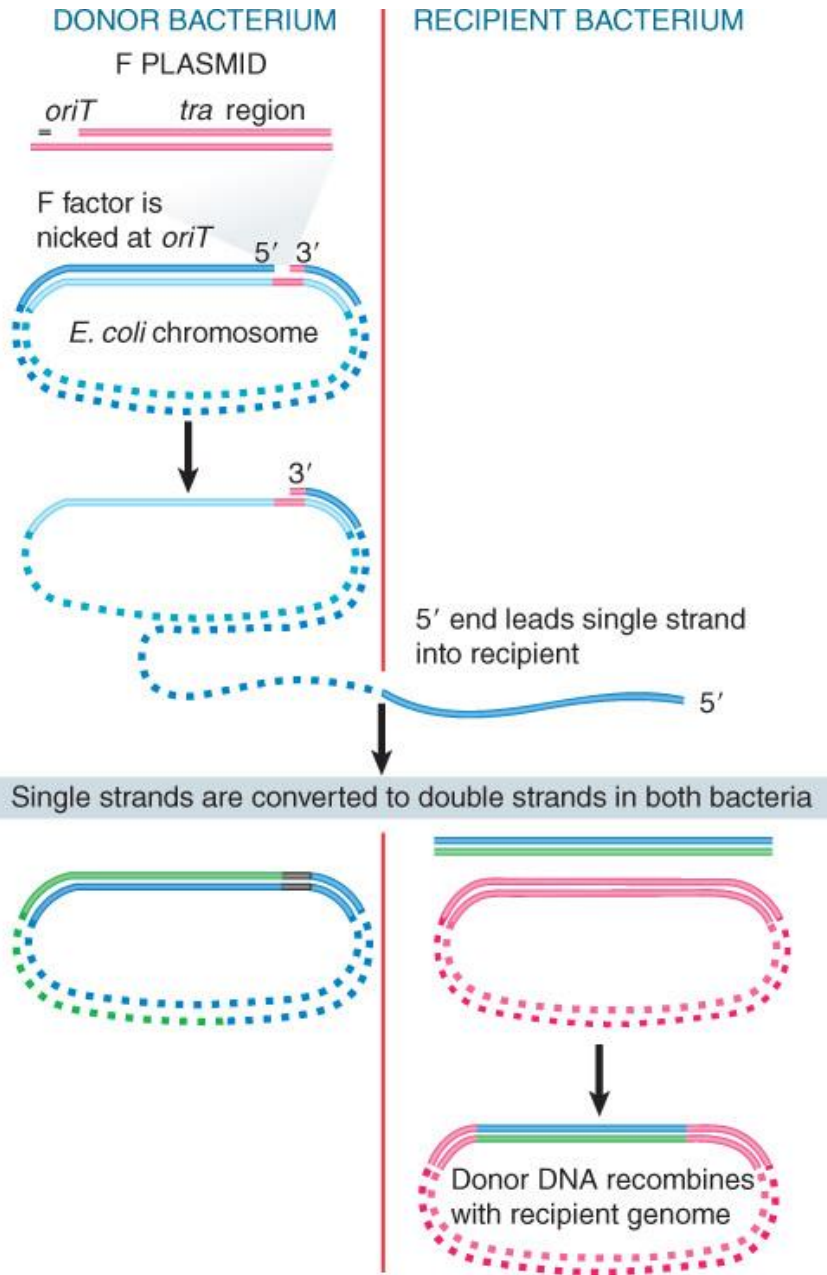
Partners interacting at conjugation

# Conjugative DNA Transfer

## Rolling Circle Replication

**Figure 12.22** Transfer of DNA occurs when the F factor is nicked at *oriT* and a single strand is led by the 5' end into the recipient. Only one unit length is transferred. Complementary strands are synthesized to the single strand remaining in the donor and to the strand transferred into the recipient.

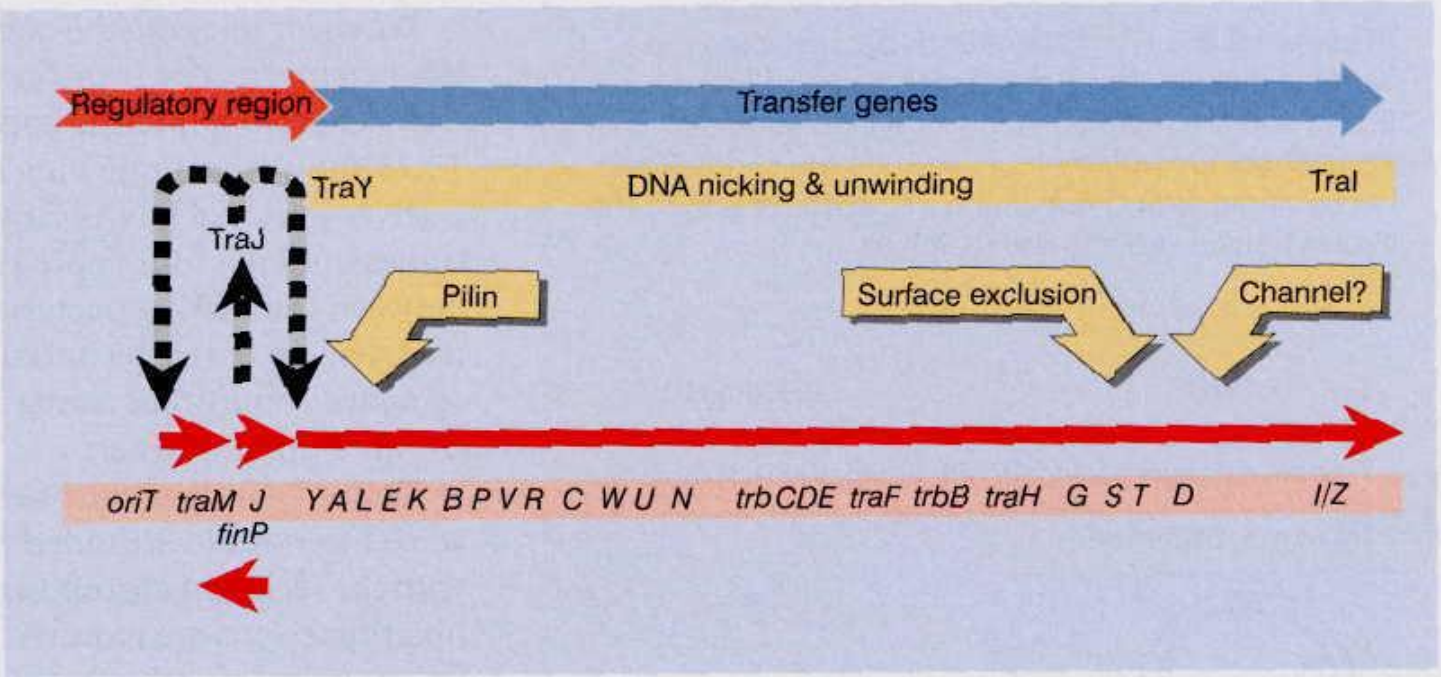




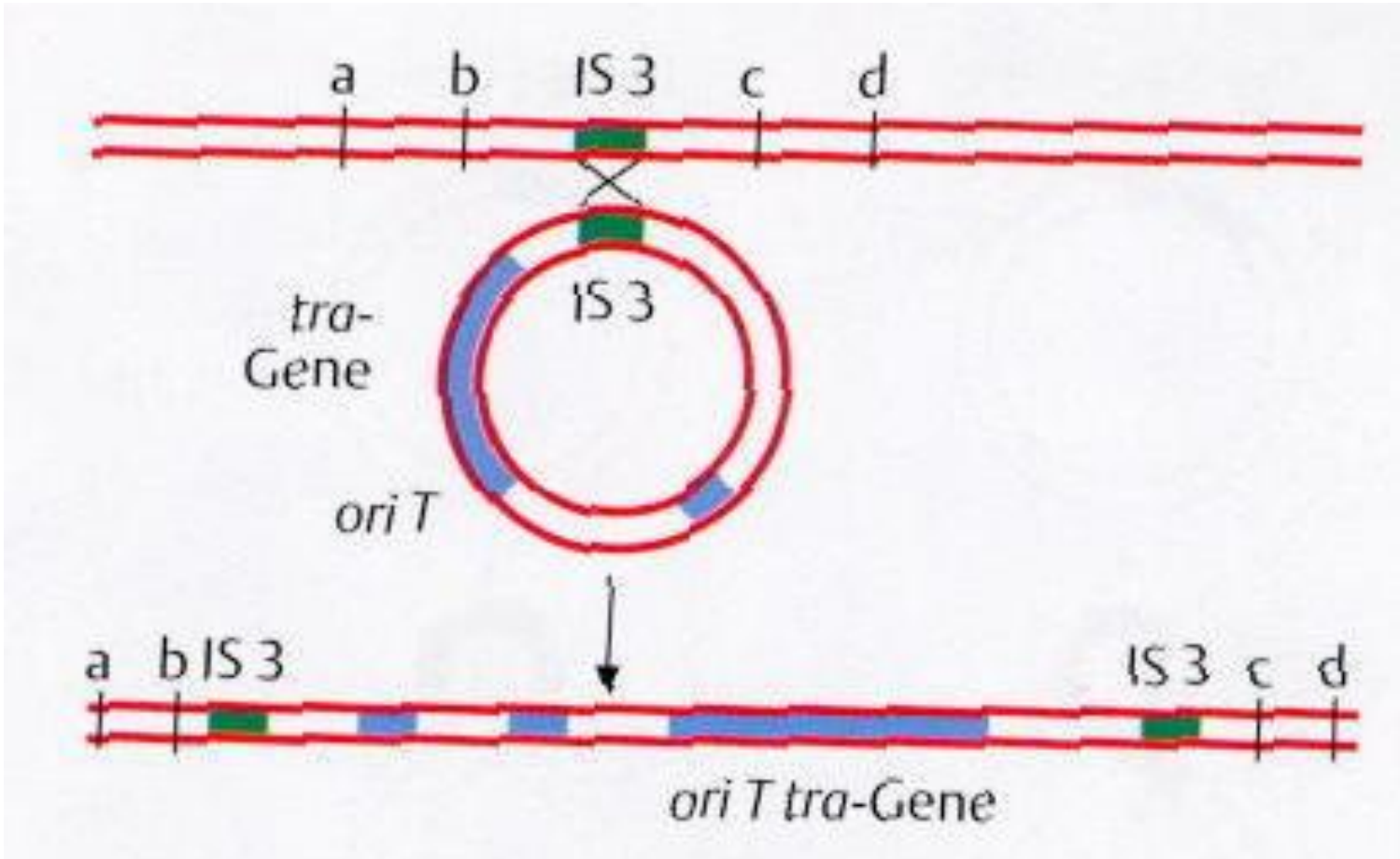
Transfer of chromosomal DNA occurs when an integrated F plasmid is nicked at *oriT*.

## Rolling circle replication

**Figure 12.20** The *tra* region of the F plasmid contains the genes needed for bacterial conjugation.

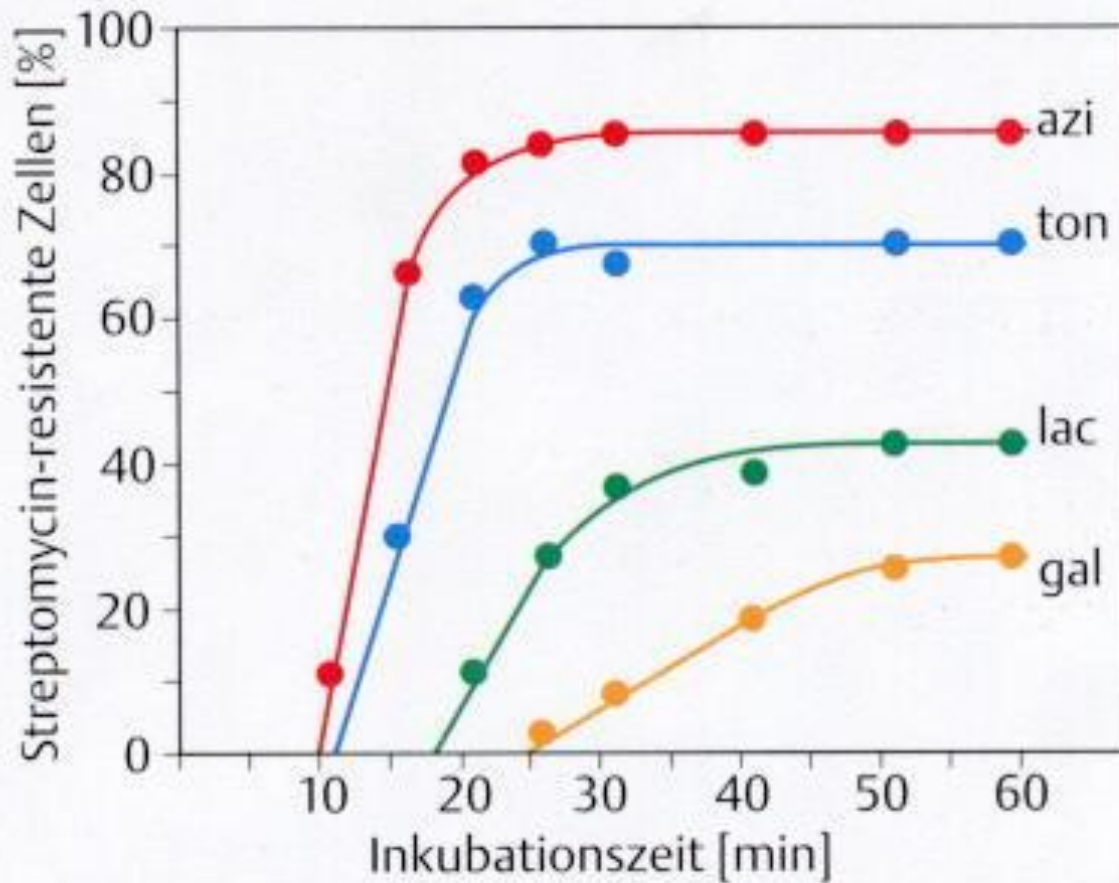




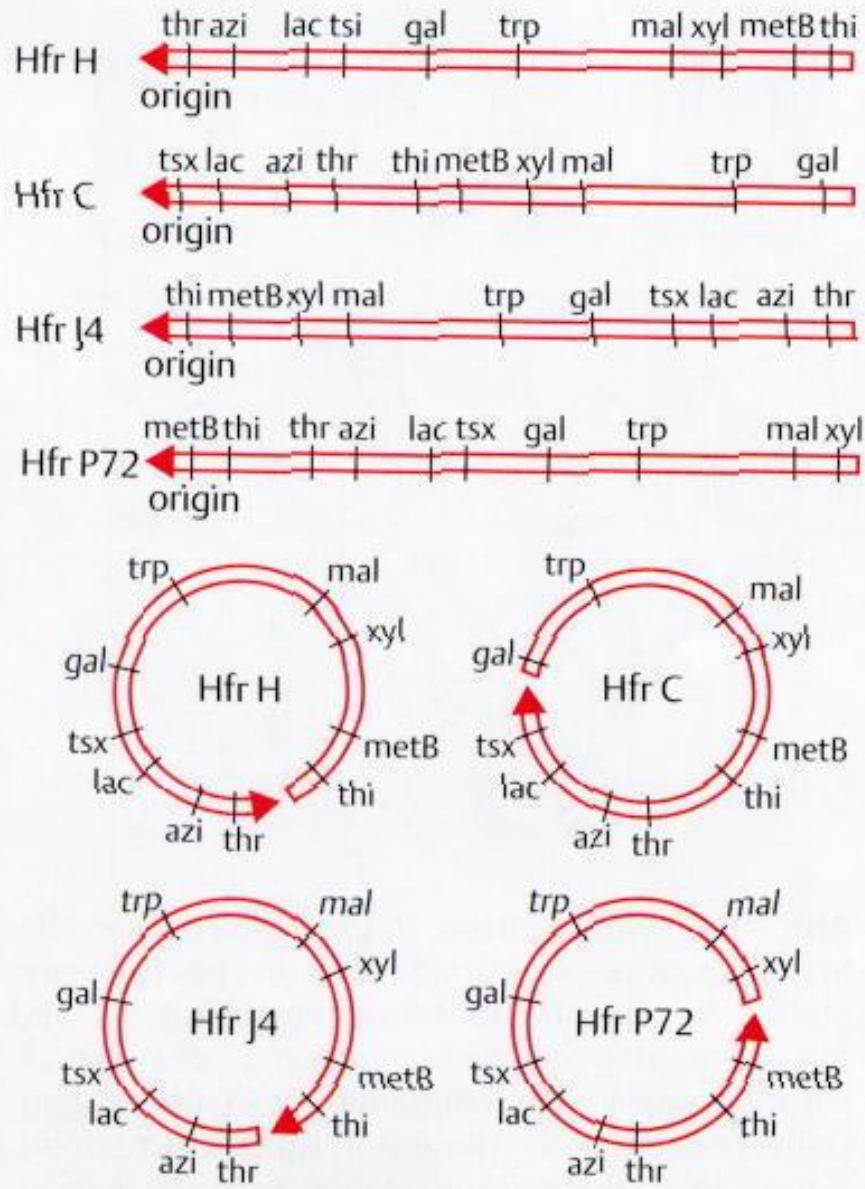


Scheme of integration of F-plasmid into bacterial (E.coli) chromosome

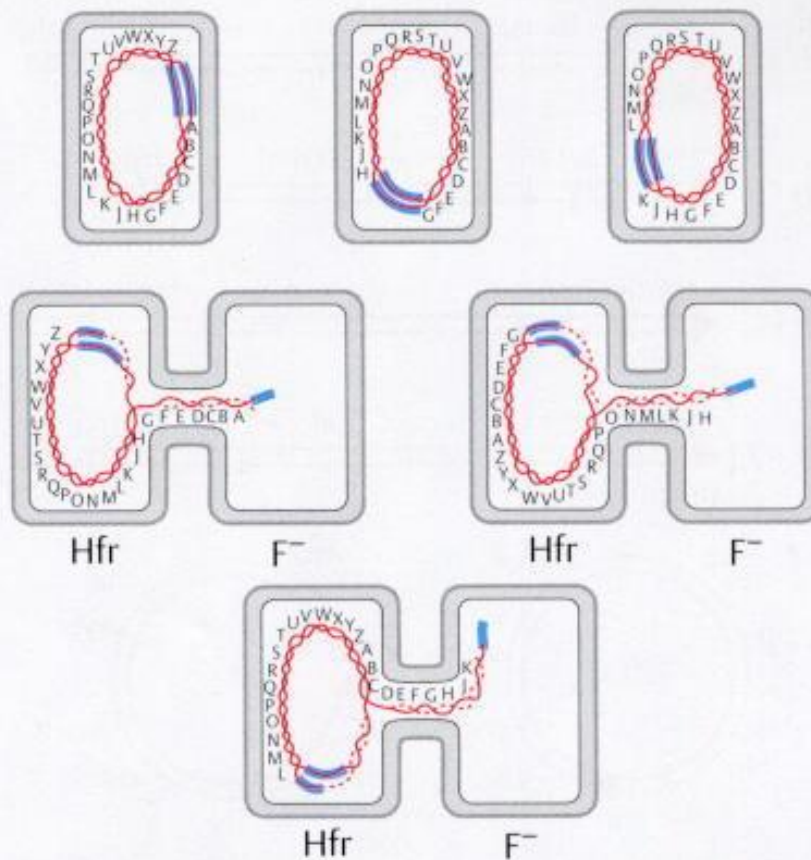
Interrupted Transfer:



**Abb. 4.21** Das Experiment der „unterbrochenen Paarung“.



**Abb. 4.22 Reihenfolge des Marker-Transfers bei einigen verschiedenen Hfr-Stämmen.**

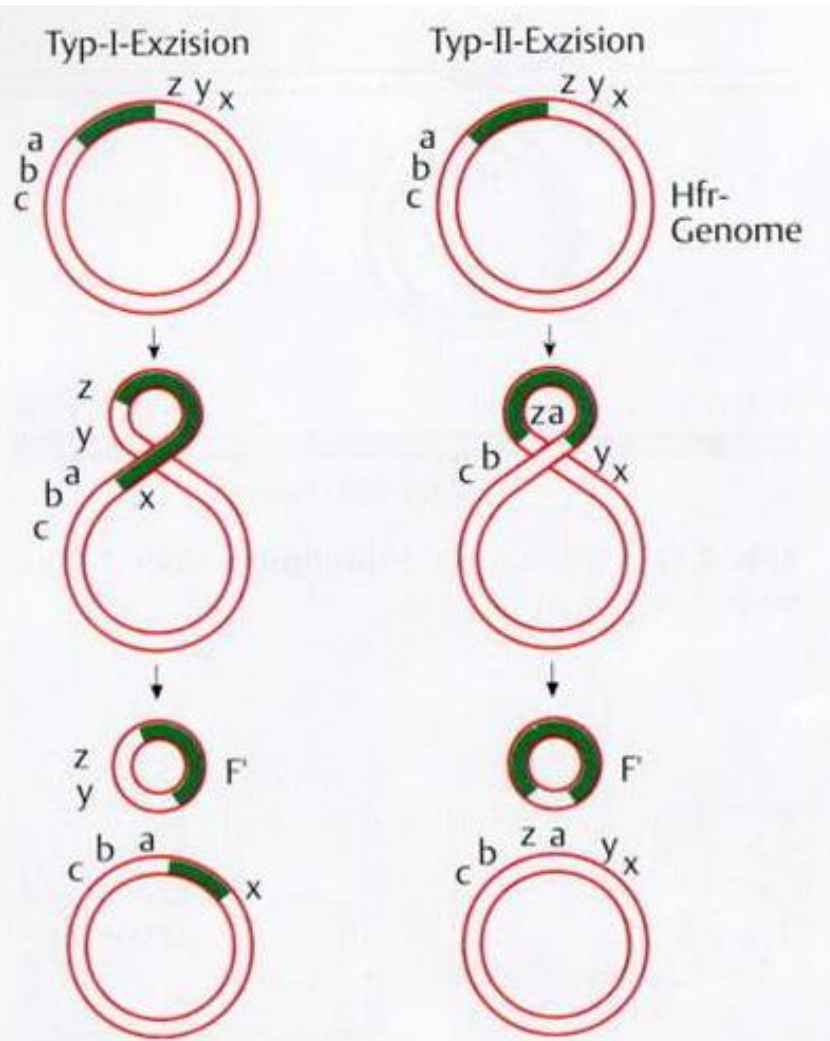


**Abb. 4.20 Konjugation.** In unterschiedlichen Hfr-Stämmen ist das F-Plasmid (blau) an verschiedenen Stellen des Hauptchromosoms eingebaut. Es wird mit dem daranhängenden Strang des Hauptchromosoms in die Empfänger-Zelle übertragen. Dabei findet DNA-Synthese statt (gestrichelte Linie). Daraus folgt: Bei der Konjugation kann die Reihenfolge, in der die Gene übertragen werden, verschieden sein, abhängig von der Art des untersuchten Hfr-Stammes. Die DNA in der Empfänger-Zelle ist nicht eingezeichnet.



# Excision of F-Plasmid from Hfr Genomes :

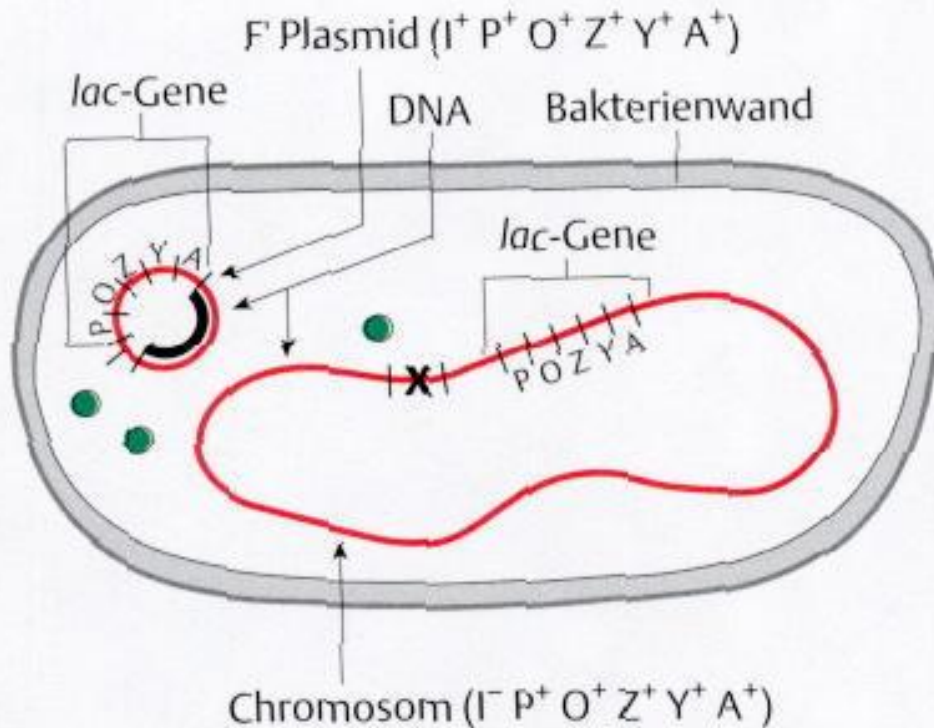
Formation of Plasmids containing chromosomal fragments  $\rightarrow$  F'



**Abb. 4.19** Exzisionswege bei der Bildung von F'-Plasmiden.

Beachte:

1. Bei der **Typ-I-Exzision** bleibt ein Teil des Plasmids im Hauptchromosom zurück. Das entstandene Exzisionsprodukt kann als Plasmid in der Zelle replizieren, wenn es noch mindestens die plasmidalen Replikationsfunktionen und den *ori V* (s. Abb. 4.11) besitzt. Falls die *tra*-Gene im Hauptchromosom zurückgeblieben sind, hat das Plasmid die Fähigkeit zum Konjugationstransfer verloren. Der chromosomale DNA-Abschnitt im F'-Plasmid entspricht einer Folge von genetischen Elementen, die ursprünglich auf einer Seite des integrierten Plasmids lagen.
2. Das unterscheidet die Typ-I- von der Typ-II-Exzision, bei der chromosomale DNA-Abschnitte von beiden Seiten des integrierten F-Plasmids ausgeschnitten werden. Bei der **Typ-II-Exzision** bleiben keine plasmidalen Sequenzen im Hauptchromosom zurück.



Formation of „partial diploids“

**Abb. 4.37 Merodiploide Zelle vom Typ I/F<sup>1+</sup>.**  
 Das *lac*-Gen ist im Verhältnis zu groß gezeichnet. Es nimmt in Wirklichkeit nur den Platz von etwa 0,15% des *E. coli*-Chromosoms ein. Das Wildtyp-*lac I*-Gen des Plasmids produziert einen aktiven Repressor (grüne Kugeln), der sich frei in der Zelle befindet und deshalb sowohl am chromosomalen *lac*-Operator als auch am plasmidalen *lac*-Operator angreifen kann. Zur Bedeutung der genetischen Elemente P und O siehe Text.

# Transformation

Uptake of DNA by cells

## Natural Transformation

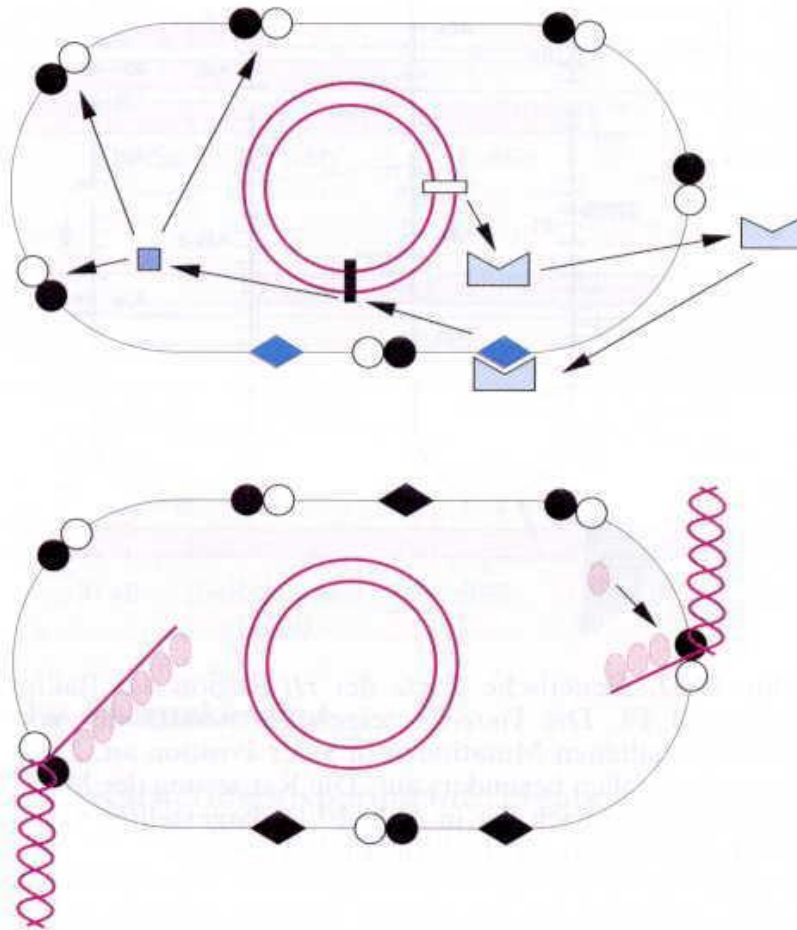
**Competence:**

Specific physiological conditions  
Expression of DNA binding proteins

**Transport:** of DNA into cell:

specific active transport mechanisms  
random events

Integration of DNA into genomic DNA by recombination  
or autonomous replication (plasmids)



**Abb. 10.18.** Transformationsmechanismus bei Bakterien. *Oben:* Vom Bakteriengenom (*Kreis*) werden Kompetenzfaktoren (*blaues Viereck*) kodiert, die an membrangebundene Rezeptoren binden (*Raute*) und dadurch weitere Gene induzieren. Hierdurch werden membrangebundene DNA-bindende Proteine und Nucleasen (*Kreise*) aktiviert, die extrazelluläre doppelsträngige DNA binden und abbauen. *Unten:* Ein Einzelstrang dieser DNA kann durch DNA-bindendes Protein (*Ellipsen*) gegen Abbau geschützt werden. Dieser DNA-Einzelstrang kann in die Bakterienzelle eindringen und mit dem bakteriellen Genom rekombinieren. (Nach Watson et al. 1987)

# Transformation

Forced DNA Transfer  
mostly undefined mechanisms

Treatment of cells with ions ( $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Li}^+$ ,  $\text{Rb}^+$ )

Generation of protoplasts & Fusogenic agents

Electroporation

Mechanical transfer → Gene gun



# Parasexual recombination in lower eukaryotes

