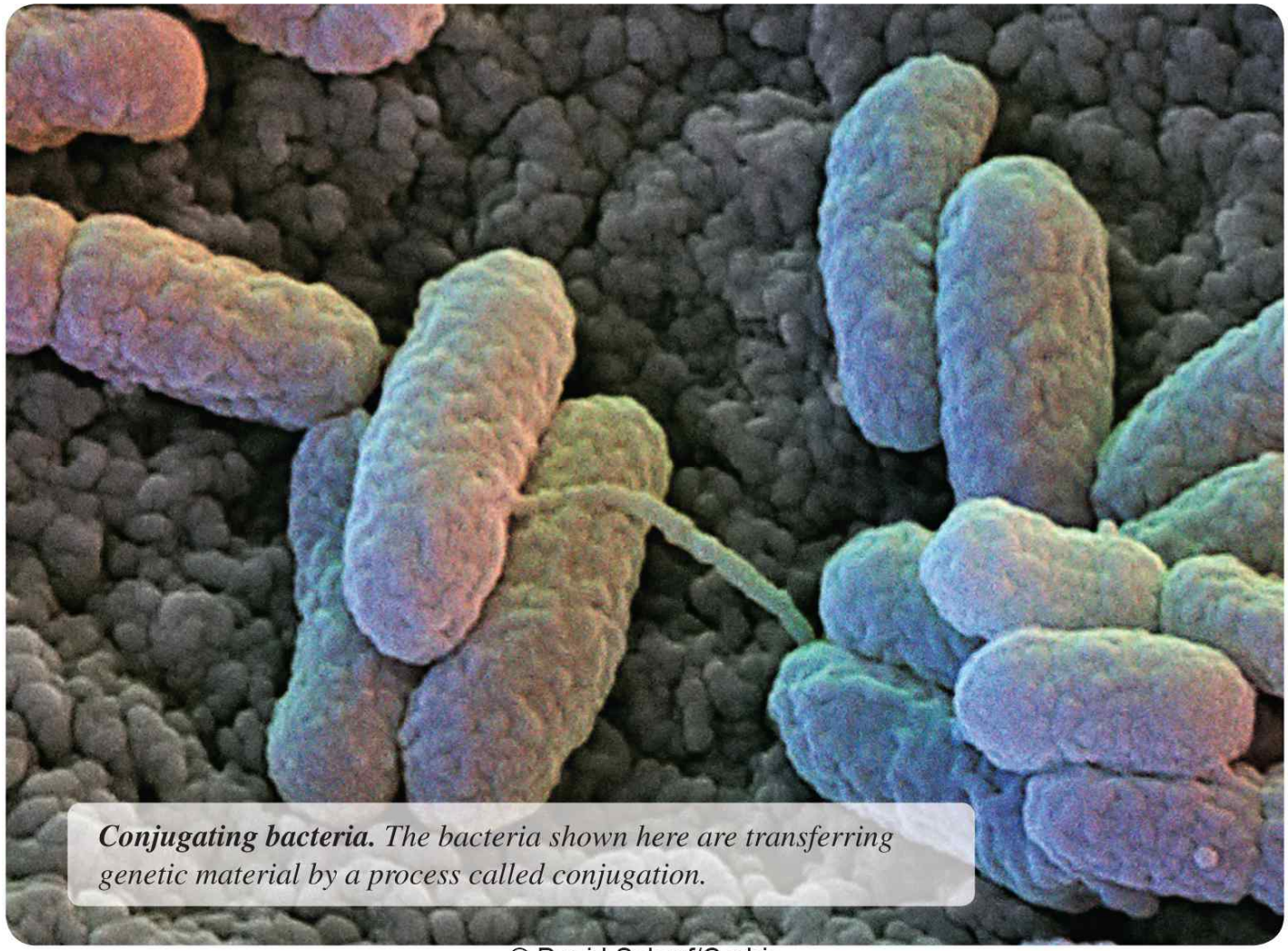
Chapter 9. Genetics of Bacteria



Conjugating bacteria. *The bacteria shown here are transferring genetic material by a process called conjugation.*

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- Bacteria and viruses account for one-quarter to one-third of **human deaths** worldwide

- Like eukaryotes, bacteria often possess **allelic differences** that affect their cellular traits
  - Example: sensitivity or resistance to antibiotics

- Bacteria are usually **haploid**
  - Makes it easier to identify loss-of-function mutations in bacteria than in eukaryotes
  - These usually recessive mutations are not masked by dominant alleles in haploid species

- Bacteria **reproduce asexually**
  - Crosses are not used in the genetic analysis of bacterial species

- **Genetic transfer**
  - A segment of bacterial DNA is transferred from one bacterium to another
  - Useful for mapping genes

9.1 Overview of Genetic Transfer in Bacteria

- The three mechanisms of genetic transfer in bacteria

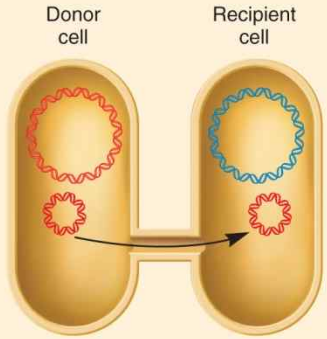
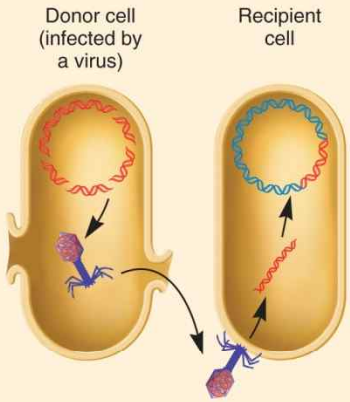
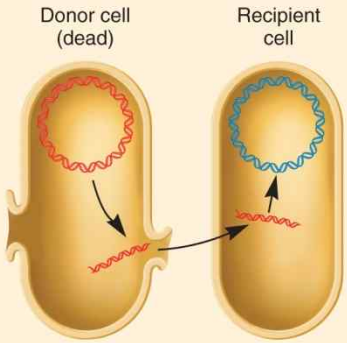
  - Transfer of genetic material from one bacterium to another can occur in three ways:
    - **Conjugation**
      - Direct transfer from one cell to another
    - **Transduction**
      - Viruses transfer DNA between bacteria cells
    - **Transformation**
      - Uptake of DNA from the environment

    - See Table 9.1

Table 9.1

TABLE 9.1

Three Mechanisms of Genetic Transfer Found in Bacteria

Mechanism		Description
Conjugation		Requires direct contact between a donor and a recipient cell. The donor cell transfers a strand of DNA to the recipient. In the example shown here, DNA known as a plasmid is transferred to the recipient cell.
Transduction		After a virus infects a donor cell, a fragment of chromosomal DNA is incorporated into a newly made virus particle. The virus then transfers this fragment of DNA to a recipient cell, which incorporates the DNA into its chromosome by recombination.
Transformation		When a bacterial cell dies, it releases a fragment of its DNA into the environment. This DNA fragment is taken up by a recipient cell, which incorporates the DNA into its chromosome by recombination.

9.2 Bacterial Conjugation

- Lederberg, Tatum, and Davis's results that showed bacteria can transfer genetic material via direct physical contact
- The steps of conjugation via F factors.
- Different types of plasmids

Conjugation

- Genetic transfer in bacteria was discovered in 1946 by **Joshua Lederberg and Edward Tatum**

- They were studying strains of *Escherichia coli* that had different nutritional growth requirements
  - **Auxotrophs** cannot synthesize a needed nutrient
  - **Prototrophs** make all their nutrients from basic components

- One auxotrophic strain, *bio*$^{-}$ *met* $^{-}$ *thr* $^{+}$ *leu*$^{+}$ *thi*$^{+}$
  - required biotin and methionine
  - could produce threonine, leucine, and thiamine

- The other auxotrophic strain, *bio*$^{+}$ *met* $^{+}$ *thr* $^{-}$ *leu* $^{-}$ *thi* $^{-}$
  - could produce biotin and methionine
  - required threonine, leucine, and thiamine

- Their experiment was to see if one strain cold pass its traits to the other
  - Determined by growth of cells on **minimal medium**
    - Agar without any added biotin, methionine, threonine, leucine or thiamine

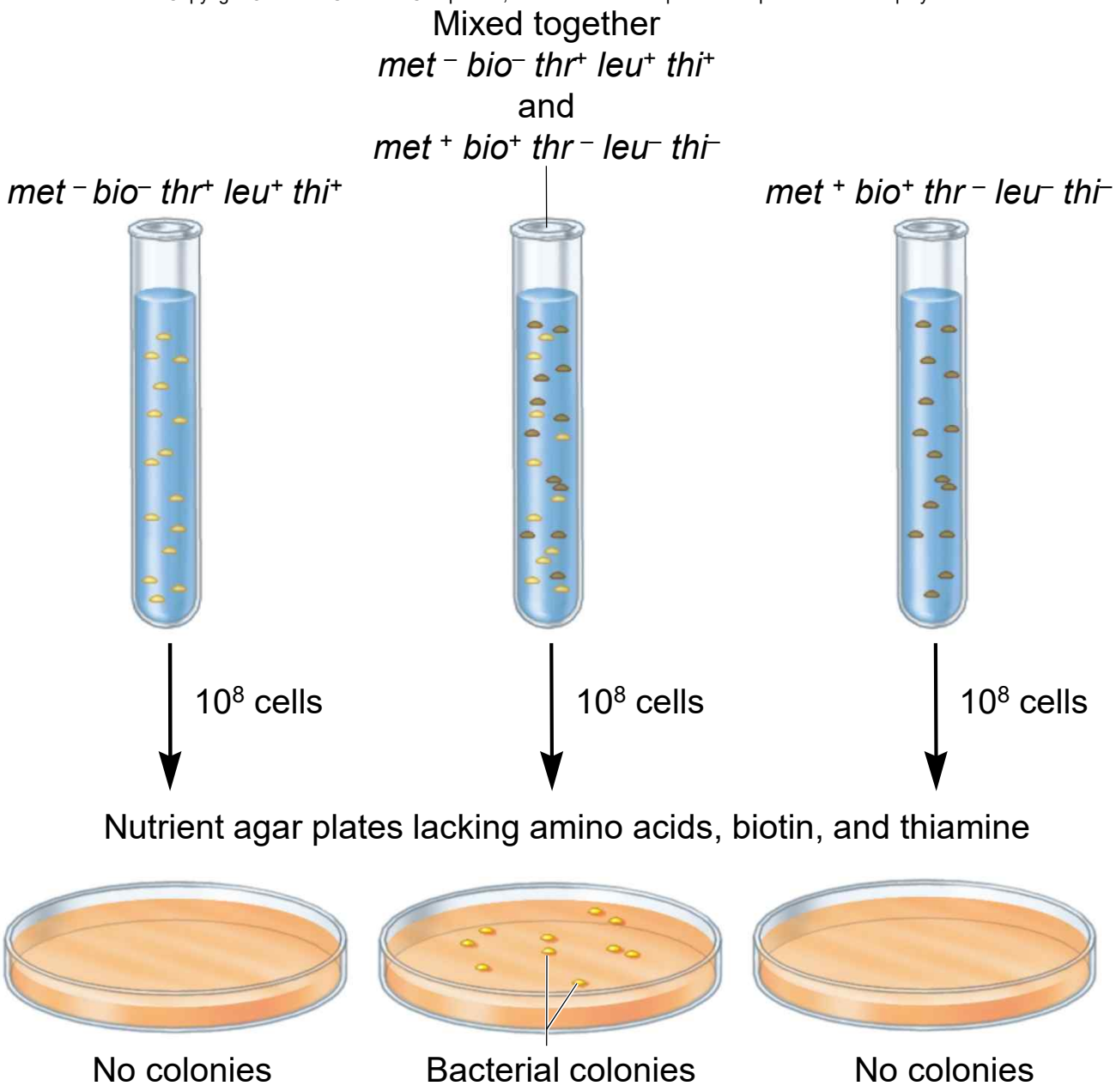


Figure 9.1

- The genotype of the bacterial cells that grew on the plates has to be *met* $^{+}$ *bio*$^{+}$ *thr* $^{+}$ *leu*$^{+}$ *thi* $^{+}$

- Lederberg and Tatum reasoned that **some genetic material was transferred** between the two strains
  - Either the *met* $^{-}$ *bio*$^{-}$ *thr* $^{+}$ *leu*$^{+}$ *thi* $^{+}$ strain got the ability to synthesize biotin and methionine (*bio*$^{+}$ *met* $^{+}$)
  - Or the *met* $^{+}$ *bio*$^{+}$ *thr* $^{-}$ *leu* $^{-}$ *thi* $^{-}$ strain got the ability to synthesize threonine and leucine and thiamine (*thr* $^{+}$ *leu*$^{+}$ *thi* $^{+}$)

  - The results of this experiment cannot distinguish between these two possibilities

- Bernard Davis showed that bacterial strains must make **physical contact** for transfer of genetic material to occur

- He used an apparatus known as **U-tube**

  - It contains a filter at the bottom which has pores
    - Large enough for passage of genetic material
    - Too small for passage of bacterial cells

  - Refer to Figure 9.2

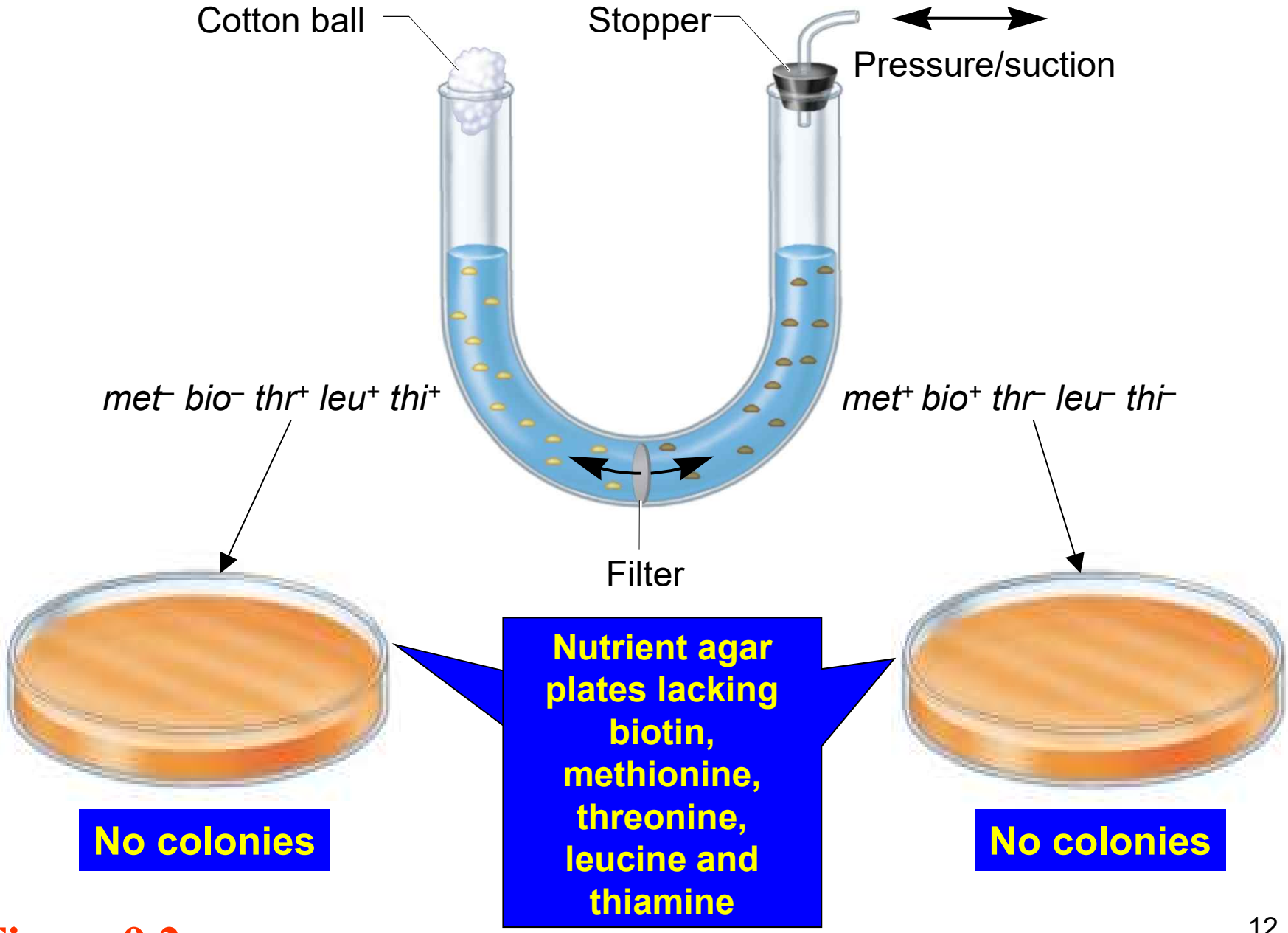


Figure 9.2

- **Conjugation** – the transfer of DNA from one bacterium to another following direct cell-to cell contact

- Many, but not all, species of bacteria can conjugate

- Only certain strains of a bacterium can act as donor cells
  - Contain a small circular piece of DNA called **F factor** (for Fertility factor)
    - Strains containing the F factor are designated F^+
    - Those lacking it are F^-
    - The F factor carries genes that allow for transfer of DNA from F^+ cell to F^- cell

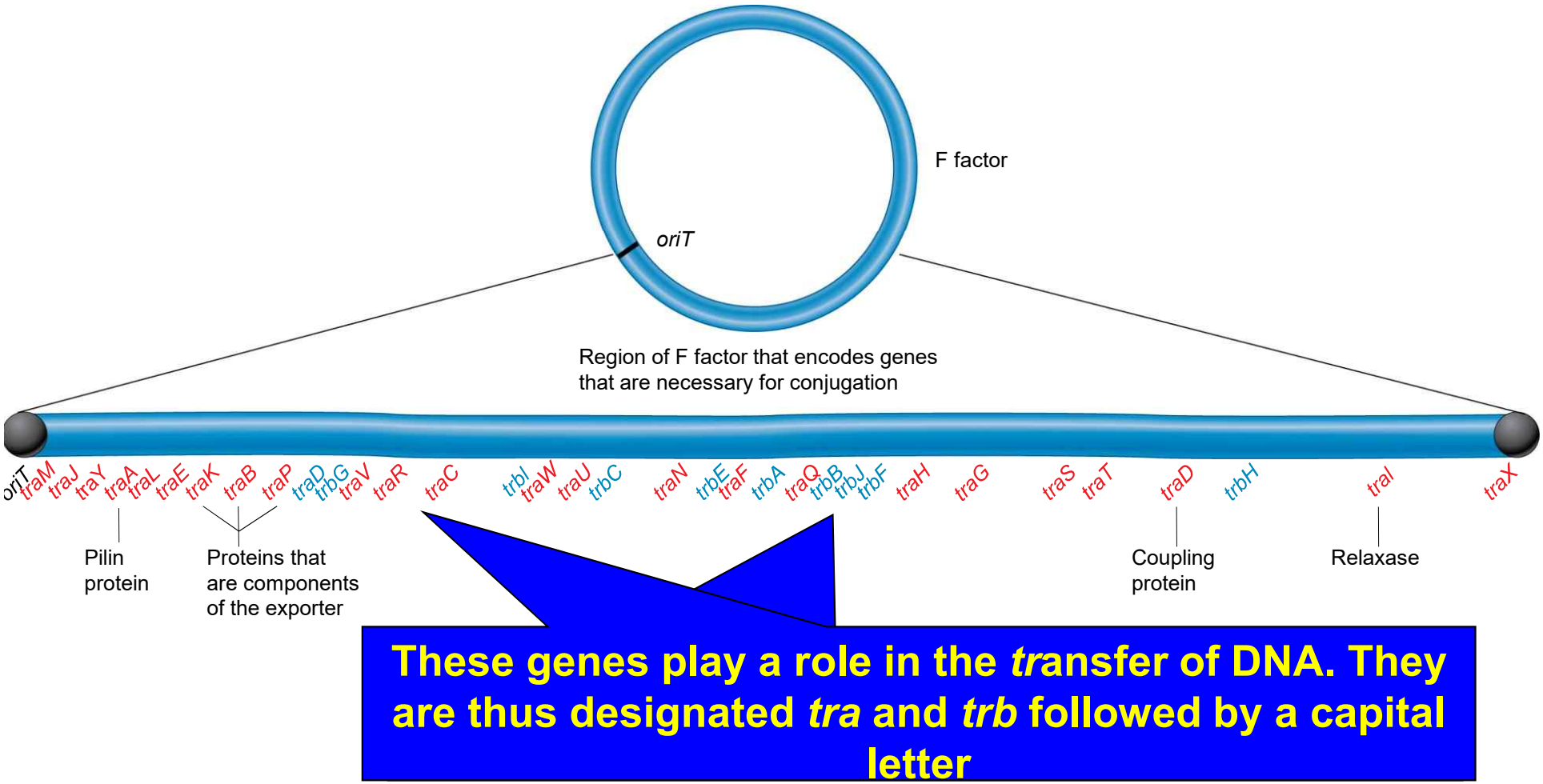
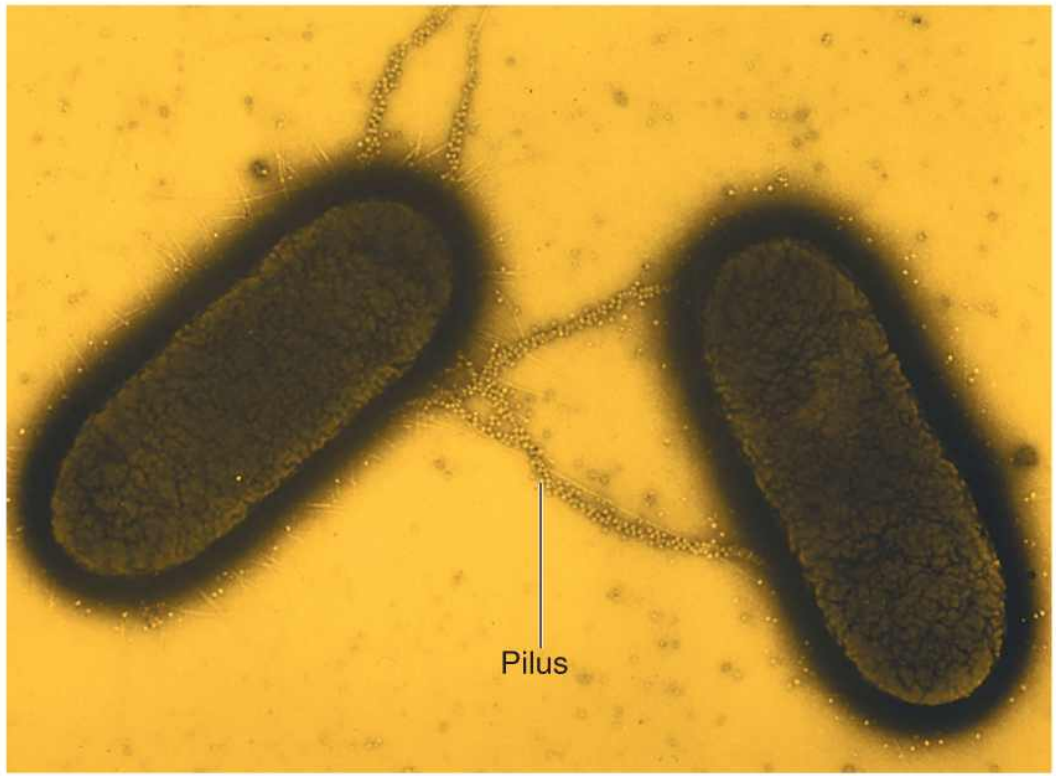

F factor
oriT
Region of F factor that encodes genes
that are necessary for conjugation
oriT traM traJ traY traA traL traE traK traB traP traD trbG traV traR traC trbI traW traU trbC traN trbE traF trbA traQ trbB trbJ trbF traH traG traS traT traD trbH traI traX
Pilin protein
Proteins that are components of the exporter
Coupling protein
Relaxase
These genes play a role in the *tra*nsfer of DNA. They are thus designated *tra* and *trb* followed by a capital letter

Figure 9.3

- Conjugation is mediated by a **sex pilus**
  - Sex pili are made only by F^+ strains
    - These pili act as attachment sites for other bacteria



(a) Conjugating *E. coli*

Figure 9.4a

Molecular Events of Conjugation

1. The sex pilus contacts the F^- cell, shortens, and a **conjugation bridge** is formed between the cells
2. A protein complex called the **relaxosome** binds the **origin of transfer** in the F factor DNA, and cuts one strand of the DNA
3. The relaxosome separates the strands of DNA, leaving the protein **relaxase** bound
4. The DNA/relaxase complex is pumped out of the donor cell
5. After transfer of one strand of the F factor, relaxase rejoins the ends to form a circle

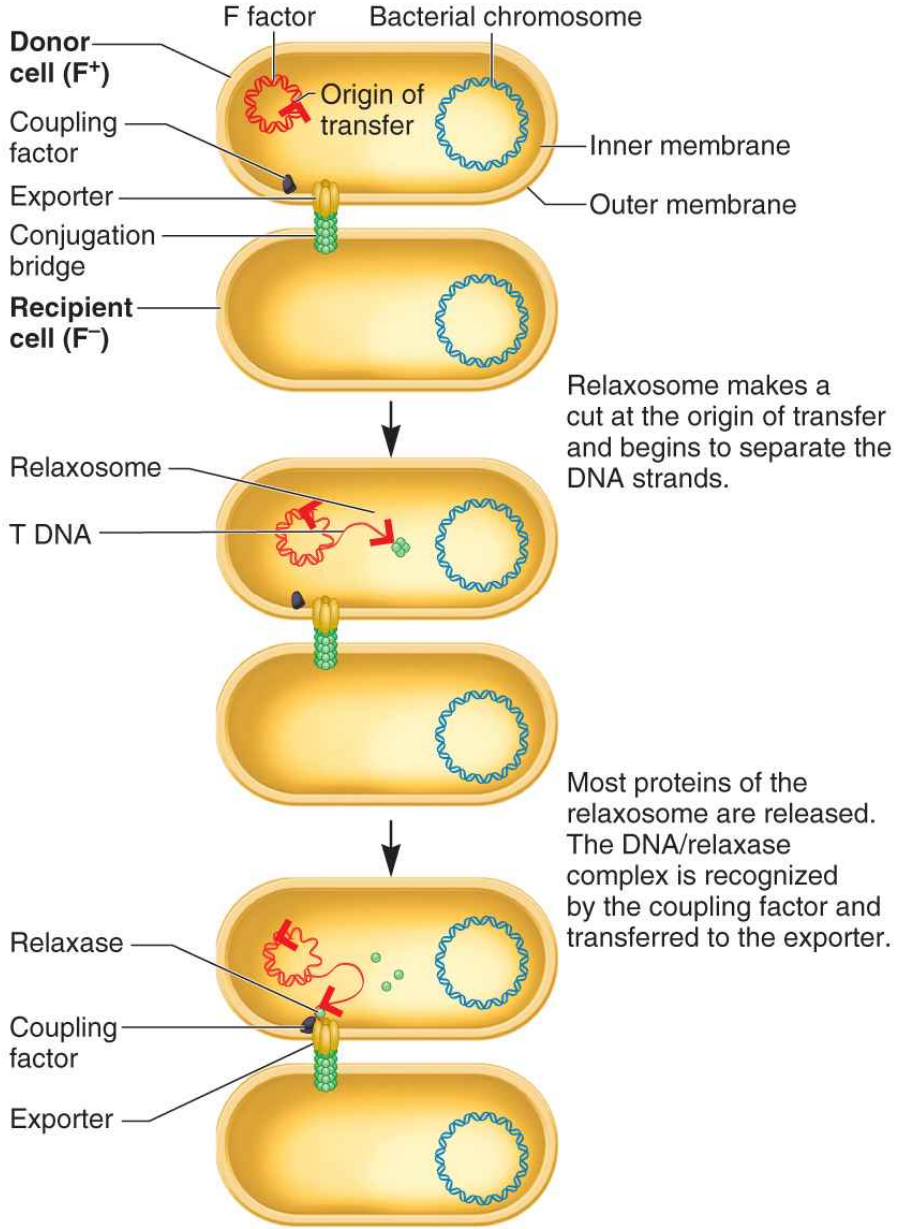


Figure 9.4b

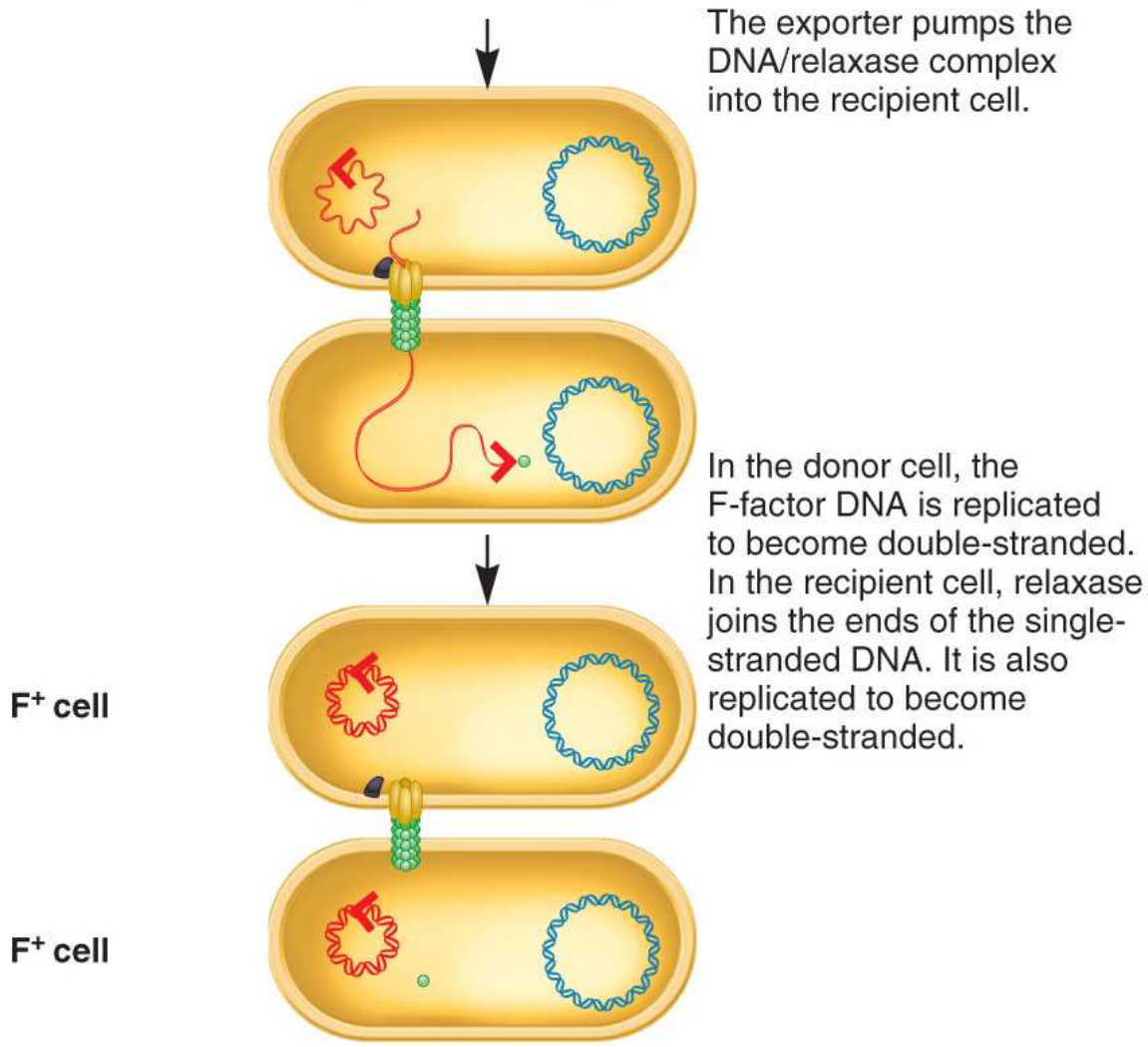


Figure 9.4b

Plasmids

- **Plasmid** – DNA that exists independently of the chromosomal DNA
  - Usually circular, but can be linear
  - Occur in bacteria and yeast
  - Range from a few thousand to 500,000 bp
  - Range from one gene to hundreds of genes
  - **Episomes** – plasmids that can integrate into the chromosome
    - Example: F factor

Types of Plasmids

- **Fertility plasmids** – F factors, allow conjugation
- **Resistance factors** – R factors, give resistance to antibiotics
- **Degradative plasmids** – allow the bacterium to metabolize an unusual substance
- **Col plasmids** – encode colicins, proteins that kill other bacteria
- **Virulence plasmids** – turn the bacterium into a pathogenic strain

9.3 Conjugation and Mapping via Hfr Strains

- How an Hfr strain is produced
- How an Hfr strain can transfer portions of the bacterial chromosome to recipient strains
- Constructing a genetic map using data from conjugation experiments

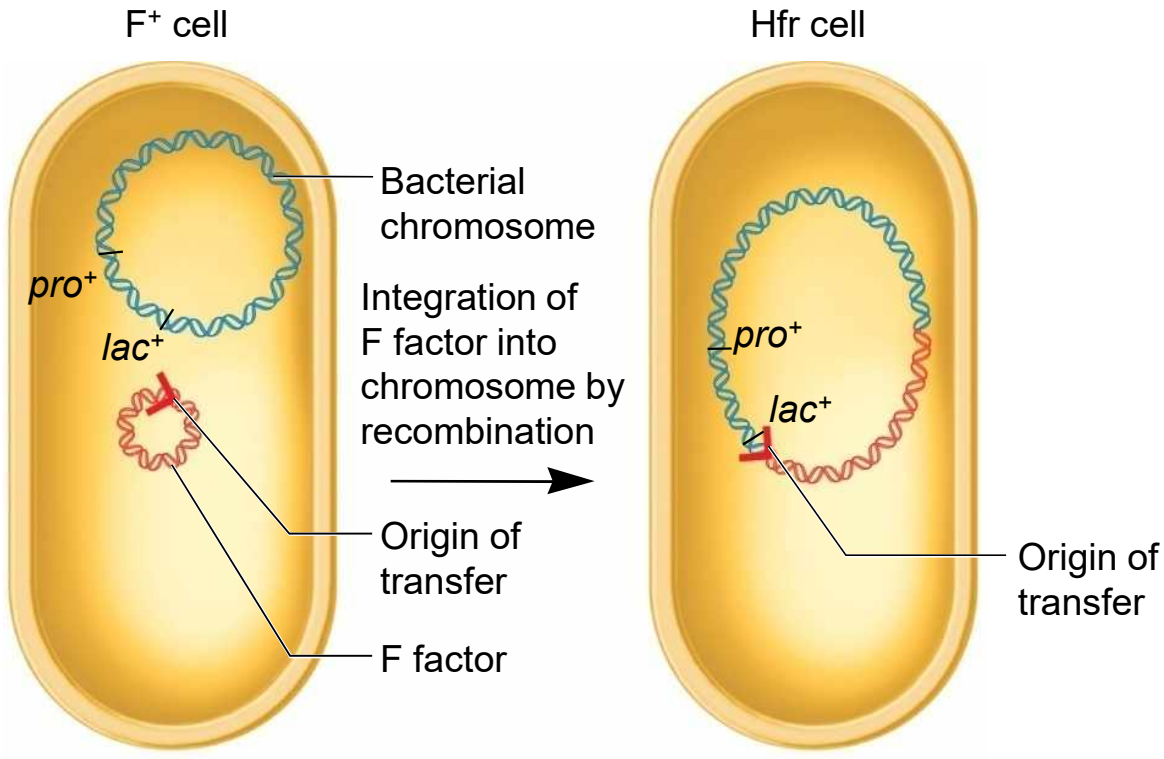
Hfr Strains

- In the 1950s, Luca Cavalli-Sforza discovered a strain of *E. coli* that was very efficient at transferring chromosomal genes
  - Called ***Hfr* strain** (for High frequency of recombination)
  - The F factor is integrated into the bacterial chromosome

- Hfr strains are derived from F^+ strains
  - F factor integrates into host chromosome
  - Multiple sites where it can integrate

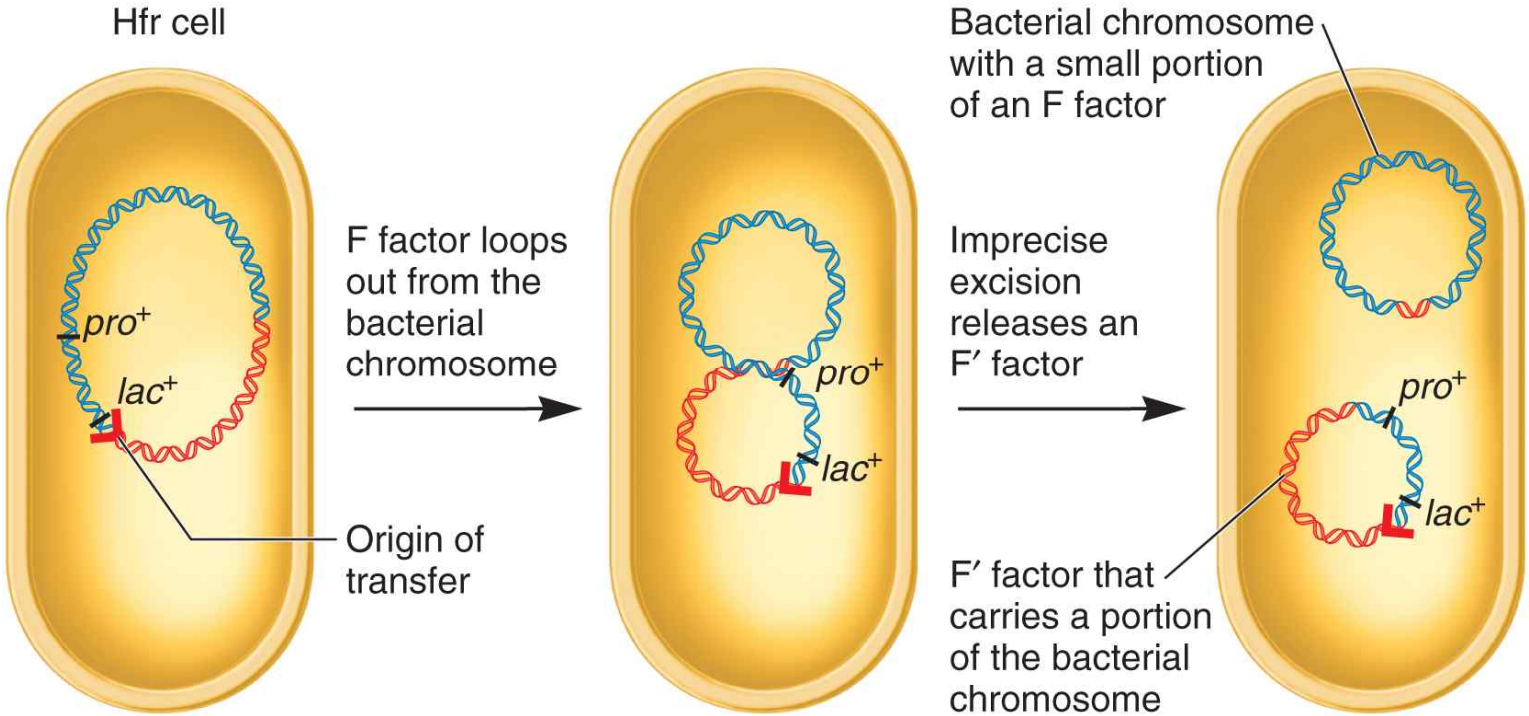
- F factor may also leave chromosome and carry genes that were once found on the bacterial chromosome
  - These types of F factors are called **F′ factors** (F prime factors)
  - F′ factors can be transferred through conjugation
    - May introduce new genes and thereby alter recipient genotype

Refer to Figure 9.5a and b



(a) When an F factor integrates into the chromosome, it creates an Hfr cell.

Figure 9.5a



(b) When an F factor excises imprecisely, an F′ factor is created.

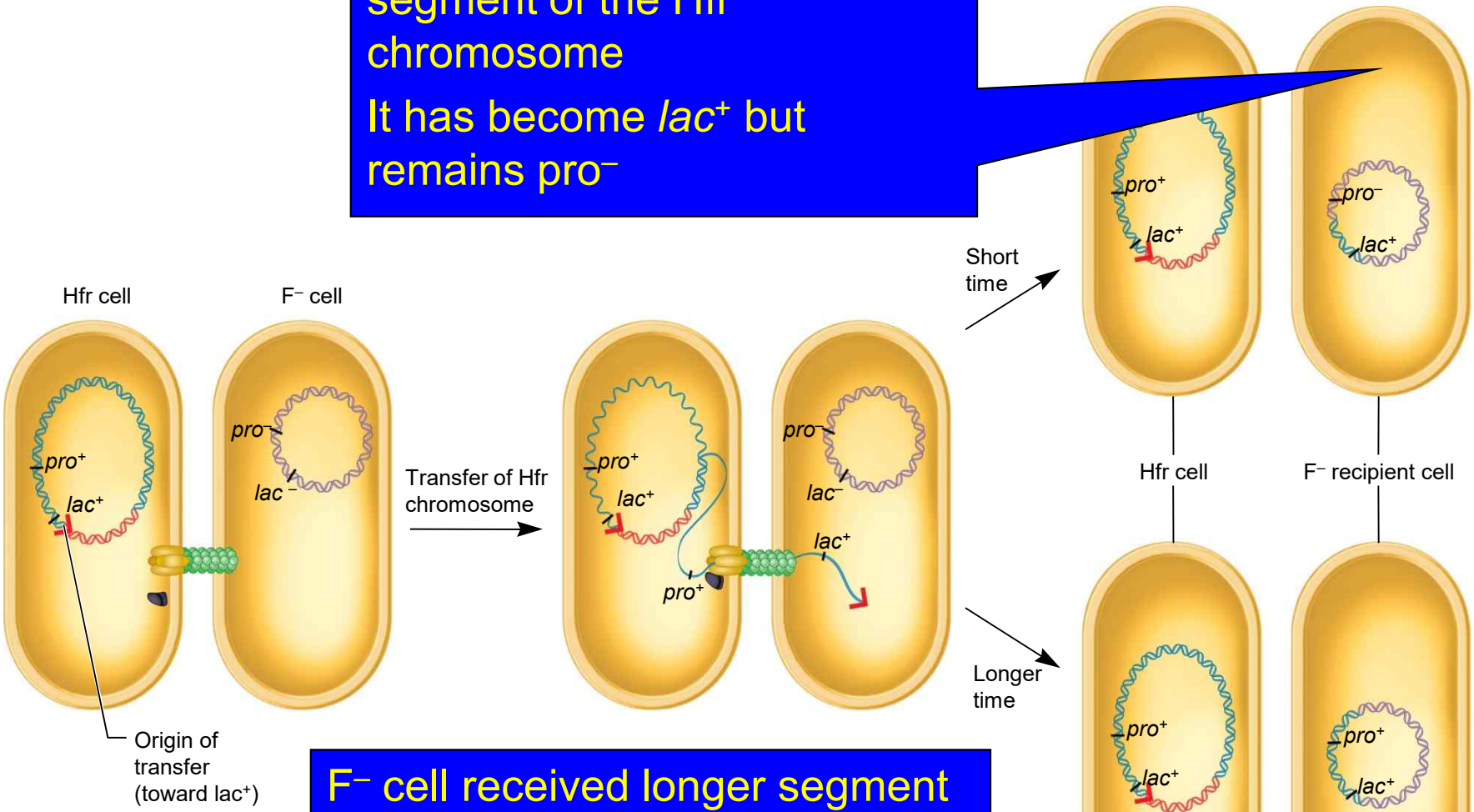
Figure 9.5b

Conjugation between an Hfr and F^- strain

1. One of the DNA strands is nicked at the origin of transfer
   - Determines the starting point and direction of the transfer
2. A strand of the DNA of the Hfr chromosome begins to enter the F^- cell
   - It would take almost 2 hrs to transfer the whole Hfr chromosome
   - But usually mating is interrupted earlier, so only a portion transfers
3. In the recipient cell, the chromosomal region can recombine with the homologous region of the recipient's chromosome
   - Thus possibly providing **new alleles**

F⁻ cell received short segment of the Hfr chromosome

It has become *lac*⁺ but remains pro⁻



F⁻ cell received longer segment of the Hfr chromosome

It has become *lac*⁺ AND pro⁺

Figure 9.6

Interrupted Mating to Map Order of Genes along the *E. coli* Chromosome

- Developed by Elie Wollman and François Jacob in the 1950s
- The order of genes along the chromosome can be deduced from how long it takes the genes to be transferred during an Hfr mating
  - Interrupting mating at different times would result in recipient cells receiving different lengths of DNA

- Wollman and Jacob started the experiment with two *E. coli* strains
  - The donor (Hfr) strain had the following genetic composition
    - *thr*$^{+}$: synthesizes threonine
    - *leu*$^{+}$: synthesizes leucine
    - *azi*^{s} : sensitive to azide (a toxic chemical)
    - *ton*^{s} : sensitive to infection by T1 (a bacterial virus)
    - *lac*$^{+}$: metabolizes lactose for growth
    - *gal*$^{+}$: metabolizes galactose for growth
    - *str* ^{s} : sensitive to streptomycin (an antibiotic)
  - The recipient (F^{-}) strain had the opposite genotype
    - *thr* $^{-}$ *leu*$^{-}$ *azi*^{r} *ton*^{r} *lac* $^{-}$ *gal* $^{-}$ *str* ^{r} (r = resistant)

- Wollman and Jacob already knew that
  - The *thr*$^+$ and *leu*$^+$ genes were transferred first, in that order
  - Both were transferred within 5-10 minutes of mating

- Therefore their main goal was to determine the times at which genes *azi*^{s}, *ton*^{s}, *lac*$^+$, and *gal*$^+$ were transferred
  - The transfer of the *str*^{s} was not examined
    - Streptomycin was used to kill the donor (Hfr) cell following conjugation
    - The recipient (F^- cell) is streptomycin resistant

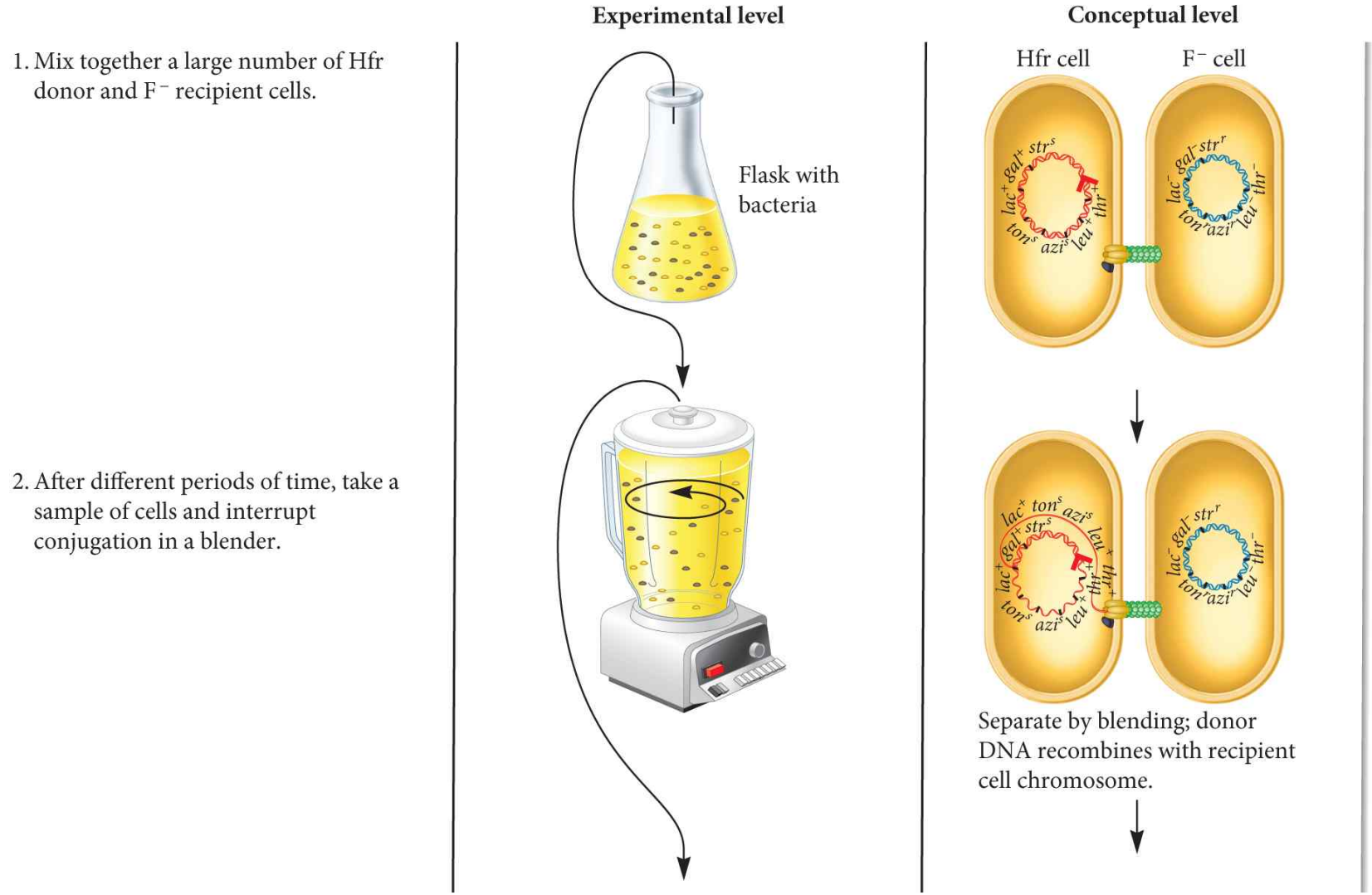
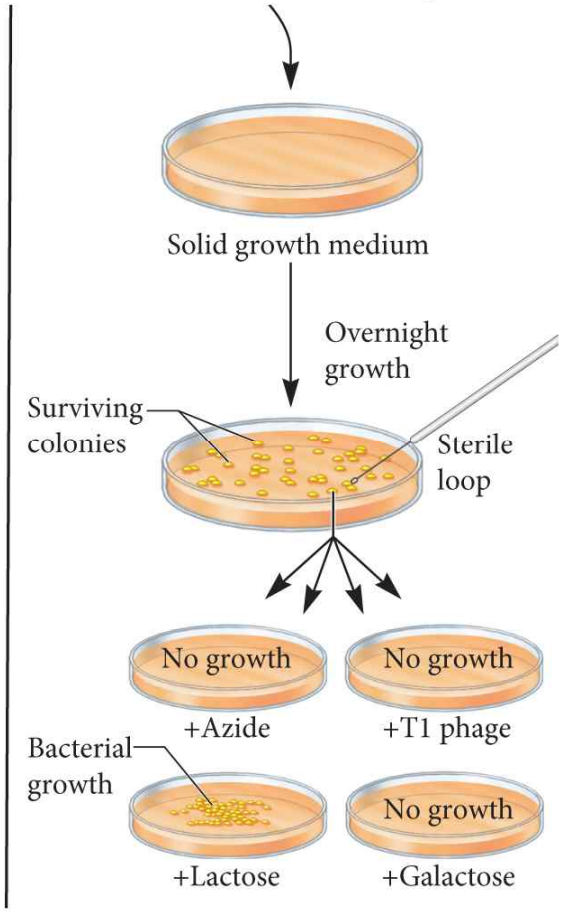


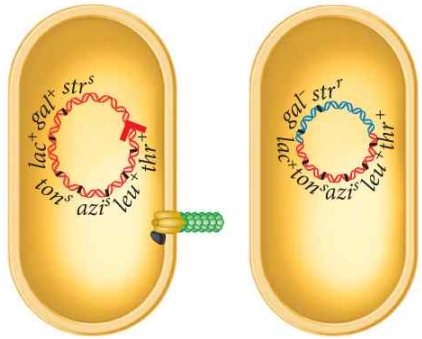
Figure 9.7

3. Plate the cells on solid growth medium lacking threonine and leucine but containing streptomycin. Note: The general methods for growing bacteria in a laboratory are described in the Appendix.

4. Pick each surviving colony, which would have to be *thr*$^+$ *leu*$^+$ *str*^{r}, and test to see if it is sensitive to killing by azide, sensitive to infection by T1 bacteriophage, and able to metabolize lactose or galactose.



In this conceptual example, the cells have been incubated about 20 minutes.



Cannot survive on plates with streptomycin

Can survive on plates with streptomycin

Additional tests

The conclusion is that the colony that was picked contained cells with a genotype of *thr*$^+$ *leu*$^+$ *azi*^{s} *ton*^{s} *lac*$^+$ *gal*$^-$ *str*^{r}.

Figure 9.7

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	***thr*$^+$ *leu*$^+$**	***azi*^{s}**	***ton*^{s}**	***lac*$^+$**	***gal*$^+$**
5	—	—	—	—	—

At 5 minutes no change in the phenotypes/genotypes of the recipient cells

Conclude - no genes transferred

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	***thr*$^+$ *leu*$^+$**	***azi*^{s}**	***ton*^{s}**	***lac*$^+$**	***gal*$^+$**
5	—	—	—	—	—
10	100	12	3	0	0

After 10 minutes

All cells are *thr*$^+$ *leu*$^+$ - *5* to10 minutes from oriT and closest to oriT due to highest number of cells converted

azi^s – 5 to10 minutes from oriT but farther than *thr* and *leu*

Fewest cells are *ton*^{s} - 5 -10 minutes from oriT but farther than *thr, leu* and *azi* due to lower percentage of cells converted

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	***thr*$^+$ *leu*$^+$**	***azi*^{s}**	***ton*^{s}**	***lac*$^+$**	***gal*$^+$**
5	—	—	—	—	—
10	100	12	3	0	0
15	100	70	31	0	0

At 15 minutes no changes

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	***thr*$^+$ *leu*$^+$**	***azi*^{s}**	***ton*^{s}**	***lac*$^+$**	***gal*$^+$**
5	—	—	—	—	—
10	100	12	3	0	0
15	100	70	31	0	0
20	100	88	71	12	0

At 20 minutes

Some cells are now *lac*$^+$ - 15 to 20 minutes from oriT

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	***thr*$^+$ *leu*$^+$**	***azi*^{s}**	***ton*^{s}**	***lac*$^+$**	***gal*$^+$**
5	—	—	—	—	—
10	100	12	3	0	0
15	100	70	31	0	0
20	100	88	71	12	0
25	100	92	80	28	0.6

At 25 minutes start to see *gal* being transferred

gal is 20 to 25 minutes from oriT

Minutes that Bacterial Cells were Allowed to Mate Before Blender Treatment	Percent of Surviving Bacterial Colonies with the Following Genotypes				
	thr$^+$ *leu*$^+$	*azi*^{s}	*ton*^{s}	*lac*$^+$	*gal*$^+$
5	—	—	—	—	—
10	100	12	3	0	0
15	100	70	31	0	0
20	100	88	71	12	0
25	100	92	80	28	0.6
30	100	90	75	36	5
40	100	90	75	38	20
50	100	91	78	42	27
60	100	91	78	42	27

- From these data, Wollman and Jacob constructed the following genetic map:

- They also identified various Hfr strains in which the F factor had been integrated at different places in the chromosome
  - Comparison of the order of genes among these strains demonstrated that the *E. coli* chromosome is circular

- Conjugation experiments have been used to map more than 1,000 genes on the *E. coli* chromosome

- The *E. coli* genetic map is 100 minutes long
  - Approximately the time it takes to transfer the complete chromosome in an Hfr mating

  - Refer to Figure 9.8

Arbitrarily assigned the starting point

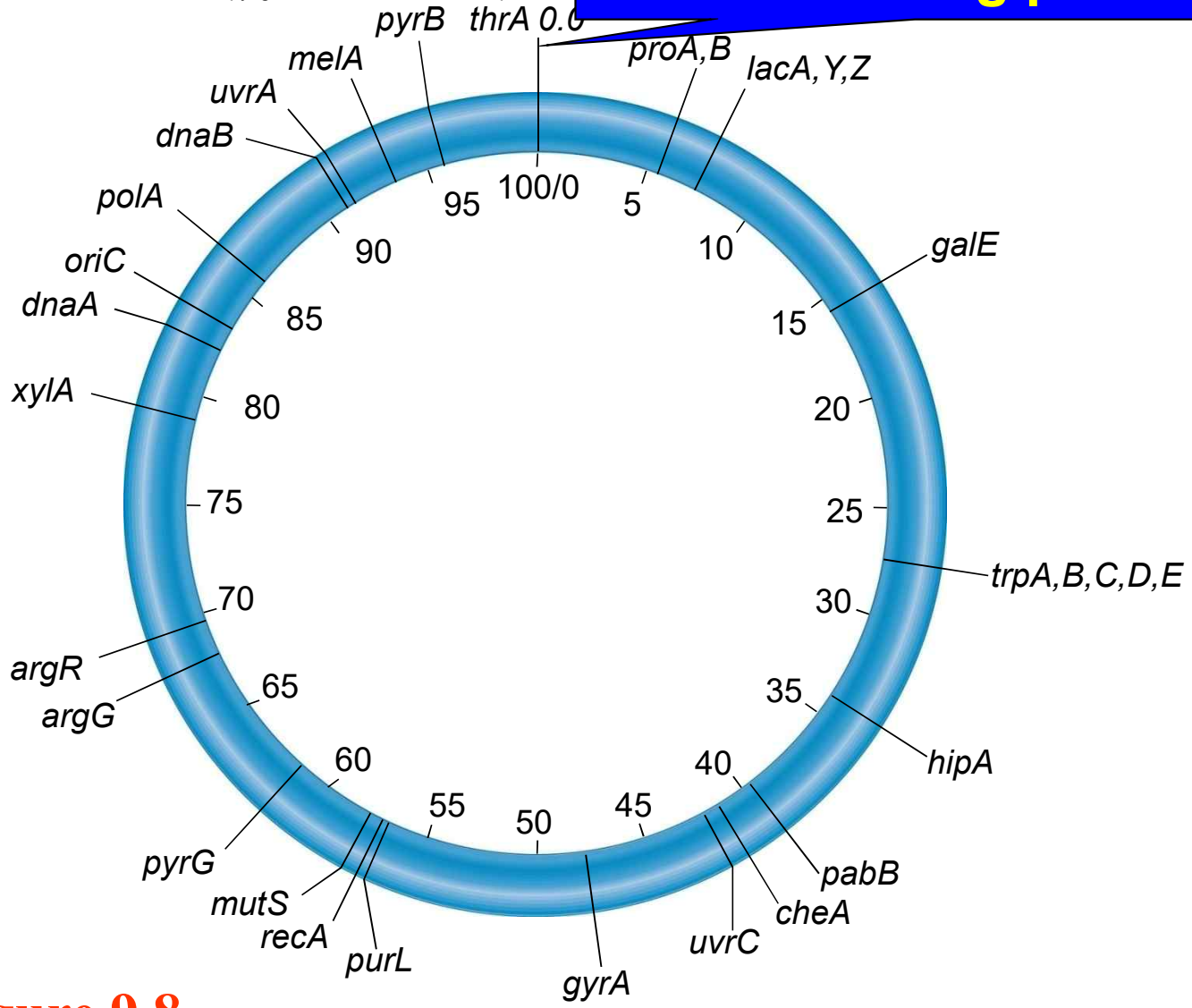


Figure 9.8

- The distance between genes is determined by comparing their times of entry during an interrupted mating experiment
  - Get approximate time of entry by extrapolating back to the origin

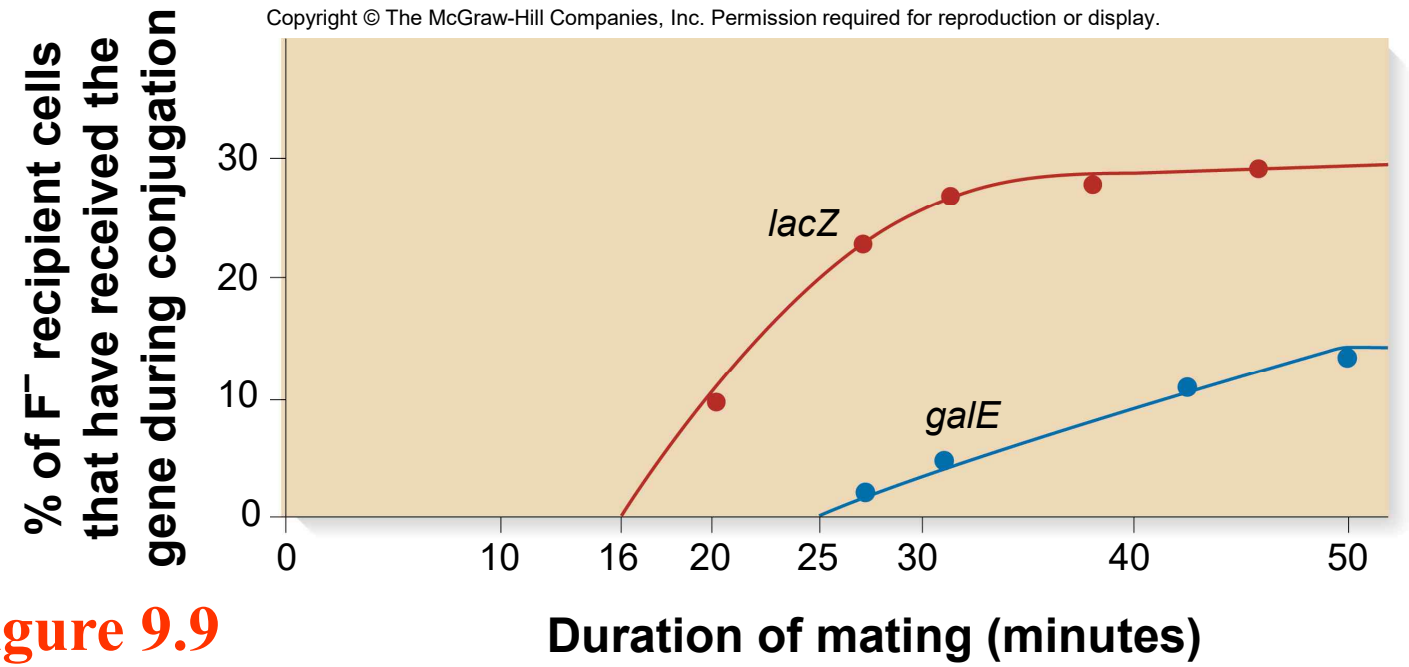


Figure 9.9

- Therefore these two genes are approximately 9 minutes apart along the *E. coli* chromosome

9.4 Bacterial Transduction

- The steps of bacterial transduction

- How transduction can be used to map genes

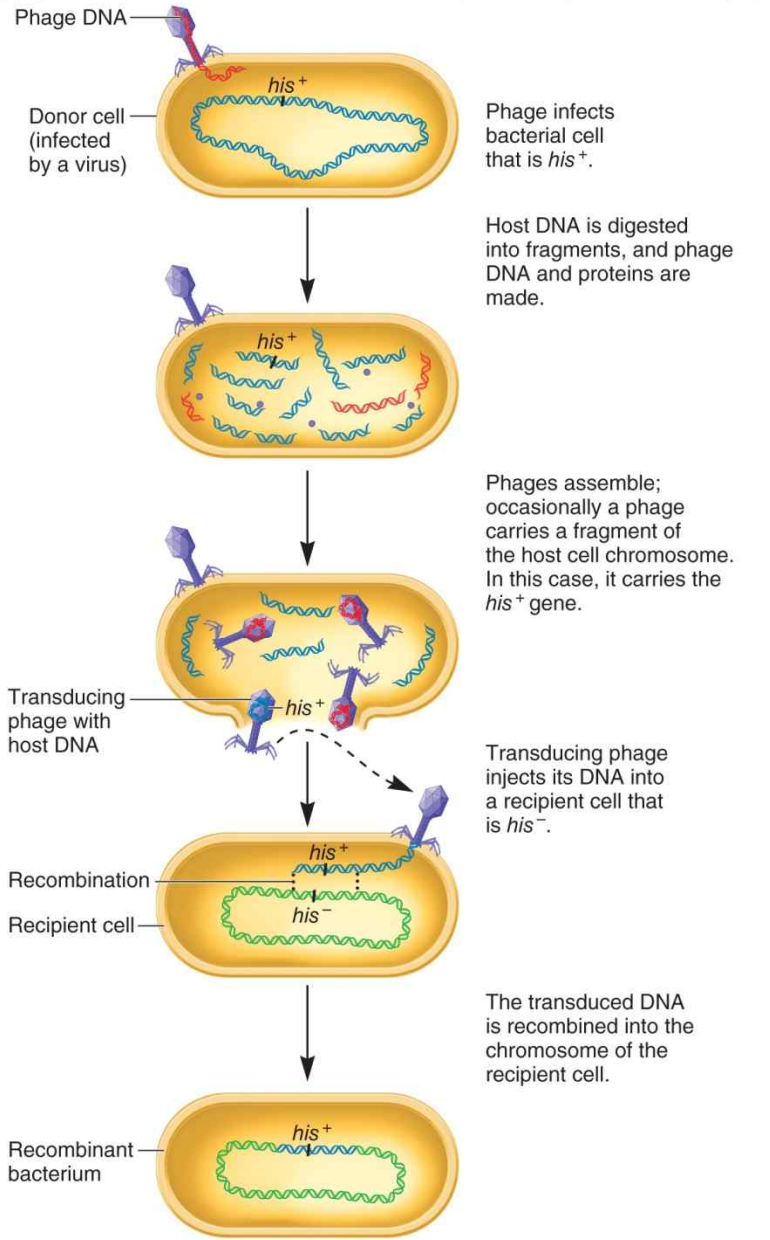
- **Transduction** is the transfer of DNA from one bacterium to another via a bacteriophage

- A **bacteriophage** is a virus that specifically attacks bacterial cells
  - It is composed of genetic material surrounded by a protein coat

- Examples of phages that can transfer bacterial DNA:
  - P22, which infects *Salmonella typhimurium*
  - P1, which infects *Escherichia coli*

- When a bacteriophage infects a cell it can cause the host DNA to become fragmented

- The pieces of the host chromosome can be packaged with the bacteriophage proteins



The recombinant bacterium's genotype has changed from *his*$^-$ to *his*$^+$.

Figure 9.10

Cotransduction Mapping

- There is a maximum size to the DNA that can be packaged by bacteriophages during transduction
  - P1 can pack up to 2-2.5% of the *E. coli* chromosome
  - P22 can pack up to 1% of the *S. typhimurium* chromosome

- **Cotransduction** refers to the packaging and transfer of two closely-linked genes
  - It is used to determine the order and distance between genes that lie fairly close together
  - Only useful for genes **within 2 minutes** of each other

- Select for the transduction of one gene
  - They then monitor whether a second gene is cotransduced

- Consider for example the following two *E. coli* strains
  - The donor strain with genotype arg^{+} met^{+} str^{s}
  - The recipient strain with genotype arg^{-} met^{-} str^{r}

- Refer to Figure 9.11

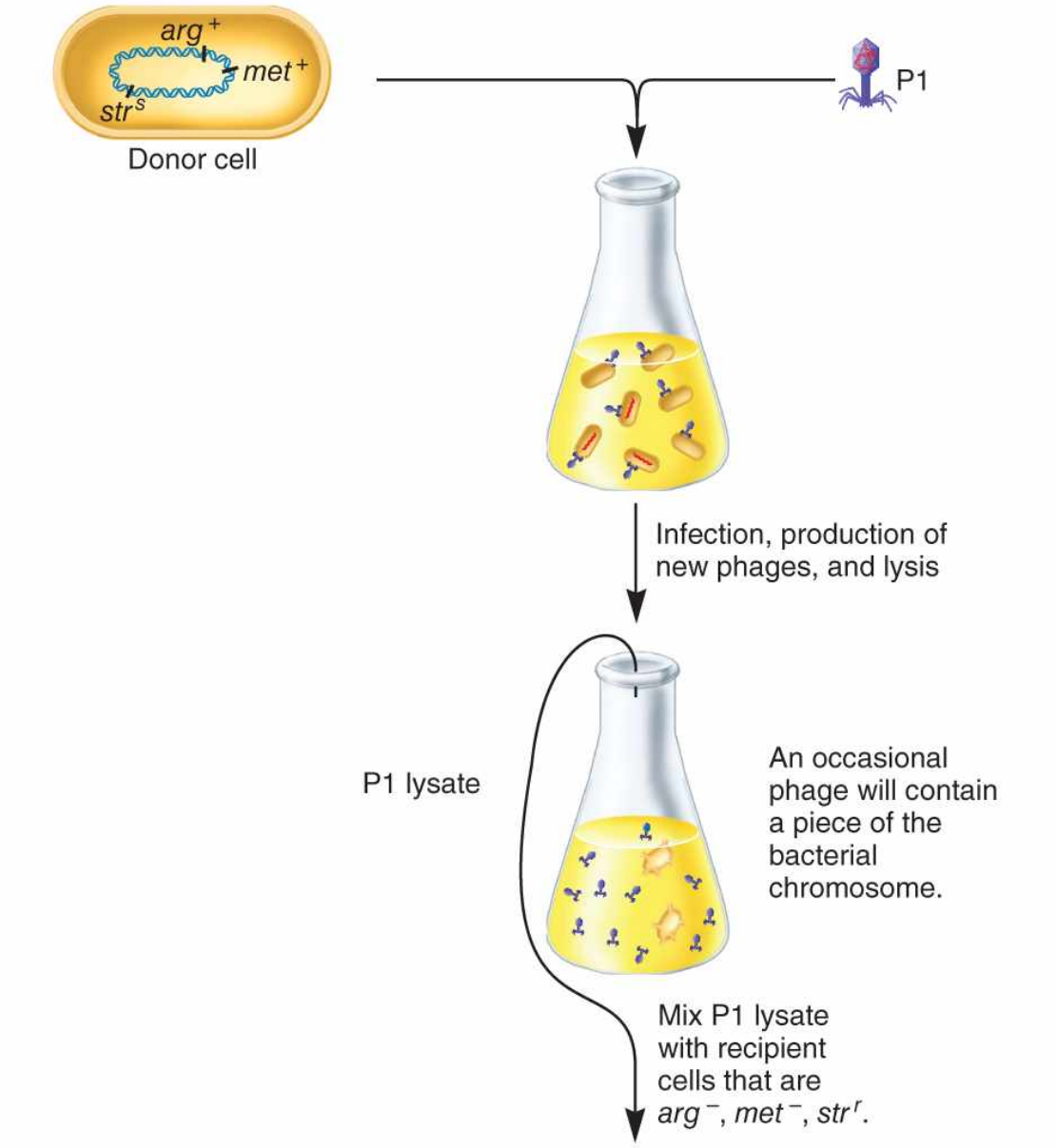
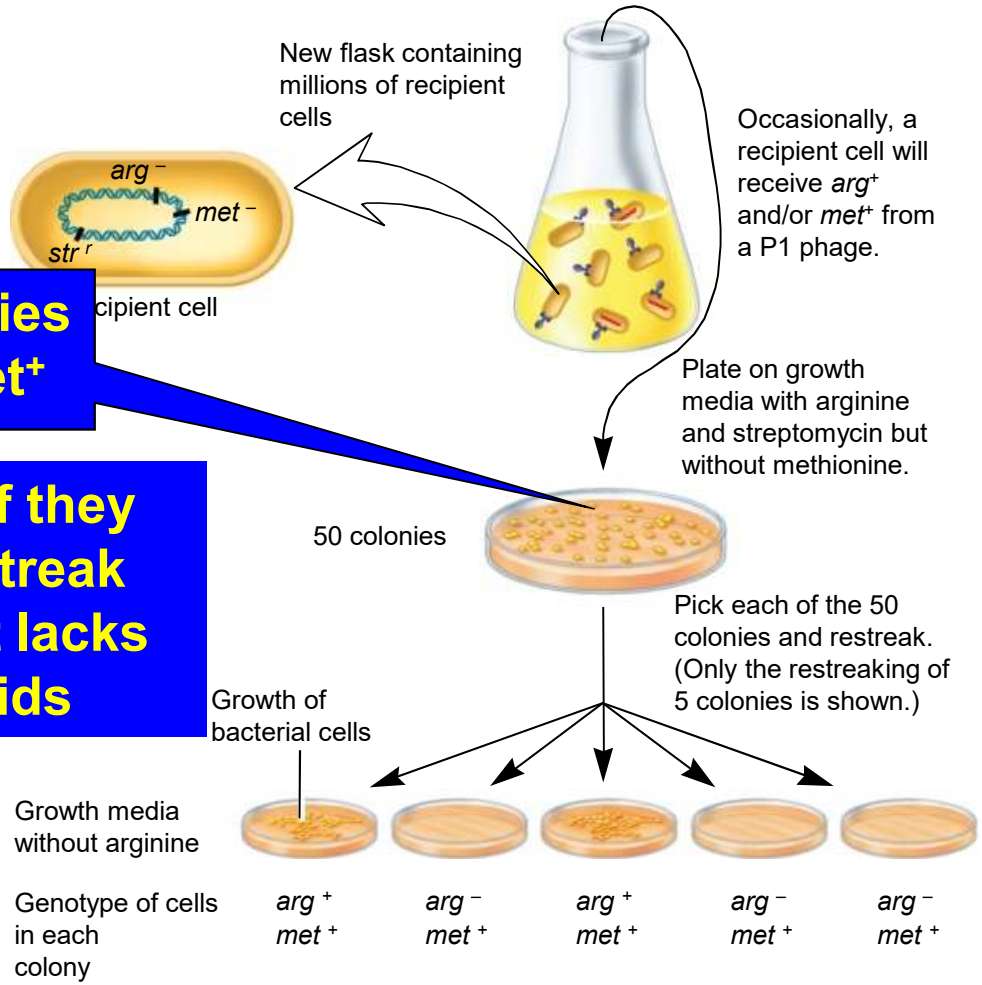


Figure 9.11



These colonies must be met+

To determine if they are also arg+ streak onto plate that lacks both amino acids

Results				
Selected gene	Nonselected gene	Number of colonies that grew on		Cotransduction frequency
		media + arginine	media – arginine	
met +	*arg* +	50	21	0.42

21/50

Figure 9.11

Formula for Cotransduction Mapping

- T. T. Wu derived a relationship between cotransduction frequency and map distances obtained from conjugation experiments

Cotransduction frequency = $(1 - d/L)^3$

- where
  - d = distance between two genes in minutes
  - L = the size of the transduced DNA (in minutes)
    - » For P1 transduction, this size is ~ 2% of the *E. coli* chromosome, which equals about 2 minutes

- Let's use the equation in our example,

$$0.42 = (1 - d/2)^3$$

$$(1 - d/2) = \sqrt[3]{0.42}$$

$$1 - d/2 = 0.75$$

$$d/2 = 0.25$$

$$d = 0.5 \text{ minutes}$$

Therefore, the distance between the *met*$^+$ and *arg*$^+$ genes is approximately 0.5 minutes

- **Transduction experiments**
  - Provide very accurate mapping data for genes that are fairly close together

- **Conjugation experiments**
  - Usually used for genes that are far apart on the chromosome

9.5 Bacterial Transformation

- The steps of bacterial transformation

- Transformation is the process by which a bacterium will take up extracellular DNA released by a dead bacterium

- It was discovered by Frederick Griffith in 1928 while working with strains of *Streptococcus pneumoniae*

- There are two types
  - **Natural transformation**
    - DNA uptake occurs without outside help
  - **Artificial transformation**
    - DNA uptake occurs with the help of special techniques

- Natural transformation occurs in a wide variety of bacteria

- Bacteria able to take up DNA are called **competent cells**
  - They carry genes that encode proteins called **competence factors**
    - These proteins facilitate the binding, uptake and subsequent incorporation of the DNA into the bacterial chromosome

- Refer to Figure 9.12

Steps of Transformation

1. Large fragment of DNA binds to surface of cell
2. Endonuclease enzyme outside the cell cuts up the DNA
3. One strand of the DNA is degraded, and the other enters the cell
4. DNA must be incorporated into the chromosome to be stably inherited
   - This can occur by **homologous recombination**
5. This results in **heteroduplex** DNA...
6. Which is repaired by DNA repair enzymes
   - Sometimes transforming the recipient to the new genotype

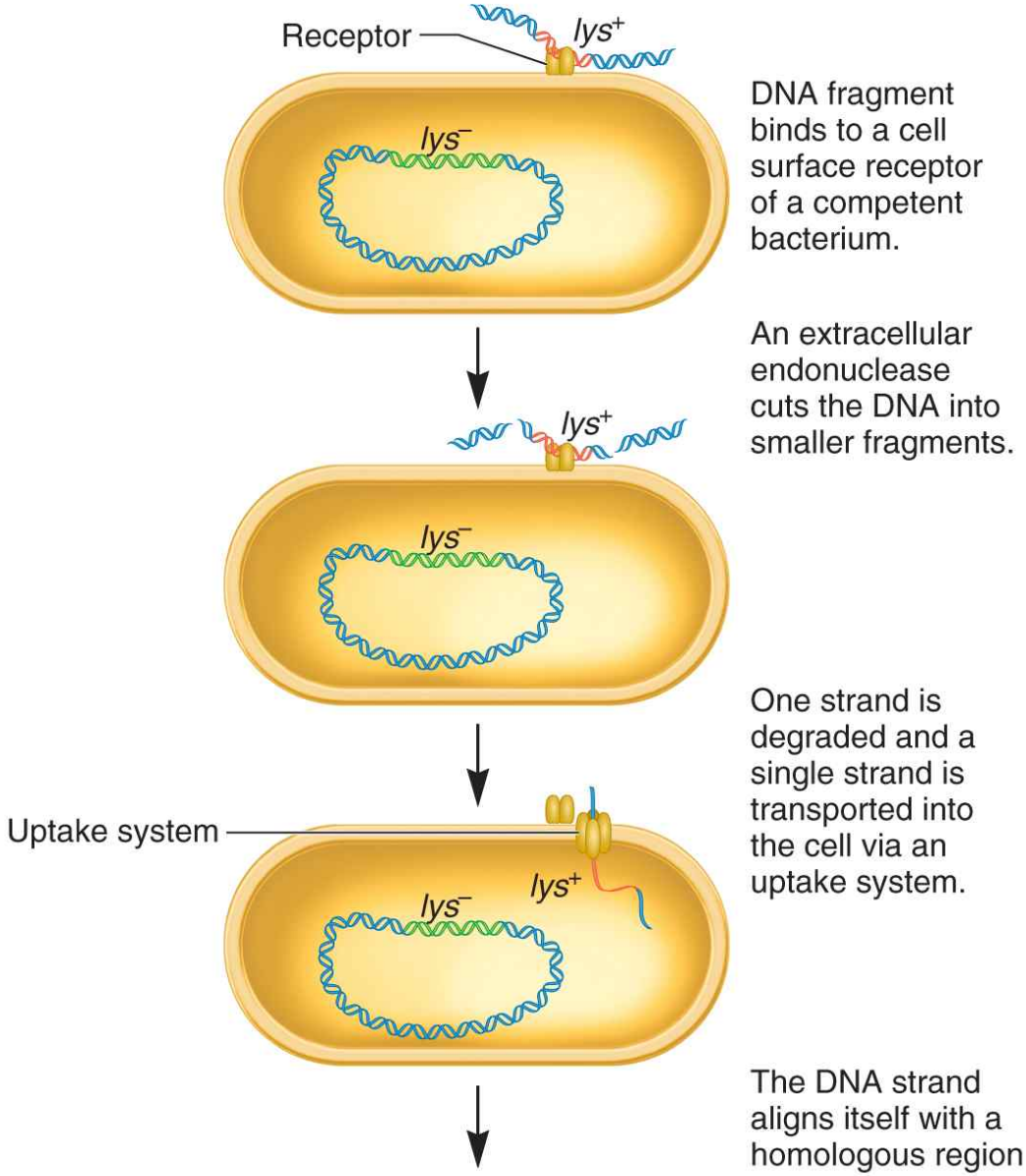


Figure 9.12

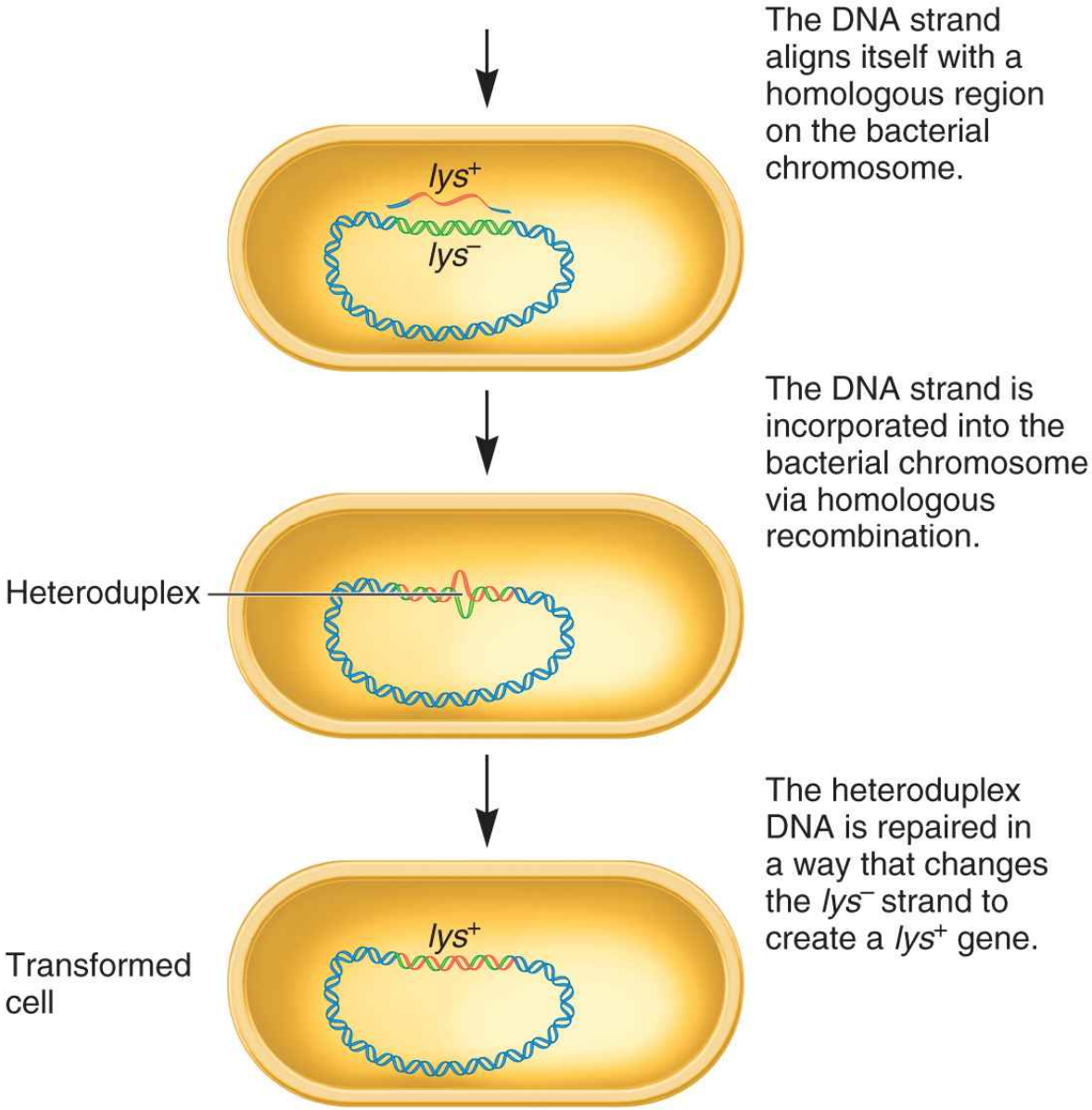


Figure 9.12

- Sometimes, the DNA that enters the cell is not homologous to any genes on the chromosome
  - It may be incorporated at a random site on the chromosome
  - This process is termed **nonhomologous** or **illegitimate recombination**

9.6 Medical Relevance of Bacterial Gene Transfer

- Definition of horizontal gene transfer.

- The impact of bacterial horizontal gene transfer in medicine

- **Horizontal gene transfer** is the transfer of genes from one organism to another
  - May occur between the same or different species

- Vertical gene transfer is transfer of genes from mother to daughter cell or from parents to offspring

- A sizable fraction of bacterial genes are derived from horizontal gene transfer

- Horizontal gene transfer has dramatically contributed to the phenomenon of **acquired antibiotic resistance**

- Bacterial resistance to antibiotics is a serious problem worldwide
  - Many strains of *Staphylococcus aureus* strains are resistant to methicillin and penicillin
    - Example: **MRSA** – “mersa”, methicillin-resistant *Staphylococcus aureus*
      - Causes skin infections that are difficult to treat