Chapter 13

Bacterial Genetics

Sections to study

13.1 The enormous diversity of bacteria

13.2 Bacterial genomes

13.3 Bacteria as experimental organisms

13.4 Gene transfer in bacteria

13.5 Bacterial genetic analysis

13.1 The enormous diversity of bacteria

- Outnumber all other organisms on earth.
- 10,000 species identified.

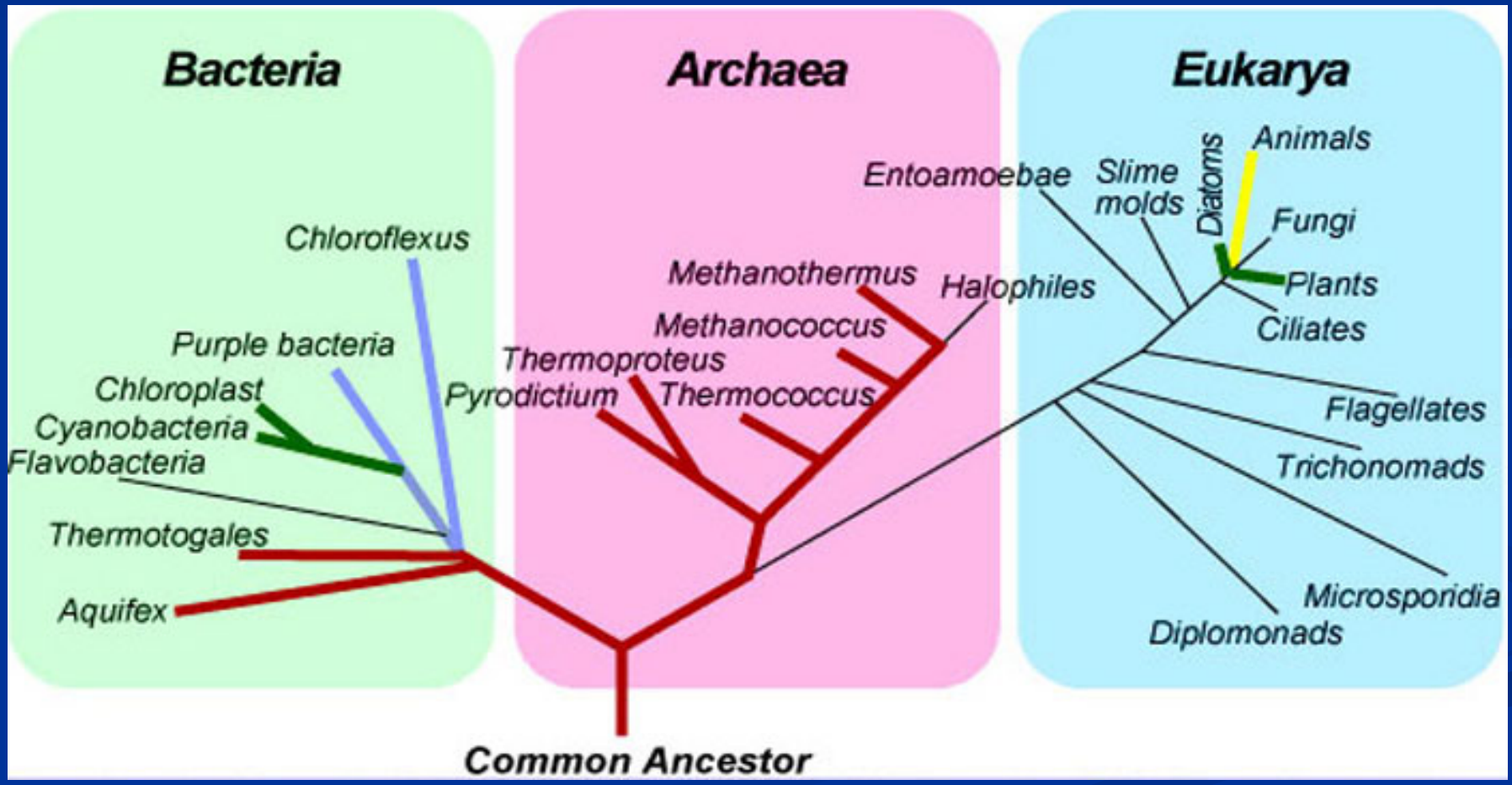
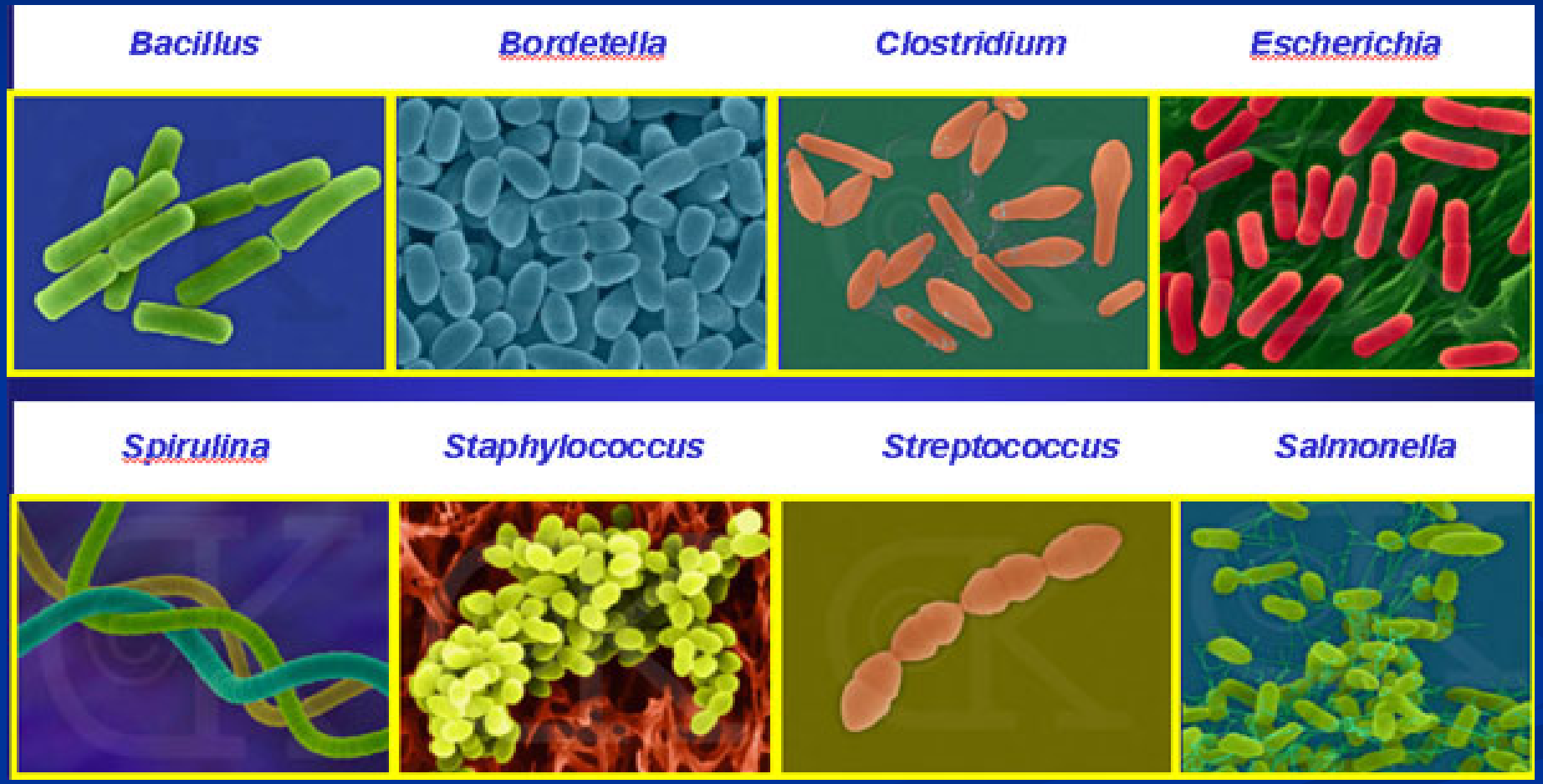


Fig 13.1

Bacteria vary in size and shape

- Smallest 200 nm in diameter, the largest 500 μm in length.



Common features of bacteria

- Lack defined nuclear membrane. Chromosomes fold to form a **nucleoid body**.
- Lack membrane bound organelles.
- Most have a **cell wall**. Some has a mucus-like coating called a **capsule**.
- Many move by **flagella**.

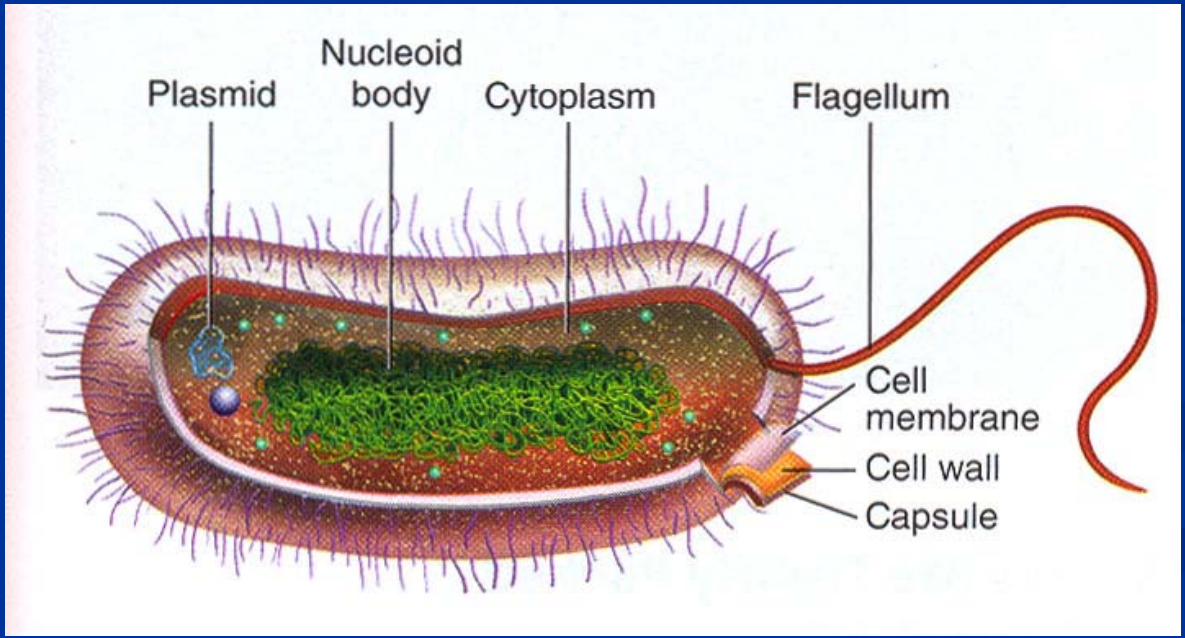
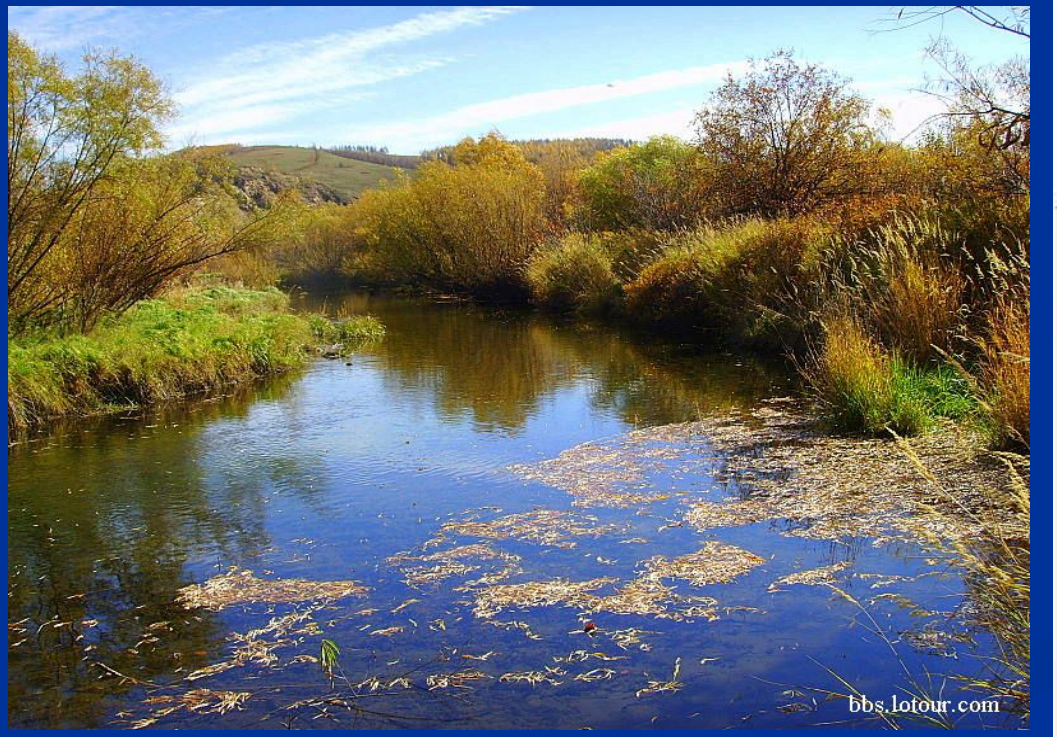
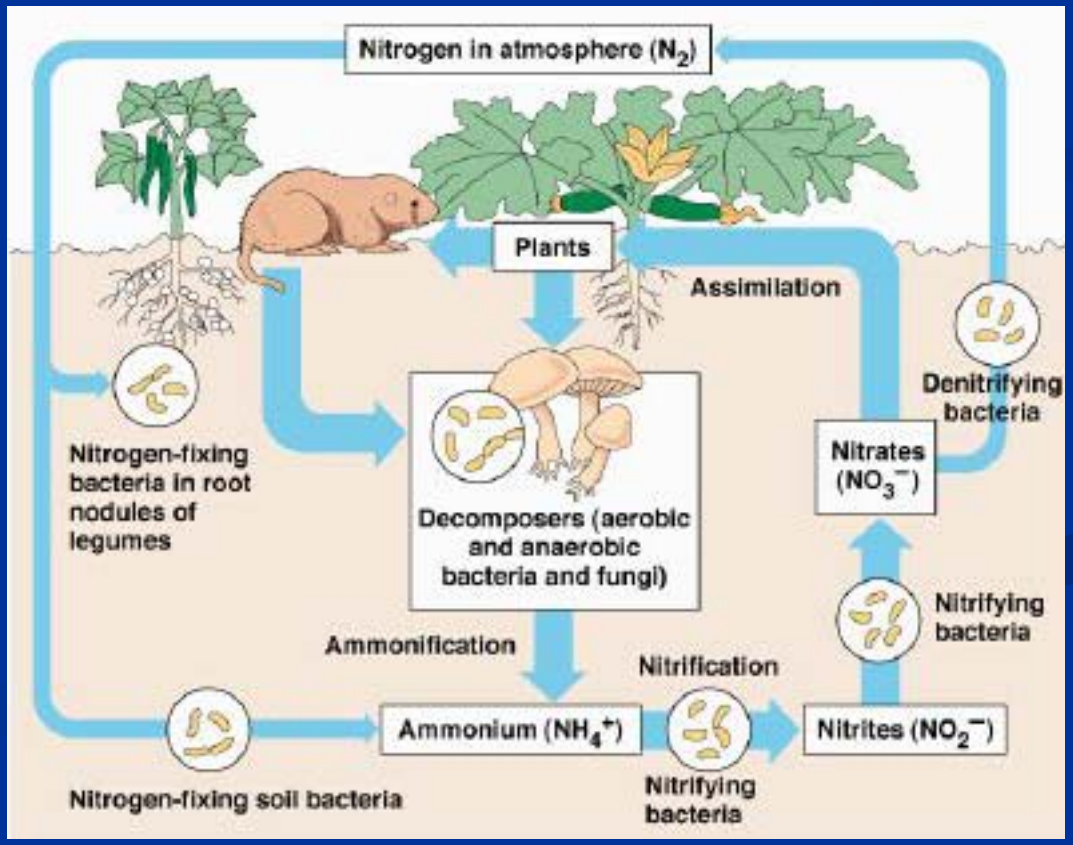


Fig 13.3

Bacteria have diverse metabolisms

- Remarkable metabolic diversity allows them to live almost anywhere.
  - Habitats range from land, to aquatic, to parasitic.
  - Can obtain energy from sunlight or breaking down chemicals.
- Bacteria are crucial to the maintenance of earth's environment.
  - Can fix nitrogen, decompose oil and other chemicals.





Bacteria must be grown and studied in cultures

Culture: The visible accumulation of microorganisms in or on a nutrient medium. Also, the propagation of microorganisms with various media.

- **On agar plate – A single bacterium can multiply to 10^7 – 10^8 cells in less than a day.**
- **In liquid media – *E. coli* grows to concentration of 10^9 cells/ml within a day.**

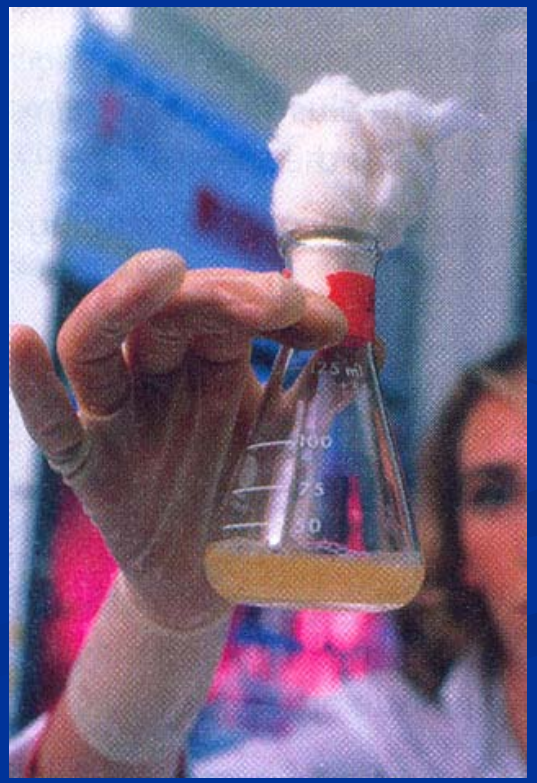
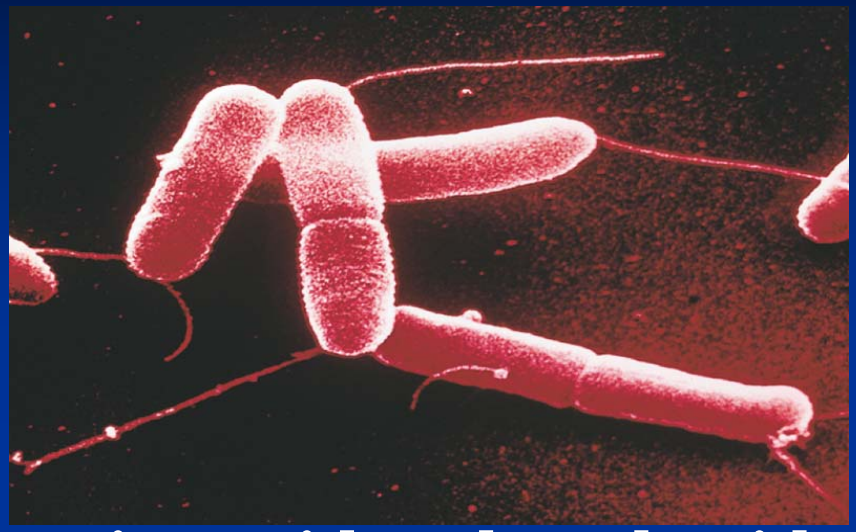


Fig 13.11

Escherichia coli: A versatile model organism



- Inhabitant of intestines in warm blooded animals.
- Strains in laboratory are not pathogenic.
- Makes all the enzymes it needs for amino acid and nucleotide synthesis.
  - **Prototroph** – A microorganism that grows on minimal media. It is usually wild type.
- Rapid multiplication makes it possible to observe very rare genetic events.
  - Divides about once every hour in minimal media and every 20 minutes in enriched media.

13.2 Bacterial genomes

- 4-5 Mb long
- Circular
- Condenses by supercoiling and looping into a densely packed nucleoid body.
- Chromosomes replicate inside cell and cell divides by **binary fission**.

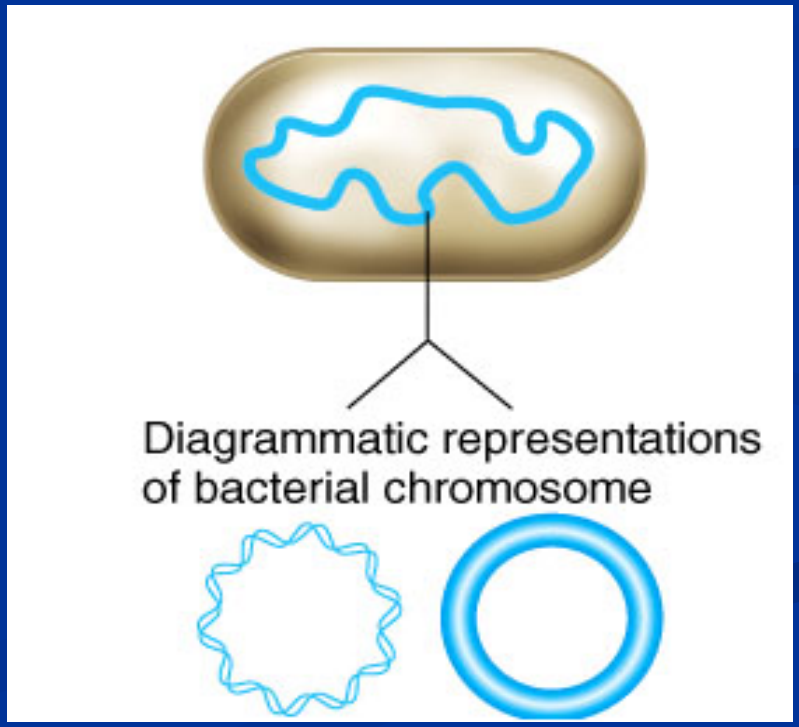
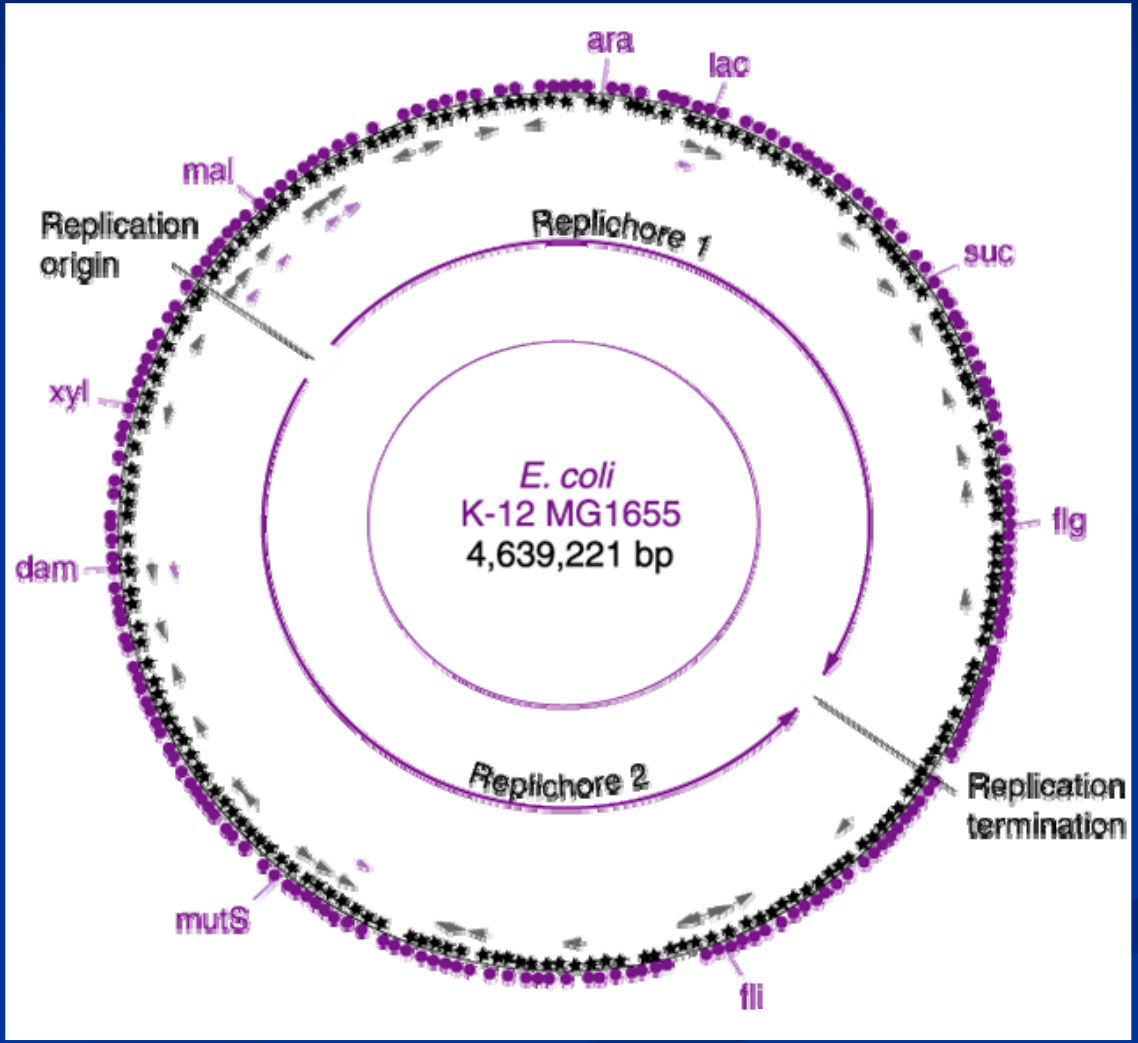


Fig. 13.4, 13.5

Genes are tightly packed in bacterial genomes

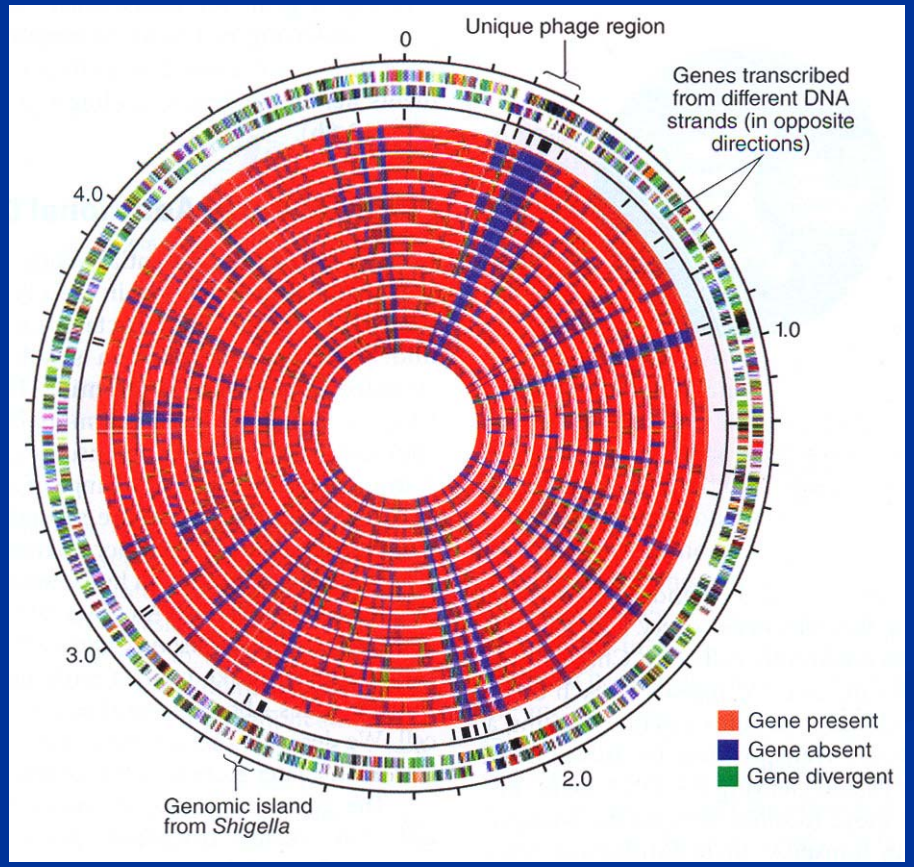
The genome of *E. coli* K12 strain:

- 4.6 million base pairs.
- 4288 genes, 40% of which we do not know what they do.
- Almost no repeated DNA.
- 90% of genome encodes protein.
- The largest class: 427 genes have a transport function.
- **Bacteriophage** sequences found in 8 places (must have been invaded by viruses at least 8 times during history).



Individual *E. coli* strains contain only a subset of the *E. coli* pangenome

- **Core genome**: Genes shared by all bacterial strains of a given species.
  - ~ 1000 genes for *E. coli*.
- **Pangenome**: The core genome of a bacterial species plus all genes found in some strains but not others.
  - ~ 15,000 genes for *E. coli*.



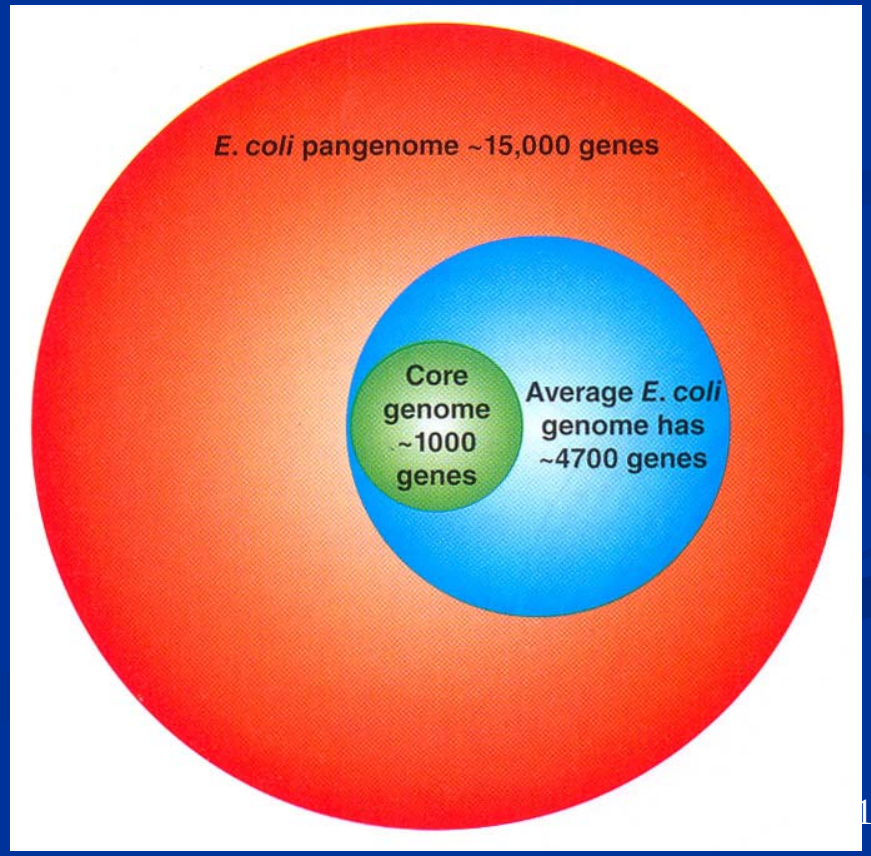


Fig. 13.6, 13.7

Bacterial genomes contain transposable elements

- **Transposable elements** place DNA sequences at various locations in the genome.
- Many of the spontaneous mutations in *E. coli* result from **IS transposition** into a gene.

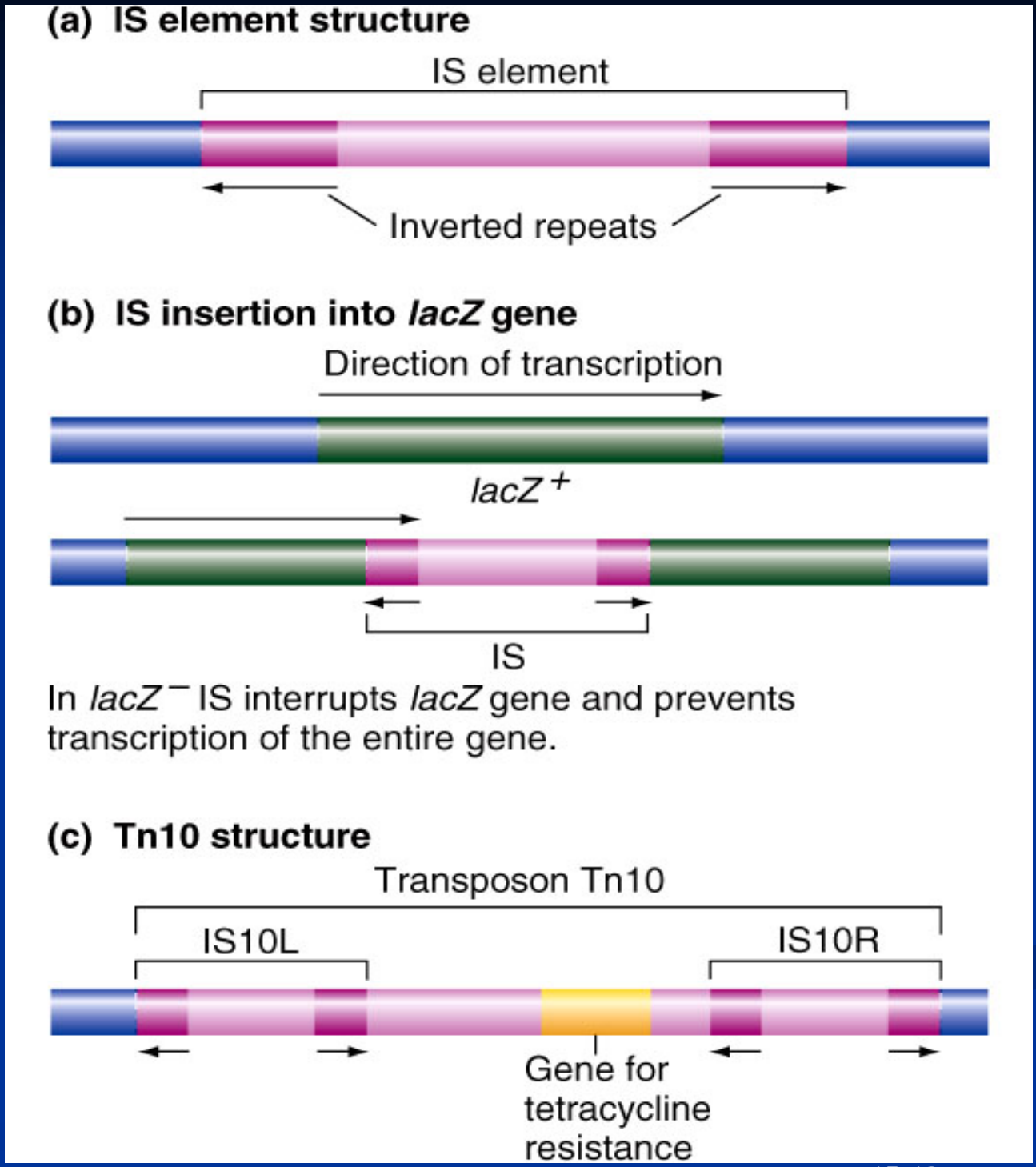
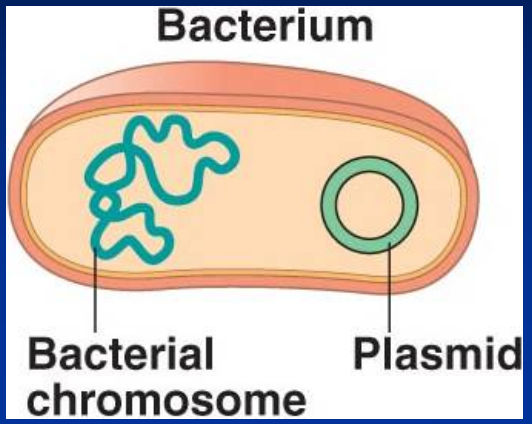


Fig. 13.8

Plasmids carry additional DNA

Plasmids: Small circles of double-stranded DNA that can replicate in bacterial cells independently of the chromosome.



- **Plasmids vary in size ranging from 1 kb – 3 Mb.**
- **Plasmids are not needed for reproduction or normal growth, but they can be beneficial.**

Fig. 13.9

Some plasmids contain multiple antibiotic resistance genes

- Plasmids can carry genes that confer resistance to antibiotics and toxic substances.
- Plasmids can transfer genes from one bacteria to another.

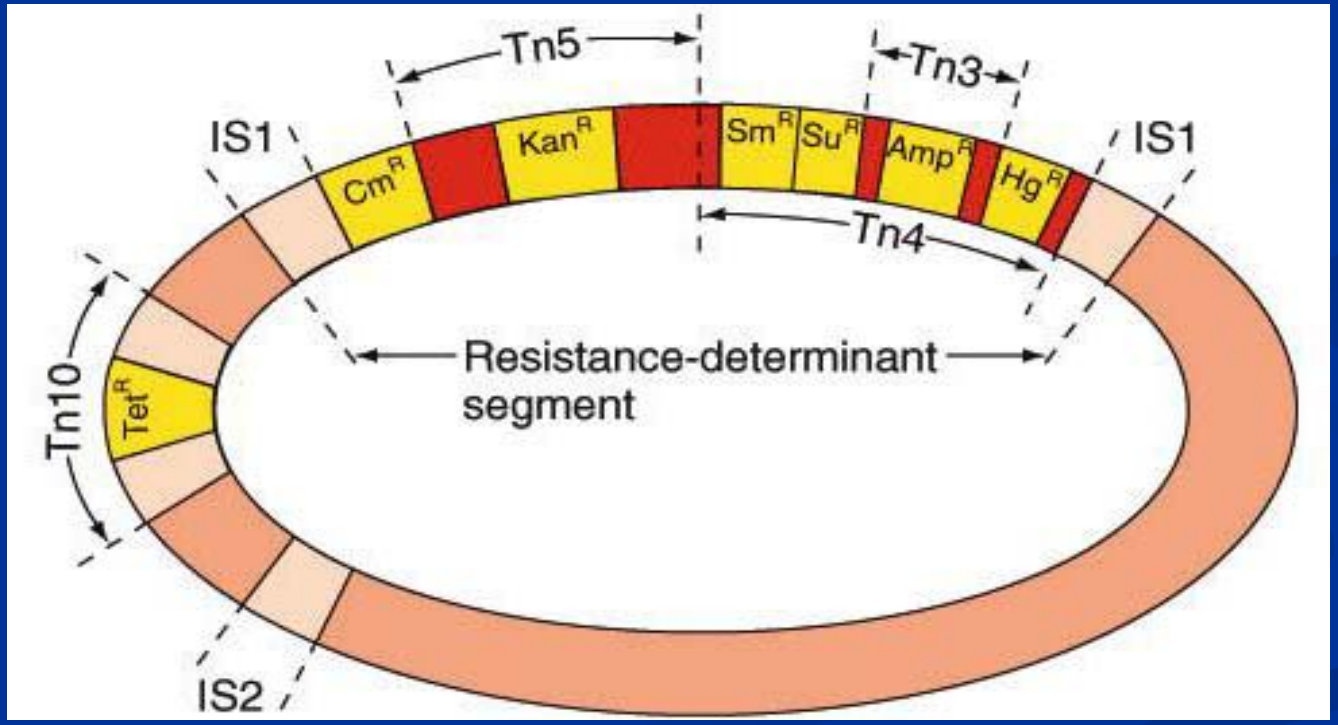
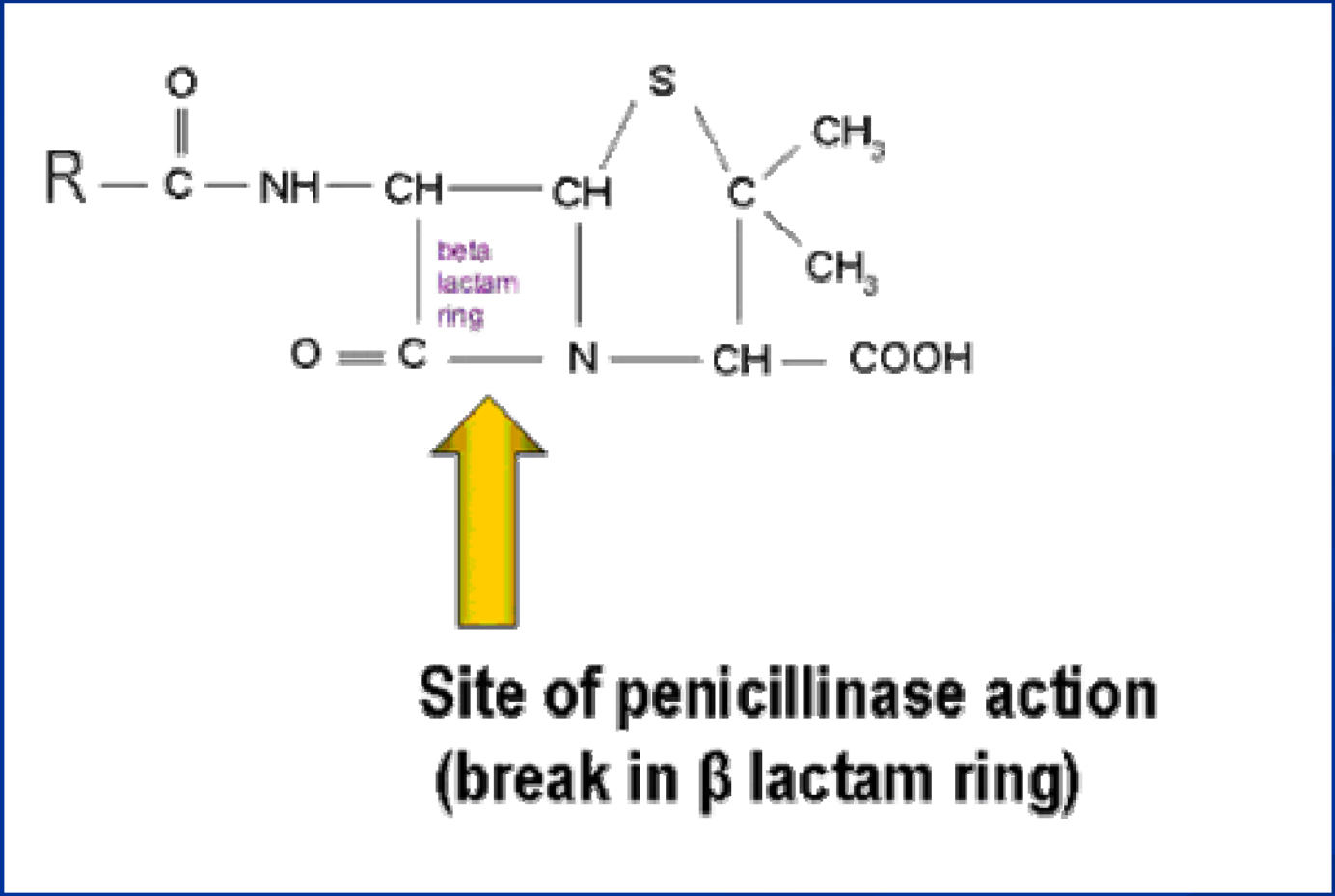
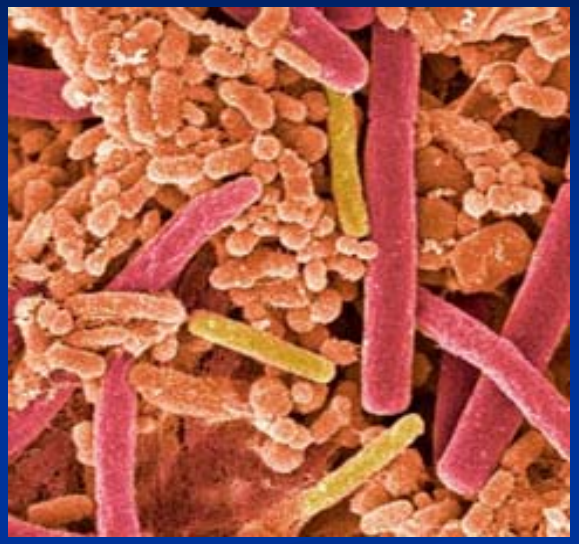


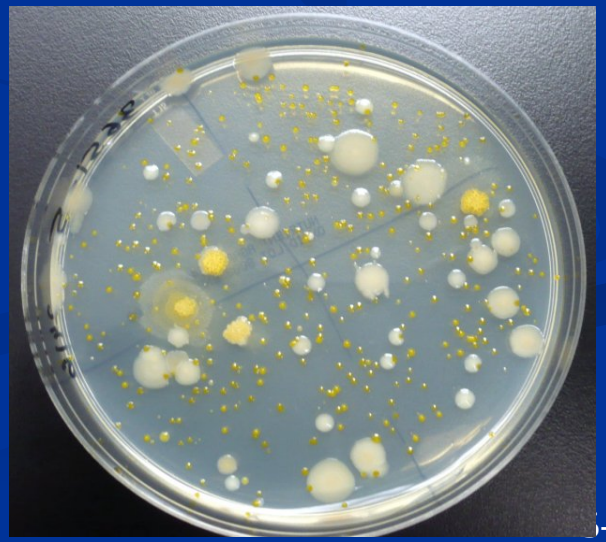
Fig 13.10

Some R plasmids carry *bla* (*Amp*^{r}) gene, which encodes β-lactamase (or penicillinase, β-内酰胺酶), a penicillin-degrading enzyme.



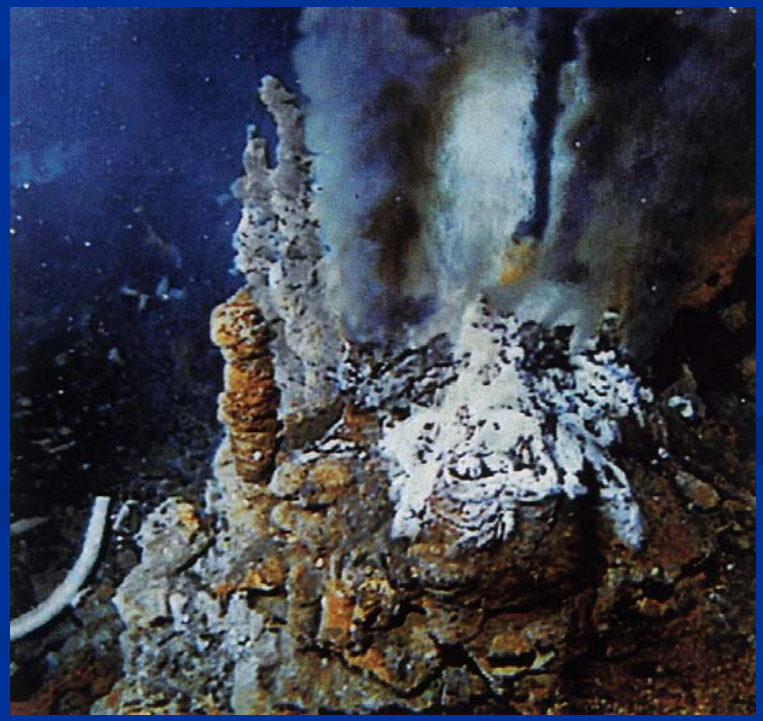
Metagenomics explore the collective genomes of microbial communities





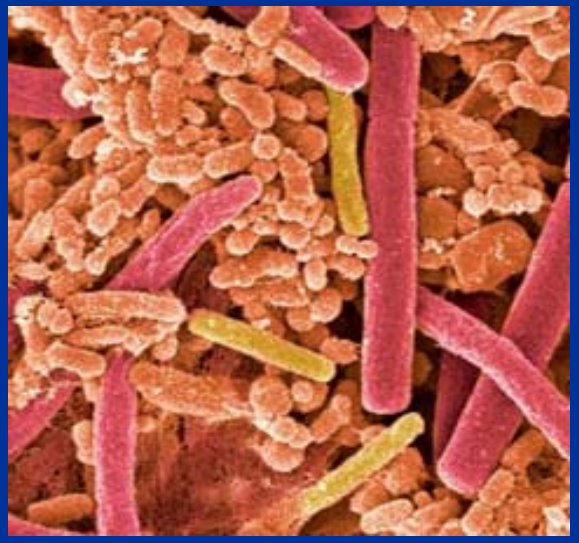
Pure culture

- **Microorganisms that live in extreme and unusual environments are often difficult to culture.**
  - **Hot springs**
  - **Deep sea sediments**
  - **Mining sites**



An example: Bacteria in soil

- 1700 16S rRNA sequences analyzed.
- 847 distinct types.



Total DNA

↓ PCR, cloning

↓ DNA sequencing

16S rRNA

- **Metagenomics (宏基因组学): The analysis of genomic DNA from a microorganism community bypassing the need to isolate and culture individual microbial species.**

Extract all DNA from microbial community in sampled environment

DETERMINE WHAT THE GENES ARE
(Sequence-based metagenomics)

- Identify genes and metabolic pathways
- Compare to other communities
- and more…

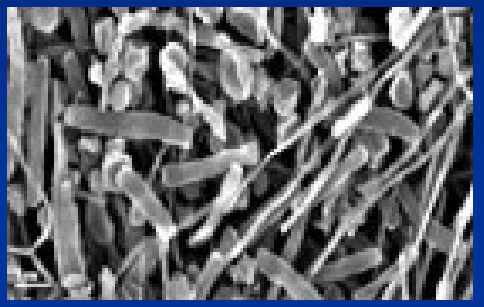
DETERMINE WHAT THE GENES DO
(Function-based metagenomics)

- Screen to identify functions of interest, such as vitamin or antibiotic production
- Find the genes that code for functions of interest
- and more…

- **Metagenome (宏基因组): All the genetic material present in an environmental sample, consisting of the genomes of many individual organisms.**

Antarctic bacterioplankton (DRI)

hypersaline mats (UCol)



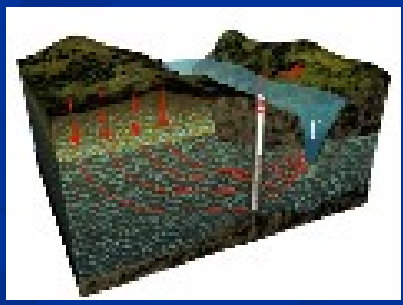
Korarchaeota enrichment

Farm soil (Diversa)

termite hindgut (CalTech)

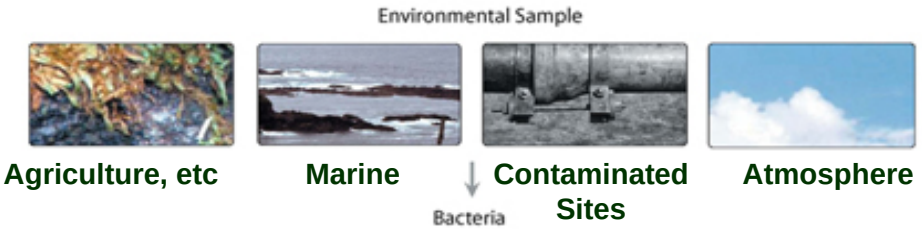
planktonic archaea (MIT)

Alaskan soil (UW)



groundwater (ORNL)

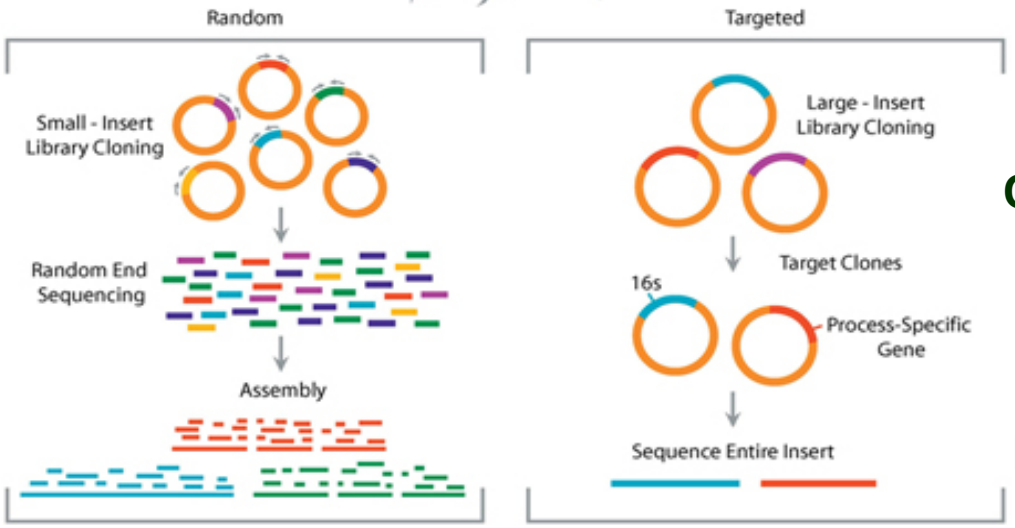
Collect environmental samples



Rem: no culturing

Extract total genomic DNA

Construction of small insert libraries



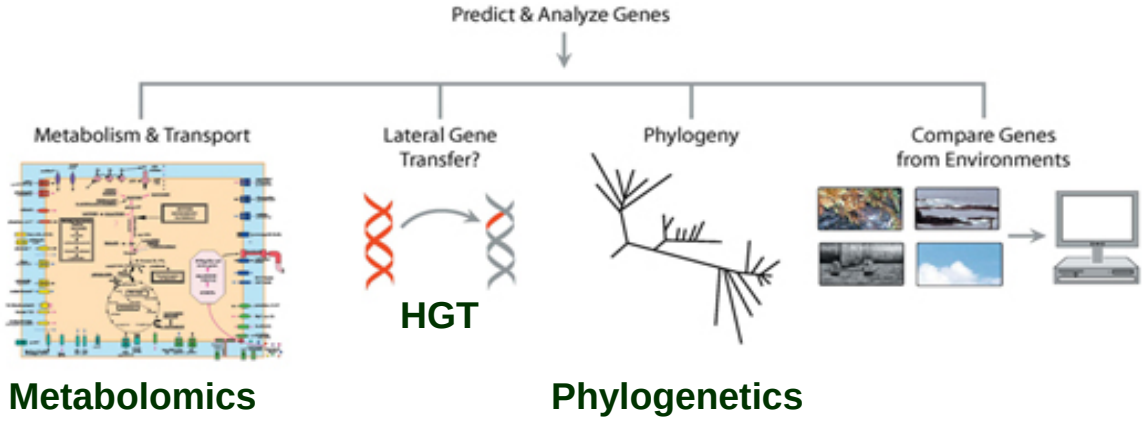
Construction of large insert libraries

Massive sequencing

Massive sequencing

Binn sequences

(%GC, codon usage, sequence coverage, n-mer presence, etc)



Metabolomics

Phylogenetics

Providing basic answers to:

1. Who is out there?

What types of organisms exist? What are their numbers?

Which organisms comprise a community?

2. What are they doing?

A community is more than just a list of organisms

What processes does each member contribute?

How do they communicate and interact?

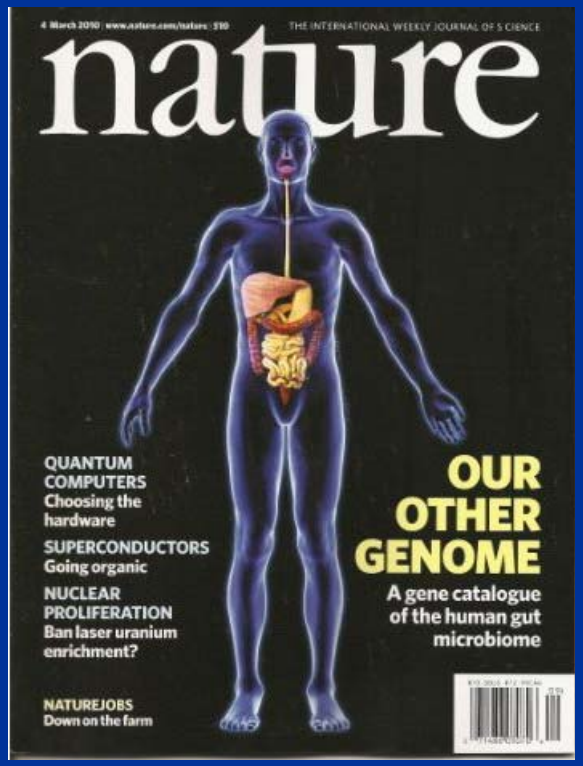
Metagenomics described as at least as important as the invention of the microscope*

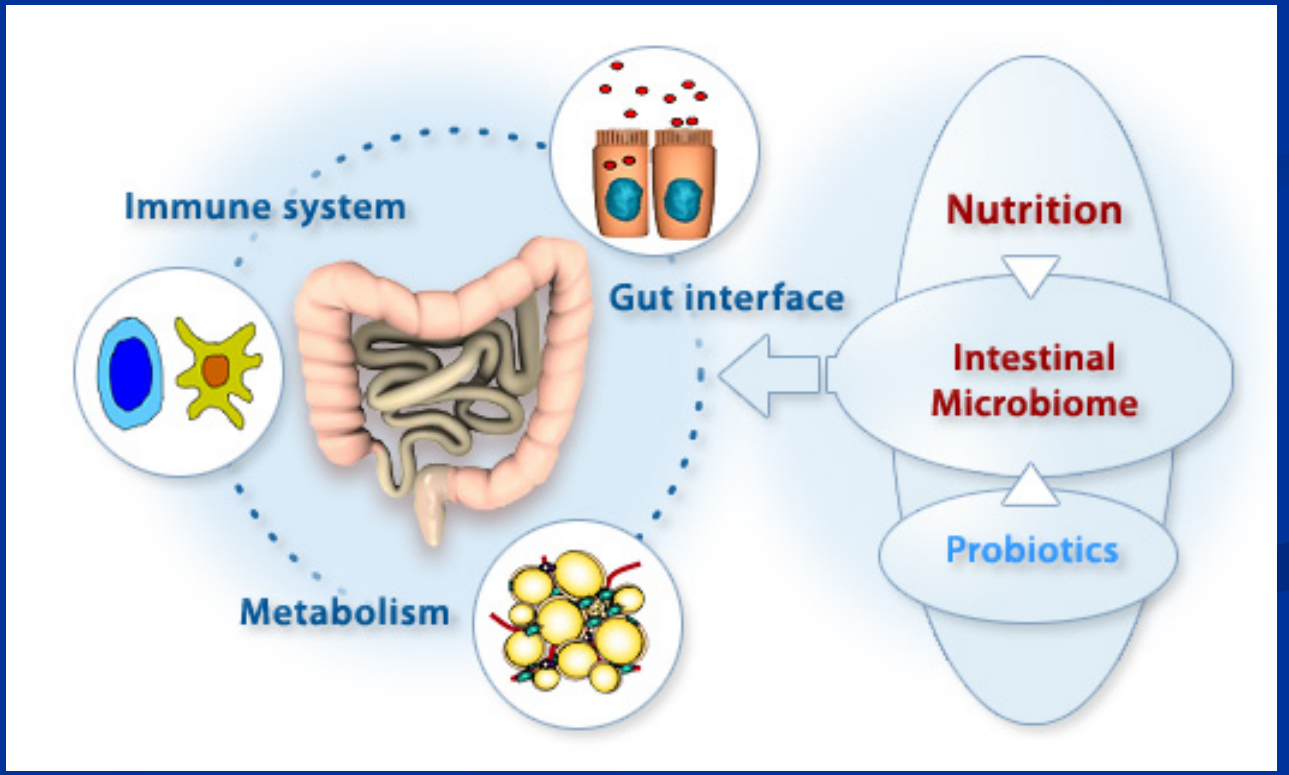
Metagenomics is revolutionizing fundamental biological concepts

****www.scienceagogo.com/news/metagenomics.shtml, 2007***

The human microbiome（人体微生物基因组)

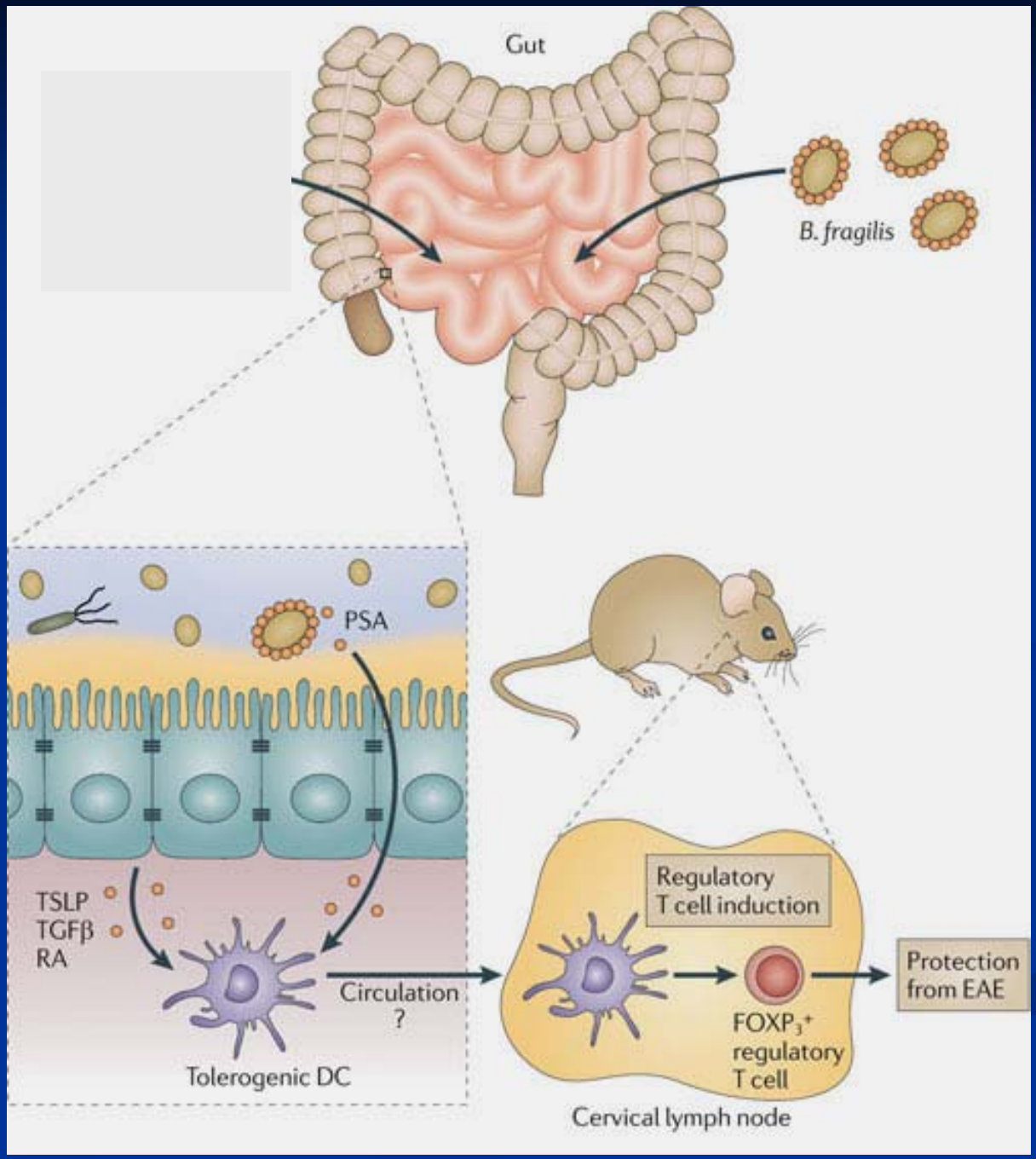
- ~ 5000 different bacterial spp. exist in our bodies, with > 100 trillion bacterial cells (our body has several trillion cells).





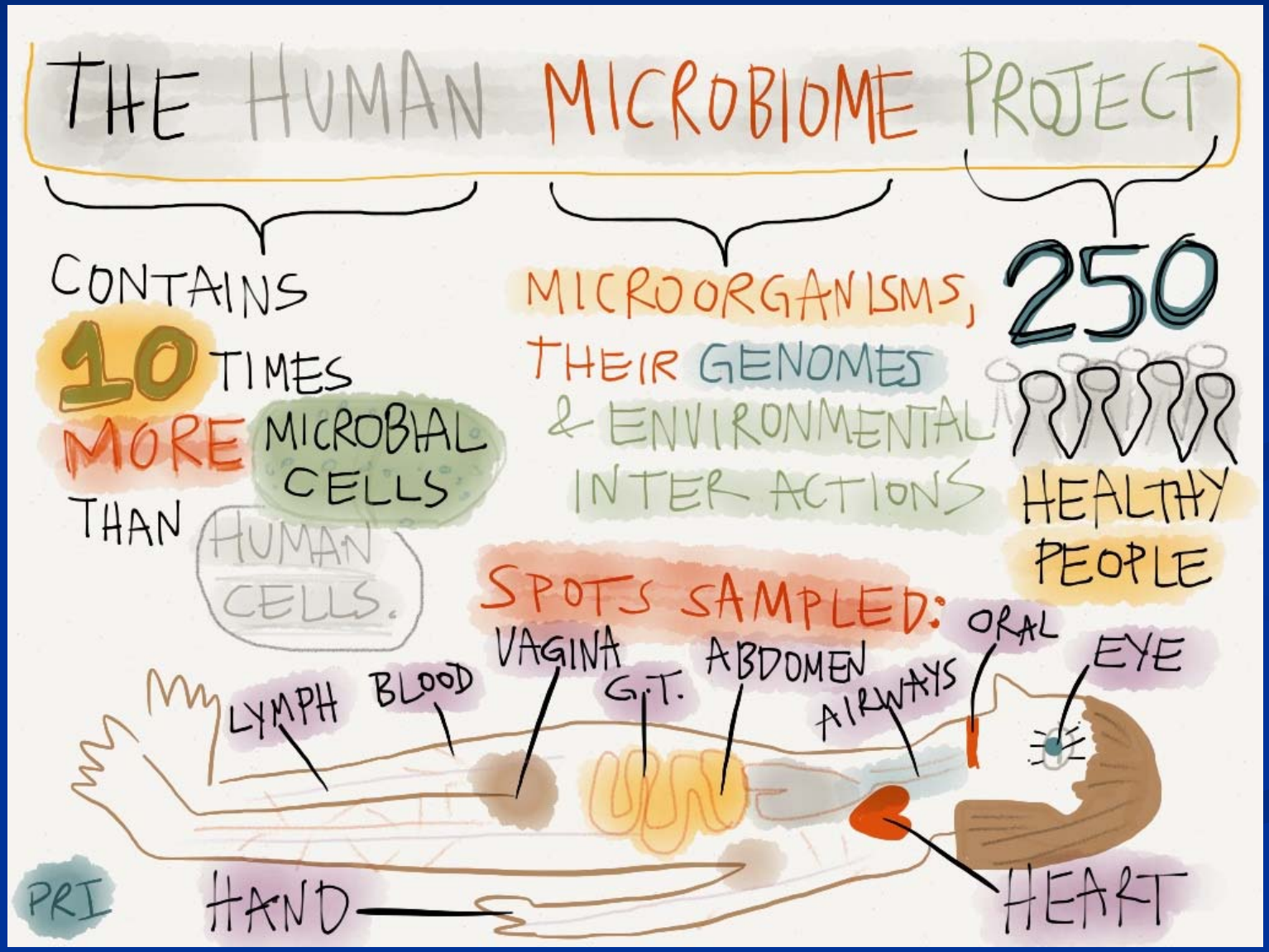
Gut bacteria direct the development of animal immune system

- **The development of regulatory T cells in mice is induced by polysaccharide A (PSA), which locates on the surface of bacteria *Bacteroides fragilis* (脆弱拟杆菌).** (Mazmanian SK et al. *Cell* 122:107-118, 2005)



The Human Microbiome Project (HMP)

- 2007-12, funded by NIH. To explore the relationship between microbes and human disease.

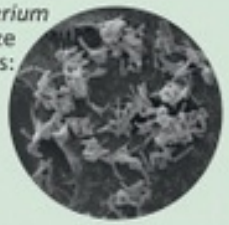


A map of diversity in the human microbiome

Streptococcus dominates the oral cavity with *S. mitis* > 75% in the cheek

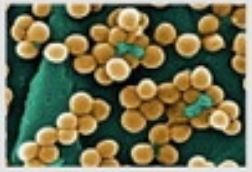
Propionibacterium acnes lives on the skin and nose of most people

Many *Corynebacterium* species characterize different body sites:
C. matruchoti the plaque
C. accolens the nose
C. croppenstedtii the skin



Lactobacillus species (*L. gasseri, L. jensenii, L. crispatus, L. iners*) are predominant but mutually exclusive in the vagina

Staphylococcus epidermidis colonizes external body sites



Several *Prevotella* species are present in the gastrointestinal tract. *P. copri* is present in 19% of the subjects and dominates the intestinal flora when present

Bacteroides is the most abundant genus in the gut of almost all healthy subjects

Campylobacter includes opportunistic pathogens, but members live in the oral cavities of most healthy people in the cohort

E. coli is present in the gut of the majority of healthy subjects but at very low abundance

Key:

- Commensal microbes
- Potential pathogens

The four most abundant phyla

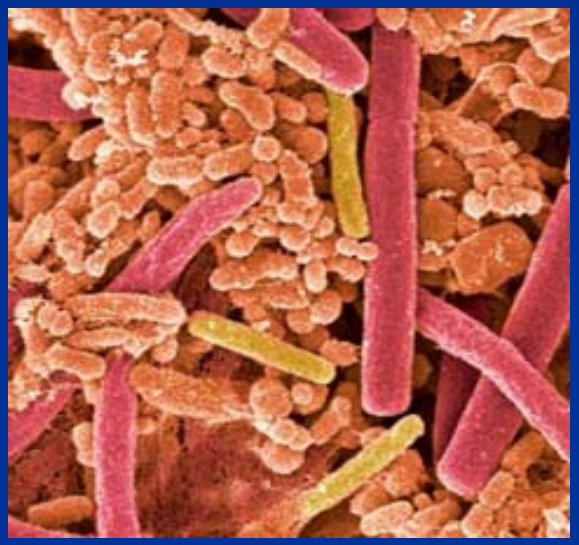
- Actinobacteria
- Bacteroidetes
- Firmicutes
- Proteobacteria

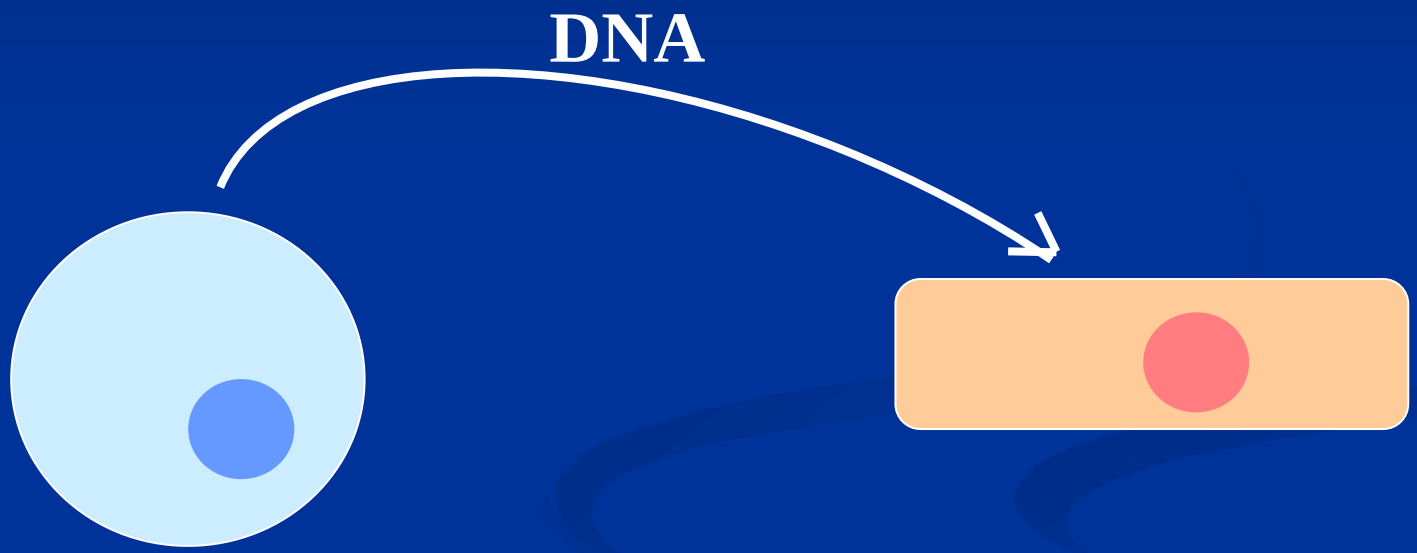
Low abundance phyla

- Chloroflexi
- Cyanobacteria
- Euryarchaeota
- Fusobacteria
- Lentisphaerae
- Spirochaetes
- Synergistetes
- Tenericutes
- Thermi
- Verrucomicrobia

TRENDS in Genetics

13.4 Gene transfer in bacteria





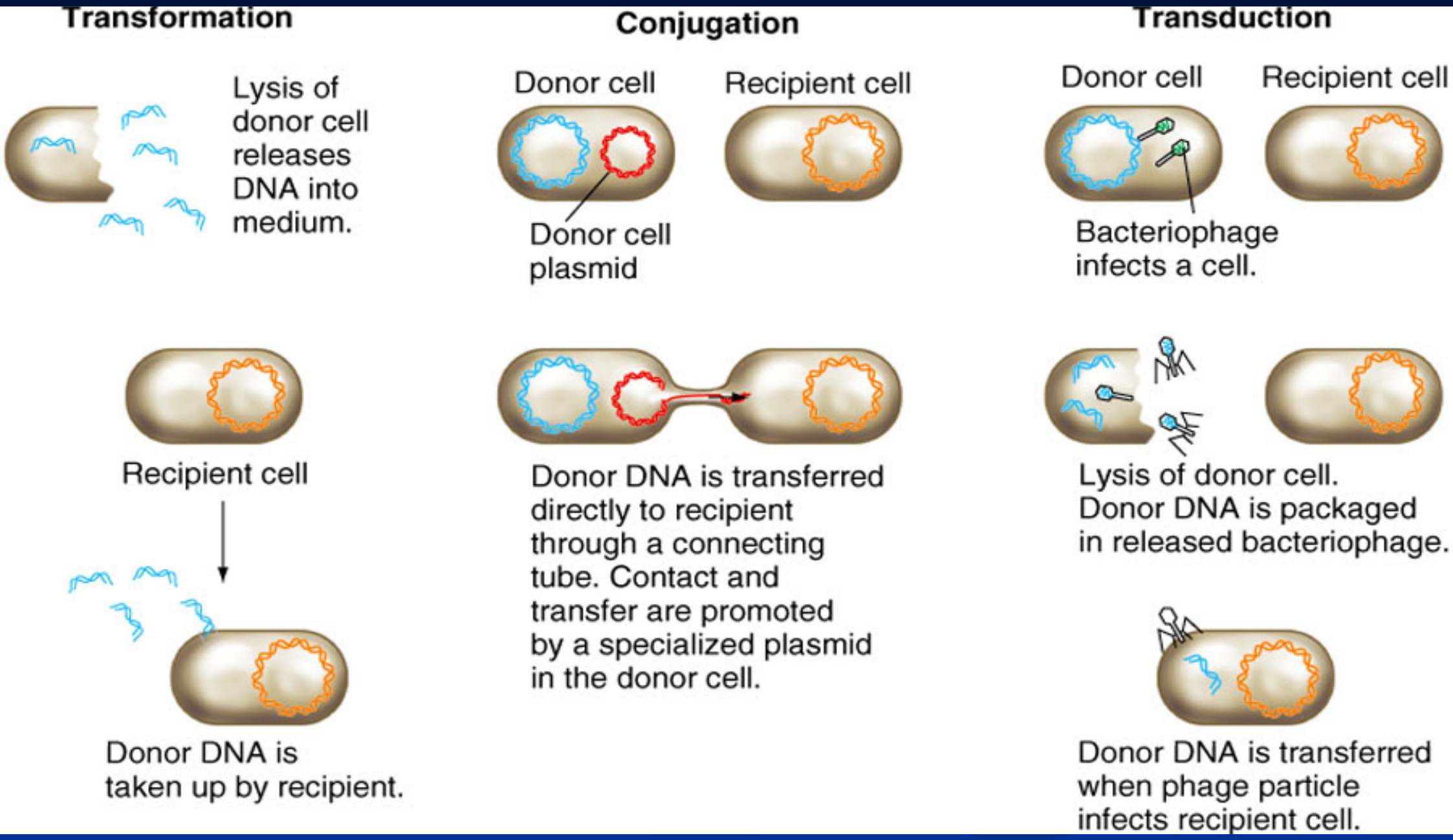

Transformation
Lysis of donor cell releases DNA into medium.
Recipient cell
Donor DNA is taken up by recipient.
Conjugation
Donor cell
Recipient cell
Donor cell plasmid
Donor DNA is transferred directly to recipient through a connecting tube. Contact and transfer are promoted by a specialized plasmid in the donor cell.
Transduction
Donor cell
Recipient cell
Bacteriophage infects a cell.
Lysis of donor cell. Donor DNA is packaged in released bacteriophage.
Donor DNA is transferred when phage particle infects recipient cell.

Fig. 13.12

1. Transformation

- **Transformation**: Fragments of donor DNA enter the recipient cell and alter its genotype.
  - **Natural transformation** – occurs in the natural environment.
  - **Artificial transformation** – occurs in the laboratory.
    - **Competent cells**: Cells that are able to take up DNA from the medium. (Treat cells by suspending in calcium at cold temperatures)
    - **Electroporation** – mix donor DNA with recipient bacteria and subject to very brief high-voltage shock.

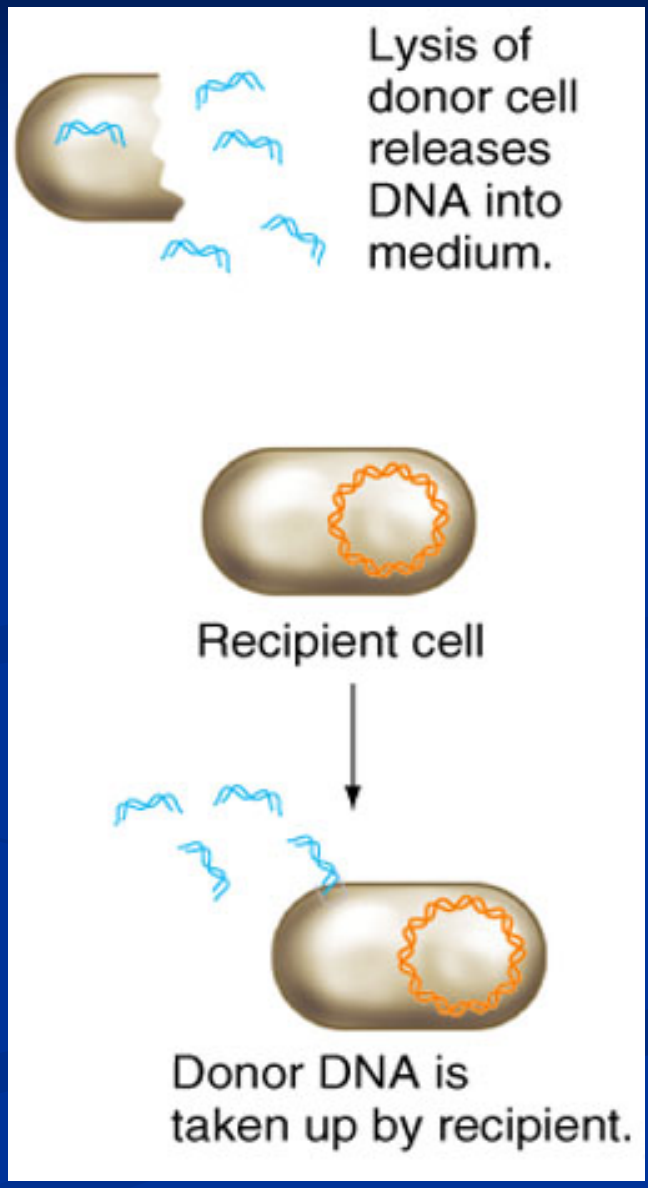


Figure 13.13

Natural transformation in *B. subtilis*

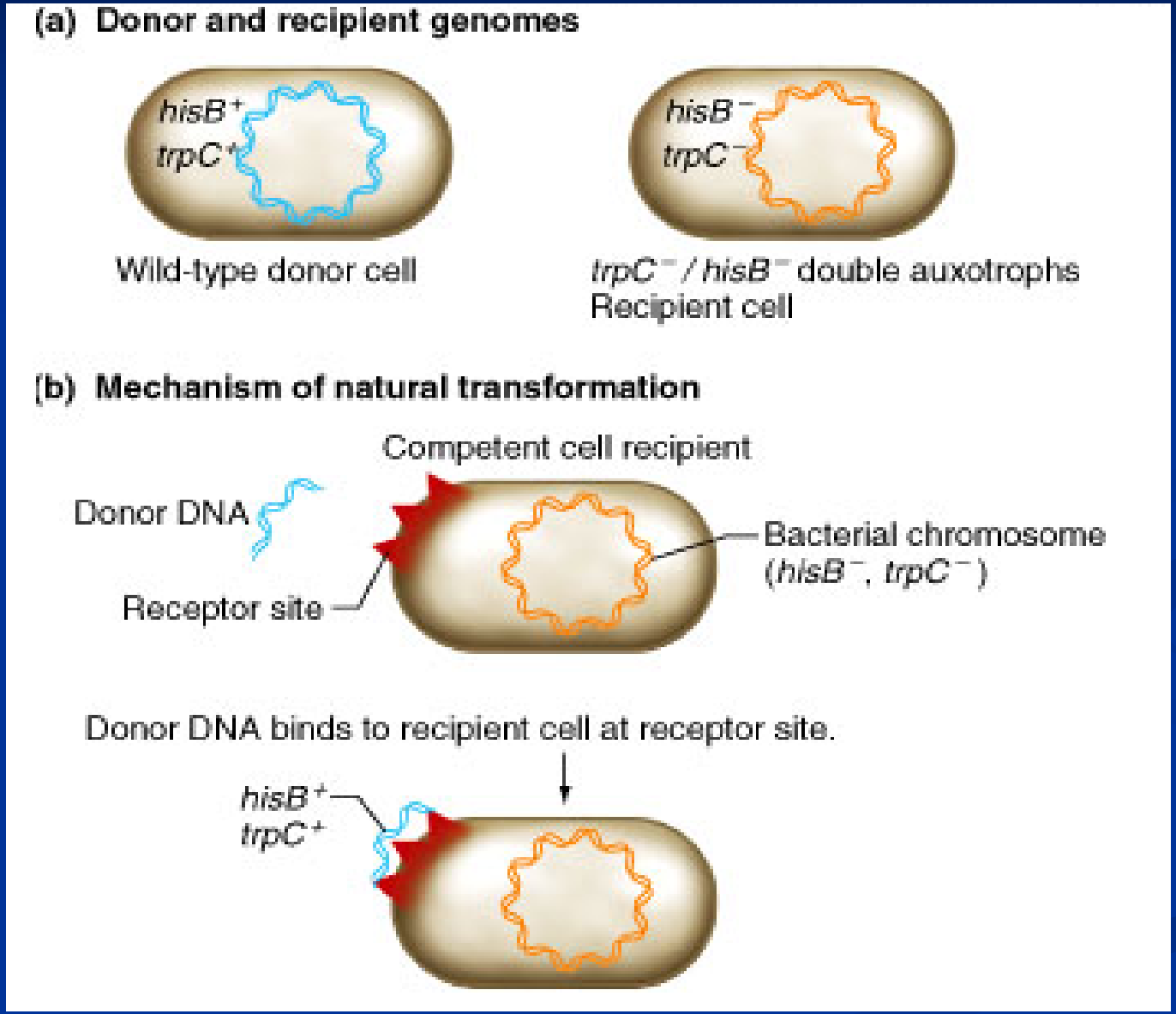


Fig. 13.13

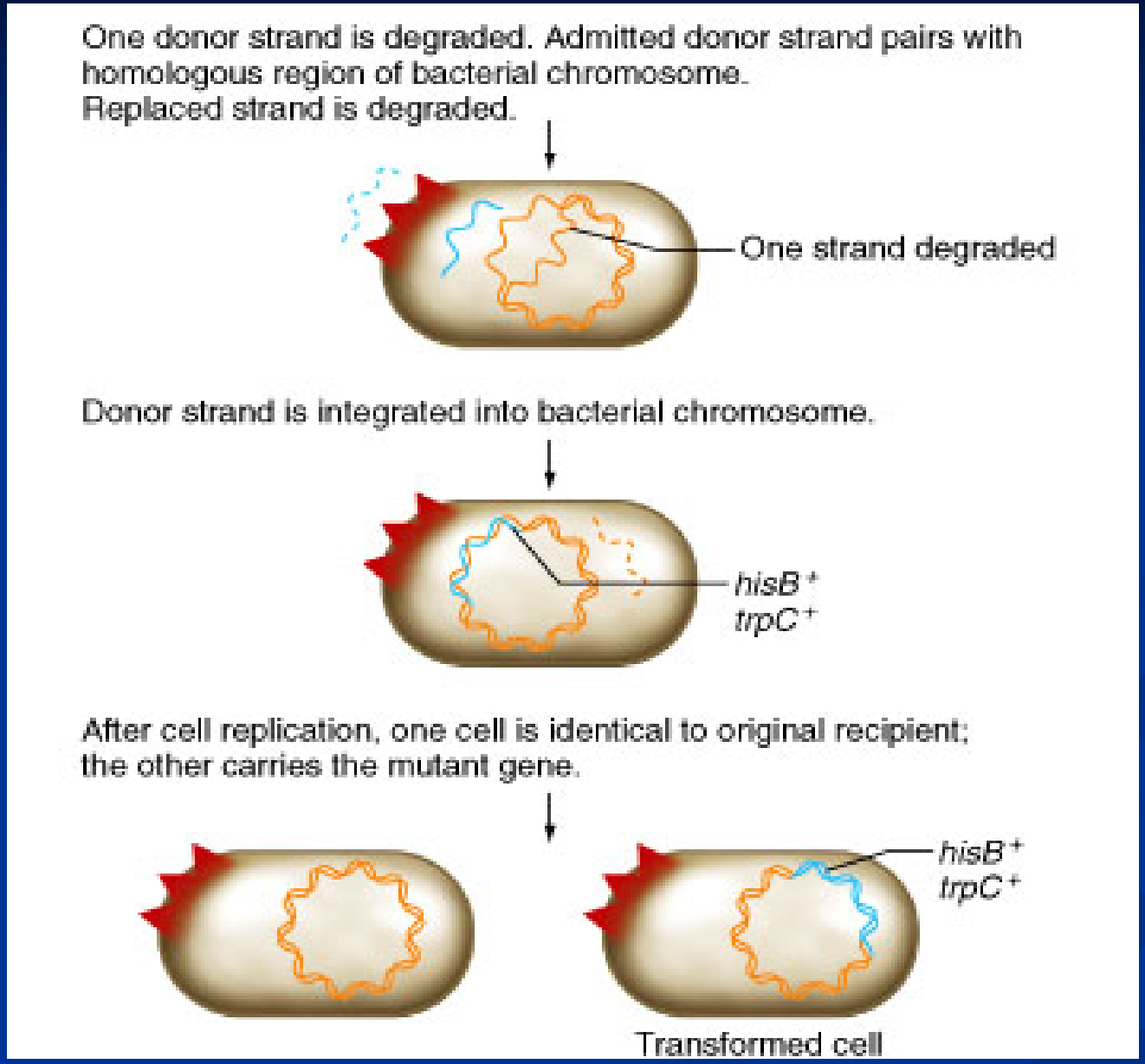

One donor strand is degraded. Admitted donor strand pairs with homologous region of bacterial chromosome.
Replaced strand is degraded.
One strand degraded
Donor strand is integrated into bacterial chromosome.
hisB+
trpC+
After cell replication, one cell is identical to original recipient; the other carries the mutant gene.
hisB+
trpC+
Transformed cell

Fig. 13.13

About 40% of His^+ transformants are also Trp^+.

Cotransformation: The simultaneous transformation of two or more genes.

- **The closer the genes lie, the more frequent they will be cotransformed.**

2. Conjugation

- **Late 1940s, Joshua Lederberg and Edward Tatum.**

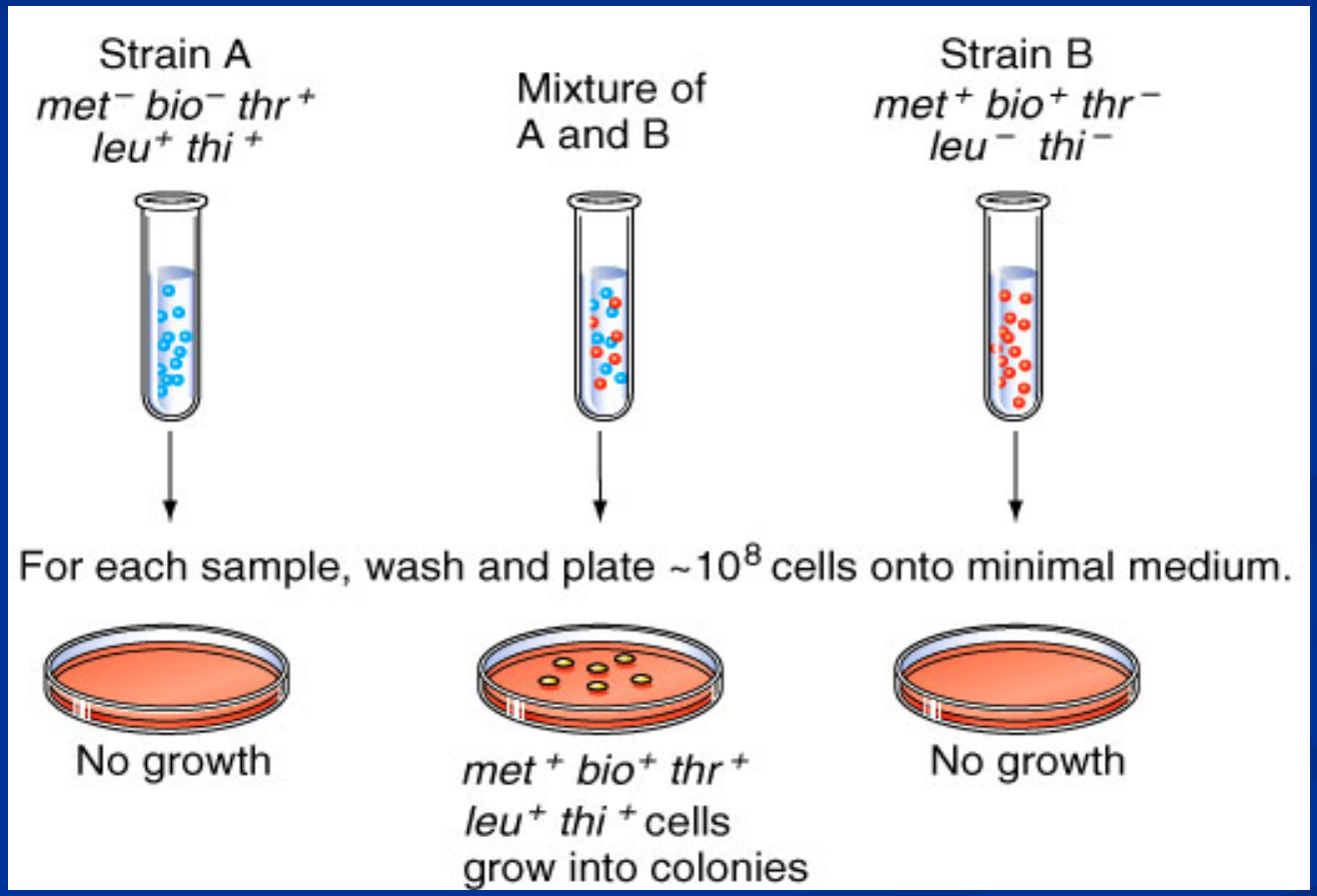
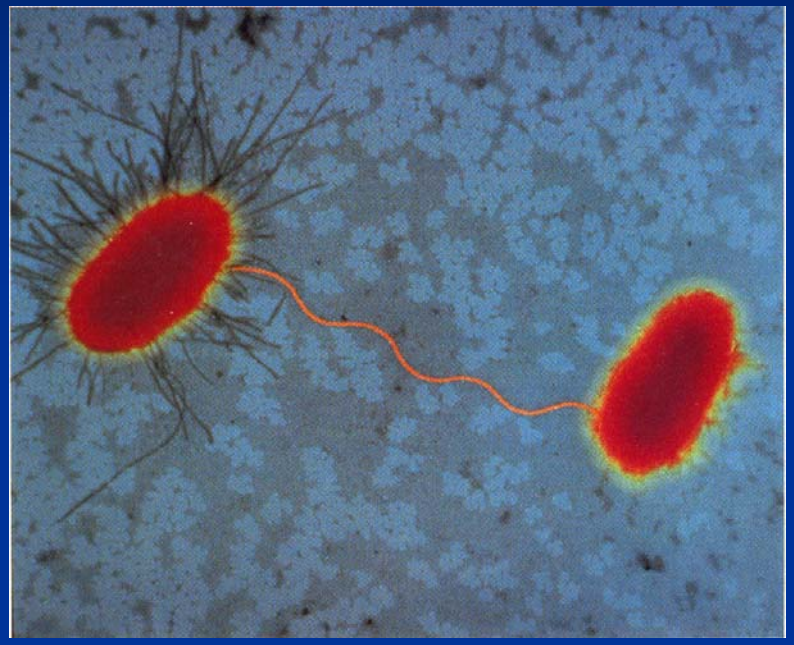
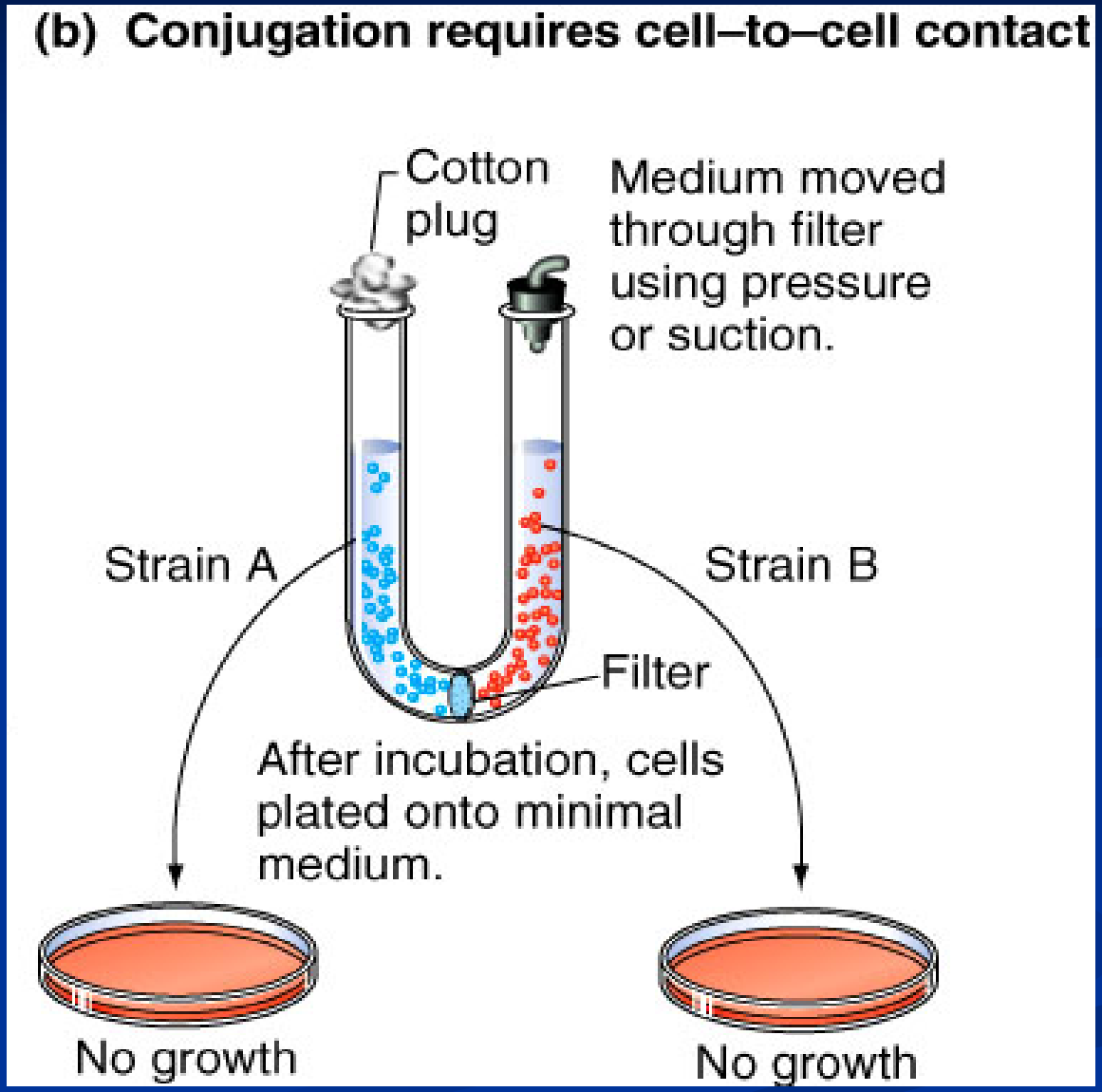


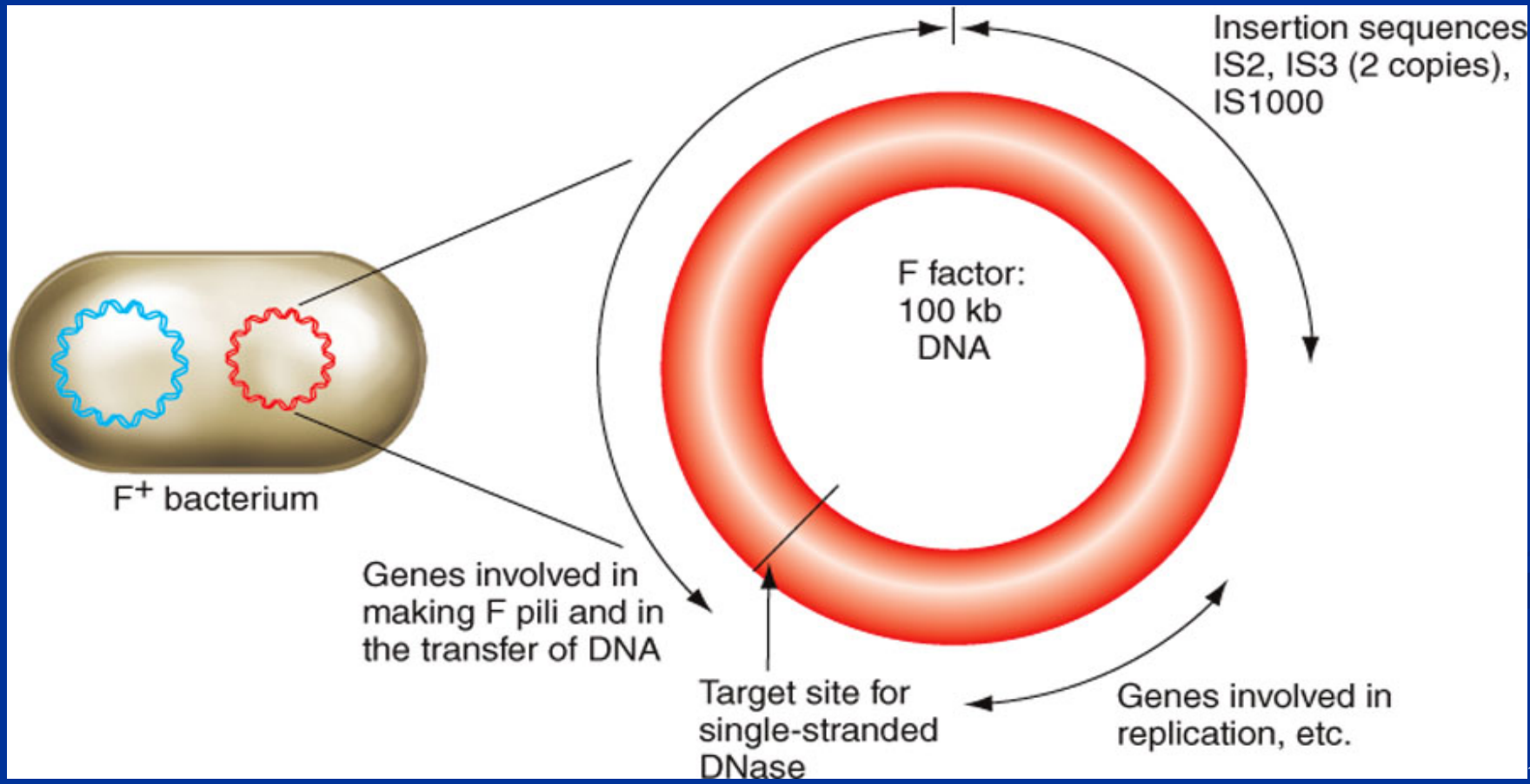
Fig. 13.14



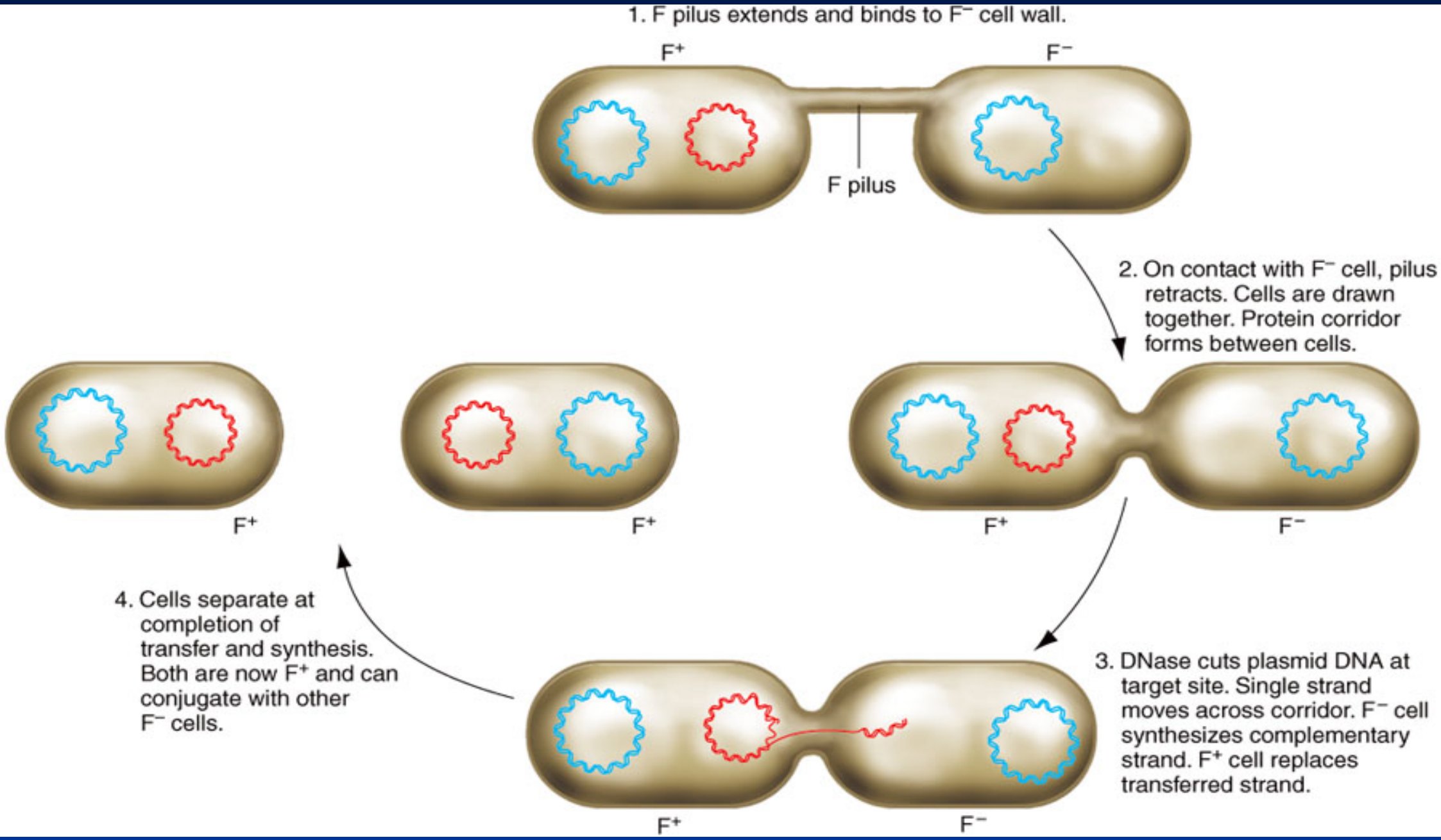

(b) Conjugation requires cell–to–cell contact
Cotton plug
Medium moved through filter using pressure or suction.
Strain A
Strain B
Filter
After incubation, cells plated onto minimal medium.
No growth
No growth

The F plasmid and conjugation

- Conjugation is mediated by **conjugative plasmids** in donor strains.
- **F plasmid**: A plasmid in *E. coli* that could mediate conjugation and transfer genes.
- F^+ (donor) and F^- (recipient, lacks F plasmid) strains.
- **Exconjugant** – recipient cell in conjugation.



The process of conjugation



The F plasmid occasionally integrates into the *E. coli* chromosome

- **Hfr strain**: (high frequency of recombination), An bacterial strain that contain an integrated F plasmid on their chromosomes. They can transfer host genes to a recipient bacterial strain with high efficiency via conjugation.
- **Episomes**: Plasmids that can integrate into host chromosome.

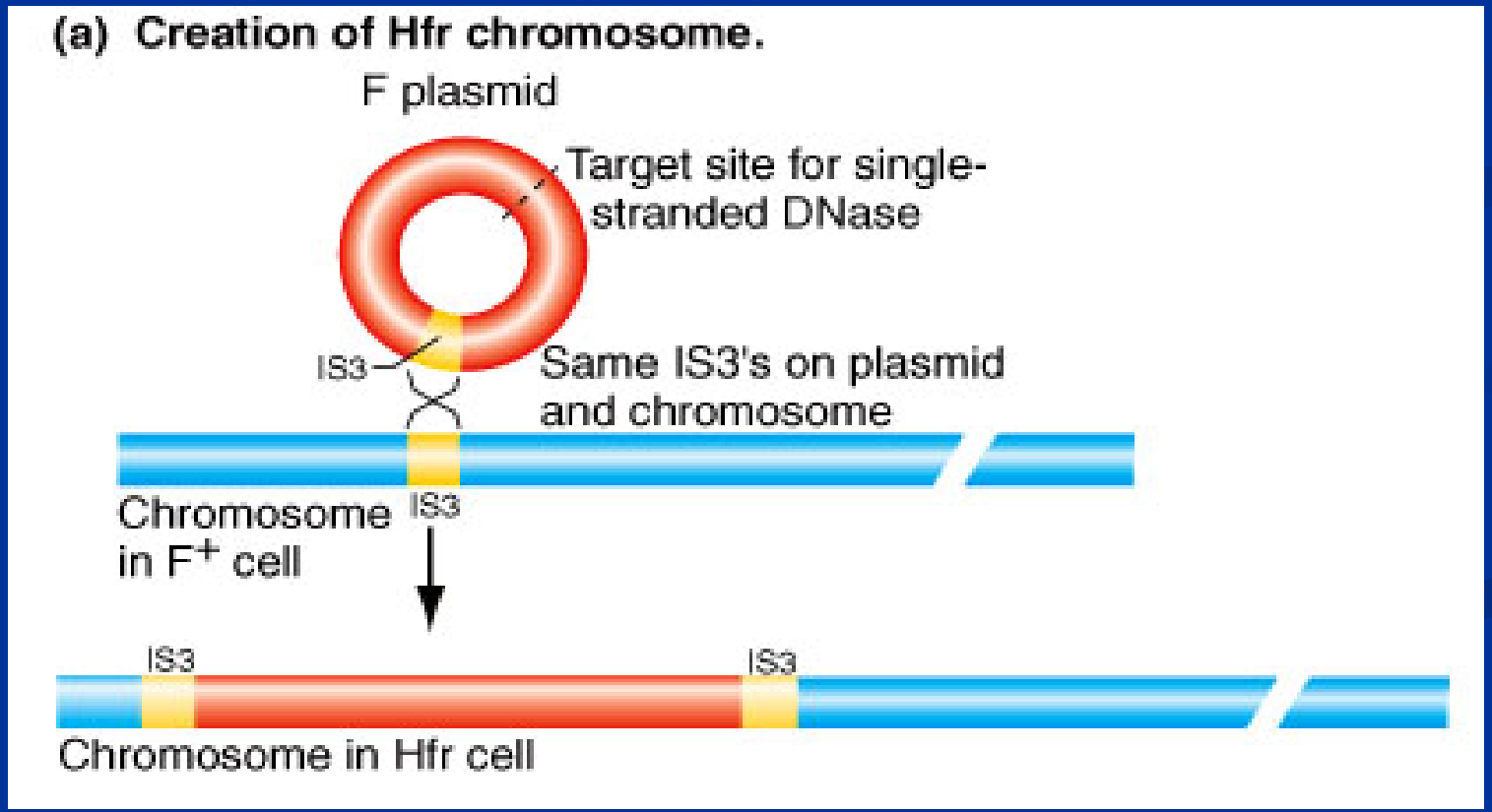


Fig 13.17

- 20-30 different strains of Hfr cells
- Integrated plasmid can initiate DNA transfer by conjugation, but may transfer some of bacterial chromosome as well.

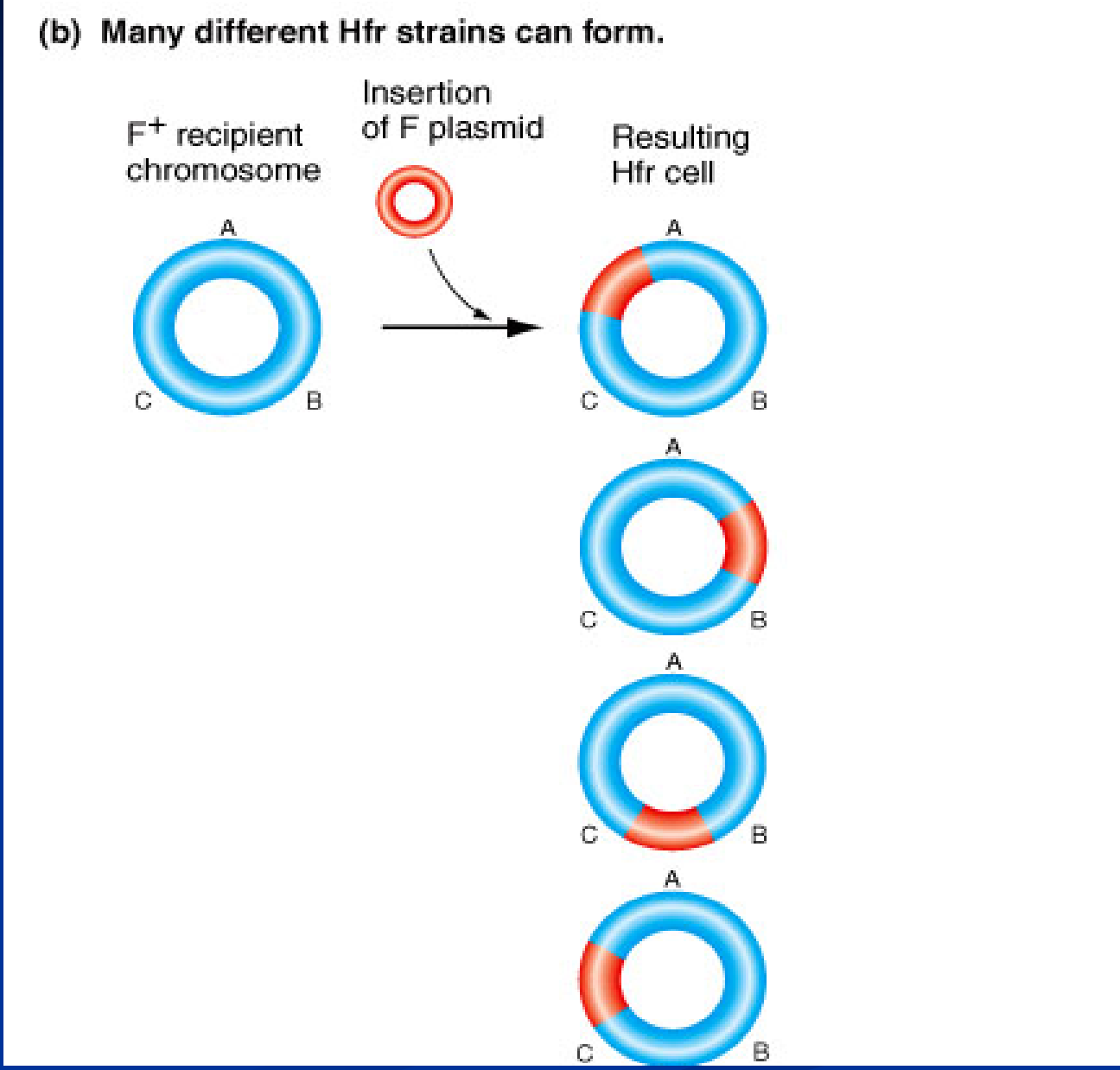


Fig 13.18

Gene transfer in a mating between Hfr donors and F^- recipients

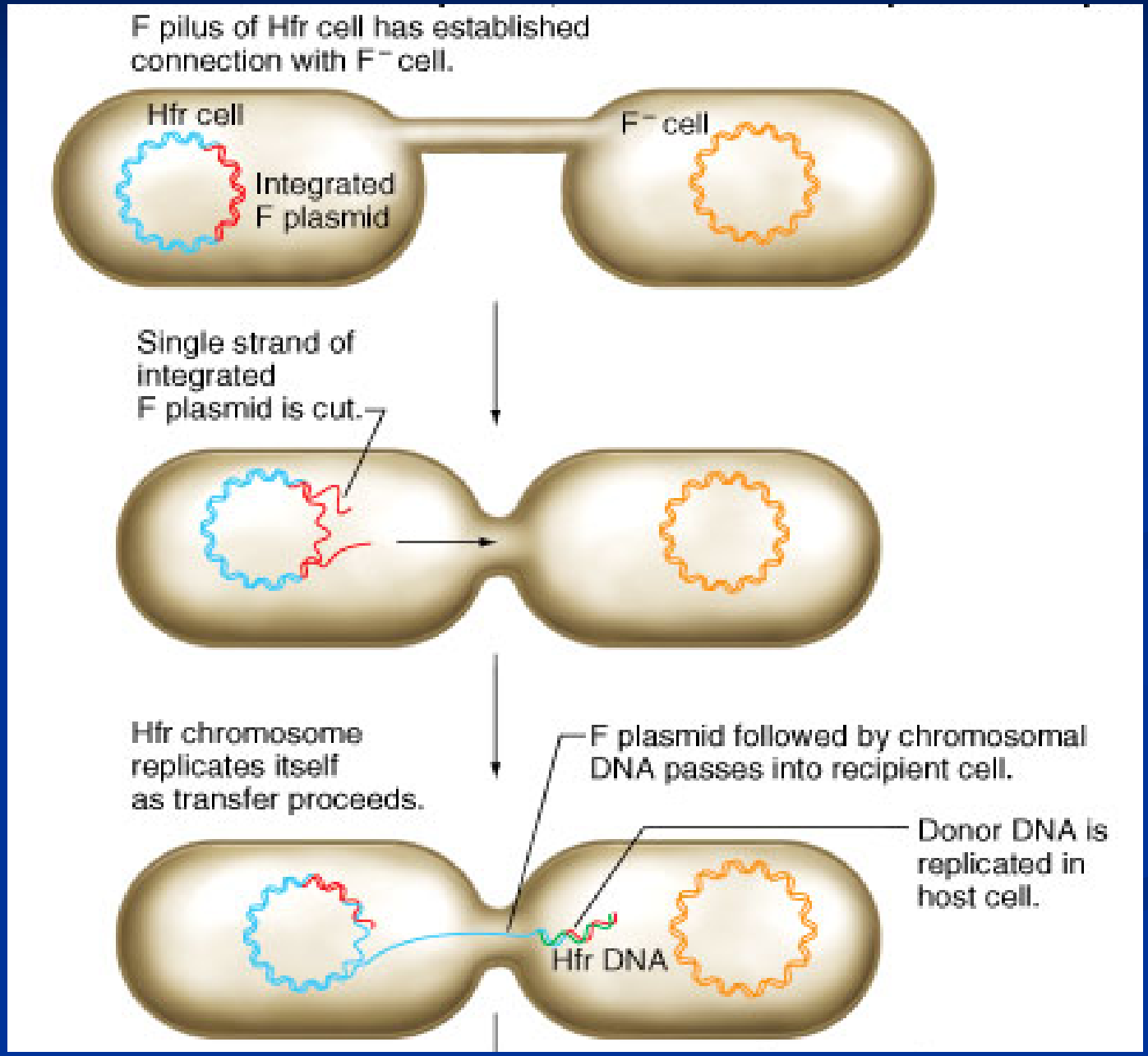
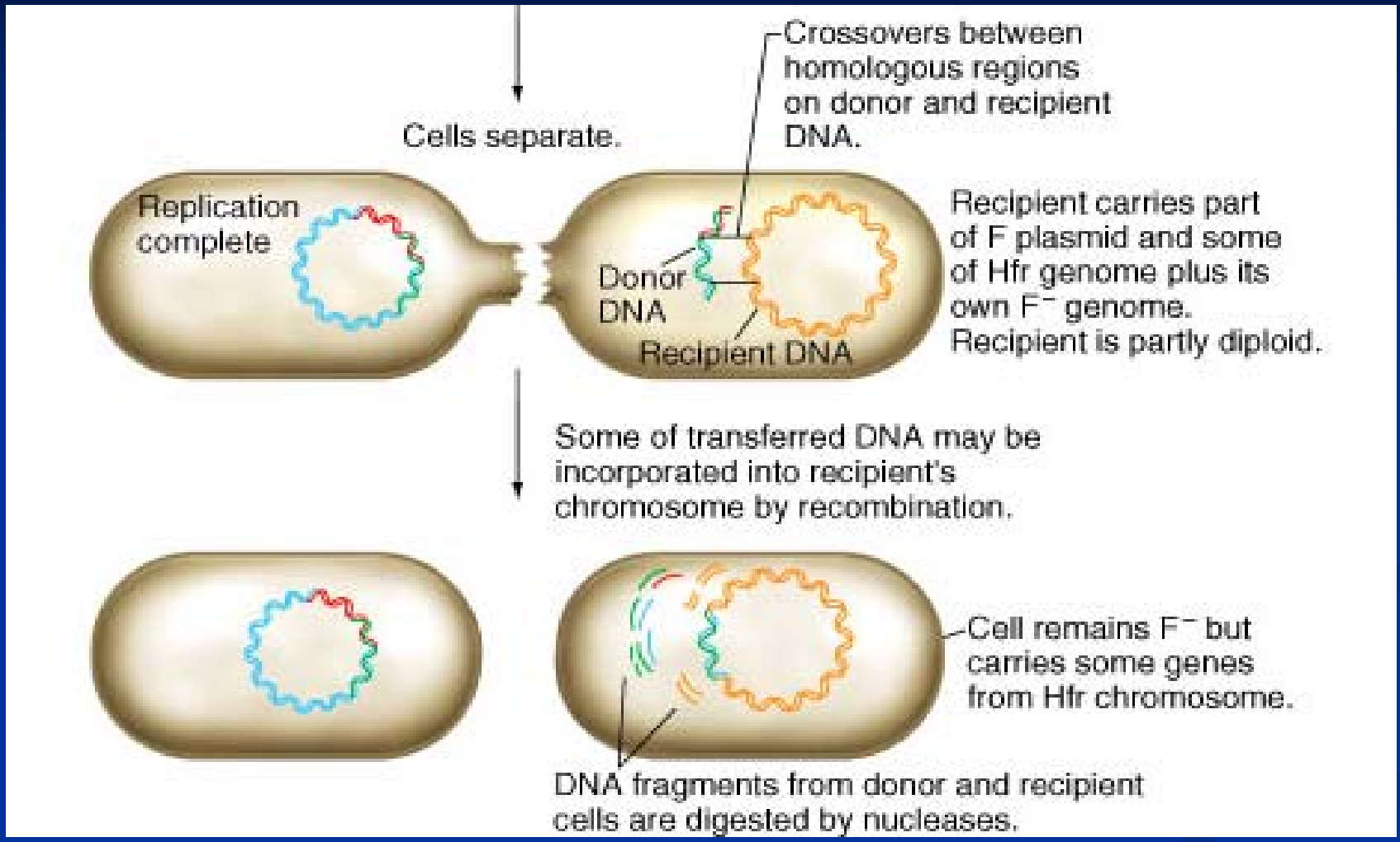


Fig 13.19



Living cells must have even number of crossovers (2, 4,…).

Fig 13.19

Wollman-Jacob interrupted-mating experiment: Mapping genes in Hfr × F⁻ crosses

Elie Wollman
François Jacob

HfrH strain (*str^{s} thr^{+} azi^{r} ton^{r} lac^{+} gal^{+}*)

F⁻ strain (*str^{r} thr^{-} azi^{s} ton^{s} lac^{-} gal^{-}*)

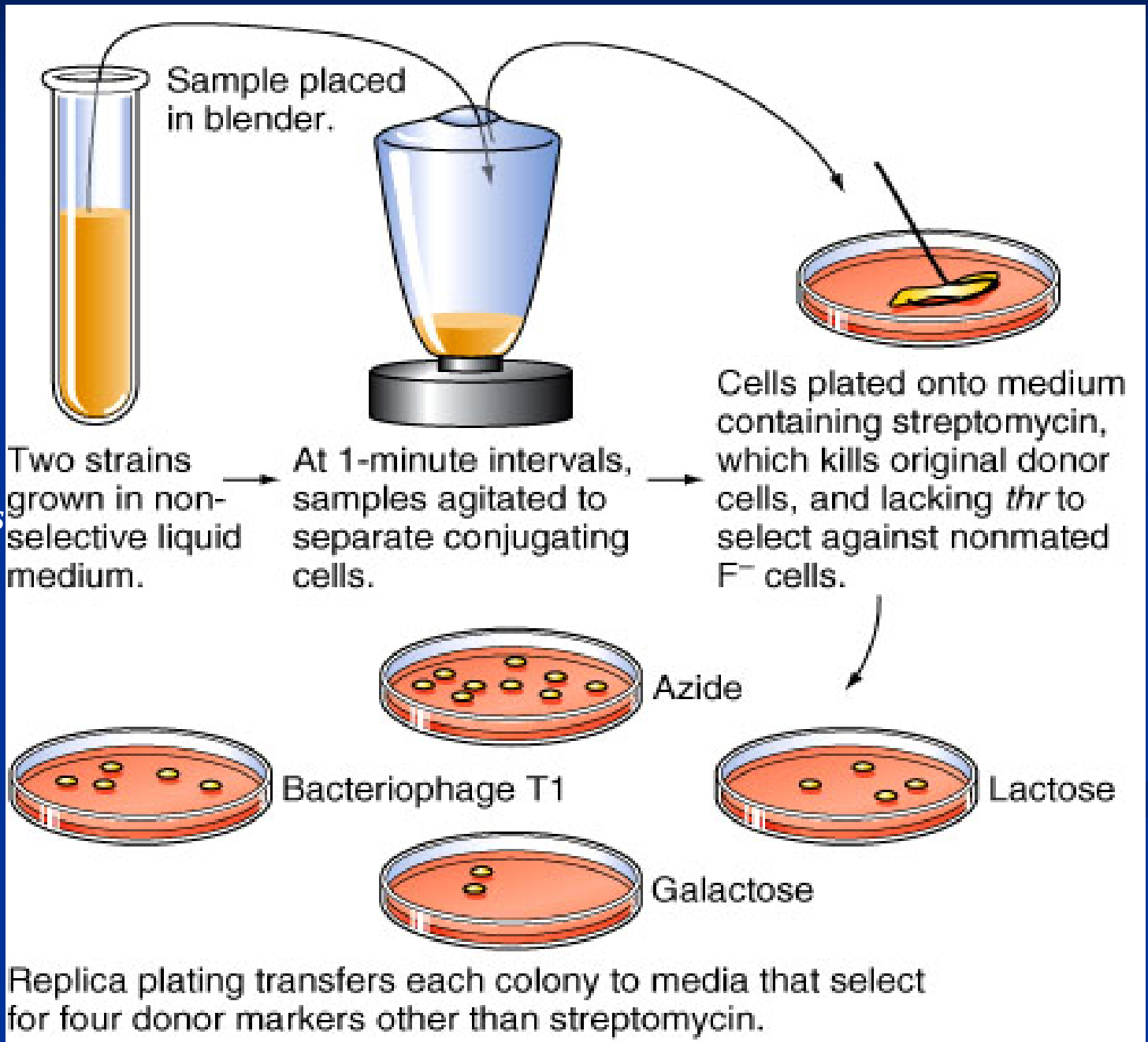


Fig 13.20

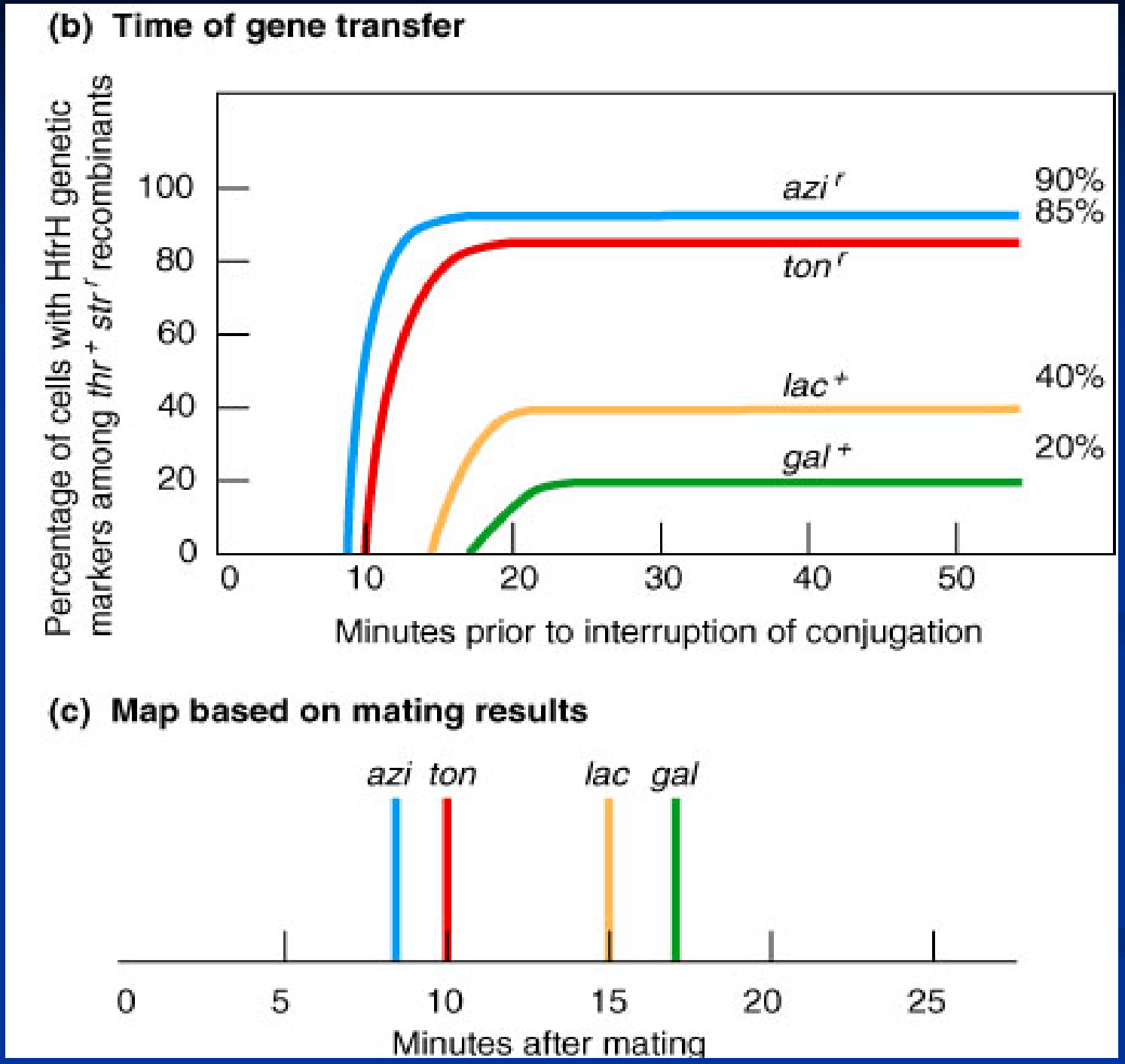

(b) Time of gene transfer
Percentage of cells with HfrH genetic markers among thr+ strr recombinants
100
80
60
40
20
0
azi r
ton r
lac +
gal +
90%
85%
40%
20%
0
10
20
30
40
50
Minutes prior to interruption of conjugation
(c) Map based on mating results
azi
ton
lac
gal
0
5
10
15
20
25
Minutes after mating

Fig 13.20

F′ plasmid formation and transfer

F′ plasmid: An F plasmid that carries a piece of bacterial chromosomal DNA.

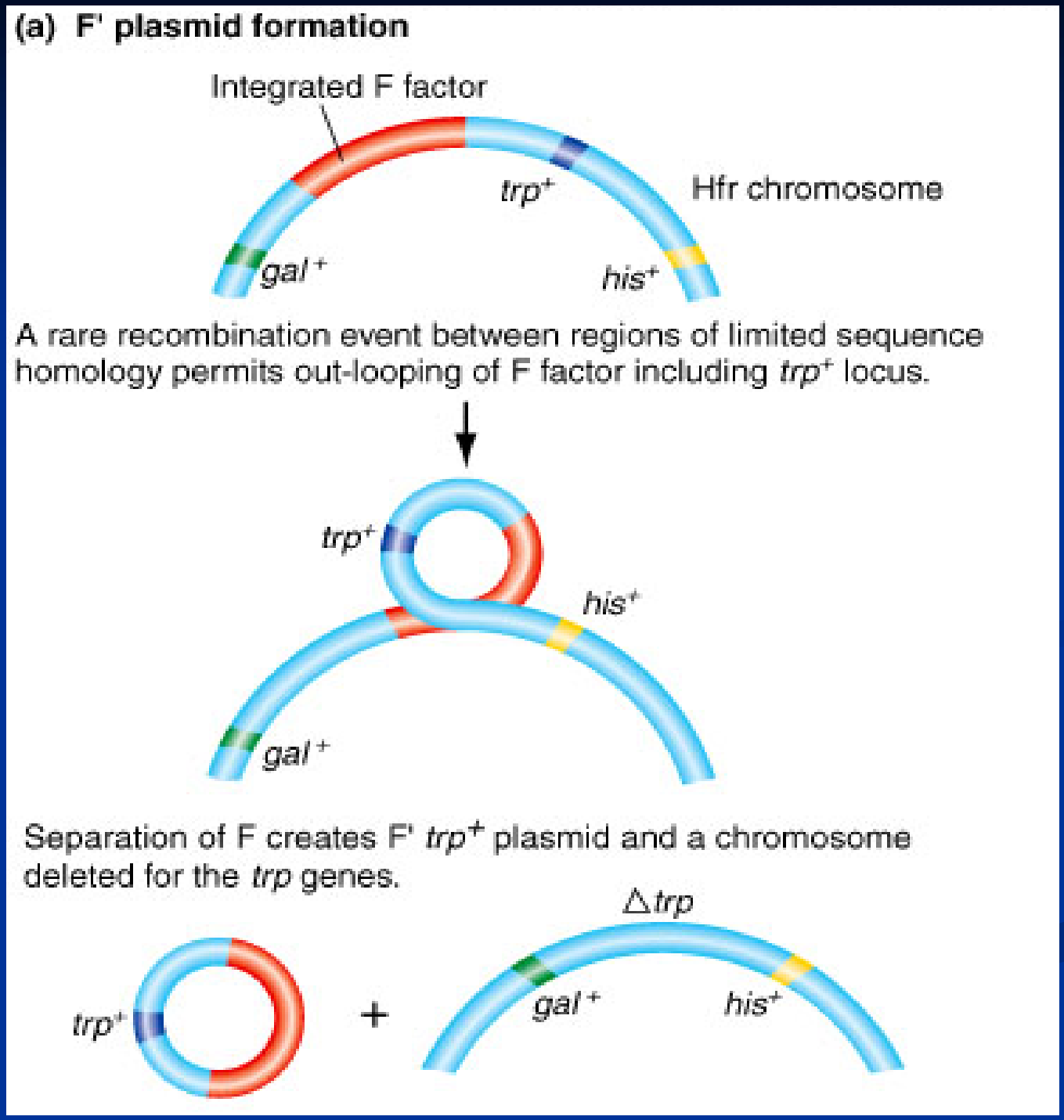


Fig 13.21

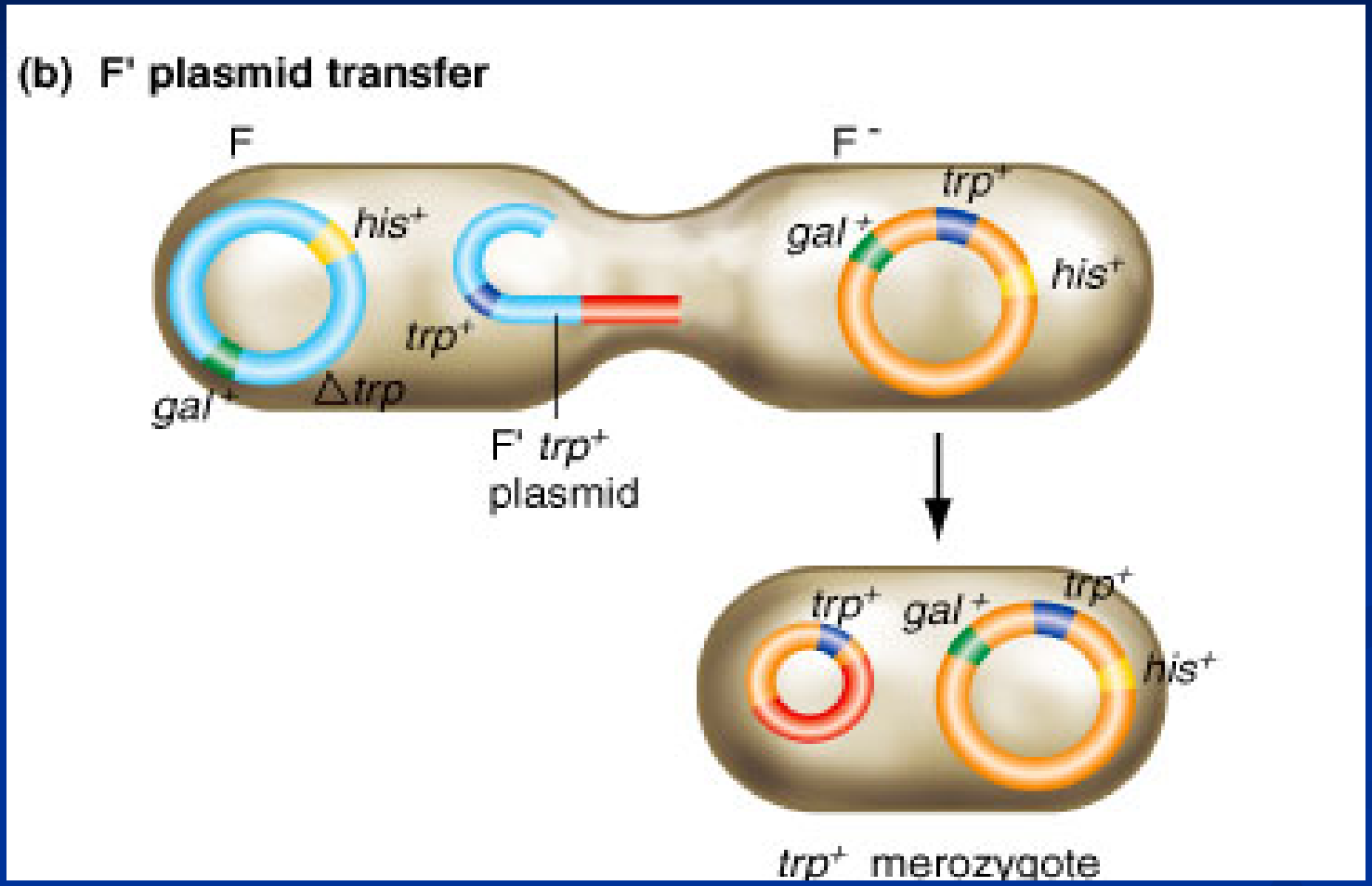

(b) F' plasmid transfer
F
F⁻
his+
trp+
gal+
his+
trp+
gal+
△trp
F' trp+
plasmid
trp+
gal+
trp+
his+
trp+ merozygote

Fig 13.21

3. Transduction

Transduction: Bacteriophages incorporate some of bacterial host chromosome into their own genomes and transfer it to other cells.

- **Bacteriophages**
  - **Widely distributed in nature**
  - **Infect, multiply, and kill bacterial host cells**

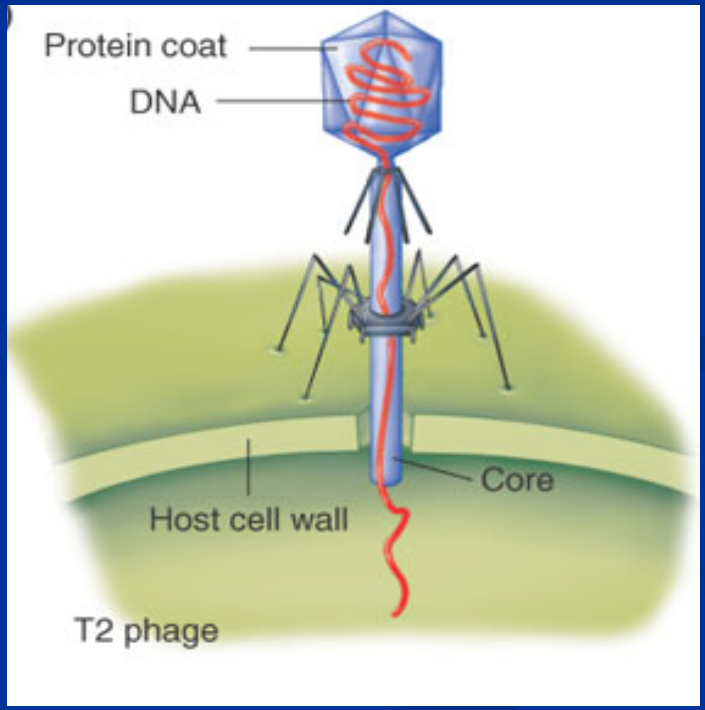


Fig 6.6

- **Bacteriophage particles are produced by the lytic cycle.**

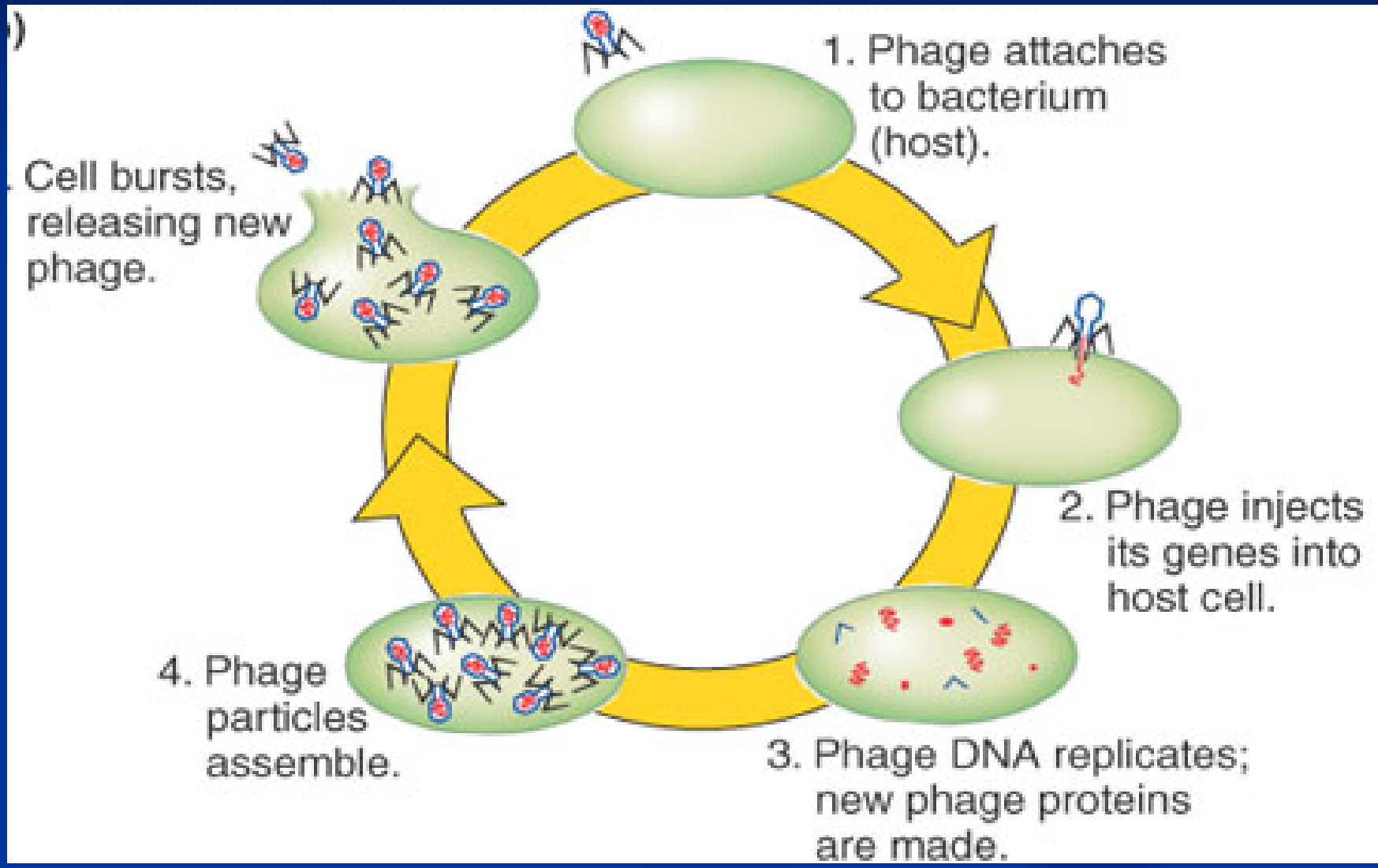


Fig 6.6

Generalized transduction

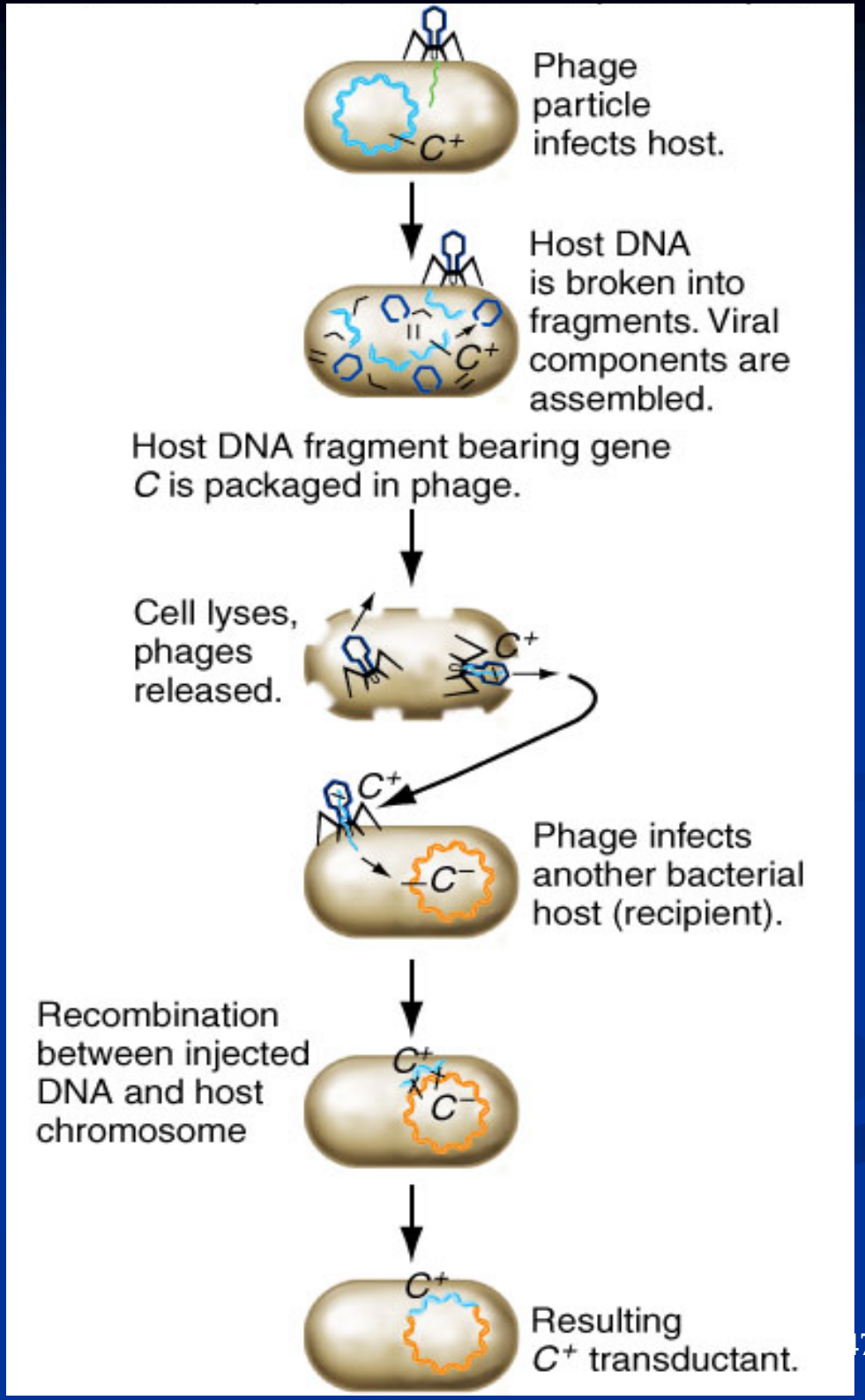


Fig 13.22

Mapping genes by cotransduction

- Mapping genes that are close on the chromosome.
- **Bacteriophage P1** often used for generalized transduction.
- 90 kb can be transduced corresponding to about 2% recombination or 2 minutes.
- First find approximate location of gene by mating mutant strain to different Hfr strains.
- Then use P1 transduction to map a new mutation.

(a) Donor: *thyA*$^+$ *lysA*$^+$ *cysC*$^+$

↓ make P1 lysate; infect recipient

Recipient: *thyA*$^-$ *lysA*$^-$ *cysC*$^-$

Selected marker	Unselected marker
thy$^+$	47% *lys*$^+$; 2% *cys*$^+$
lys$^+$	50% *thy*$^+$; 0% *cys*$^+$

(b)

Fig 13.23

(Specialized transduction) *Temperate phage* can integrate into bacterial genome through lysogenic cycle creating a prophage

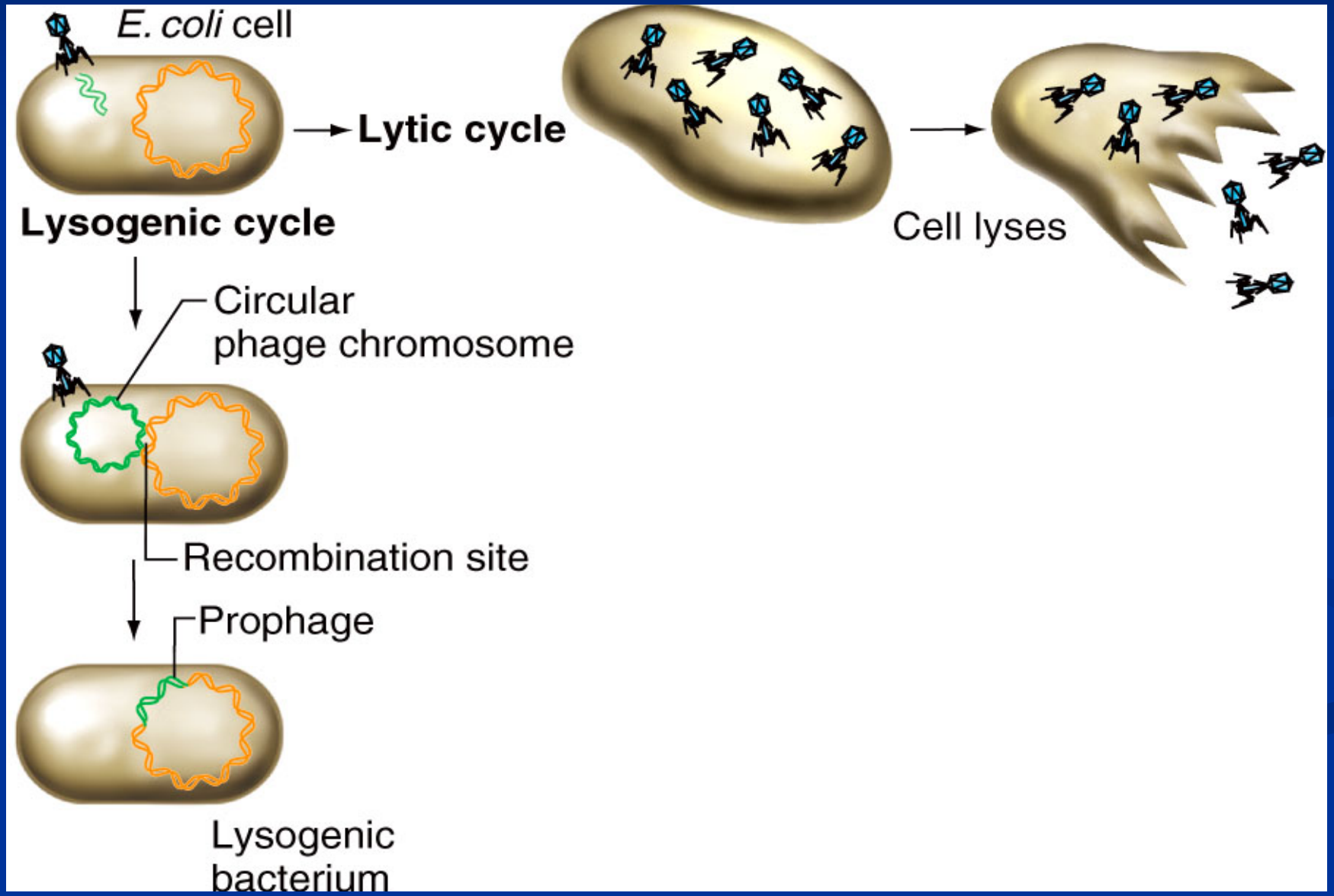


Fig 13.24

Recombination between *att* sites on the phage and chromosomes allows integration of the prophage

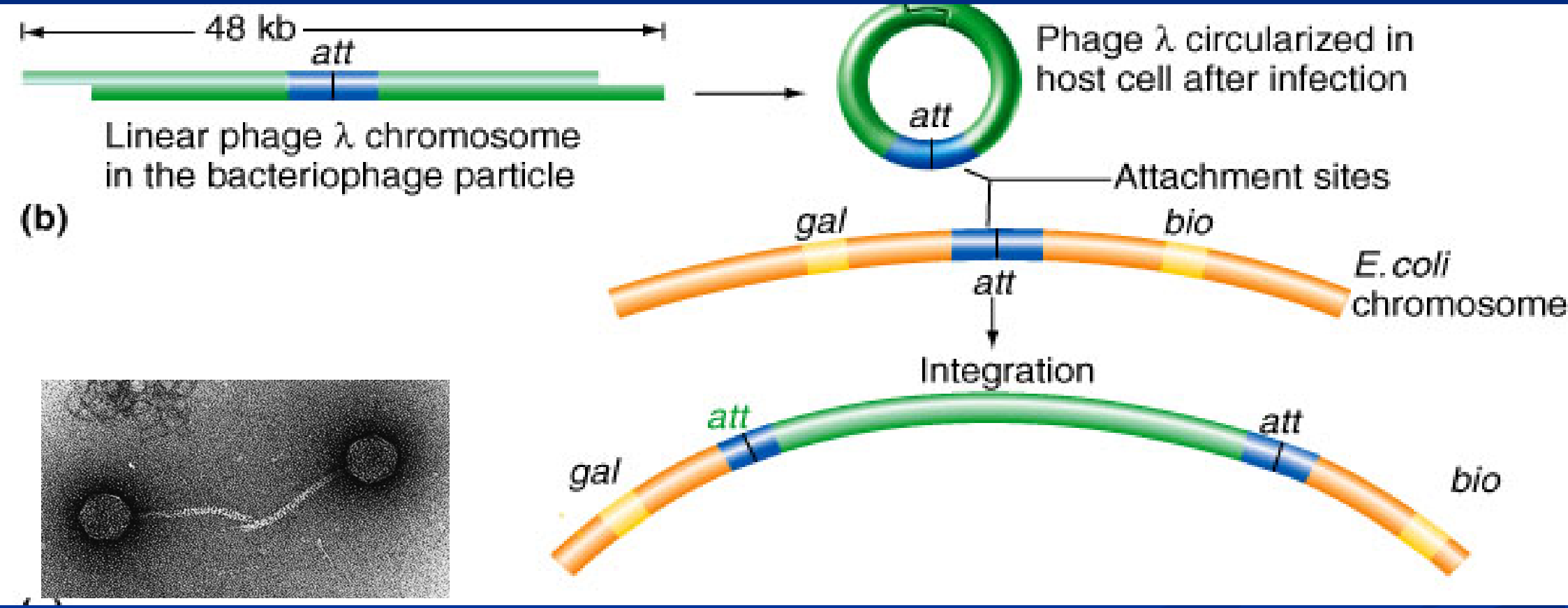


Fig 13.25, 13.26

Errors in prophage excision produce specialized transducing phage

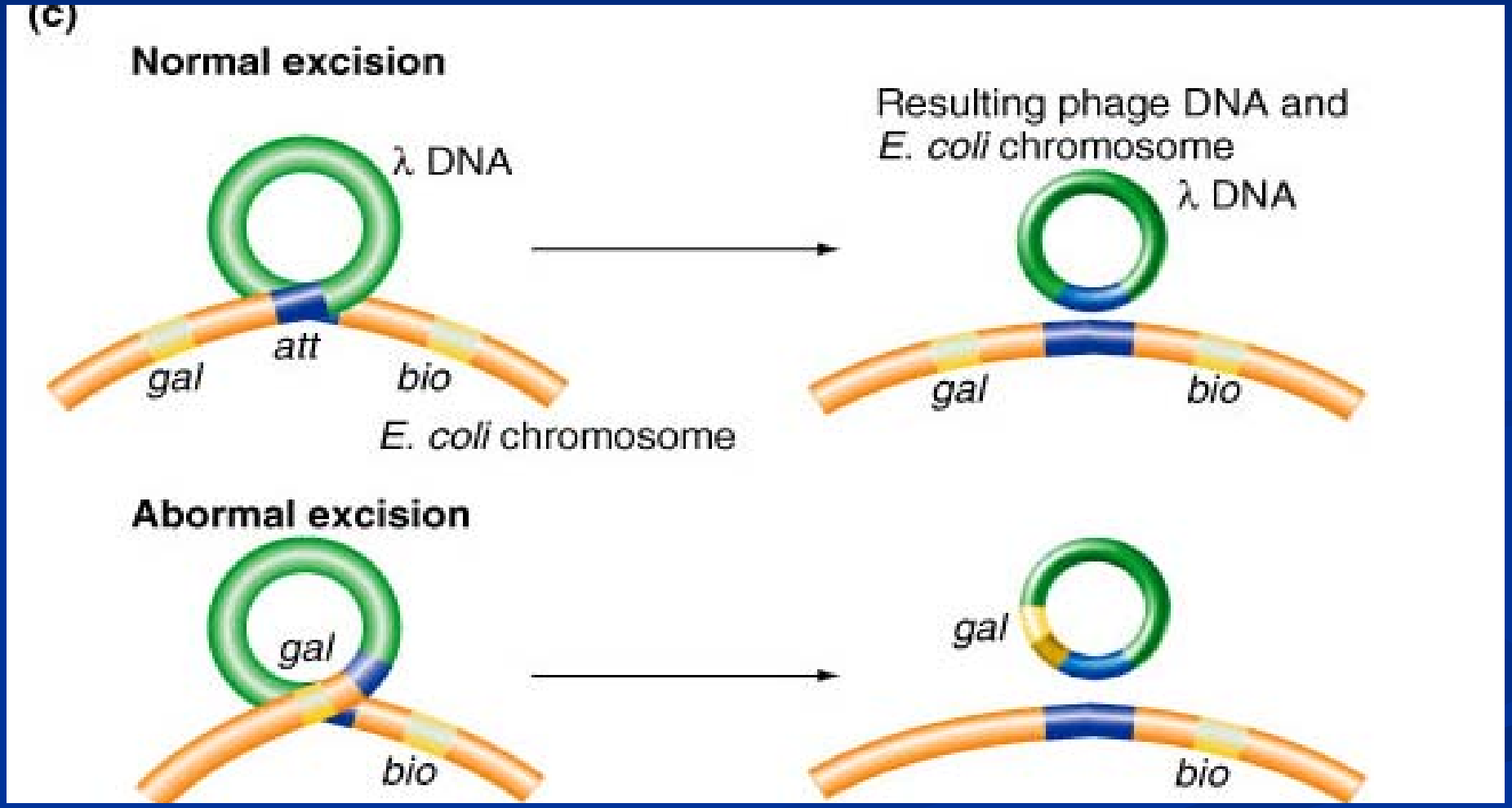


Fig 13.26

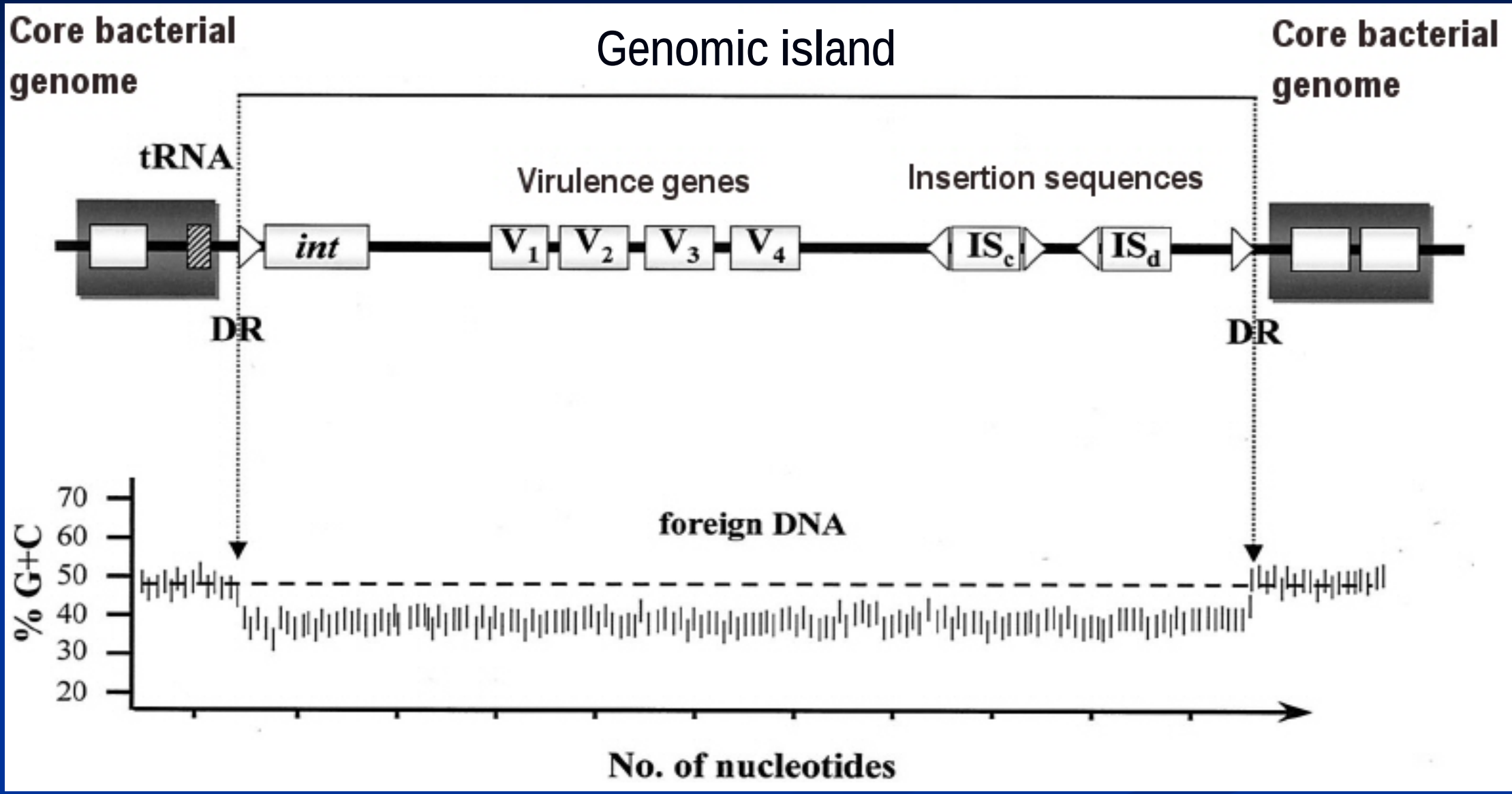
Comparison of generalized and specialized transduction

- Generalized transducing phages can transfer any bacterial genes or sets of genes contained in the right size DNA fragment into the bacterial genome. Specialized transducing phages can transfer just those genes near the site where the phage inserted into the bacterial genome.
- Generalized transducing phages pick up donor bacterial DNA during the lytic cycle. Specialized transducing phages pick up donor bacterial DNA during the transition from the lysogenic to the lytic cycle.

Lateral gene transfer has significant evolutionary implications

- Important for the rapid adaptation of bacteria to a changing environment and the development of pathogenic strains.
  - Bacterial genomes could pick up DNA from different sources.
- Genomic islands (基因组岛): Large segments of DNA (10-200 kb) show properties that they originated from transfer of foreign DNA into a bacterial cell.
  - Different G+C content.
  - Each end contains direct DNA repeats.
  - Found at the sites where tRNA genes are located.
  - Encodes integration enzymes related to known bacteriophage integration enzymes and sites for these enzymes.

Genomic island

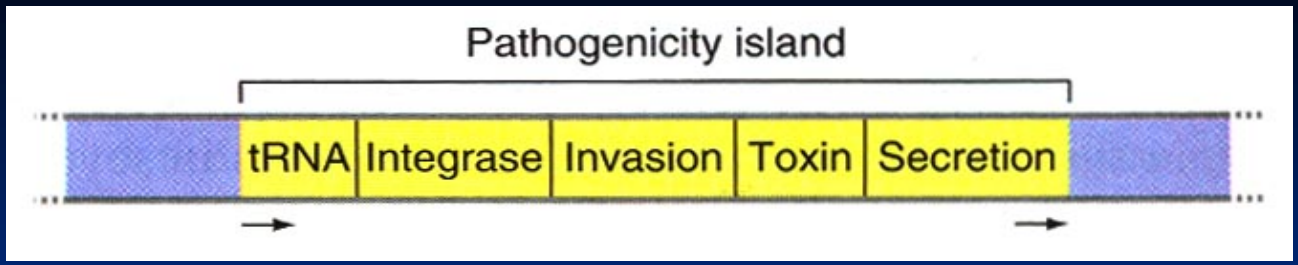


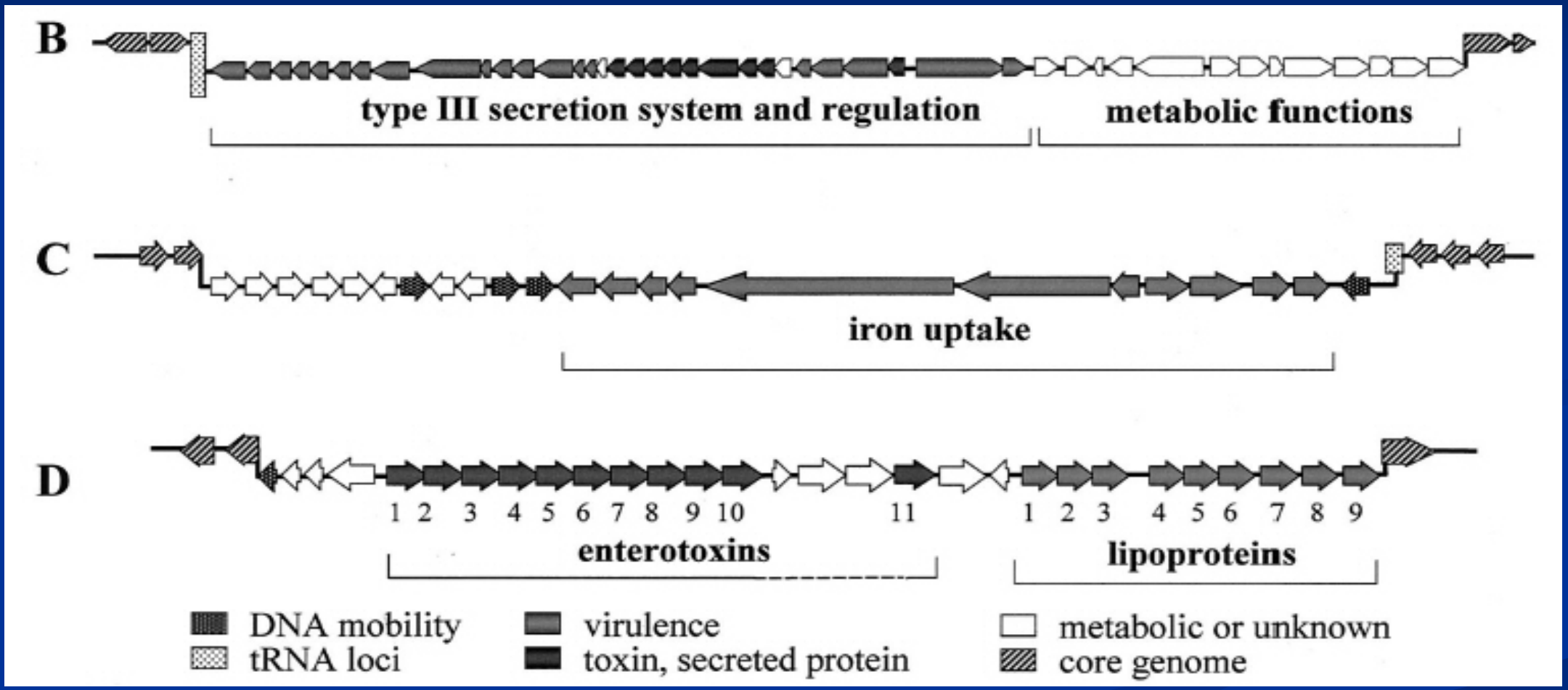
- **Genomic islands carry genes with new functions.**
  - **Genes encoding new metabolic enzymes, antibiotic resistance, toxins, or enzymes to degrade poisonous substances in the environment.**

Most pathogens contain pathogenicity islands

- **Pathogenicity islands (毒力岛)**: Segments of DNA in disease-causing bacteria that encode several genes involved in pathogenesis. They appear to be transferred into the bacteria from a different species.
  - A subtype of genomic islands that encode pathogenicity determinants. Contain genes including toxins, adhesion molecules (to host cells), or secretion systems.

Fig. 14.27





B. The SP-1 island of *Salmonella typhimurium* (typhus and food poisoning)
C. The HPI island of *Yersinia enterocolitica*
D. The vSAL island of multiple drug-resistant *Staphylococcus aureus* (MRSA)

- **Lateral transfer of a “package” of genes can transform a nonpathogenic bacteria into a pathogenic bacteria.**
  - ***Vibrio cholerae***
  - ***E. coli* O157:H7**

13.5 Bacterial genetic analysis

- **Bacteria multiply rapidly.**
  - **On agar plate – A single bacterium can multiply to 10^7 – 10^8 cells in less than a day.**
  - **In liquid media – *E. coli* grows to concentration of 10^9 cells/ml within a day.**
- **The power of bacterial genetics is the potential for studying rare events.**

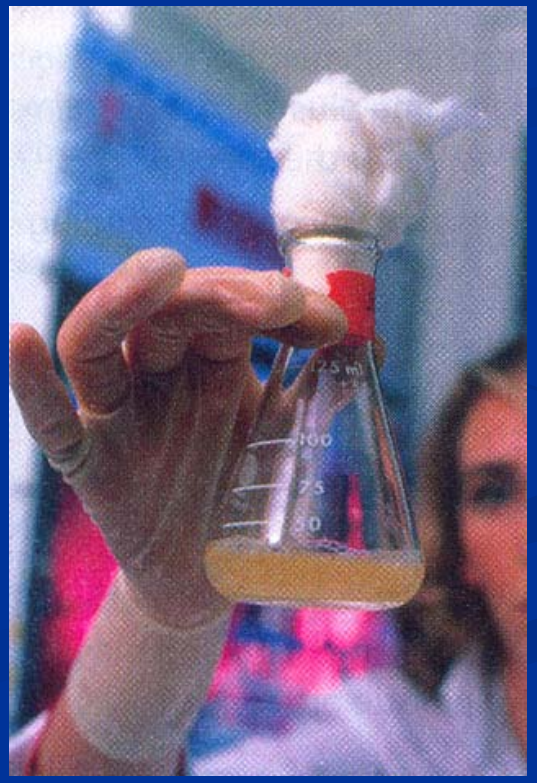


Fig 13.11

Finding mutations in bacterial genes

- Mutations affecting **colony morphology**.
- Mutations conferring **resistance** to antibiotics or bacteriophages.
- Mutations that create **auxotrophs**.
  - **Auxotrophs**: Mutants that are unable to grow on minimal medium unless supplemented with a growth factor.
- Mutations affecting the **ability of cells to break down and use complicated chemicals** in the environment.
- Mutations in **essential genes** whose protein products are required under all conditions of growth.

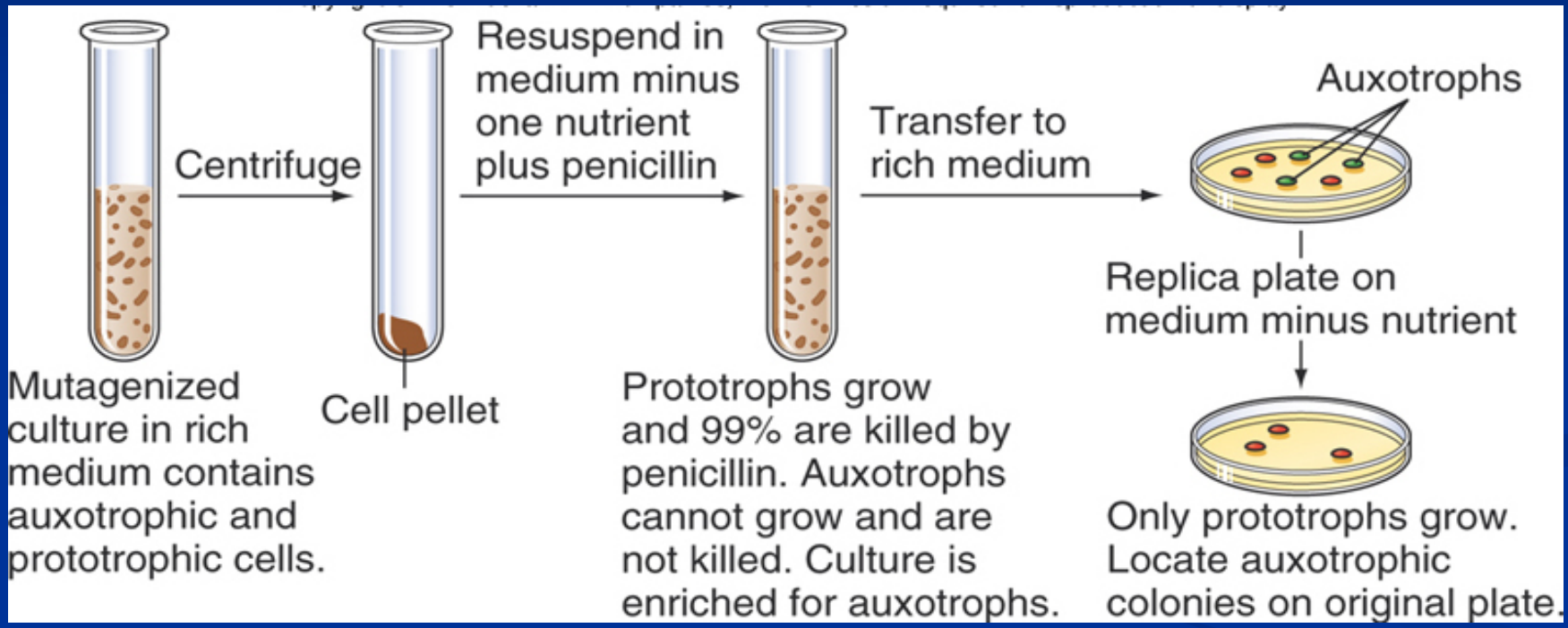
Nomenclature in bacterial genetics

- **Phenotype is written with a capital letter and no italics.**
  - **Leu$^-$: requires leucine for growth.**
  - **Lac$^+$: grows on lactose.**
  - **Str^r : is resistant to streptomycin.**
- **Genes: written with three lower case, italicized letters.**
  - **4 *leu* genes: *leuA*, *leuB*, *leuC*, and *leuD*.**
- **Alleles:**
  - **Wild-type '+'. e.g., *leuA*$^+$ is wild-type leucine gene**
  - **Mutant gene '–'. *leuA*$^-$ is a mutant.**
  - ***str*^{s} (sensitive to streptomycin) and *str*^{r} (resistant).**

Genetic screens to identify mutants

- **Genetic screen**: An examination of each individual in a population for its phenotype.
- Genetic screens provide a way to observe mutations that occur very rarely such as spontaneous mutations (1 in 10^6 to 1 in 10^8 cells).
- Techniques to simplify screens:
  - **Treatments with mutagens** – increase frequency of mutations
  - **Enrichment procedures** – increase the proportion of mutant cells by killing wild-type cells
  - **Testing for visible mutant phenotypes** on a petri plate
  - **Replica plating** – simultaneous transfer of thousands of colonies from one plate to another

Penicillin enrichment for auxotrophic mutants



Recombinant plasmid libraries simplify gene identification

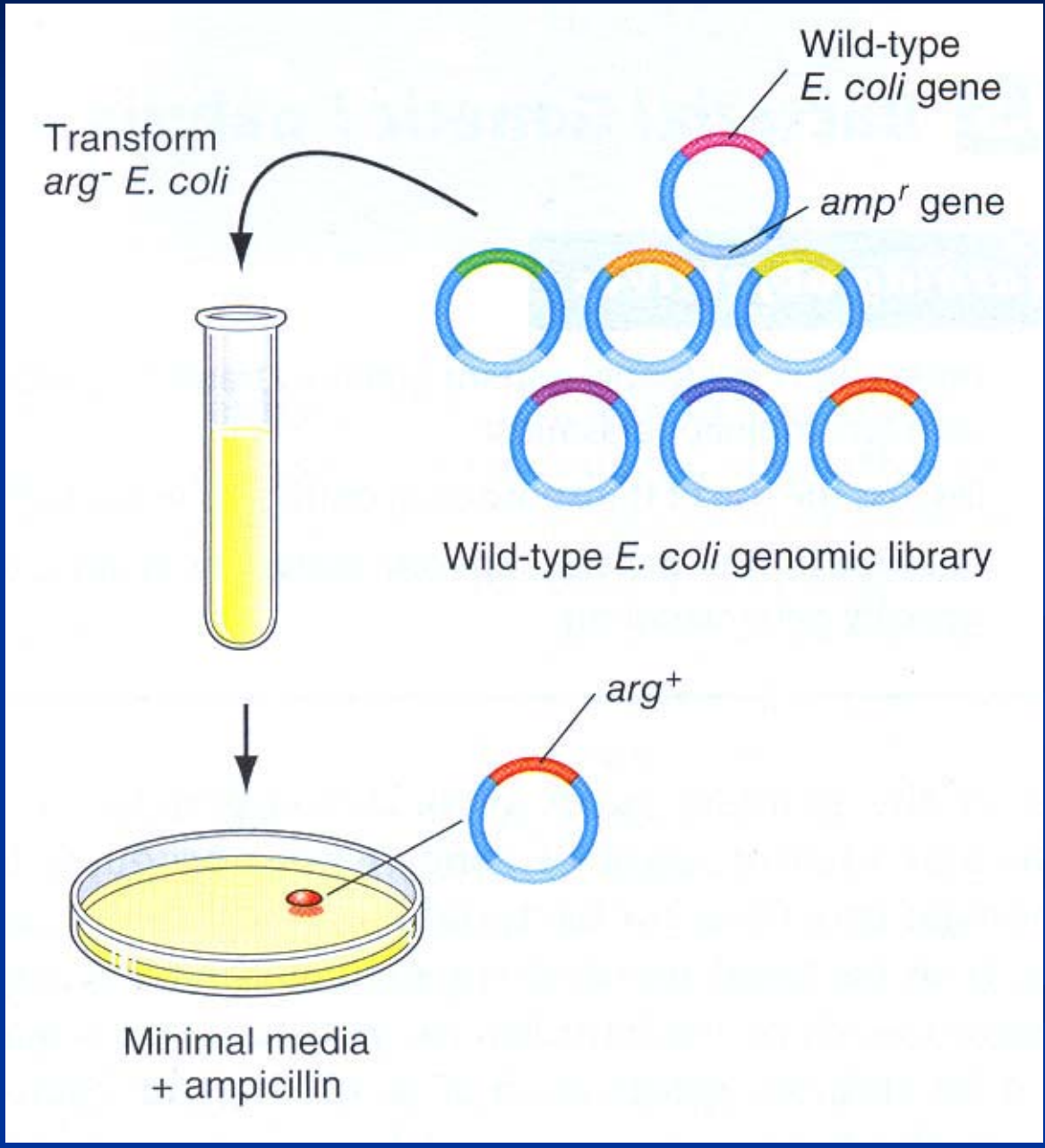
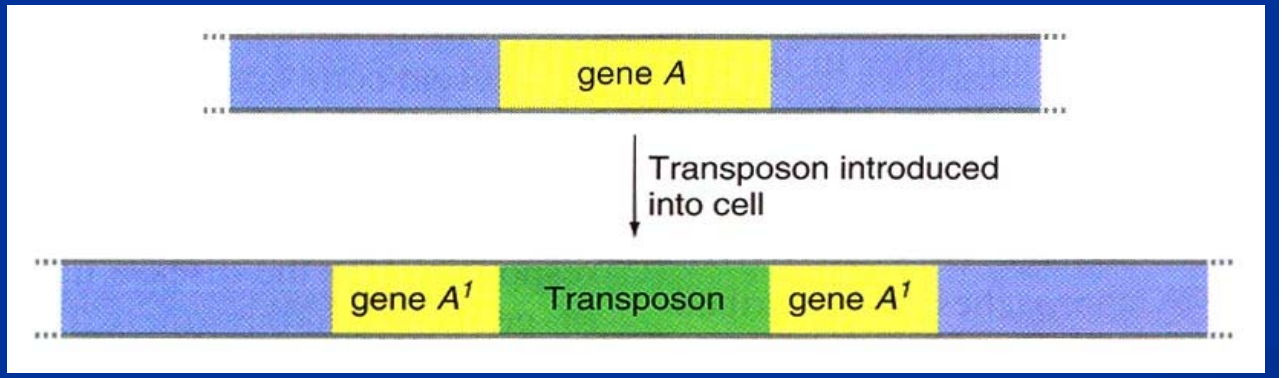


Fig. 13.28

Transposons are useful tools in bacterial genetic analysis

- Transposons can be used as gene-tagging mutagens.
- Transposon mutagenesis
  - Introduce transposon into cell.
  - Select for cells in which transposition has occurred.
  - Screen population of cells for mutant phenotype.



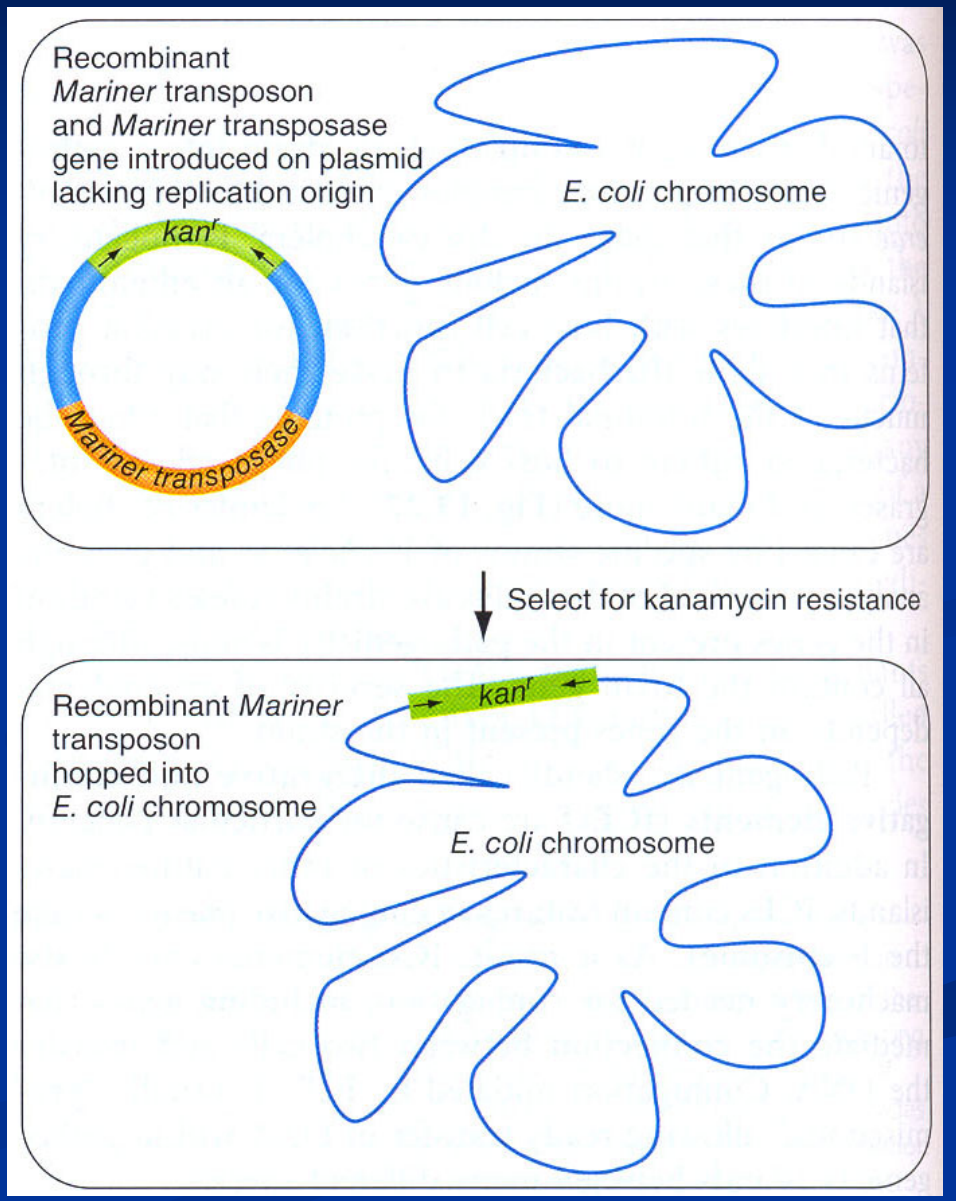
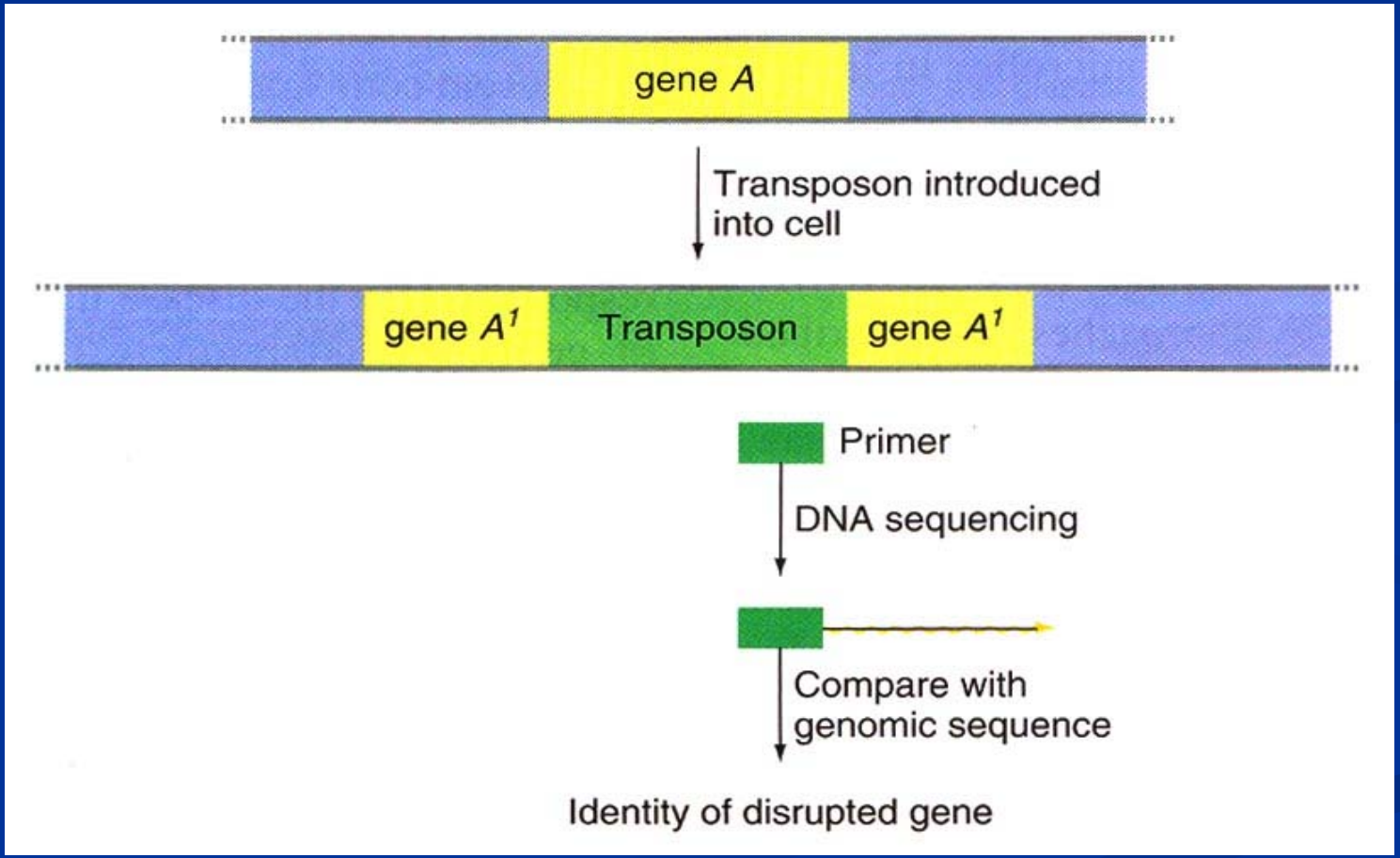


Fig. 13.29

- **Locate disrupted gene on the chromosome.**
  - **PCR amplification using primers in transposon.**
  - **Sequence PCR product and compare with *E. coli* genome database.**



Reverse genetics to determine function of unknown gene

- **Gene knockout** using **recombinant DNA technology** and **homologous recombination** in cell.

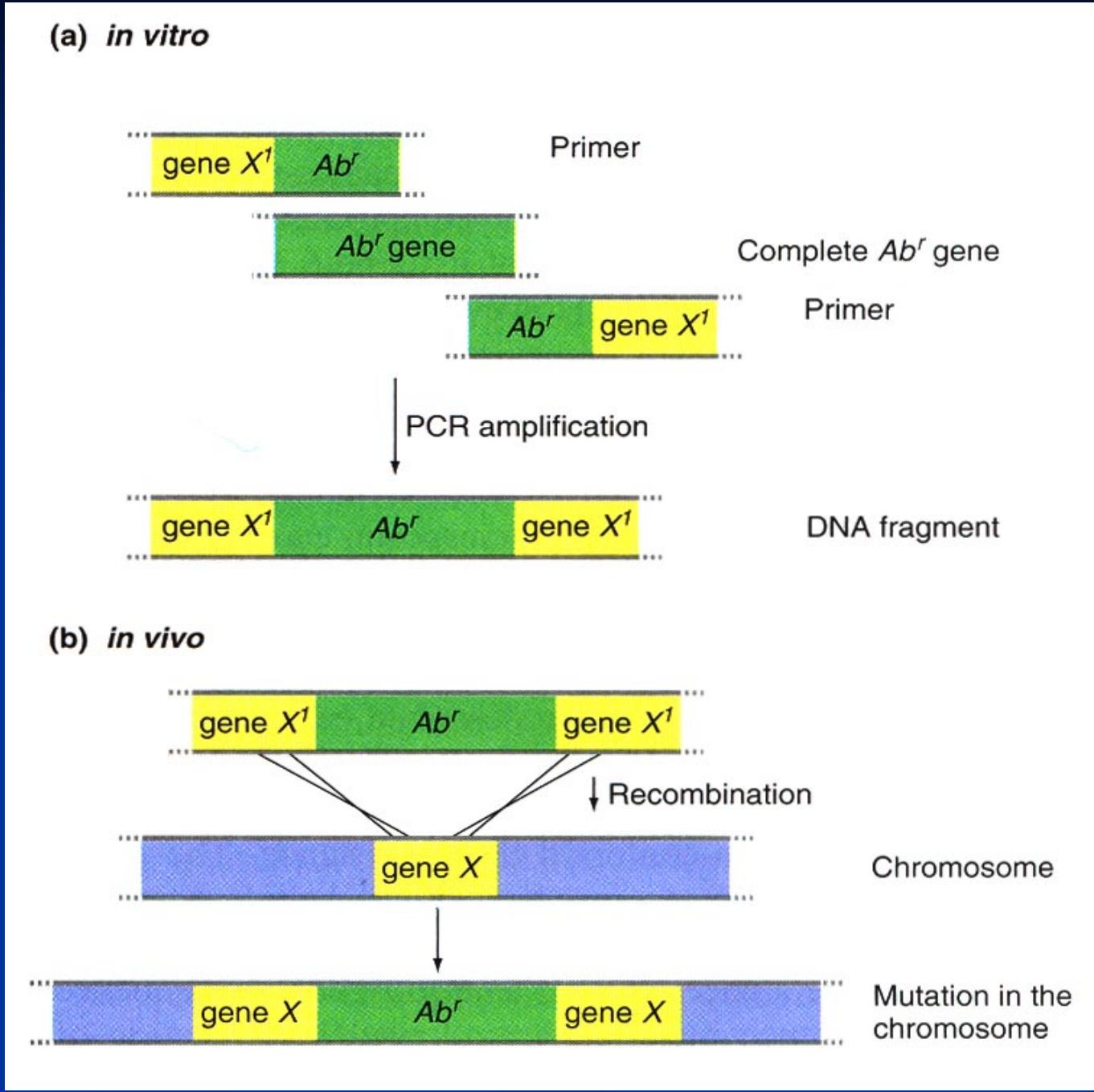
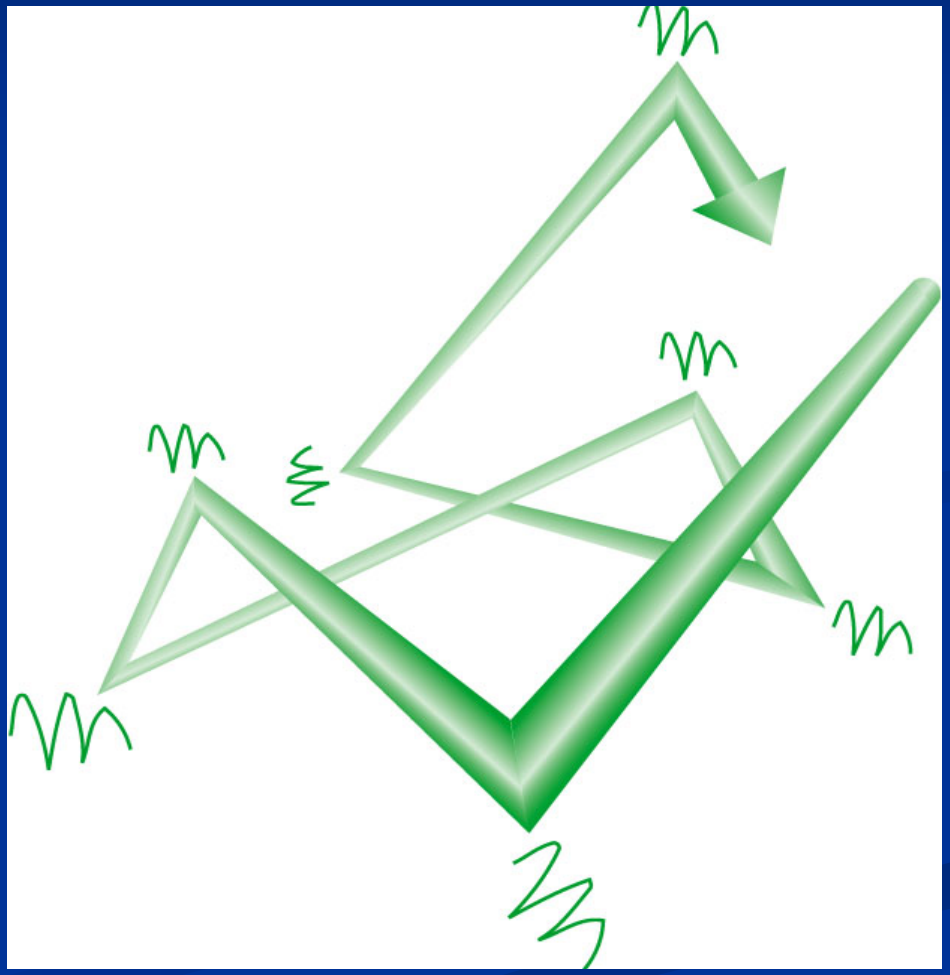


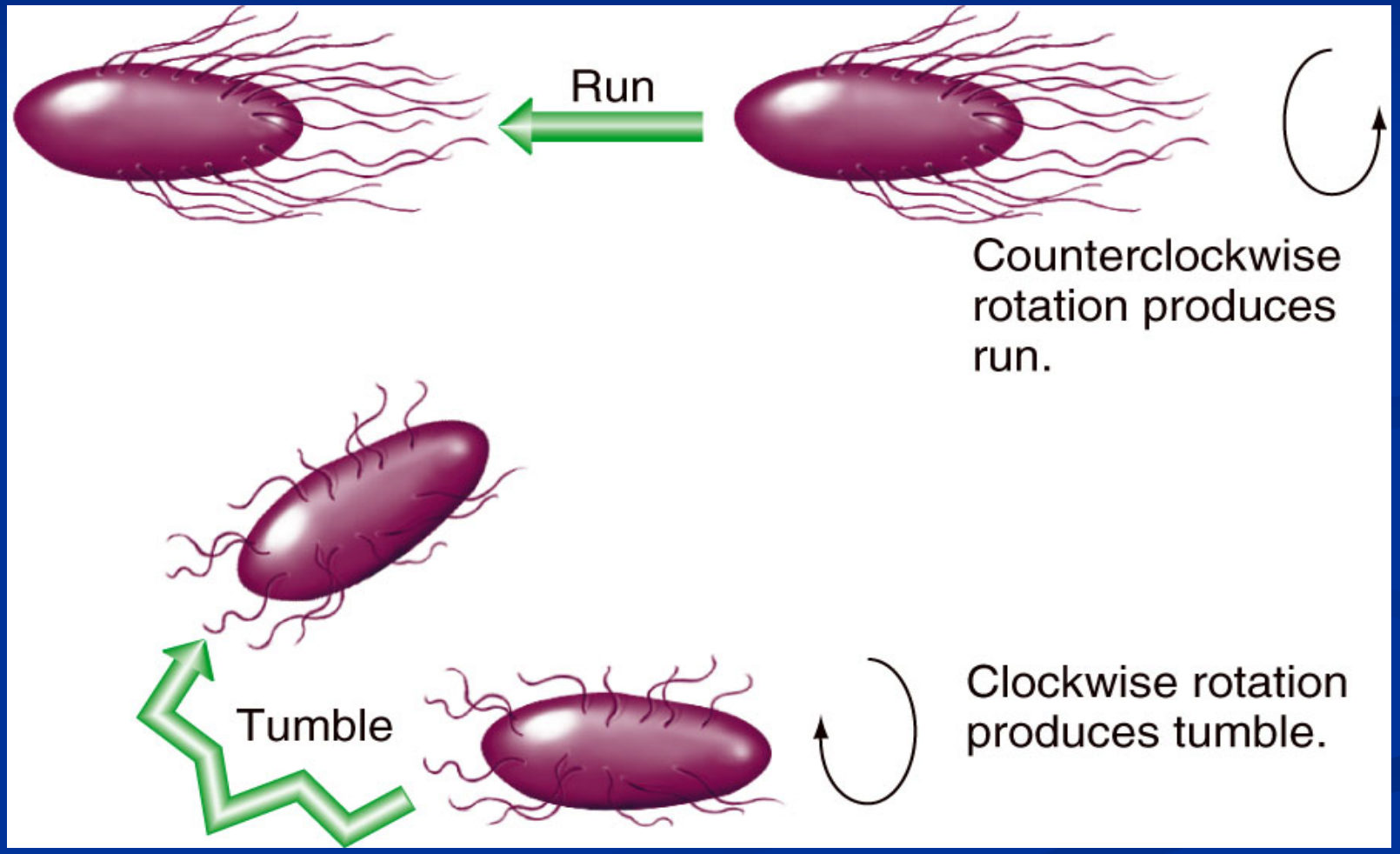
Fig 13.31

An example: Genetic dissection helps explain how bacteria move

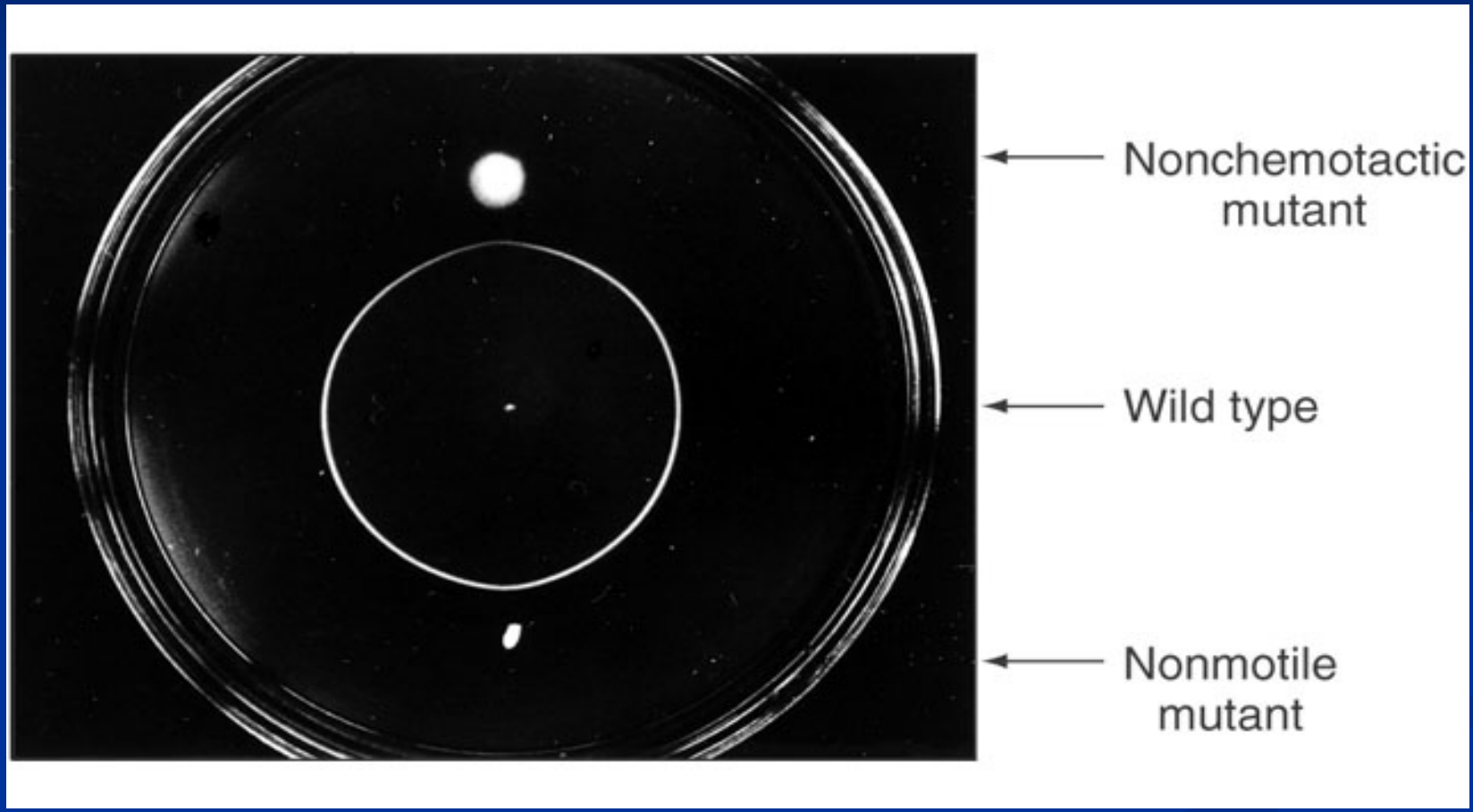
- How bacteria move to achieve chemotaxis?
  - Straight run and tumble in a random walk
  - Addition of attractant or repellent causes biased random walk.



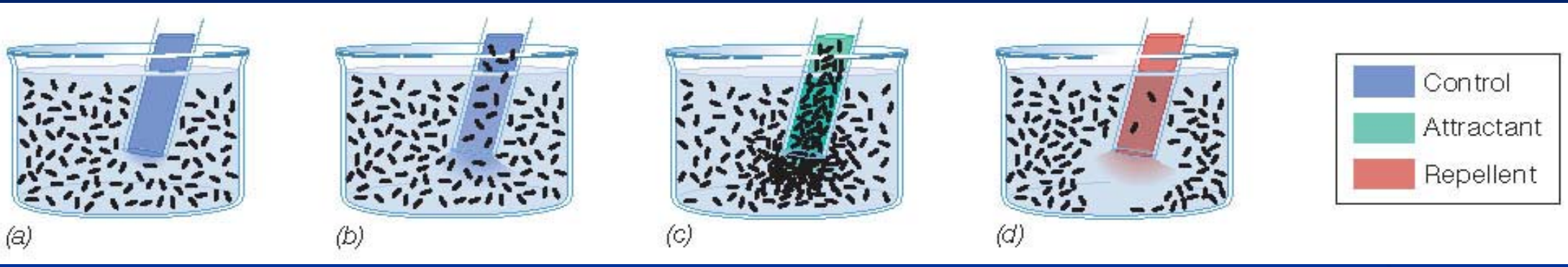
- **Counterclockwise movement is achieved when flagella bundle.**
- **Tumble is achieved when flagella are not bundled.**

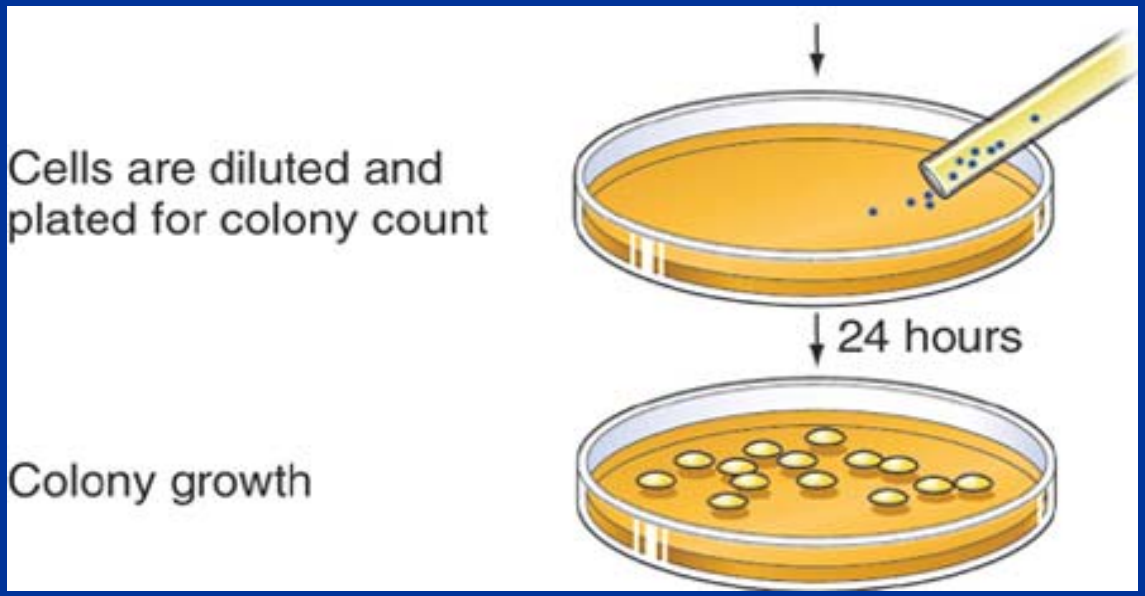


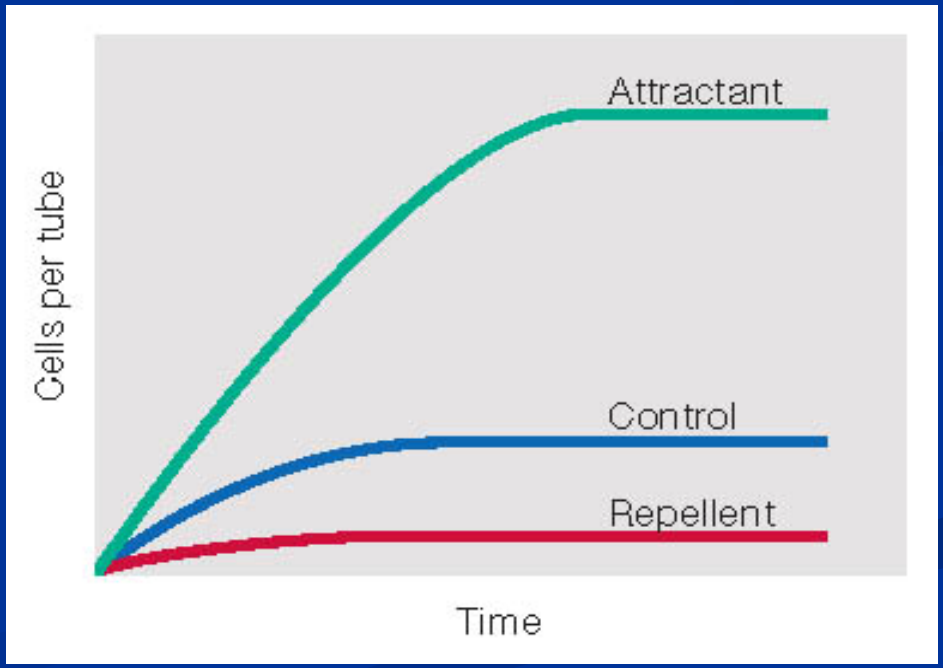
Isolating bacterial mutants that cannot move towards food



The capillary test for chemotaxis



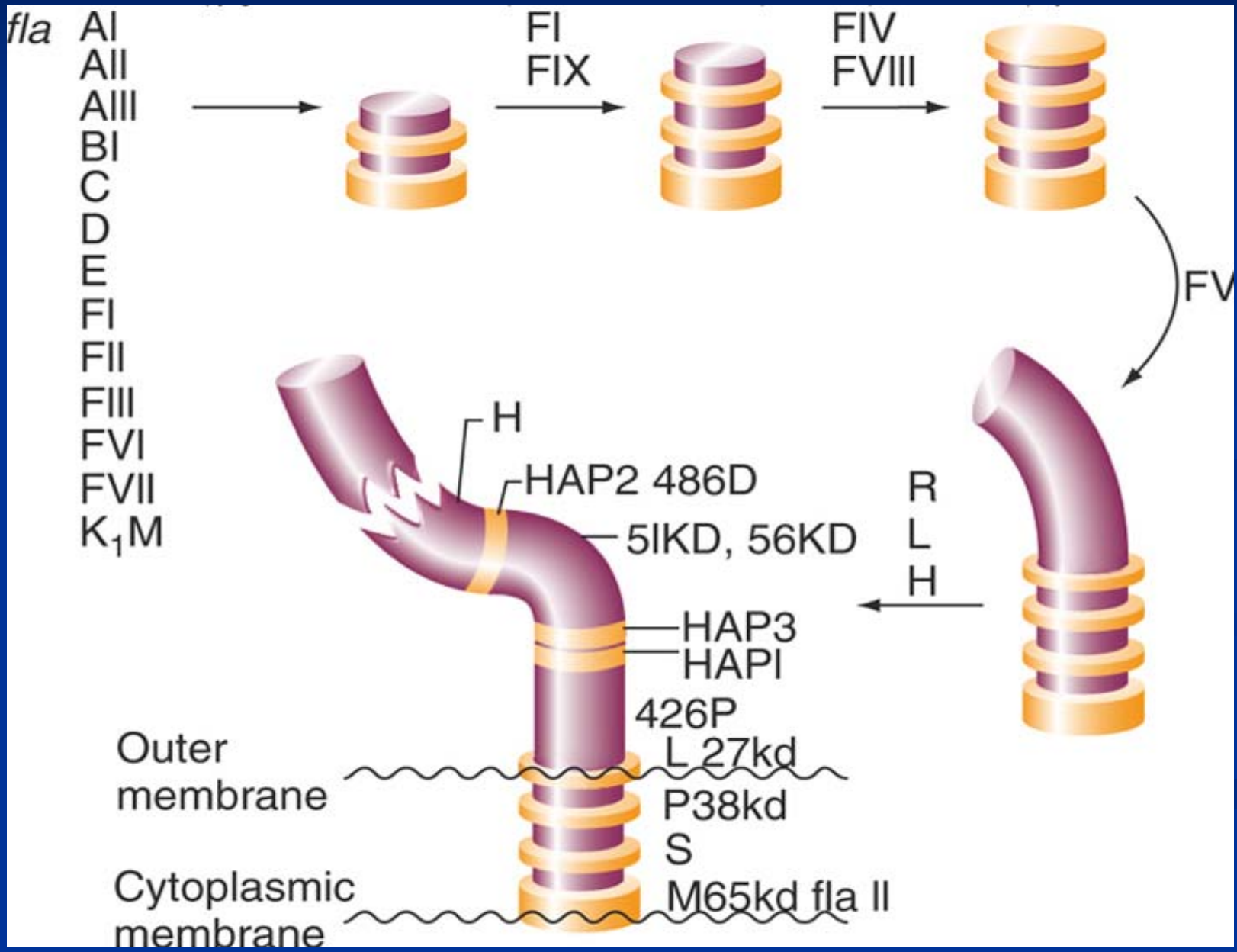




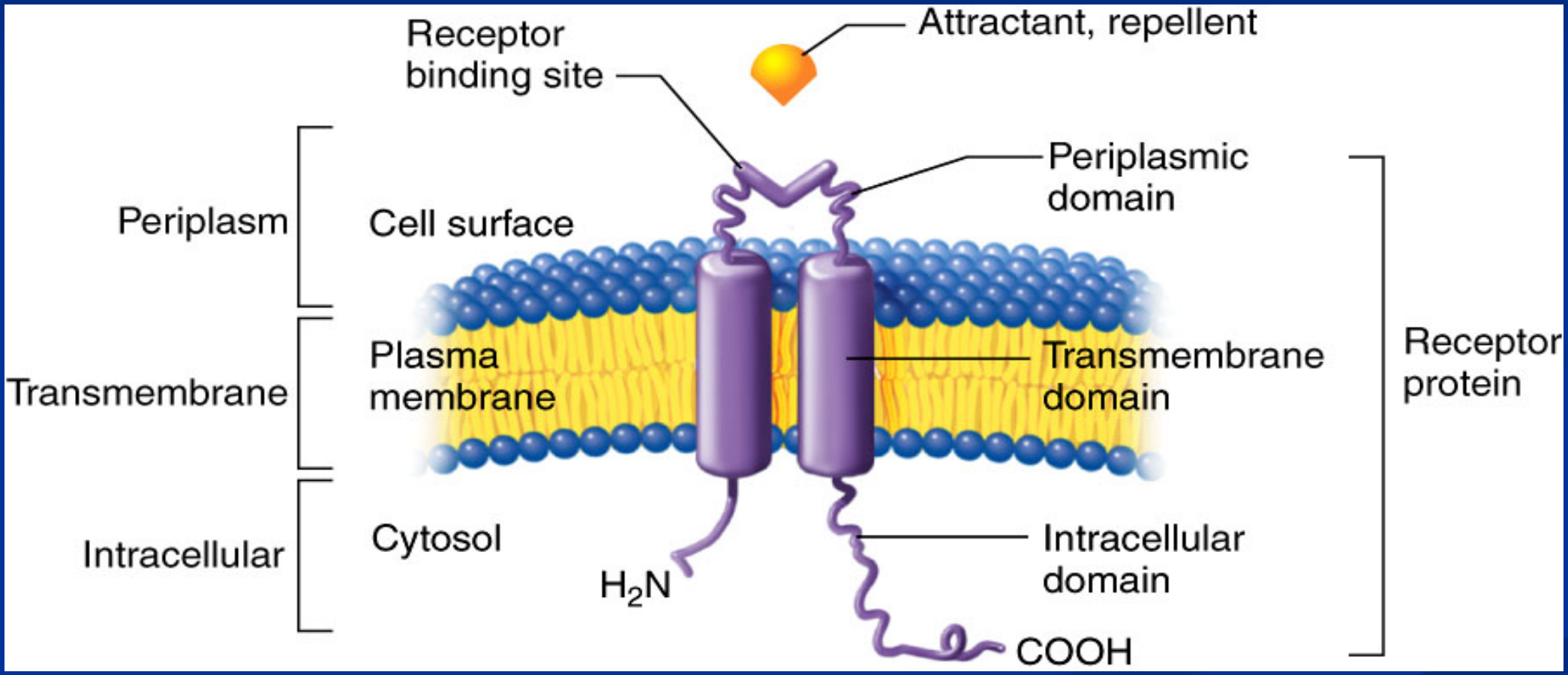
Chemotaxis mutants

- **Flagellum mutants**
  - More than 20 *fla* genes are required to generate a flagellum. Mutants prevent production of functional flagella.
- **Motor mutants**
  - Mot genes are required to turn the flagellum. Mutants are paralyzed.
- **Signal transduction mutants**
  - Mutants prevent proper relay of messages from cell surface to motor where frequency and direction of rotation takes place.
  - *che* (chemotaxis) mutants have flagella that move only in one direction.
- **Receptor mutants**
  - Mutants in receptors that bind particular chemicals.

More than 20 genes are needed to generate a bacterial flagellum



Bacteria have cell surface receptors that recognize particular attractants or repellents



The molecular basis of bacterial chemotaxis