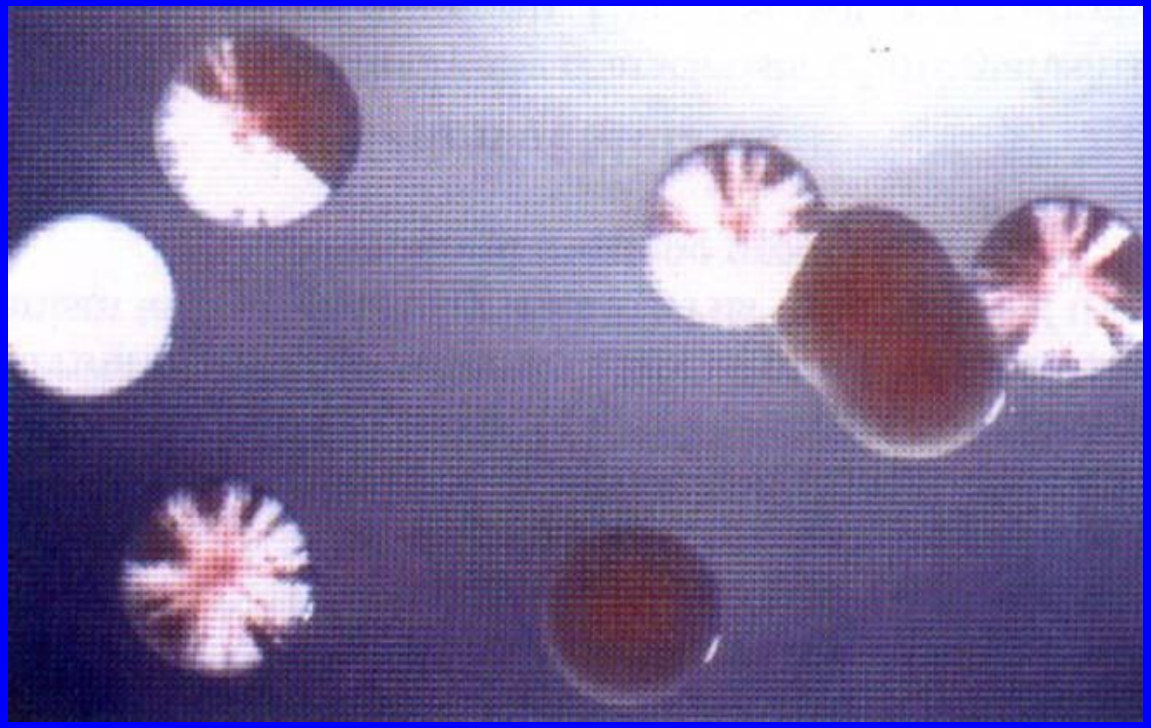
Chapter 8

Microbial genetics



Chapter outline

8.1 Mutations and Mutants
8.2 Genetic Recombination
8.3 Genetic Transformation
8.4 Transduction
8.5 Conjugation
8.6 Plasmids
8.7 Transposons and Insertion Sequences
8.8 Comparative Prokaryotic Genomics

Concepts

- DNA replication is a very complex process involving a variety of proteins and a number of steps. It is designed to operate rapidly while minimizing errors and correcting those that arise when the DNA sequence is copied.
- The actual transfer of genetic material between bacteria usually takes place in one of three ways: direct transfer between two bacteria temporarily in physical contact (conjugation), transfer of a naked DNA fragment (transformation), or transport of bacterial DNA by bacteriophages (transduction)

Importance of study on microbial genetics

- simple systems
  - studying genetic phenomena
- useful tools
  - decipher the genetics mechanisms
- molecular cloning
  - isolate and duplicate specific genes from other organisms
  - genes are manipulated and placed in a microorganism where they can be induced to increase in numbers

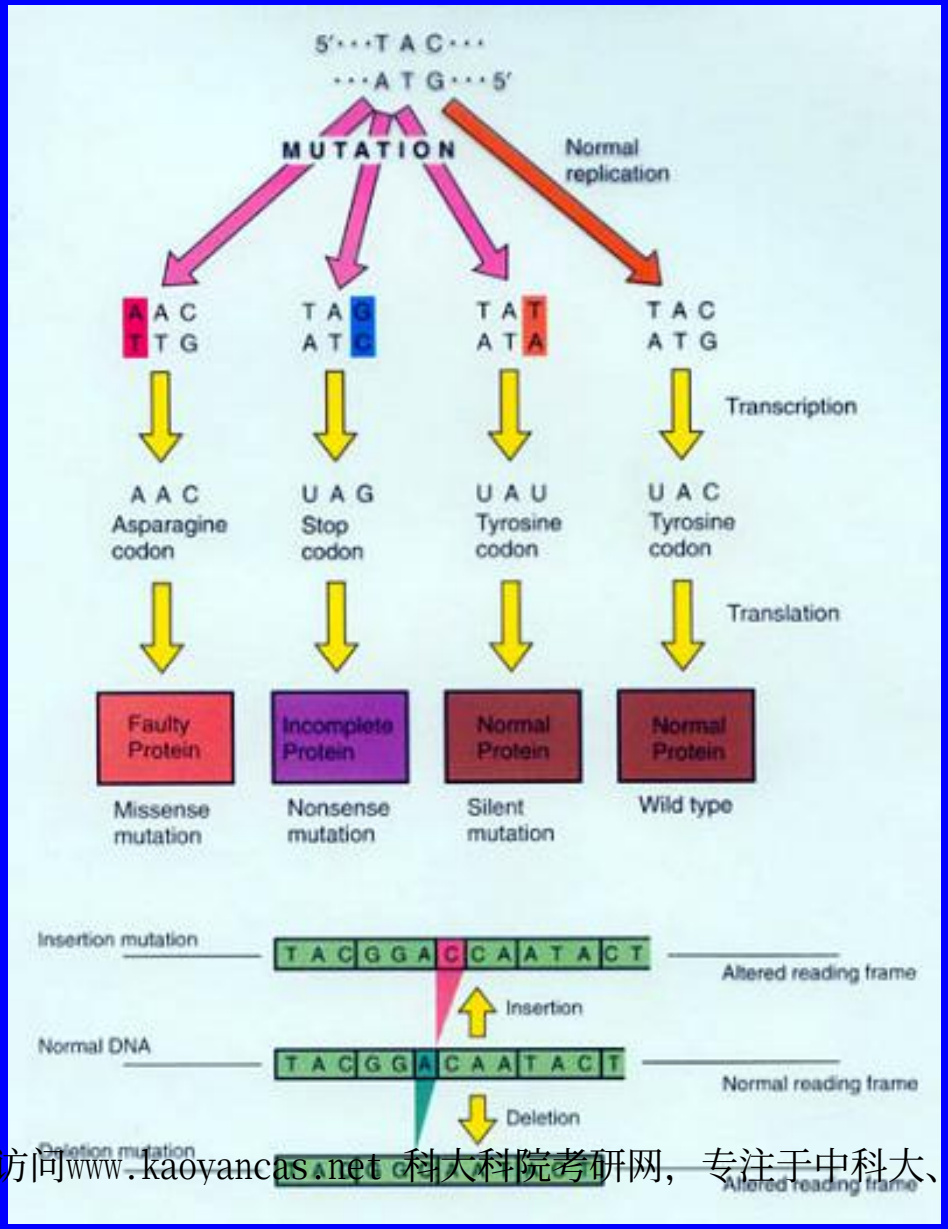
- ❖ Value in industry
- Antibiotics
- Increase yields and improve manufacturing processes
- ❖ Diseases
- Understanding the genetics of disease-causing microorganisms
- ❖ Genetic transfer in prokaryotes
- How genes can be transferred from one organism to another, even from one species to another.

8.1 Mutation and recombination

Mutation is an inherited change in the base sequence of the nucleic acid comprising the genome of an organism. Mutation usually brings about only a very small amount of genetic change in a cell.

Mutant an organism whose genome carries a mutation. Depending on the mutation, a mutant may or may not show an altered phenotype from its parent.

Different kinds of point mutation



Mutation

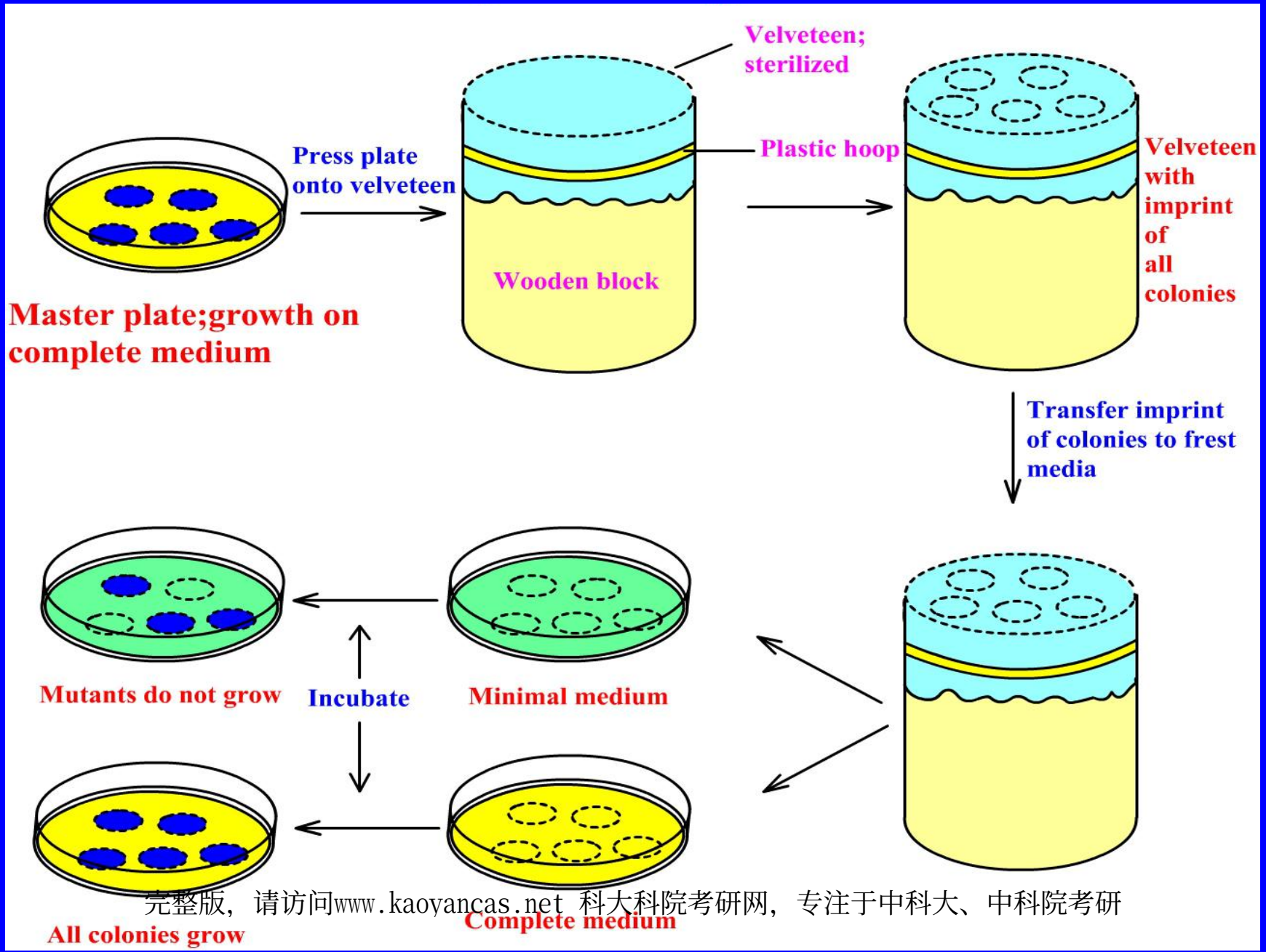
- Selectable
- Nonselectable
- Spontaneous
- Induced

Nutritional mutants

replica plating method

- The photograph on the left shows the master plate
- The colonies not appearing on the replica plate are marked with an X
- The replica plate lacked one nutrient (leucine) present in the master plate
- Therefore, the colonies marked with an X are leucine auxotrophs

Replica plating method for detection of nutritional mutants



Mutagens

While the spontaneous rate of mutation is very low, there are a variety of chemical, physical, or biological agents that can increase the mutation rate, and are therefore said to induce mutations. These agents are referred to as mutagens.

8.2 Genetic recombination

Genetic recombination is the process by which genetic elements contained in two separate genomes are brought together in one unit.

This mechanism may enable the organism to carry out some new functions and result in adaptation to changing environments.

Genetic recombination usually involves much larger changes. Entire genes, sets of genes, or even whole chromosomes, are transferred between organisms.

A number of prokaryotes have been found to be naturally transformable

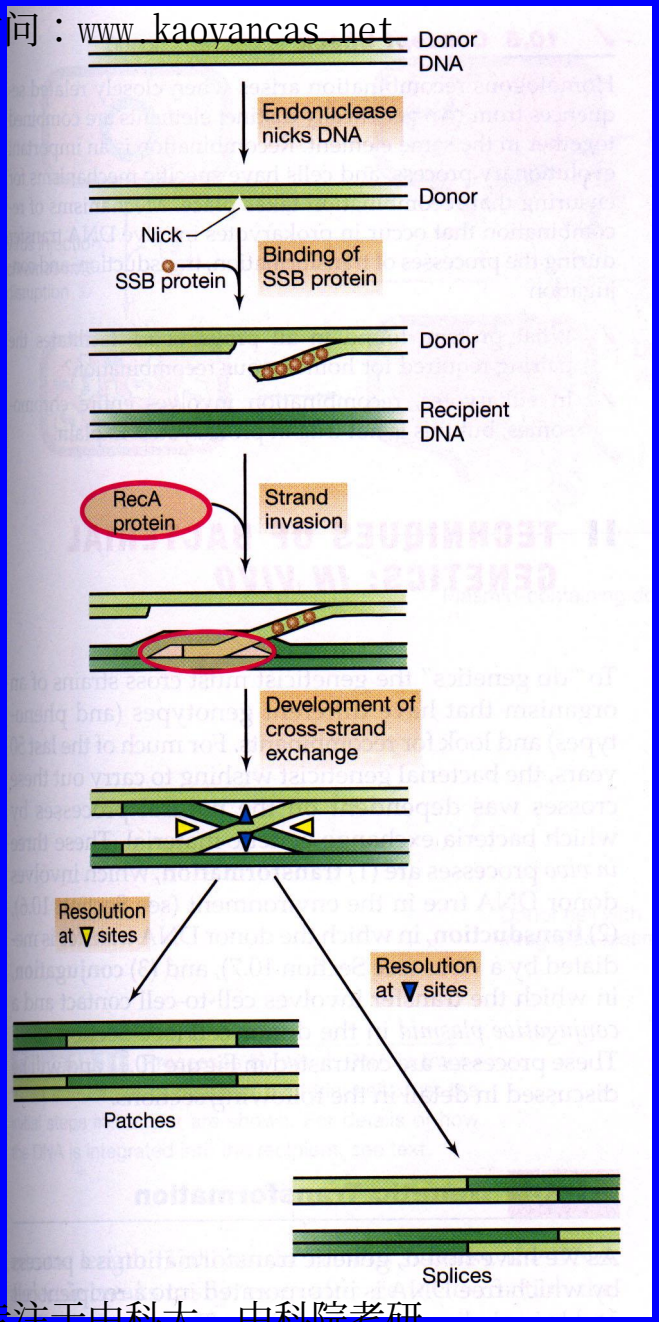
- certain species of G^+ and G^- Bacteria
- some species of Archaea

However, even within transformable genera, only certain strains or species are transformable

A simplified version of one molecular mechanism of recombination. Homologous DNA molecules pair and exchange DNA segments.

The mechanism involves breakage and reunion of paired segments. Two of the proteins involved, a single-stranded binding (SSB) protein and the RecA protein.

Note that there are two possible outcomes, depending on which strands are cut during the resolution process. In one outcome the recombinant molecules have patches, whereas in the other the two parental molecules appear to have been cut and then spliced together.



Detection of Recombination

Recombinants must be phenotypically different from the parents.

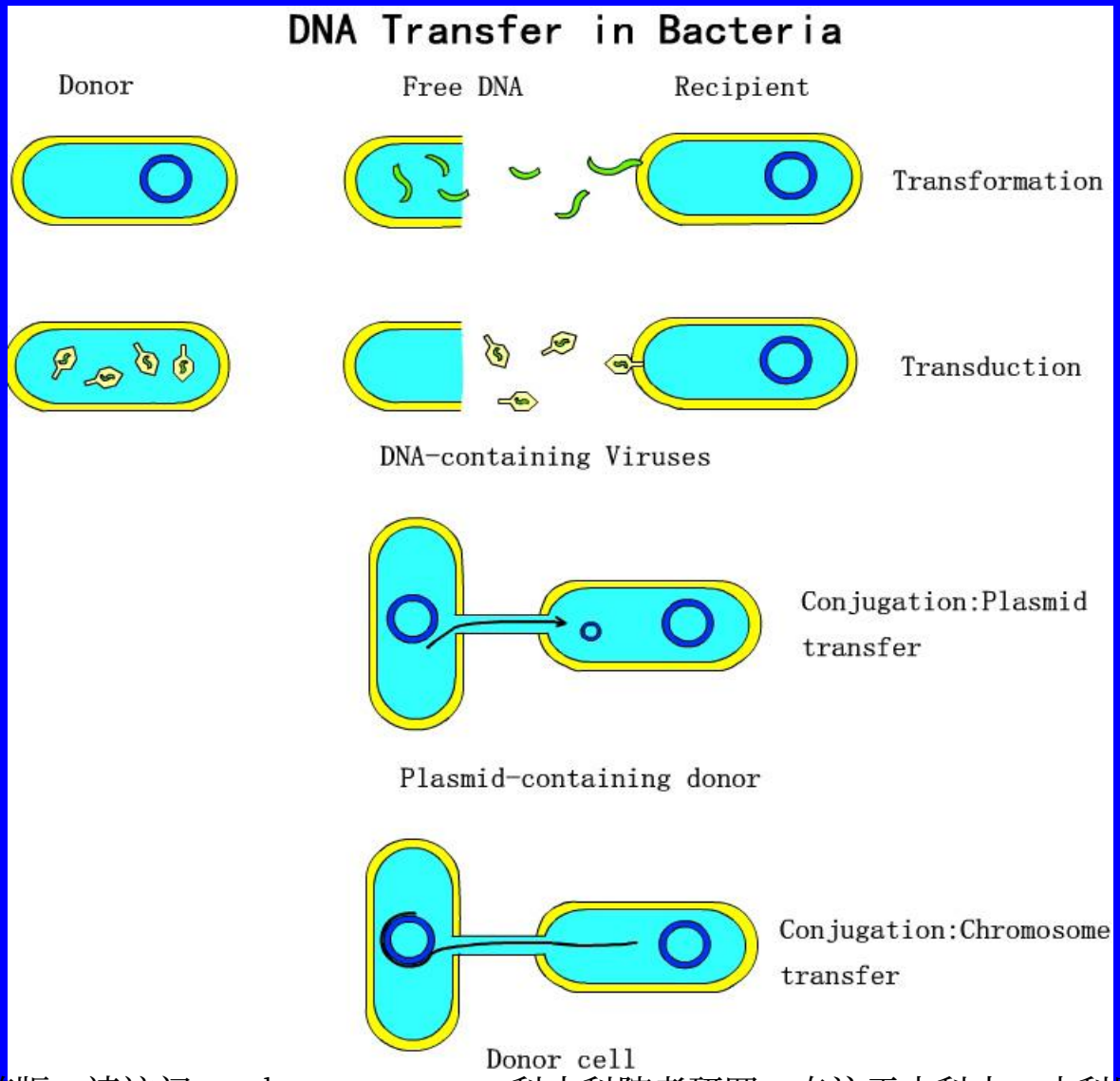
- selectable characteristics

For instance, the recipient may not be able to grow on a particular medium, and genetic recombinants are selected that can.

- selectable and nonselectable markers

such as drug resistance, nutritional requirements, and so on

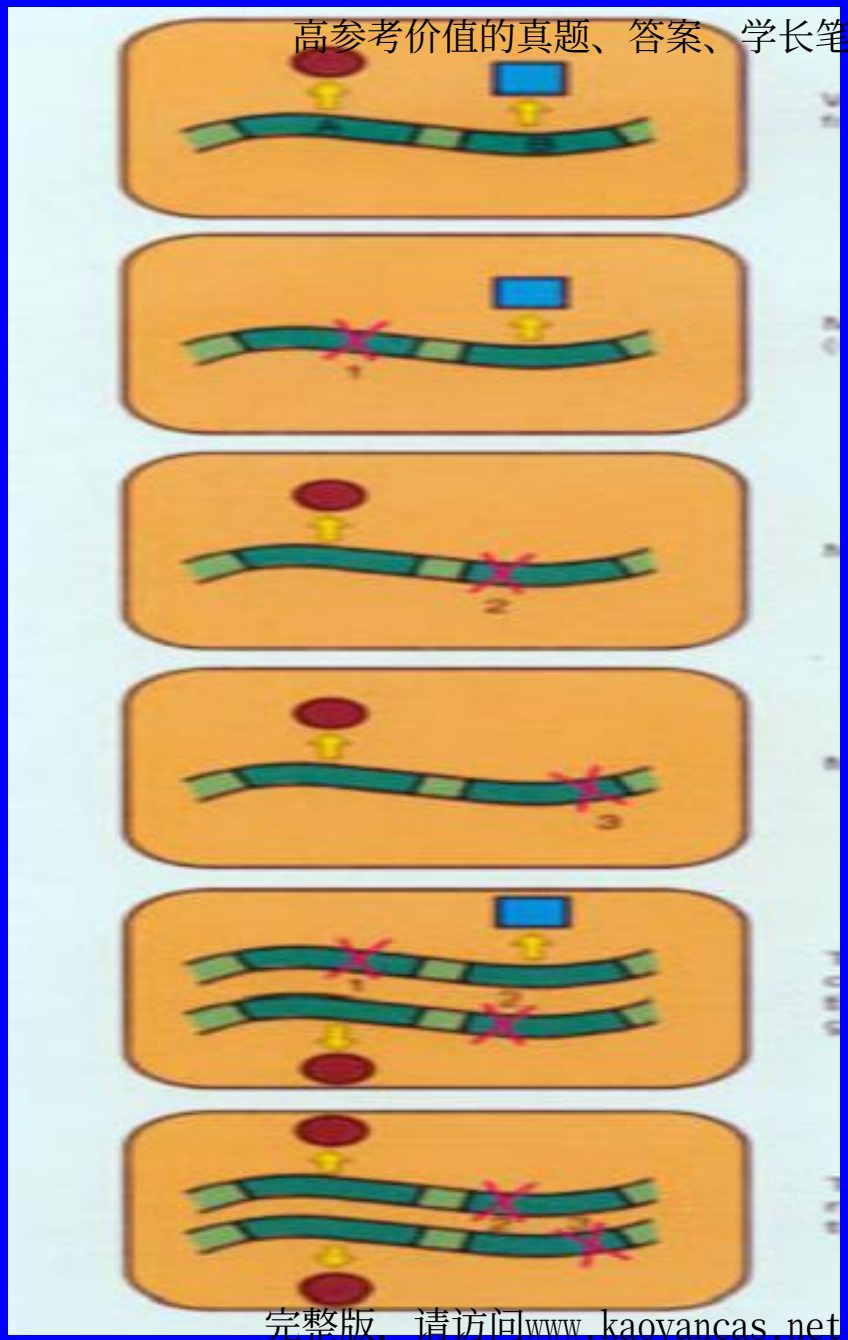
Processes by which DNA is transferred from donor to recipient bacterial cell. Just the initial steps in transfer are shown.



Complementation

When two mutant strains are genetically crossed (mated), homologous recombination can yield a wild-type recombinant unless both of the mutations include changes in exactly the same base pairs.

If two different Trp^- Escherichia coli (strains that require the amino acid tryptophan in the medium) are crossed and Trp^- recombinants are obtained, it is clear that the mutations in the two strains did not include the same base pairs. However, this experiment cannot detect whether the mutations were in the same gene. This can be determined by a type of experiment called a complementation test.



Wild type cell: both gene are functional and cell is Trp$^+$

Mutant x: cell contains mutation 1 and is Trp-(requires tryptophan for growth)

Mutant y: cell contains mutation 2 and is Trp -

Mutant z: cell contains mutation 3 and is Trp-

Trans test of mutations 1 and 2: complementation occures (cell is Trp+), therefore mutation are in separate genes

Trans test of mutations 2 and 3: no complementation occures (cell is Trp-), therefore mutations are in the same gene

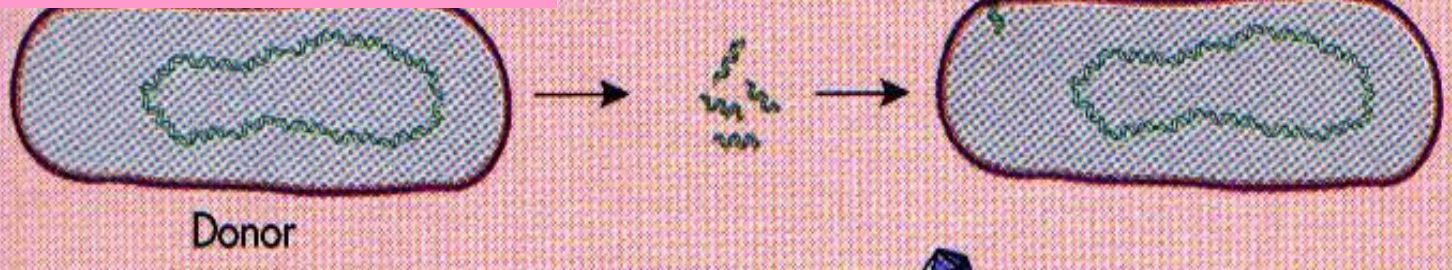
Three main processes of genetic recombination in prokaryotes fragments of homologous DNA from a donor chromosome are transferred to a recipient cell

(1) Transformation, which involves donor DNA free in the environment

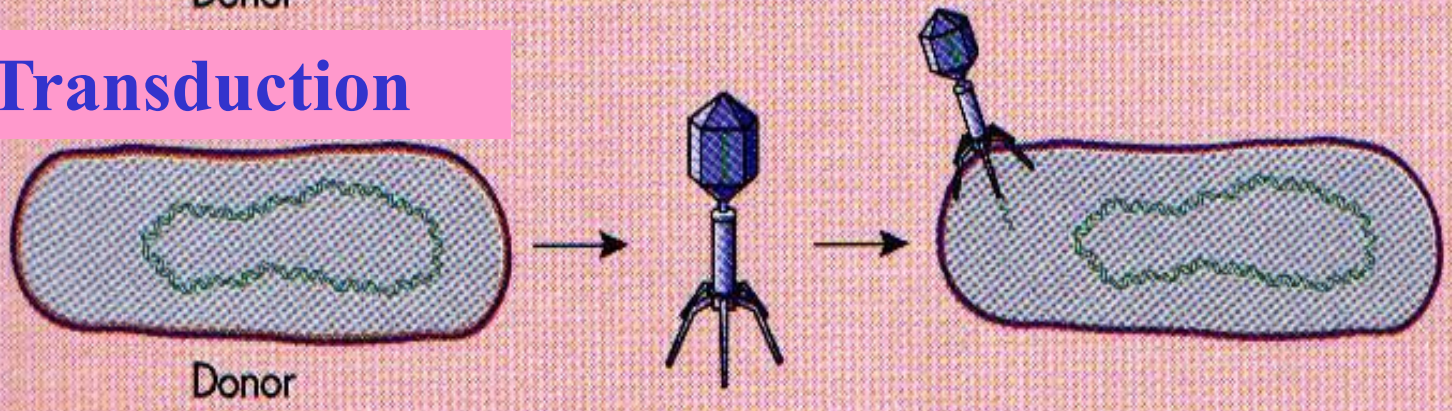
(2) Transduction, in which the donor DNA transfer is mediated by a virus

(3) Conjugation, in which the transfer involves cell-to-cell contact and a *conjugative plasmid* in the donor cell

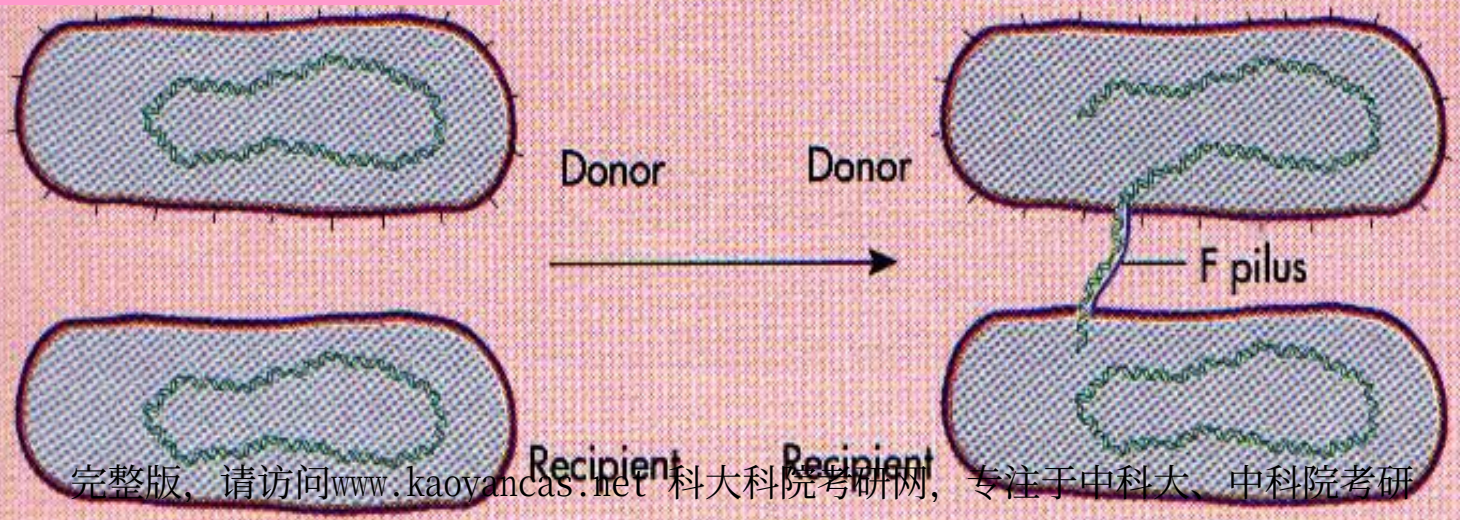
Transformation



Transduction

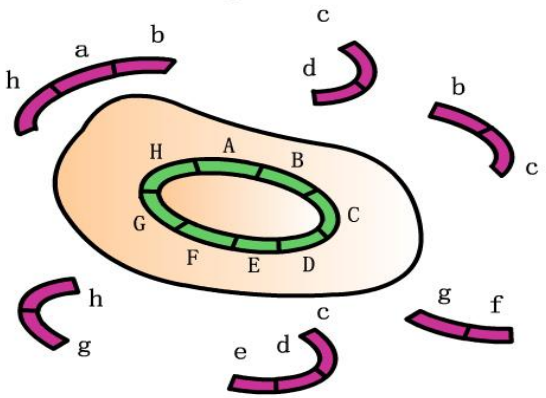


Conjugation

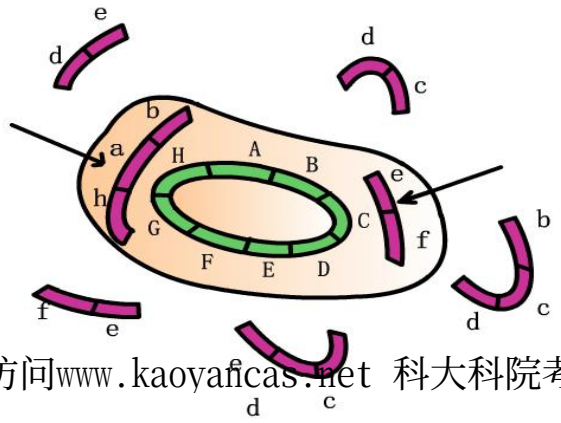


8.3 Genetic Transformation

1. NAcked DNA fragments from disintegrated cells in the area of a potential recipient cell. This cell must be of the correct genus and be in a state of competence, a proper physiologic condition, topermit entry of the DNA fragments.

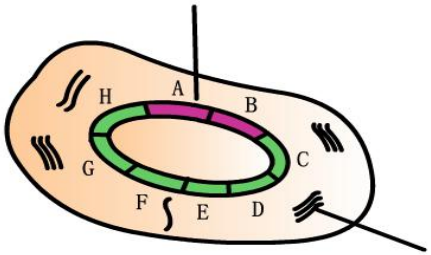


2. Entry of nacked DNA into compentent cell



3. Recombination

Some DNA fragments replace (recombine with) original host cell DNA. the resultant recombinant cell is said to have been genetically transformed and will now express the foreign genes it has recreived and pass them on to all its offspring



DNA that has not recombined is broken down by enzymes.

1. Genetic transformation is a process by which free DNA is incorporated into a recipient cell and brings about genetic change.

2. The discovery of genetic transformation in bacteria was one of the outstanding events in biology, as it led to experiments demonstrating that DNA is the genetic material.

3. This discovery became the keystone of molecular biology and modern genetics.

4. A number of prokaryotes have been found to be naturally transformable, including certain species of both gram-negative and gram-positive Bacteria and some species of Archaea.

Competence

- A cell that is able to take up a molecule of DNA and be transformed is said to be competent.
- Only certain strains are competent; the ability seems to be an inherited property of the organism.
- Competence in most naturally transformable bacteria is regulated, and special proteins play a role in the uptake and processing of DNA.
- These competence-specific proteins may include a membrane-associated DNA binding protein, a cell wall autolysin, and various nucleases.

Artificially Induced Competence

High efficiency natural transformation is found only in a few bacteria; *Azotobacter, Bacillus, Streptococcus,* for example, are easily transformed.

Determination of how to induce competence in such bacteria may involve considerable empirical study, with variation in culture medium, temperature, and other factors

When *E. coli* is treated with high concentrations of calcium ions and then stored in the cold, the transformation by plasmid DNA is relatively efficient.

DNA Transfer by Electroporation

For artificial induction of competence are being supplanted by a new method termed **electroporation**.

Small pores are produced in the membranes of cells exposed to pulsed electric fields.

When DNA molecules are present outside the cells during the electric pulse, they can then enter the cells through these pores. This process is called **electroporation.**

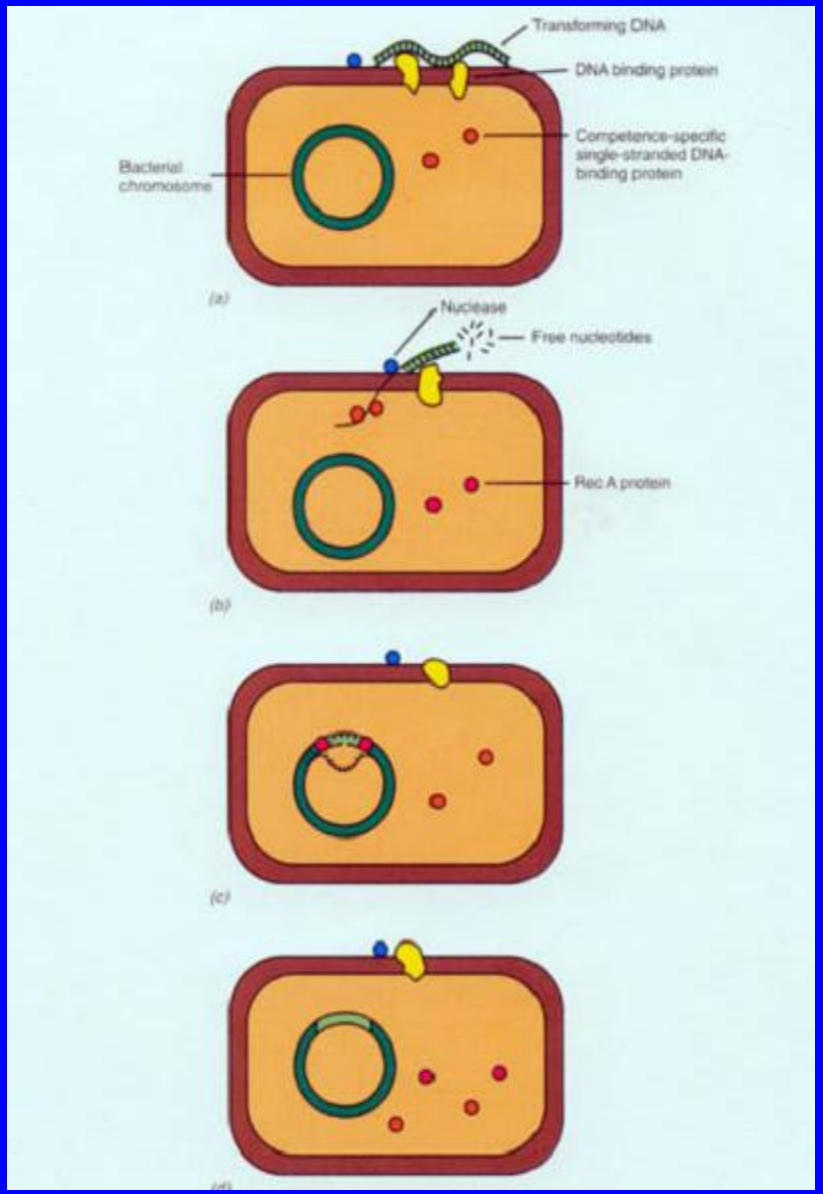
Uptake of DNA

Bacteria differ in the form in which DNA is taken up.

- During the transformation process, competent bacteria first bind DNA reversibly
- Soon, however, the binding becomes irreversible.
- Competent cells bind much more DNA than do noncompetent cells as much as 1000 times more

Integration of Transforming DNA

1. Transforming DNA is bound at the cell surface
2. After uptake, the DNA associates with a competence-specific protein that remains attached to the DNA
3. The DNA is then integrated into the genome of the recipient by recombinational processes



Mechanism of DNA transfer by transformation in a gram-positive bacterium, (a) Binding of free DNA by a membrane-bound DNA binding protein, (b) Passage of one of the two strands into the cell while nuclease activity degrades the other strand, (c) The single strand in the cell is bound by specific proteins, and recombination with homologous regions of the bacterial chromosome mediated by RecA protein occurs, (d) Transformed cell.

8.4 Transduction

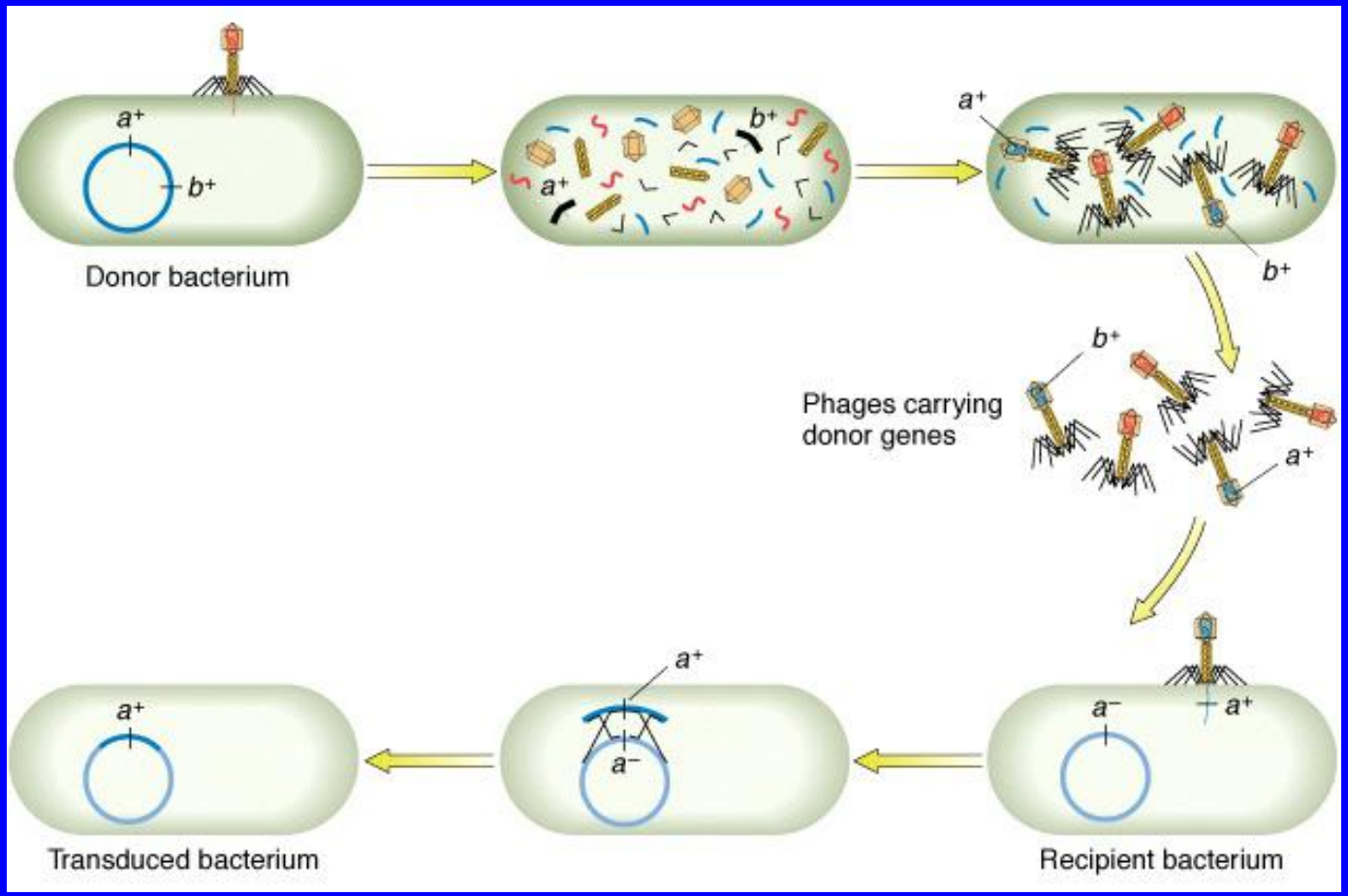
In transduction, DNA is transferred from cell to cell through the agency of viruses. Genetic transfer of host genes by viruses can occur in two ways.

Generalized transduction

And

Specialized transduction

Process of transduction



Note: Not all phages can be transducer and not all bacteria are transducible

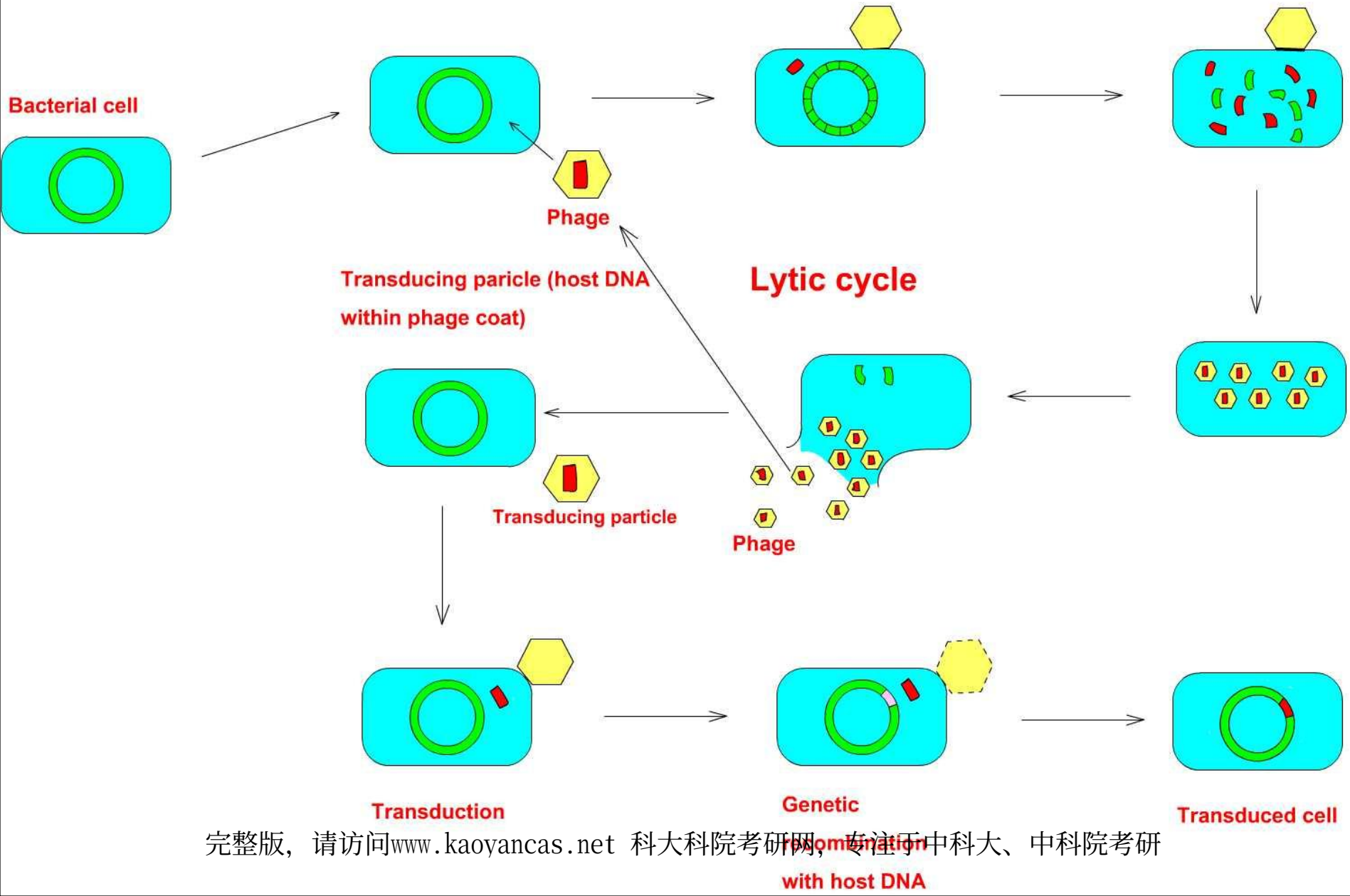
Generalized Transduction

1) Bacteria is infected with a phage
2) During a lytic infection, the enzymes is responsible for packaging viral DNA into the bacteriophage
3) On lysis of the cell, these transducing particles are released along with normal virions
4) The lysate contains a mixture of normal virions and cannot lead to a normal viral infection

In generalized transduction, virtually any genetic marker can be transferred from donor to recipient

During a lytic infection, the enzymes responsible for packaging viral DNA into the bacteriophage sometimes accidentally package host DNA. This DNA cannot replicate, it can undergo genetic recombination with the DNA of the new host.

Generalized Transduction

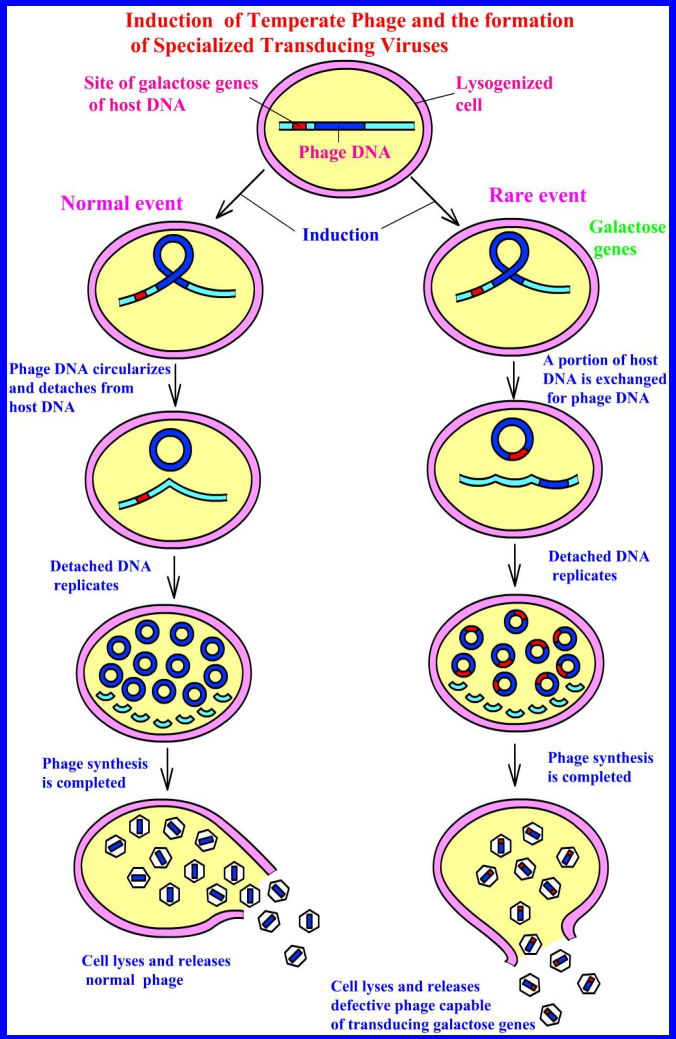


Specialized Transduction

Generalized transduction allows the transfer of DNA from one bacterium to another at a low frequency.

Specialized transduction can allow extremely efficient transfer while also allowing a small region of a bacterial chromosome to be replicated independently of the rest.

Specialized Transduction



The DNA of lambda is inserted into the host DNA at the site adjacent to the galactose genes

On induction, Under rare conditions, the phage genome is excised incorrectly

A portion of host DNA is exchanged for phage DNA, called lambda dgal *(dgal* means "defective galactose“)

Phage synthesis is completed

Cell lyses and releases defective phage capable of transducing galactose genes

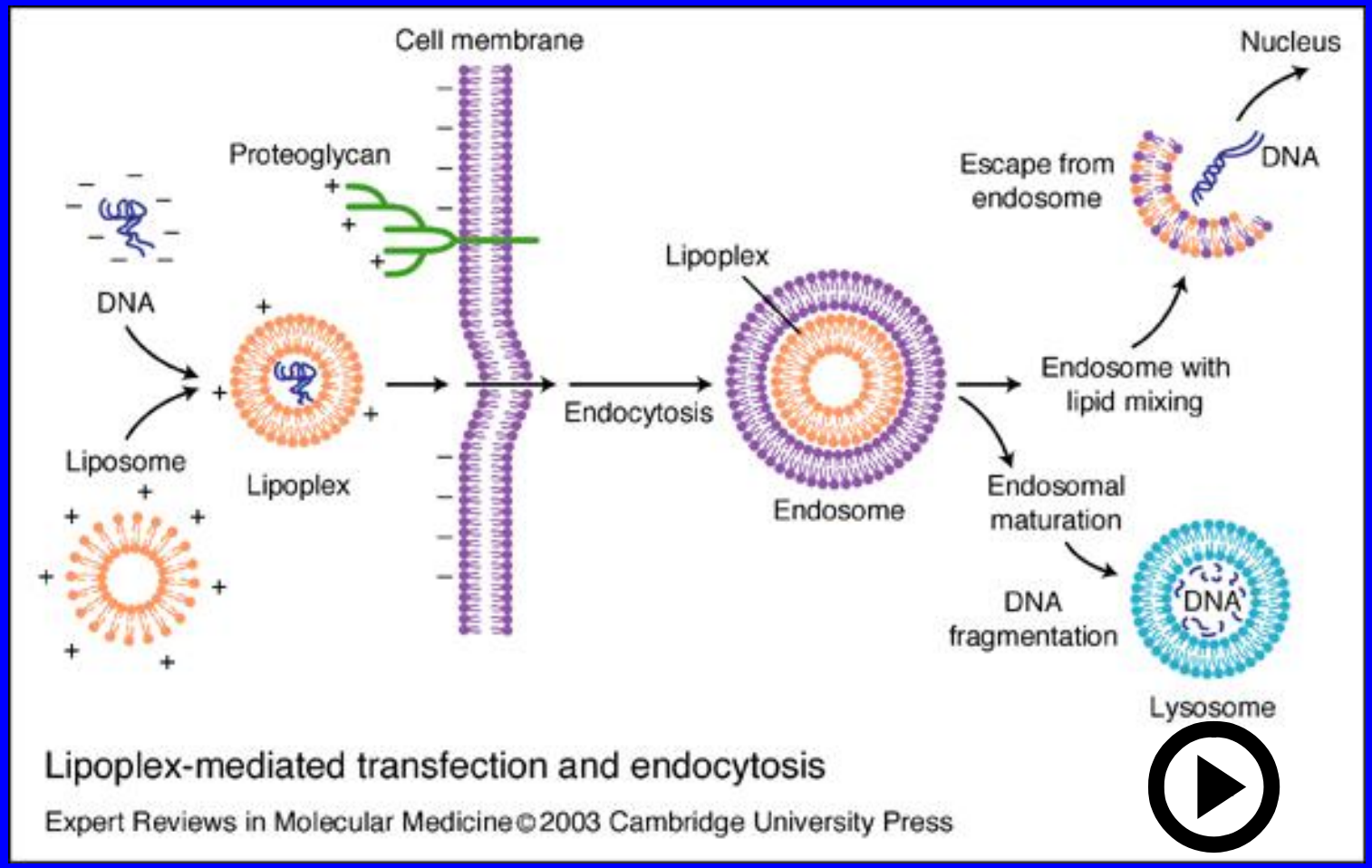
Phage Conversion

When a normal temperate phage lysogenizes a cell and its DNA is converted tothe prophage state, the lysogen is immune to further infection by the same type of phage. This acquisition of immunity can be considered a change in phenotype.

In certain cases other phenotypic alterations can be detected in the lysogenized cell, which seem to be unrelated to the phage immunity system.

Such a change, which is brought about through lysogenization by a normal temperate phage, is called phage conversion.

Transfection

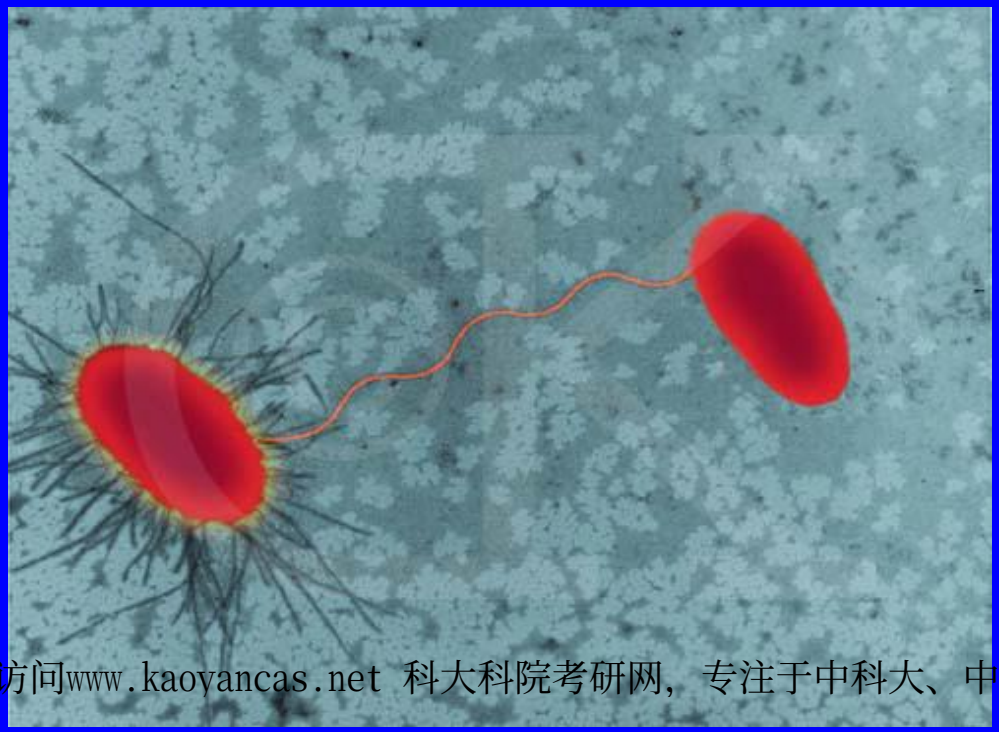


Bacteria can be transformed with DNA extracted from a bacterial virus or liposome rather than from another bacterium, a process known as **transfection**.

8.5 Conjugation

Bacterial conjugation (mating) is a process of genetic transfer that involves cell-to-cell contact.

Direct contact between two conjugating bacteria is first made via a pilus. The cells are then drawn together for the actual transfer of DNA.



Conjugation involves a donor cell, which contains a particular type of conjugative plasmid, and a *recipient* cell, which does not.

The genes that control conjugation are contained in the *tra* region of the plasmid (see Section 9.8 in your text). Many genes in the tra region have to do with the synthesis of a surface structure, the **sex pilus** . Only donor cells have these pili,

The pili make specific contact with a receptor on the recipient and then retract, pulling the two cells together. The contacts between the donor and recipient cells then become stabilized, probably from fusion of the outer membranes, and the DNA is then transferred from one cell to another.

F-

F- cells lack the F plasmid

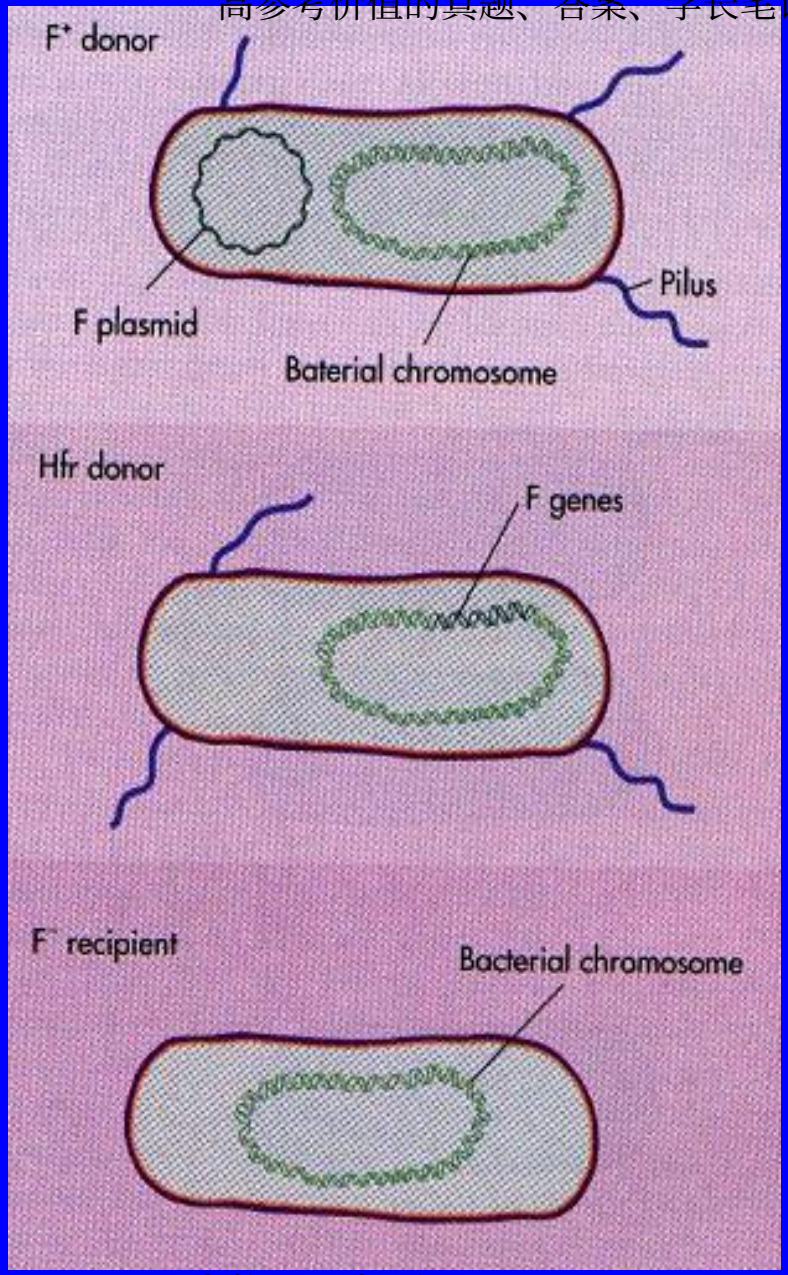
F+

Cells possessing an unintegrated F plasmid are called F^+.

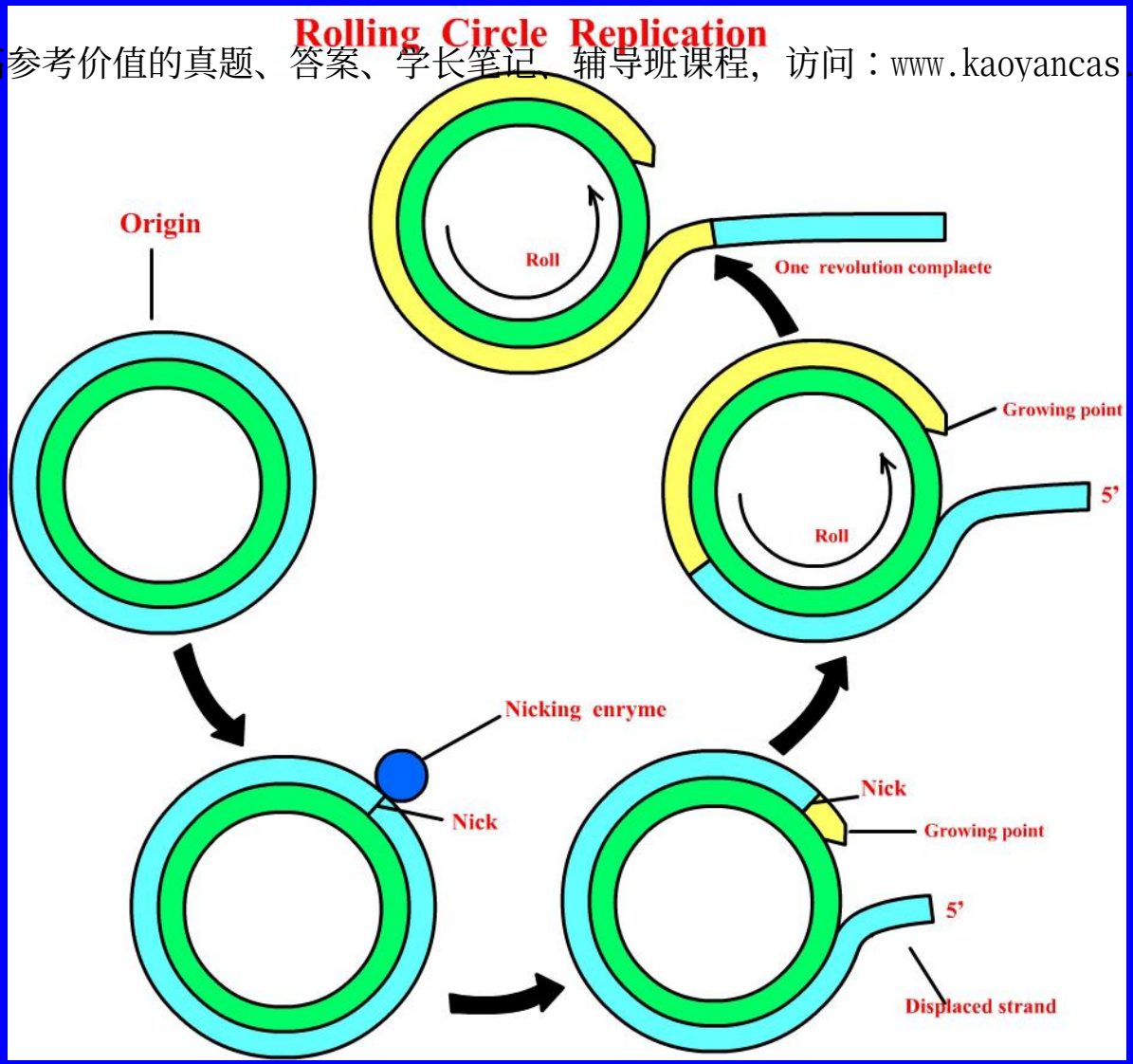
Hrf

Strains that can act as recipients for F' (or Hfr, see later in this section) are called F-.

F'



- Donor bacterial that have the fertility gene(F gene) produce F pili.
- In F^+ strains the F gene is on a plasmid.
- The F gene can be incorporated into the bacterial chromosome to produce donor strains designated Hfr (high frequency recombination strains)

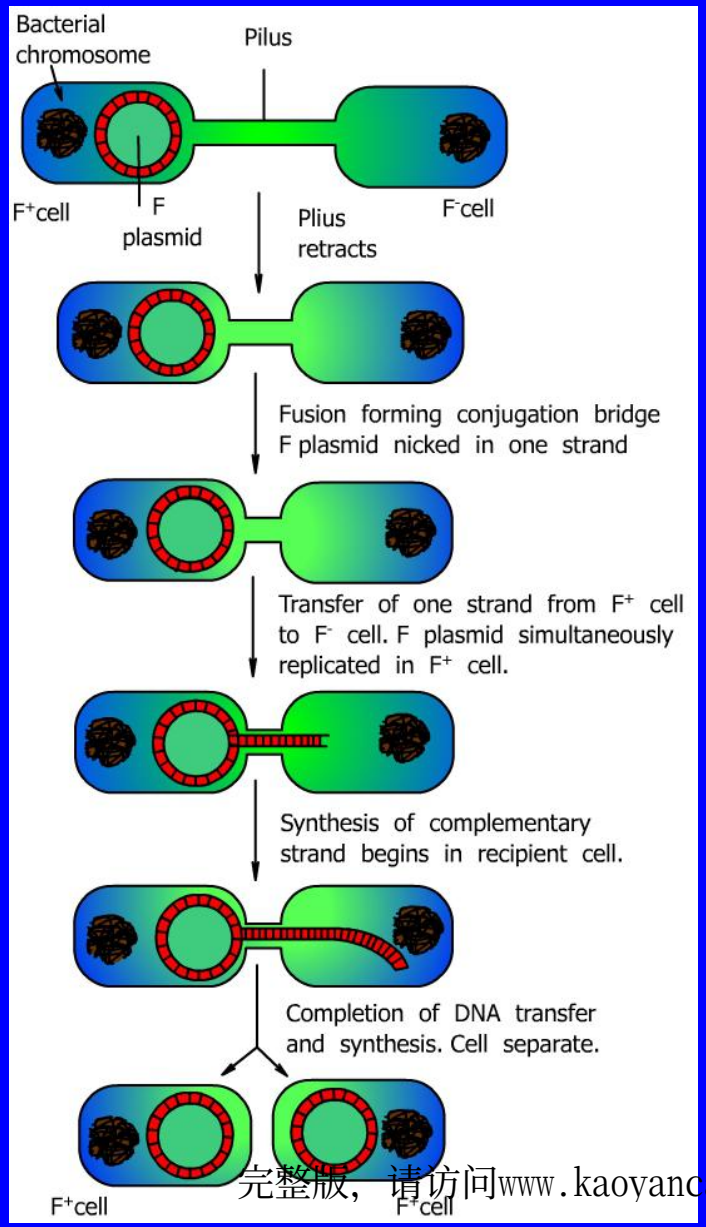


The transfer of DNA from an Hfr strain invoke copying of a single strand of DNA by the rolling circle mechanism; the single strand then moves to the recipient cell.

Result of selected conjugation

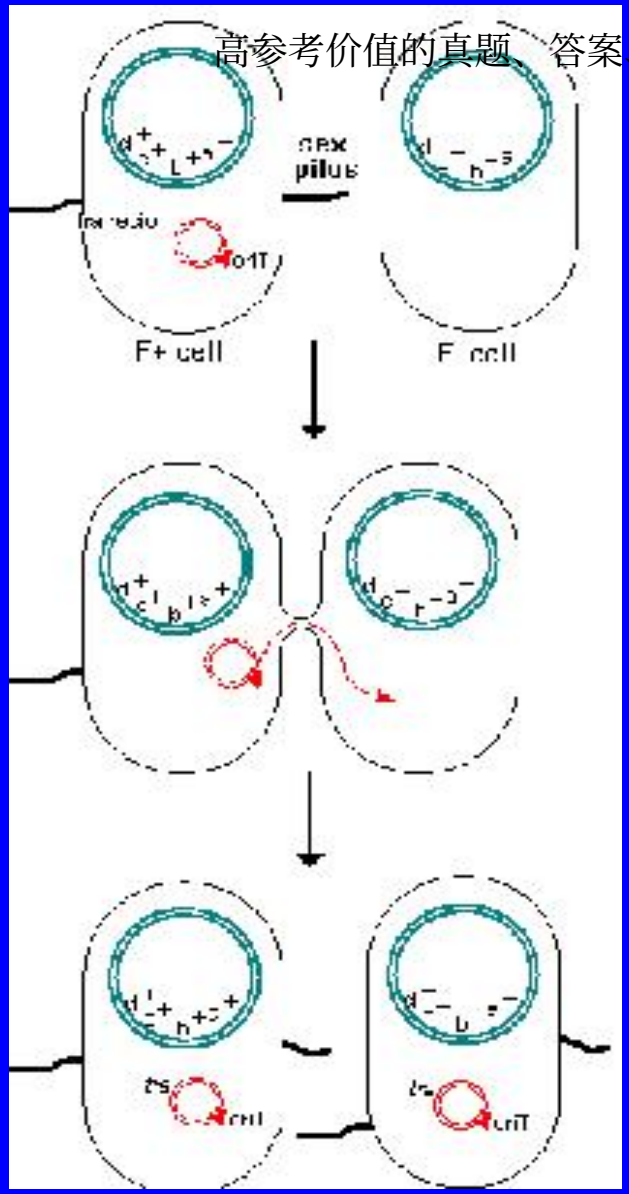
Donor	Recipient	Molecules transferred	Product
F^+	F^-	F plasmid	F^+ Cell
Hfr	F^-	Initiating segment of F plasmid and variable quantity of chromosomal DNA	F^- with variable quantity of chromosomal DNA
F^+	F^-	F^+ plasmid and some chromosomal genes it carries with it	F^+ Cell with some duplicate gene pairs: one on chromosom, one on plasmid

Mechanism of DNA Transfer During Conjugation



A mechanism of DNA synthesis in certain bacteriophages, called **rolling circle replication,** was presented here to explains DNA transfer during conjugation .

If the DNA of the donor is labeled, some labeled DNA is transferred to the recipient but only a *single* labeled strand is transferred. Therefore, at the end of the process, both donor and recipient possess completely formed plasmids.



F+ uncharged cell

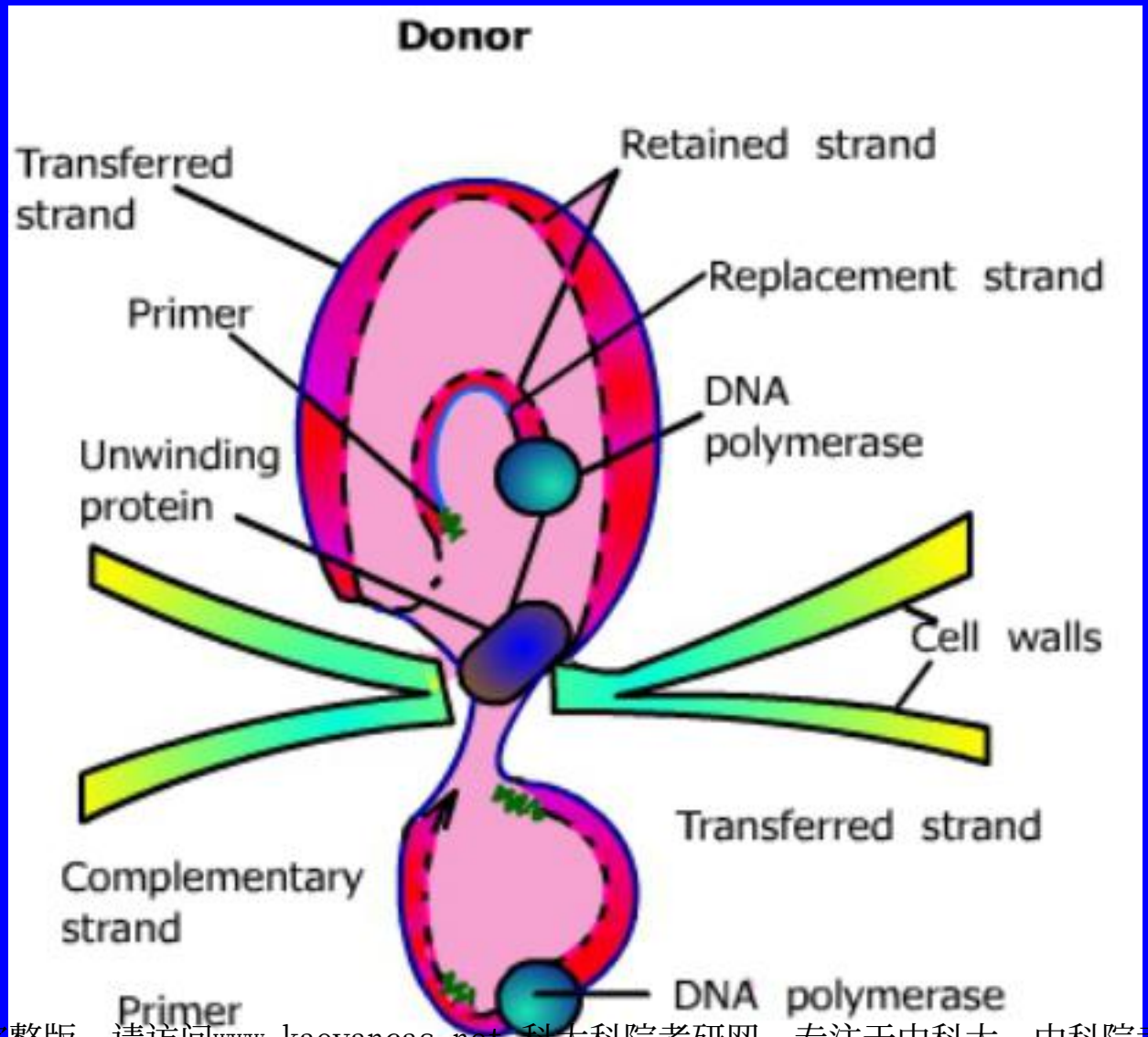
F+cell with new plasmid but no new bacterial genes

1. In the F- parent,the fertility factor is present but free from the bacterial chrosome. transfer proceed from the oriT region and then the rest of the plasmid genes are transferred

2. Only a single strand of DNA is transferred. The area that is lost is reduplicated (shown as dotted lines)so the donor remains the same genotype .the last gene to be transferred are the *trs* gene

3. The transfer of the plasmid is fairly quick so assume that it is transferred entirely 100% of the time unless otherwise told.
The F^- cell becomes F^+,there two cells can no longer mate.No baterial genes are transferred.

Details of the replication and transfer process



8.6 plasmid

- Plasmids are genetic elements that replicate independently of the host chromosome.
- Unlike viruses, plasmids do not have an extracellular form and exist inside cells simply as nucleic acid.
- However, distinguishing between viruses and plasmids can sometimes present difficulties.

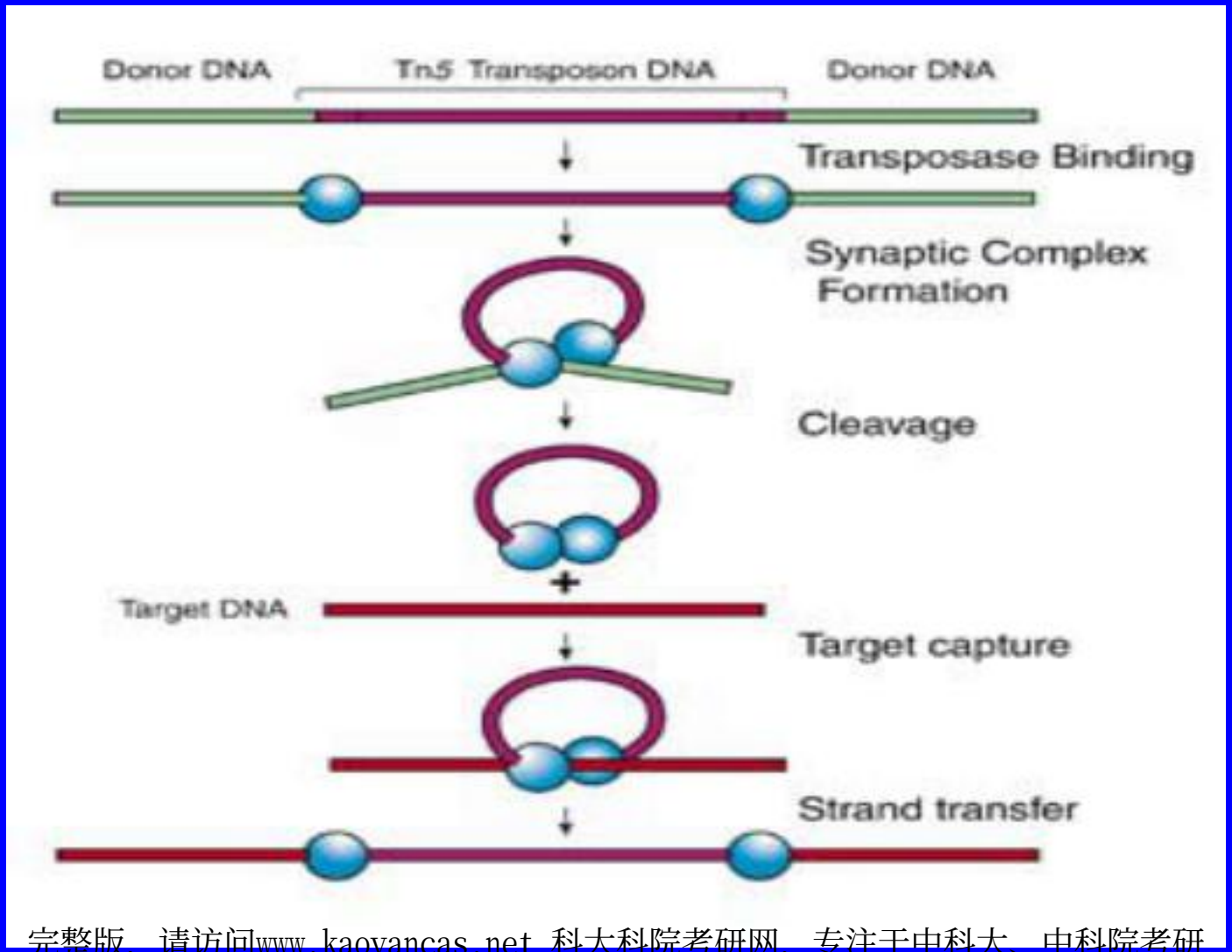
Physical Nature of Plasmids

- Most plasmids are double-stranded and circular, but many linear plasmids are also known.
- Naturally occurring plasmids vary in size from approximately 1 to 1000 kilobase pairs , less than 1/20 the size of the chromosome
- Most of the plasmid DNA isolated from cells is in the supercoiled configuration, which is the most compact form within the cell
- Plasmid DNA can generally be isolated by ultracentrifuge and electrophoresis on agarose gels

8.7 Transposons and Insertion Sequences

- Insertion sequences (IS) are the simplest type and carry no genetic information other than that required for them to move to new locations.
- IS are short segments of DNA, about 1000 nucleotides long, that can become integrated at specific sites on the genome.
- IS are found in both chromosomal and plasmid DNA, as well as in certain bacteriophages.
- IS have been characterized, and are designated by a number identifying its type IS/, IS2, IS3, and so on.

The Mechanism of Transposition



Mutagenesis with Transposable Elements

- If the insertion site for a transposable element is within a gene, insertion of the transposon will result in mutation .
- Transposons provide a facile means of creating mutants throughout the chromosome.
- The most convenient element for transposon mutagenesis is one containing an antibiotic resistance gene.

- Clones containing the transposon can then be selected by the isolation of antibiotic-resistant colonies.
- If the antibiotic-resistant clones are selected on rich medium on which all auxotrophs can grow, they can be subsequently screened on minimal medium supplemente with various growth factors to determine if a growth factor is required.

The *Escherichia coli* Chromosome

1. Transformation, transduction, and conjugation, can be used to map the locations of various genes (actually mutations in genes) on the chromosome.
2. In Escherichia coli genes were mapped to a particular region of the chromosome using conjugation.
3. By using Hfr strains with origins at different sites, it is possible to map the whole bacterial gene complement.

Escherichia coli as a Model Prokaryote

Many factors have favored the use of Escherichia coli as the workhorse for studies of biochemistry, genetics, and bacterial physiology. even E. coli viruses have served as model systems of study.

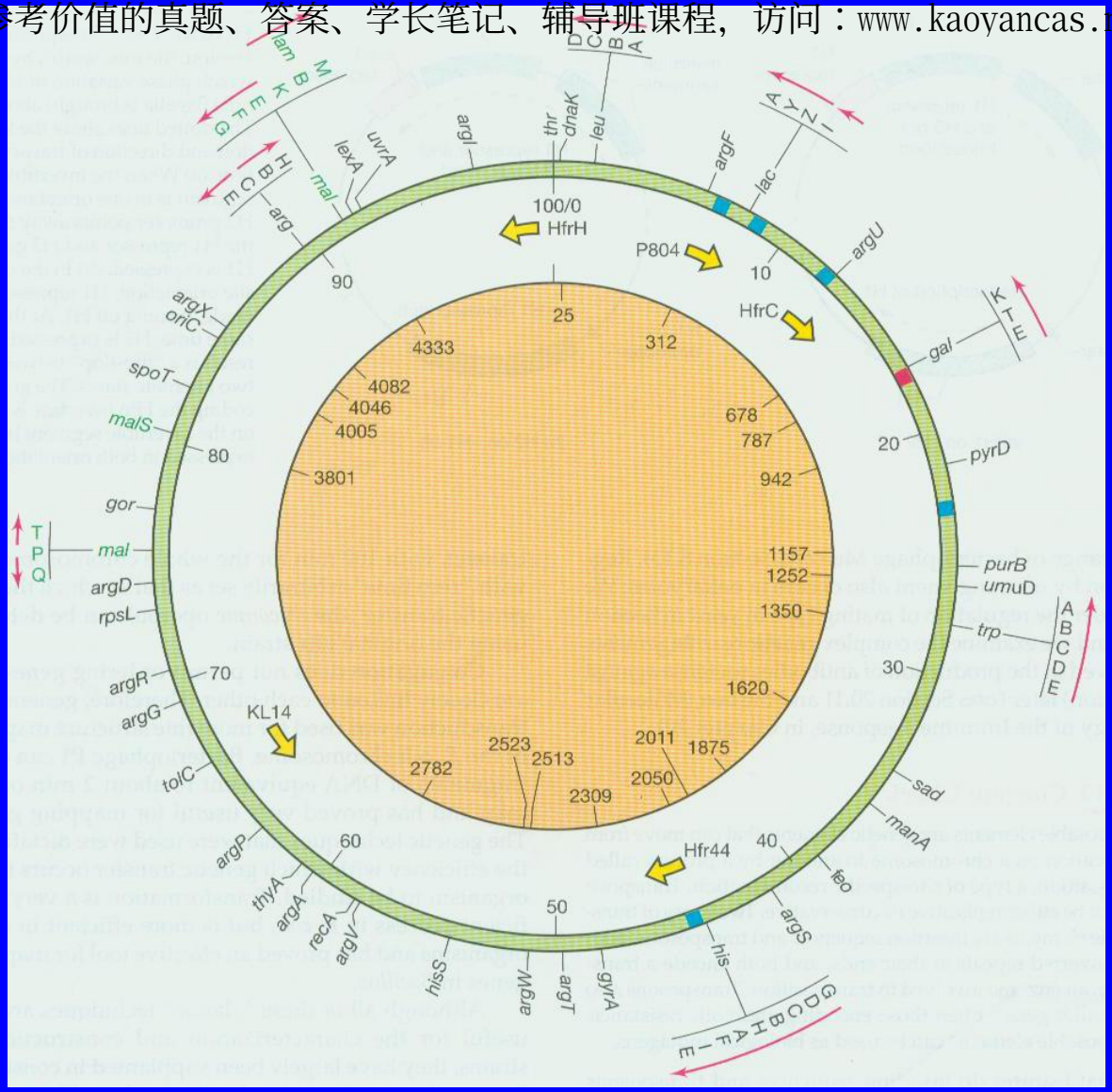
Arrangement and Expression of Genes on the *E. coli* Chromosome

Early mapping experiments and studies on the regulation of the genes that control the enzymes of a single biochemical pathway had shown that these genes were often clustered.

For instance:

- gal gene cluster at 18 min
- trp gene cluster at about 28 min
- his cluster at 44 min

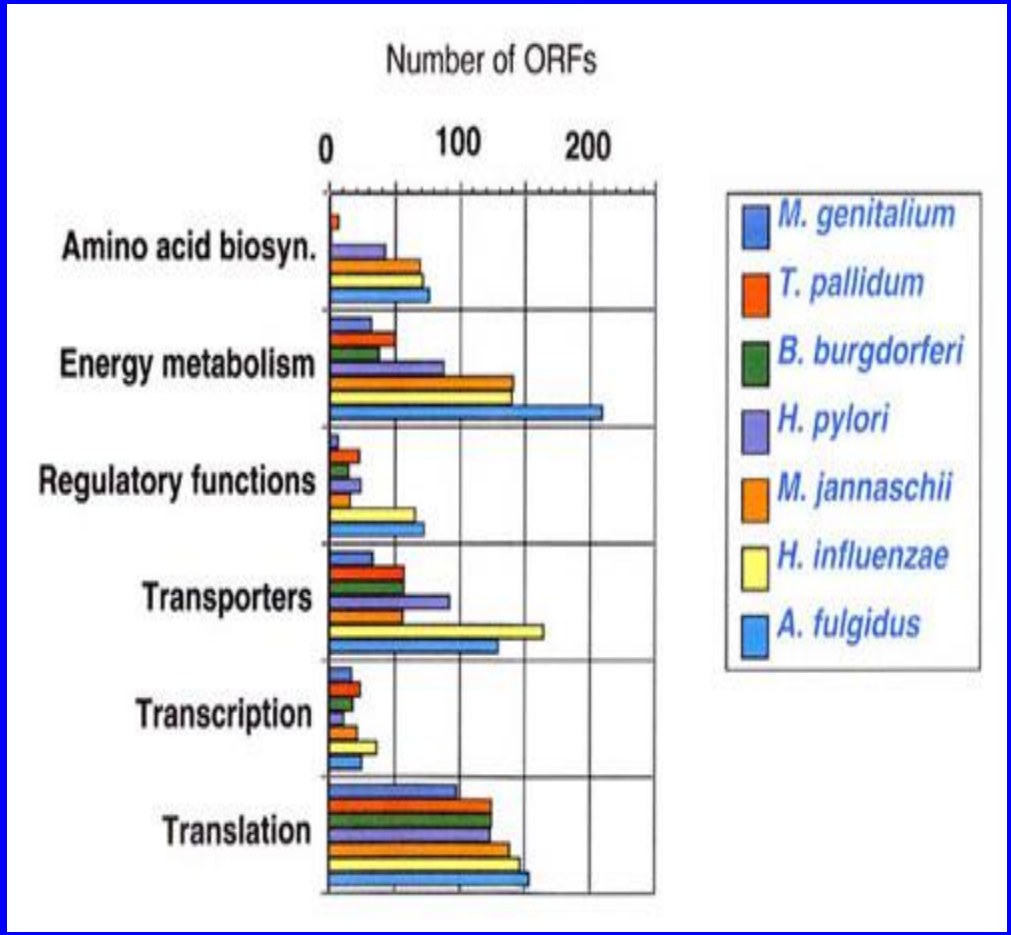
Each of these clusters is part of an operon and is transcribed as a single polycistronic mRNA



Circular Linkage Map of Chromosome of *E.coli* K-12

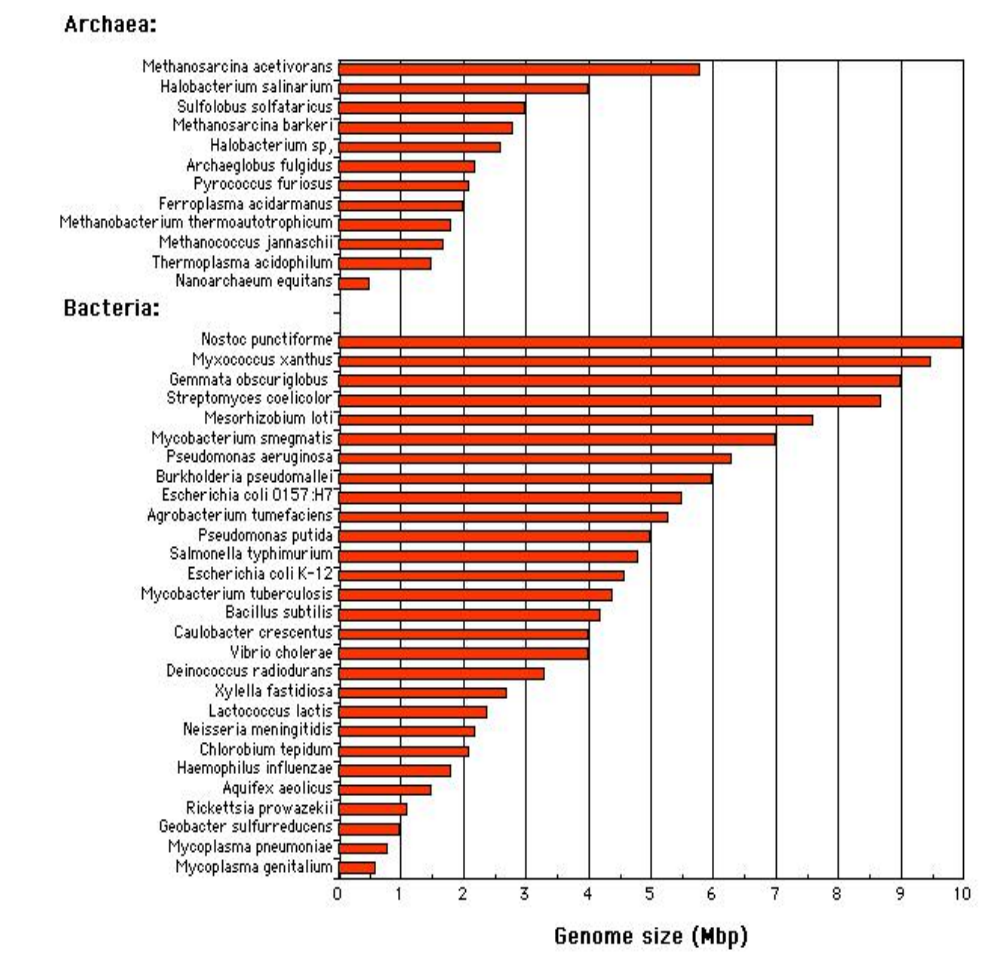
8.8 Comparative Prokaryotic Genomics

To interpret genome sequences, scientists first compare them to other entries in DNA sequence databases as shown in the figure (right). Using this concept of comparative genomics, clues to the functions of genes and how genomes change over time are discovered.



Sizes of Prokaryotic Genomes

There is tremendous diversity in the size and organization of prokaryotic genomes. The size of Bacteria chromosomes ranges from 0.6 Mbp to 10 Mbp, and the size of Archae chromosomes range from 0.5 Mbp to 5.8 Mbp.



Review question

1. Write a one-sentence definition of the term *genotype*. Do the same for the term *phenotype*. Does the phenotype of an organism automatically change when a change in genotype occurs? Why or why not? Can phenotype change without a change in genotype? In both cases, give some examples to support your answer.

2. What is site-specific mutagenesis? How can this procedure target specific genes for mutagenesis?

3. How does homologous recombination differ from site-specific recombination?

4. Why is it difficult in a single experiment using transformation to transfer a large number of genes to a cell?

5. List the similarities and differences between conjugation , transformation, and transduction

6. Strains of *Esclterichia coli* can be Hfr, F+, or F^-. What are the differences between these strains and how would they behave in a mating experiment?