Cellular & Molecular Biology
SQBS 1143

Chapter 6:
Gene Transfer in Bacteria

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Introduction

- Biologists think that sex serves the purpose of **reshuffling** (pertukaran) **genetic information** in the hope of producing offspring with combinations of genes superior to those of either parent.

- Because molecular biologists use bacteria as tools to carry most cloned genes, whether they are originally from corn or cockroaches, we must understand **how bacteria transfer** genetic information from one to another.

- Before we start, it is important to realize that sex and reproduction are not at all the same thing. In **animals**, reproduction normally involves sex, not in **bacteria**, and even in **plants**, these are two distinct processes.

- Bacteria divide by **binary fission**. First they replicate their single chromosome and then the cell elongates and divides down the middle. **No resorting** of the genes between two individuals (that is, no sex) is involved and so this is known as **asexual** or **vegetative reproduction**.

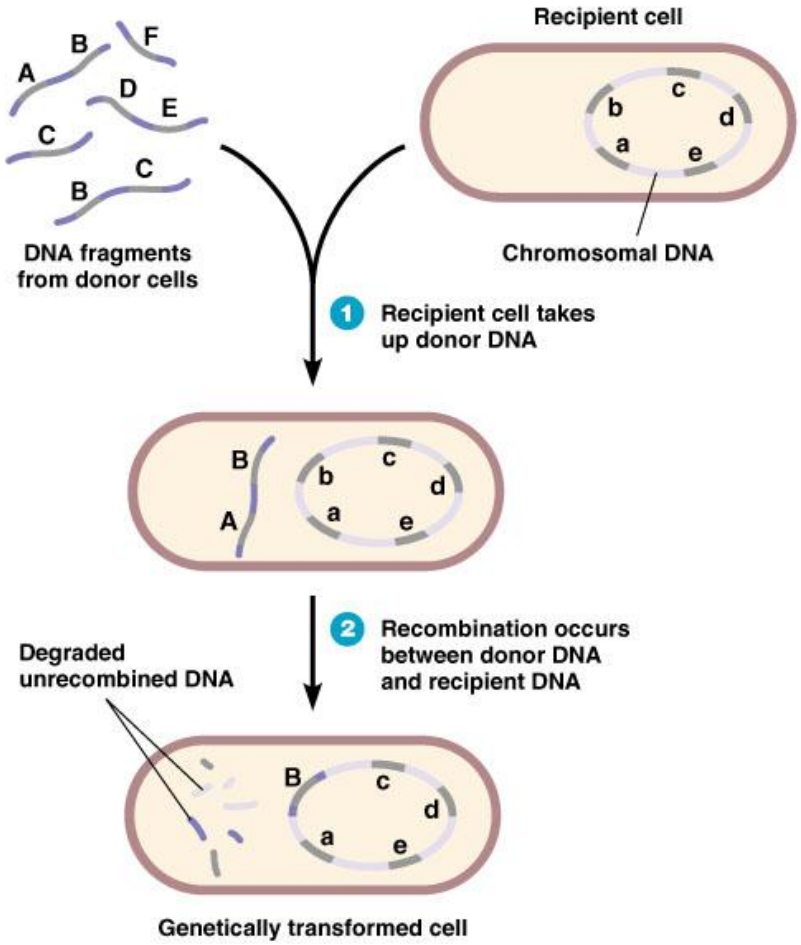
Naked DNA Transformation

- The broadest possible definition of sex means that genetic material is transferred from one partner to the other. The simplest conceivable version of sex would then consist **of transferring pure DNA from one cell to another**.

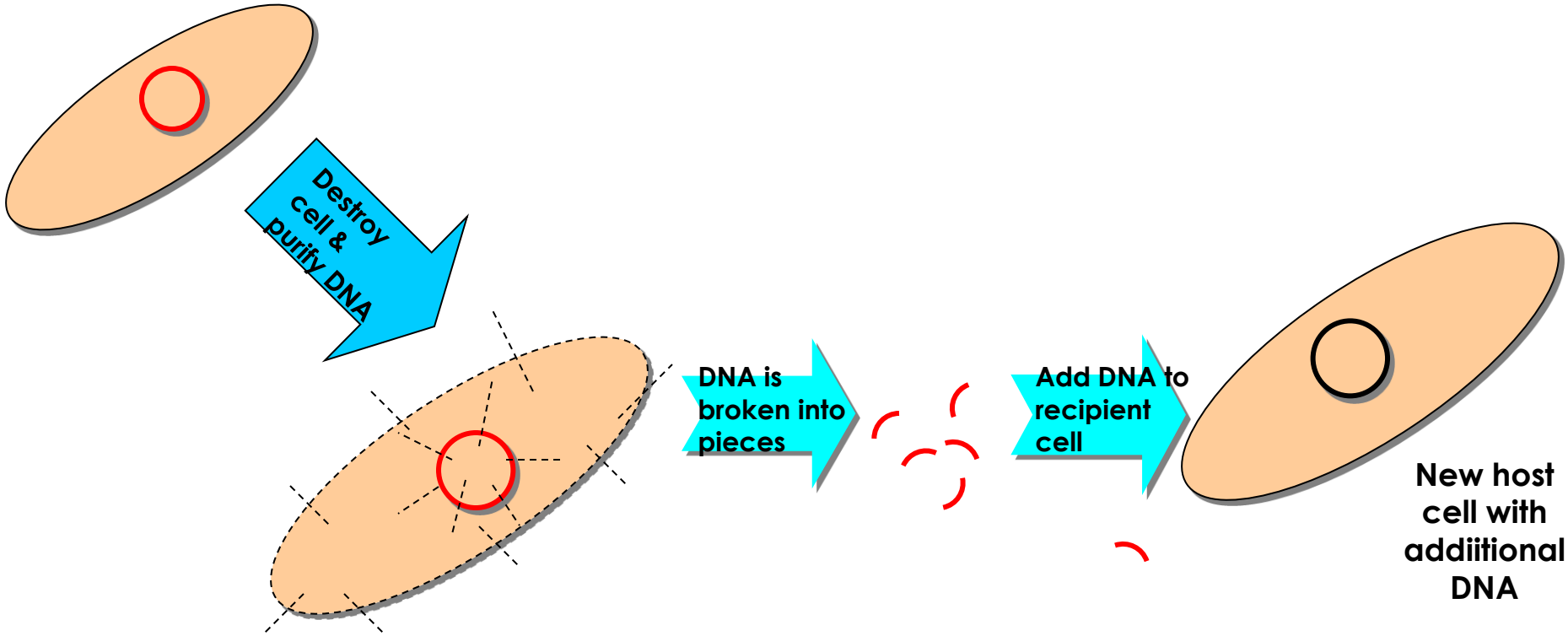
- Believe it or not, among the bacteria this is possible. **Bacterial cells** can **take up naked DNA molecules** and **incorporate the genetic information** they carry. This is referred to as **transformation**. But, please note that **no actual cell to cell contact** is allowed in transformation.

Transformation

- When a bacterial cell lyses, it releases its DNA into the environment
- DNA is transferred as naked DNA
  - DNA breaks into pieces on cell lysis
  - DNA is **taken up** by the recipient cell
  - A region of the recipient DNA is replaced by the donor DNA (**recombination**)
  - Unrecombined DNA is degraded



Principle of Transformation



Transformation is Used in Genetic Engineering

- After genes or other useful segments of DNA have been cloned in the test tube, it is almost always necessary to put them into some bacterial cell for analysis or manipulation. Thus, laboratory transformation technique is an essential tool in genetic engineering.

- Some bacteria **readily take up outside DNA**. If they can do this, they are said to be **"competent"**. Other bacteria must be modified in the laboratory before they will take up DNA.

Methods of Transformation

There are two ways of doing this:

- The older and conventional method is **to chill the bacterial cells in the presence of chemicals such as $CaCl_2$ that can damage their cell walls** and then to **heat shock** them briefly. This loosens the structure of the cell walls and allows DNA, a huge molecule, to enter.

- The modern, high-tech method is **electroshock treatment** called **electroporation**. Bacteria are placed in a machine called **electroporator** and zapped with **a high voltage discharge**.

Competence

- Transformation occurs naturally in very few genera of bacteria:
  - *Bacillus*, *Haemophilus*, *Neisseria*, *Acinetobacter*, and some strains of *Streptococcus* and *Staphylococcus*
- The recipient cell must be in a physiological state in which it can take up DNA - It must be competent!!
- **Competence:** alterations in the bacterial cell wall that make it permeable to large DNA molecules
  - Some bacteria, which are not normally competent, can be made so in laboratory

Real Life Transformation

- Does transformation actually happen in real life? **Yes**, probably it does, but only **at a very low level**. From time to time, bacteria in natural habitats die and disintegrate. In doing so, they release DNA which nearby cells may take up.

- Some bacteria simply take up any old DNA they find lying around. In practice, most bacteria need the kind of "friendly persuasion" described above before they will take up foreign DNA.

Recombination of Incoming DNA

What Happens to the DNA After Uptake?

- There are **two** possibilities, failure or success, known technically as **restriction** or **recombination**. Both of these processes apply to a wide range of either situations too, so they are dealt with in detail elsewhere.

- **Restriction** is the **destruction of the incoming foreign DNA**. Most bacteria assume that foreign DNA is more likely to come from an enemy, such as a virus, than from a friend and they chop it up into small fragments with so called **restriction enzyme**. In this case transformation fails.

- Only DNA that has been **modified** by closely related bacteria by adding the correct chemical tags is accepted as friendly. The chemical tag is normally in the form of a methyl (CH_3) group.

Recombination

- **Recombination** is the **physical incorporation of some of the incoming DNA into the bacterial chromosome**. If this happens, **some** of the host cell's genetic information is **replaced** with genes from the incoming DNA and the bacteria are permanently transformed.

- The original version of the genes is lost. If a gene enters a bacterial cell on a fragment of linear DNA, it must be recombined onto the host chromosome in order to survive.

- If the incoming DNA is part of a **plasmid** which can replicate on its own, recombination into the chromosome is not necessary. In practice, it is usually convenient to **avoid recombination**.

- Consequently, molecular biologists normally put the genes they are working onto **plasmids**.

DNA Transfer in Bacteria

- Transformation
- Transduction
- Conjugation: Plasmid Transfer
- Conjugation: Chromosome Transfer

Hitchhiking by Virus - Transduction

- When a virus succeeds in infecting a bacterial cell, it **manufactures** more virus particles, each of which should contain a new copy of the viruses own genes. But life is rarely perfect, and sometime mistakes happen, even to viruses.

- Occasionally, instead of packaging virus DNA into the virus particle, **fragments of bacterial DNA get packaged**.

- From the viewpoint of the virus, this results in a **defective particle**. Nonetheless, such a virus, carrying bacterial DNA, may **go on to infect another bacterial cell**.

- If so, instead of injecting viral genes, it injects DNA from the previous bacterial victim. This DNA can be destroyed by restriction or incorporated by recombination, as in the case of transformation (see above). If it is successfully incorporated, then **transduction** has occurred.

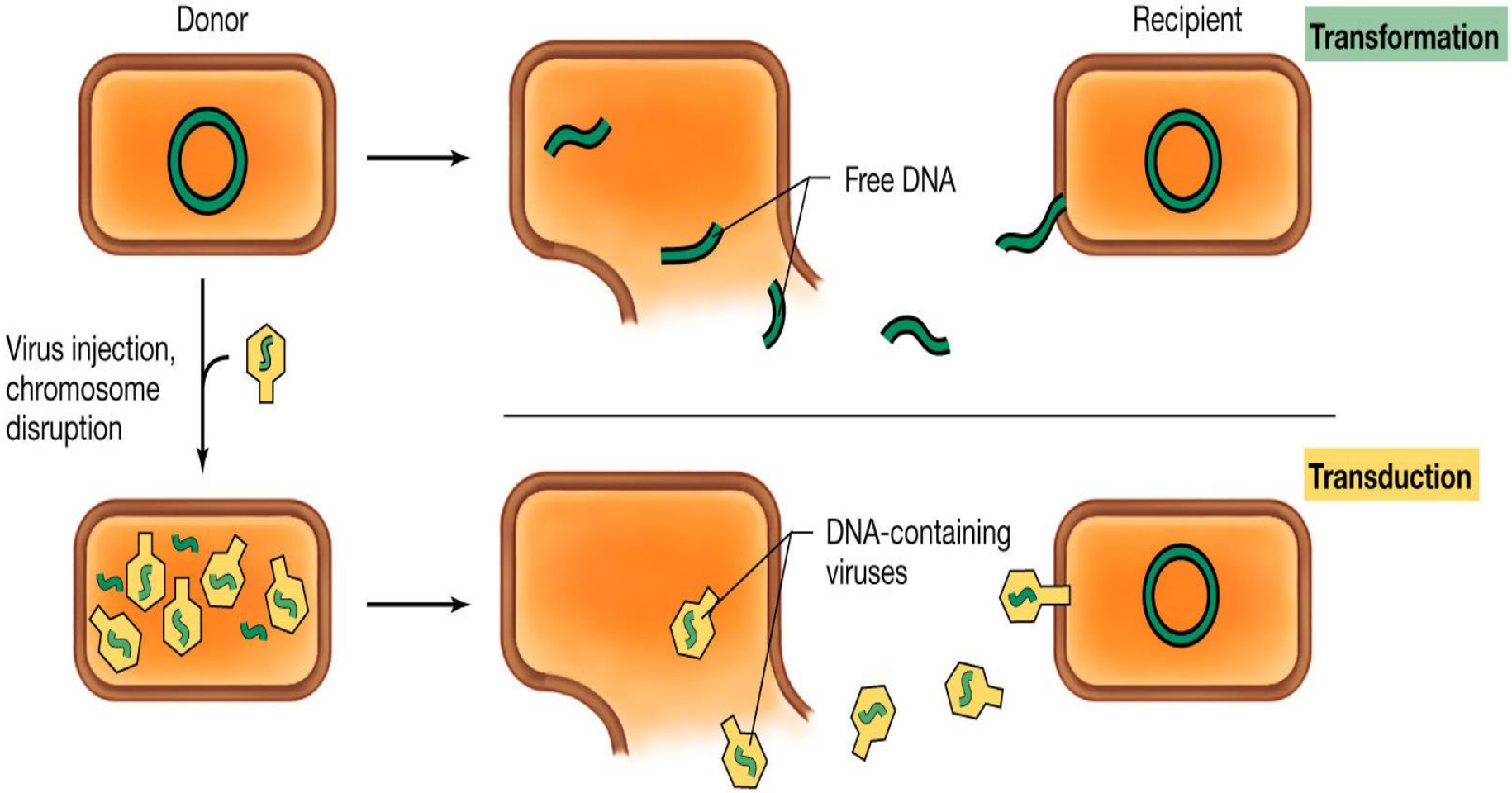
- Bacterial geneticists routinely carry out gene transfer between different bacteria by transduction using bacterial viruses, known as **bacteriophage** (or **phage** for short).

- If the bacterial strains are closely related, the incoming DNA is accepted as "friendly" and is not destroyed by restriction. In practice, transduction is the simplest way to replace a few genes of one bacteria with those of a close relative.

- To carry out a transduction, a bacteriophage is grown on a culture of the bacterial strain. These bacteria are destroyed by the phage, leaving behind only DNA which carries some of their genes and is now packaged into the phage particles.

- This phage sample can be stored in the fridge for weeks or months before use. Later, the phage are mixed with a recipient bacterial strain and the DNA is injected. Most **recipients get genuine phage DNA** and are **killed**. However, **others get donor bacterial DNA** and are **successfully transduced**.

- The best known examples are the use of phages to transduce the bacterium, *Escherichia coli*. Different bacteriophages behave differently. The two favorite bacterial phages are and **P1** and **lambda** (λ).


Donor
Recipient
Transformation
Free DNA
Virus injection,
chromosome
disruption
Transduction
DNA-containing
viruses

Viral Reproduction: (1) The Lytic Cycle

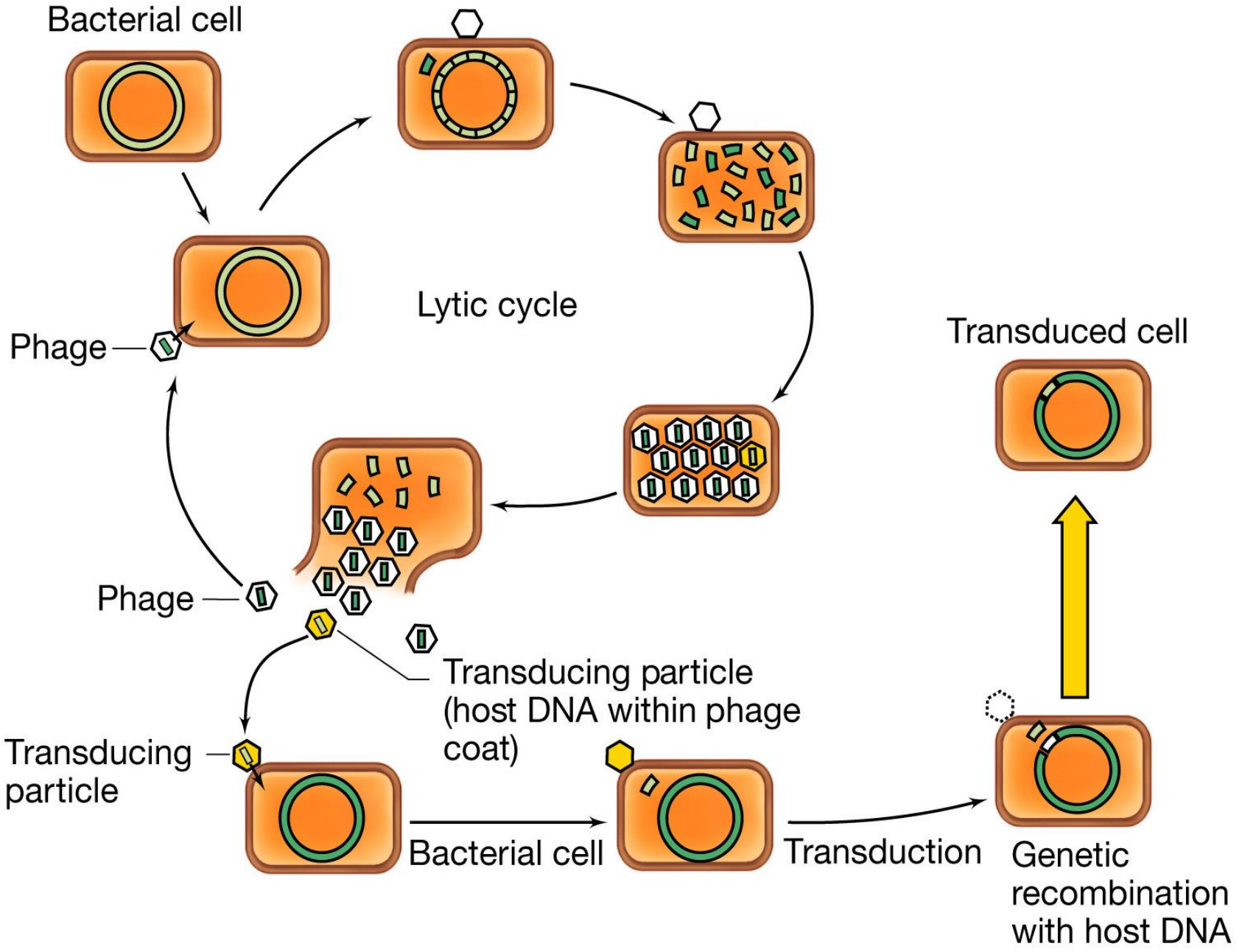
Generalized schematic for viral reproduction in a host bacterium, through the lytic cycle.

In the lytic cycle, the virus (phage) **multiplies in the host cell** and the progeny viruses are **released by lysis** of cell.

Viral Reproduction: (2) The Lysogenic Cycle

Generalized schematic for viral reproduction in a host bacterium, through the lysogenic cycle.

In the lysogenic cycle, **viral DNA is integrated into the host genome** and **replicates** as the chromosome replicates, producing lysogenic progeny cells.

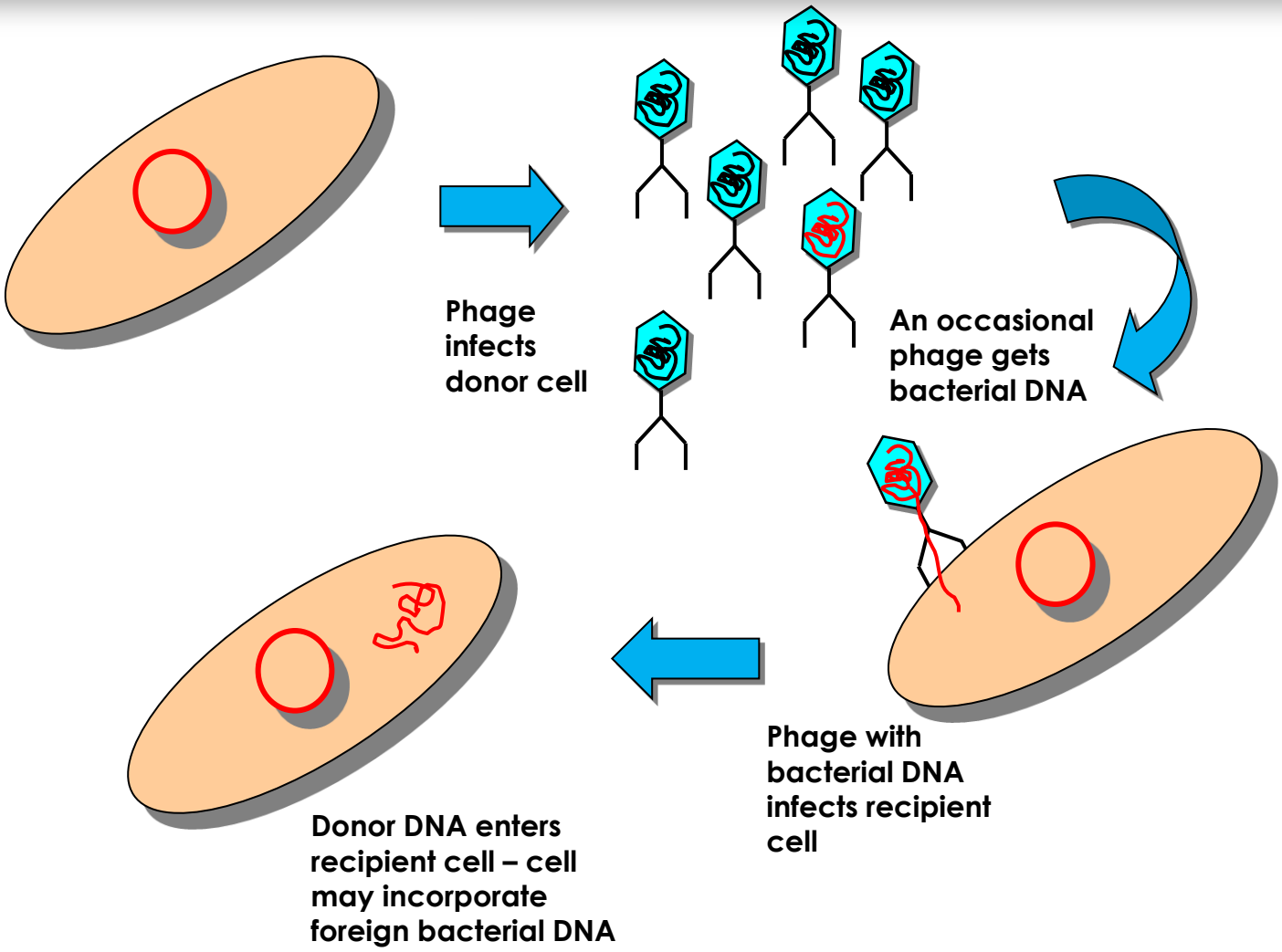

Bacterial cell
Lytic cycle
Phage
Phage
Transducing particle (host DNA within phage coat)
Transducing particle
Bacterial cell
Transduction
Transduced cell
Genetic recombination with host DNA

(1) Generalized Transduction

- In **generalized transduction**, random fragments of bacterial DNA are picked up by the virus; for example by **bacteriophage PI**. All bacterial genes have an equal chance of being transferred. P1 makes a mistake by packaging bacterial DNA instead of its own only about once every 10,000 times.

- Phage infects donor bacterial cell. An occasional phage gets bacterial DNA. Phage with bacterial DNA infects recipient bacterial cell. Donor DNA enters recipient bacterial cell. The recipient cell may incorporate foreign bacterial DNA.

Mechanism of Generalized Transduction



- Each **P1** particle **can carry 90 kb of DNA** which is equivalent to about **2 percent** of a bacterial chromosome. So, **any individual gene** will be transduced by one in 500,000 of the P1 particles resulting from any particular infection.

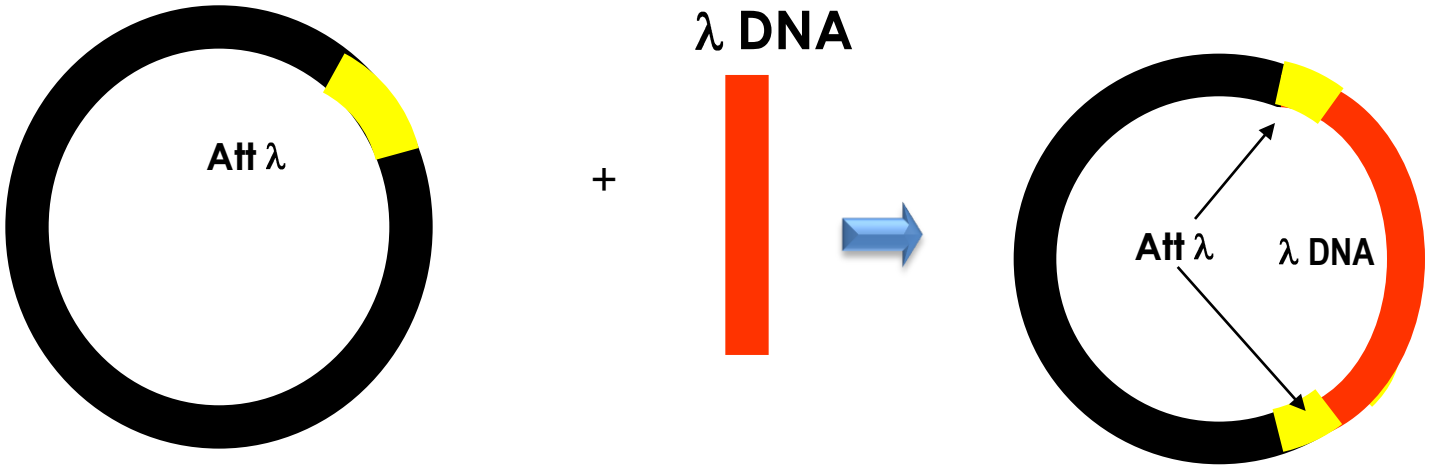
- In practice, a typical sample of P1 contains about a thousand million virus particles per milliliter. So, there is actually plenty of opportunity for transduction to happen.

(2) Specialized Transduction

- In **specialized transduction**, certain specific regions of the bacterial chromosome are favoured. For example, when bacteriophage **Lambda (λ)** infects *E. coli*, it sometimes inserts its DNA into the λ chromosome.

- This occurs at a single specific location known as the **lambda attachment site (att λ)**. When Lambda multiplies, the original donor cell is destroyed, and several hundred virus particles containing Lambda DNA are produced. Just as with P1 (explained in previous slide), **a small fraction of virus particles end up containing bacterial DNA**.

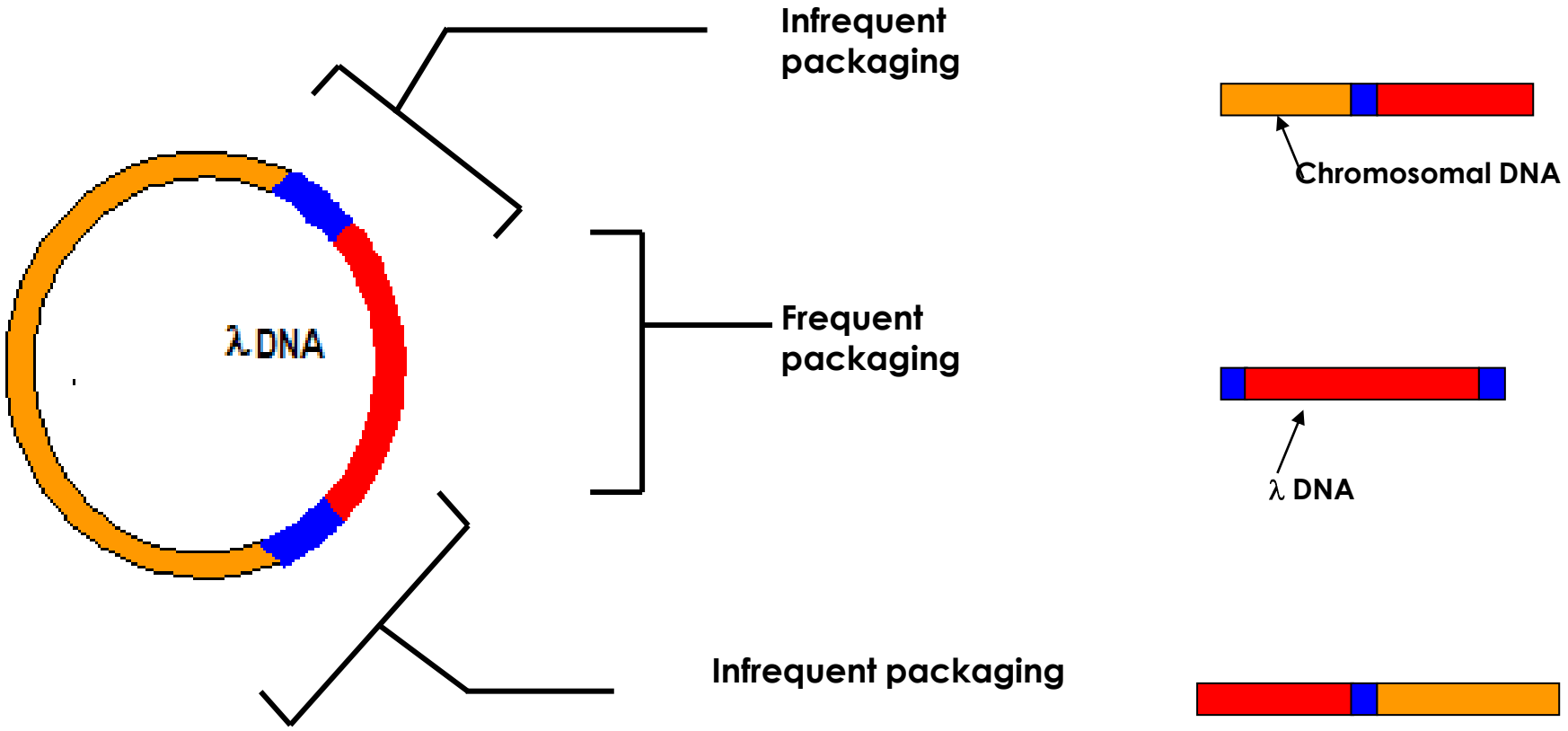
Insertion of Lambda into a Chromosome



There are two differences from the case of P1.

- First, the **transducing particles** contain a mixture of Lambda DNA and chromosomal DNA.
- Second, only chromosomal genes next to the Lambda attachment site are transduced by Lambda (see **SPECIALIZED TRANSDUCTION BY λ**).

Specialized Transduction by λ



Is There Real Sex in Bacteria? Conjugation …

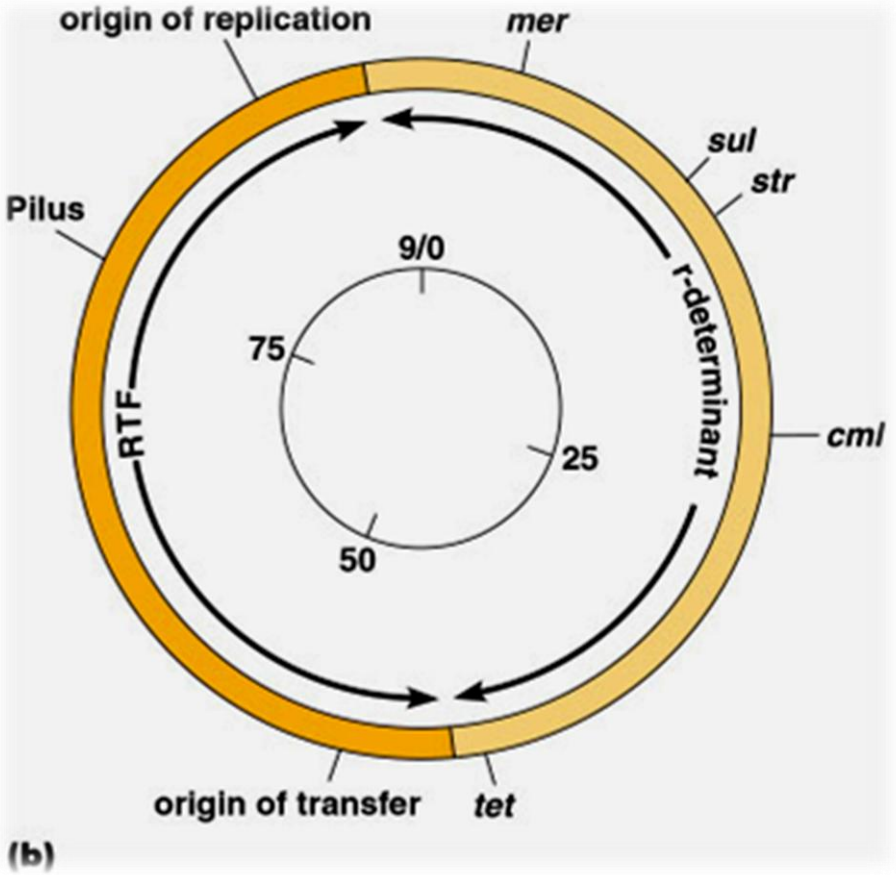
- We've given you naked DNA and viral transmission of genetic information. But, you ask, what about genuine sexual contact between bacteria?

- Yes, there is, and is known as **conjugation**. This involves two cells, a **female recipient cell** and a **male donor cell**.

- The male has a long hollow, tubular organ referred to as **sex pilus**.

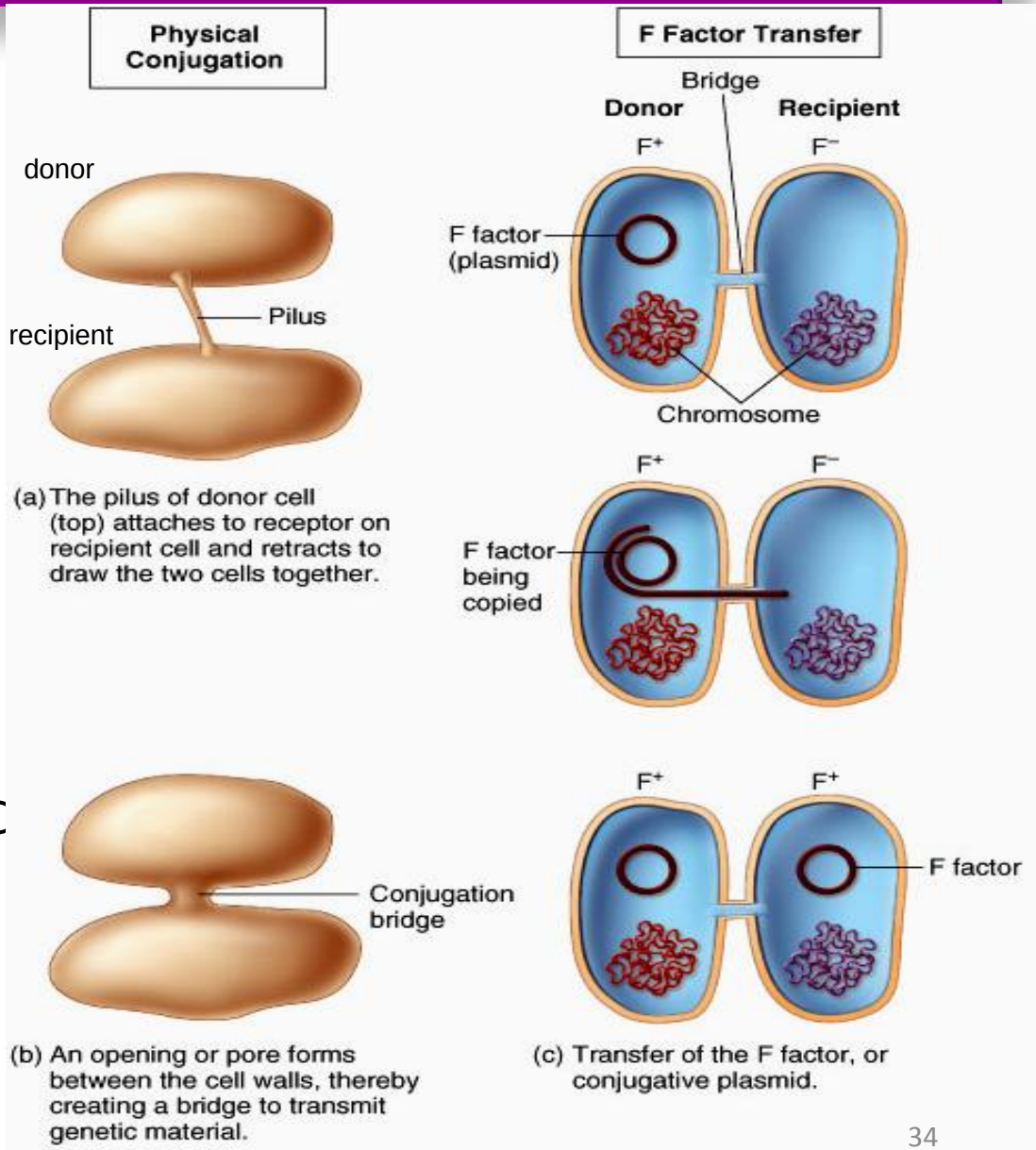
Plasmids

- Small, circular molecules of DNA
- Replicate independently of the chromosome
- Usually dispensable for growth, but under some conditions provide a selective advantage such as antibiotic resistance or a unique metabolic pathway
- **Conjugative plasmids** carry genes for conjugation including sex pili



Mechanism of Conjugation

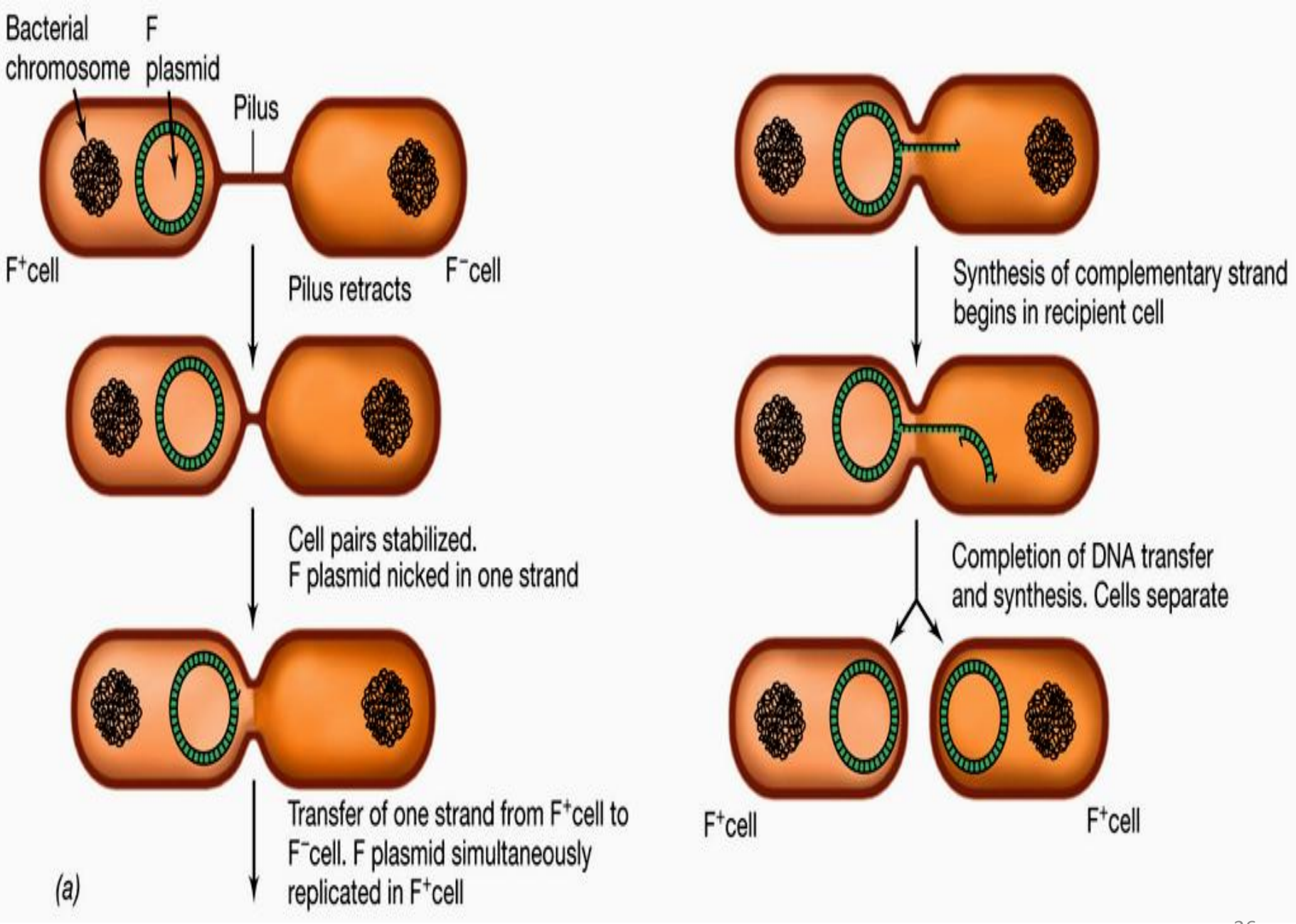
- Donor contacts recipient, attaches using sex pilus;
- F-factor initiates transfer of a copy of itself;
- Recipient is converted to a new donor cell



- The male cell uses the pilus as a grappling hook to grab the female and pull her alongside, rather like a boat hook is used to grab a dinghy.

- The two cells then form a **conjugation bridge** when they touch and DNA goes from male into the female.

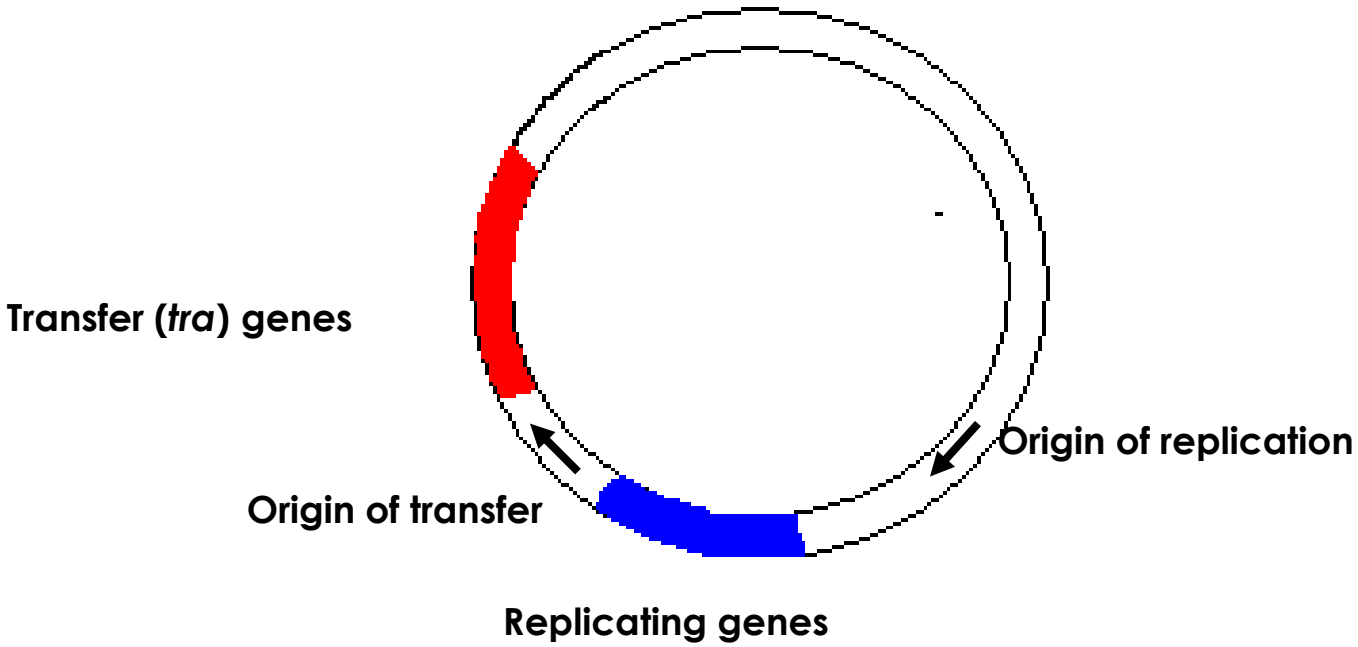
- In practice, mating bacteria snuggle together in groups of five to ten.


Bacterial chromosome
F plasmid
Pilus
F+cell
F−cell
Pilus retracts
Cell pairs stabilized.
F plasmid nicked in one strand
Transfer of one strand from F+cell to F−cell. F plasmid simultaneously replicated in F+cell
(a)
Synthesis of complementary strand begins in recipient cell
Completion of DNA transfer and synthesis. Cells separate
F+cell
F+cell

- Bacteria are **mostly female**. To be a **male** bacterium you need a personal improvement kit. This is known as the ***tra* (transfer) system** and the genes for this come on a separate DNA molecule known as a plasmid.

- Plasmids are **circular molecules** of DNA that can **replicate in bacterial cells** rather like miniature chromosomes. However, they are much **smaller** than bacterial chromosomes and are not essential for cell growth and **survival** under normal conditions.

F-Plasmid with A *tra* System



- Plasmids may carry a variety of genes that confer extra abilities on the bacteria containing them.

- Plasmids which make a cell male a called **fertility plasmids**; the most famous of these is the **F-plasmid** of *E. coli*.

- Sometimes, the male donor cells are known as **F^+** and the female recipient **F^-** to indicate that their **role in conjugation** is **determined by the presence or absence** of the F-plasmid.

Replication During Plasmid Transfer

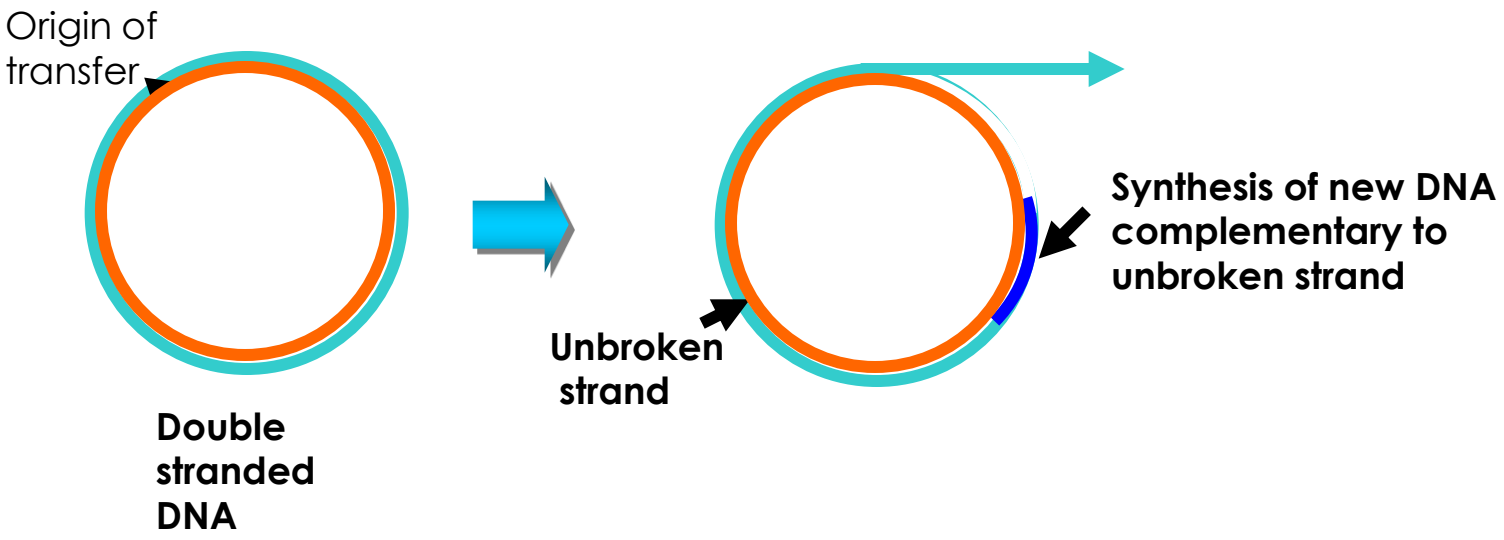
We have talked about plasmid transfer as if the whole F-plasmid simply leaves the original male cell and moves into the recipient cell. In fact, only one strand of the F-plasmid DNA is transferred. The details are as follows:

- One of the two strands of the double stranded DNA of the F-plasmid opens up at the **origin of transfer**.
- This linearized single strand of DNA moves through the conjugation bridge into the female cell.

- An **unbroken singles stranded circle** of F-plasmid DNA remains inside the donor cell. This is used as a template for the synthesis of a new second strand to replace the one that just left.

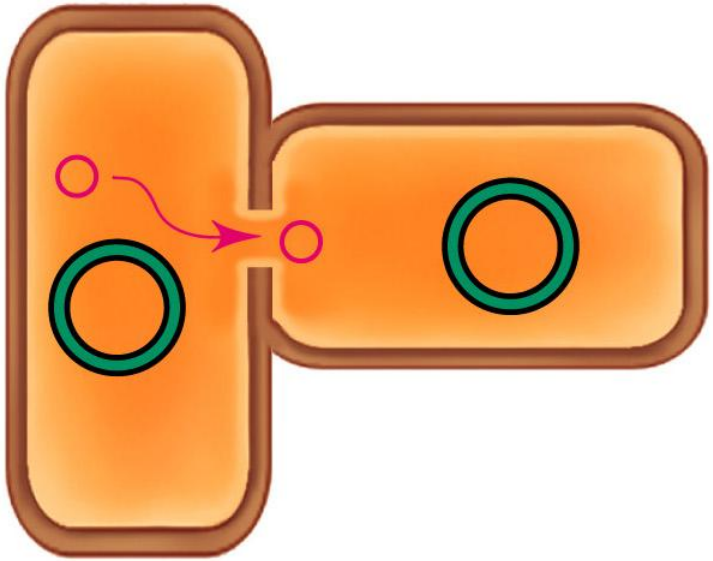
- As **the linearized single** strand of F- plasmid DNA enters the female cell, a new **complementary strand** of DNA is **made** using the incoming strand as template.

- When the female cell has received the F-plasmid it becomes F^+, in other words, a male! Consequently, bacteria stay together for an even shorter time than most Hollywood marriages!


Origin of transfer
Double stranded DNA
Unbroken strand
Synthesis of new DNA complementary to unbroken strand

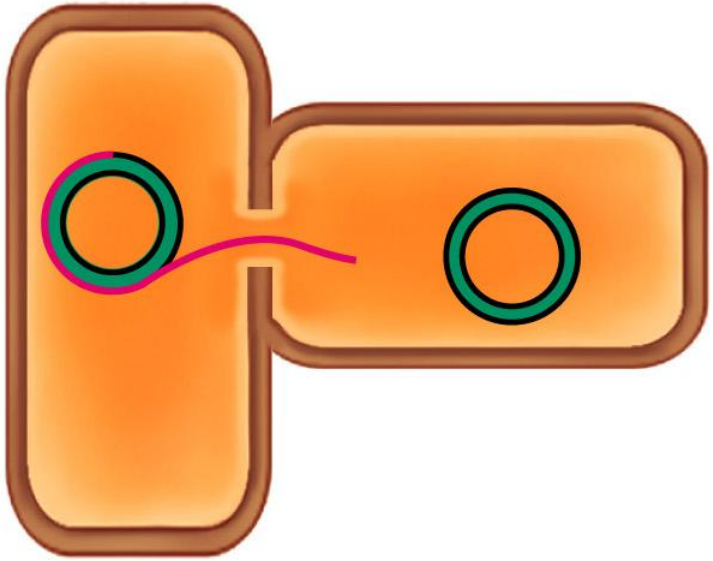
Conjugation: Plasmid transfer

Plasmid-containing donor



Conjugation: Chromosome transfer

Donor cell with integrated plasmid

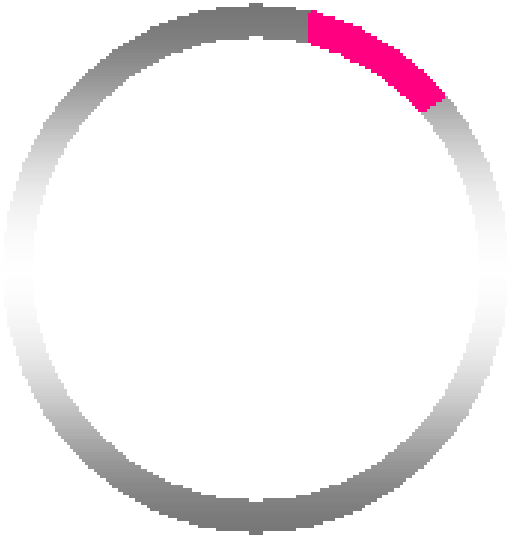


Transposition

- In order to **transfer chromosomal genes**, a plasmid must first physically integrate itself into the chromosome of the bacterium. The process of **integration needs pairs of identical or nearly identical DNA sequences**, one on the plasmid and the other on the bacterial chromosome.

- **Transposons**, or **transposable elements**, are segments of DNA that can move as a unit from one location to another. They are always inserted into other DNA so they are never free as separate molecules. They are sometimes called "**jumping genes**" because they hop around from place to place on the chromosome.

Transposon



PLASMID

CHROMOSOME

- The process of jumping from one DNA molecule to another is called **transposition**. Simple transposons cannot replicate themselves. So, a transposon is even less in control of its own destiny than is a plasmid.

- As long as the DNA molecule of which the transposon is part gets replicated, the transposon will also be replicated. If the transposon guesses wrong and inserts itself into a DNA molecule with no future, the transposon dies with it.

The Essential Parts of a Transposon

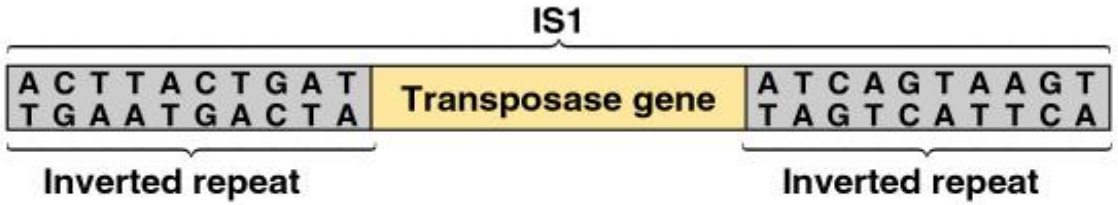
- The simplest transposons, known as **insertion sequence (IS)**, were first found in bacteria. They have two vitally important characteristics.

- First, they have **inverted repeat at either ends**. This means that the sequence of the DNA at one end the same as that at the other end as long as you read it backwards and on the either strand.

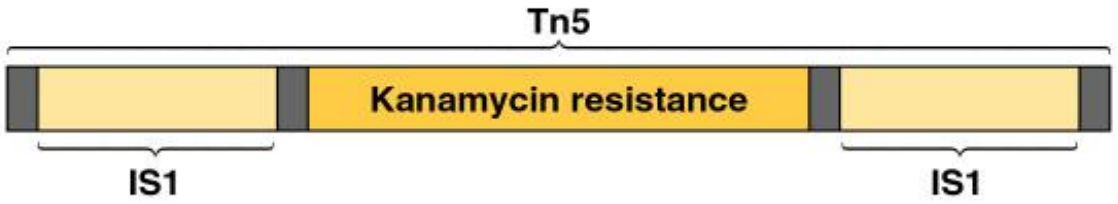
- Second, insertion sequences have just one **gene** that encodes the **transposase**, the enzyme needed for movement.

Inverted Repeats

PARTS OF AN INSERTION SEQUENCE (IS)



(a) Insertion sequence "IS1"



(b) Complex transposon "Tn5"

Transposons

- **Segments of DNA** that can **move from one region of DNA to another** and **integrate** through non-homologous recombination
- Contain information for their own transposition:
  - **Transposase enzyme** for cutting and resealing DNA
  - **Short terminal repeats** which the transposase recognizes as recombination sites
- Insertion sequences are the simplest transposons
- **Complex transposons** carry other genes, e.g. antibiotic resistance genes

- Typical insertion sequences are 750 to 1,500 base pairs (bp) long with terminal inverted repeats of 20 to 40 bp. **Insertion sequence** are found in the chromosomes of bacteria and also in the DNA of their plasmids and viruses.

- When plasmid and chromosome possess identical sequences, this allows integration of the plasmid into the host chromosome.

Composite Transposons

Structures of some bacterial transposable elements.

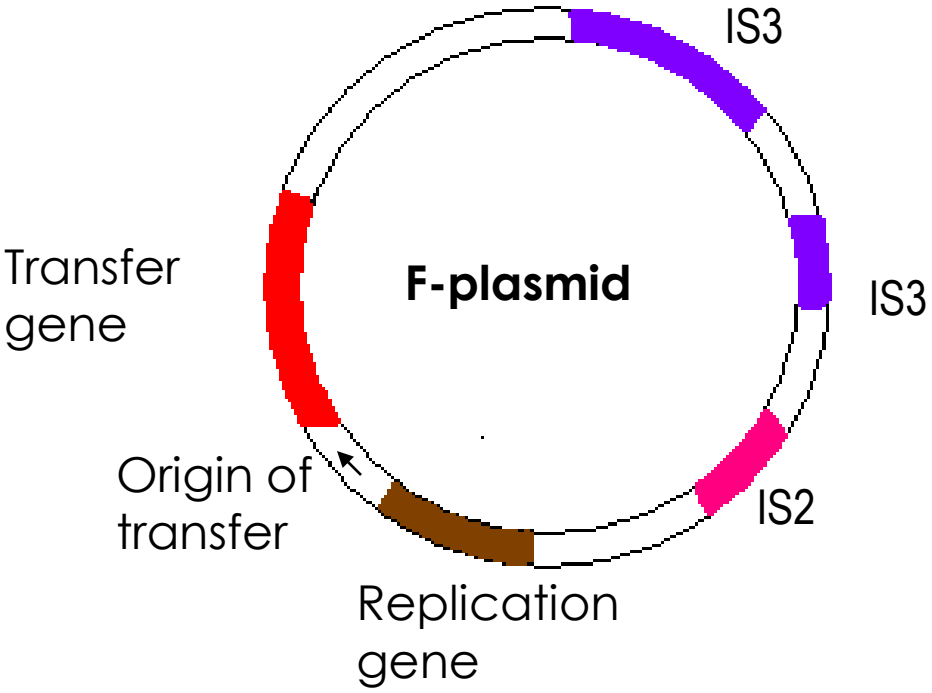
(A) A composite transposon contains antibiotic genes flanked by two insertion sequences as direct or inverted repeats. Shown here is the **Tn5 transposon**, with inverted repeats.

(B) The **Tn3 transposon**.

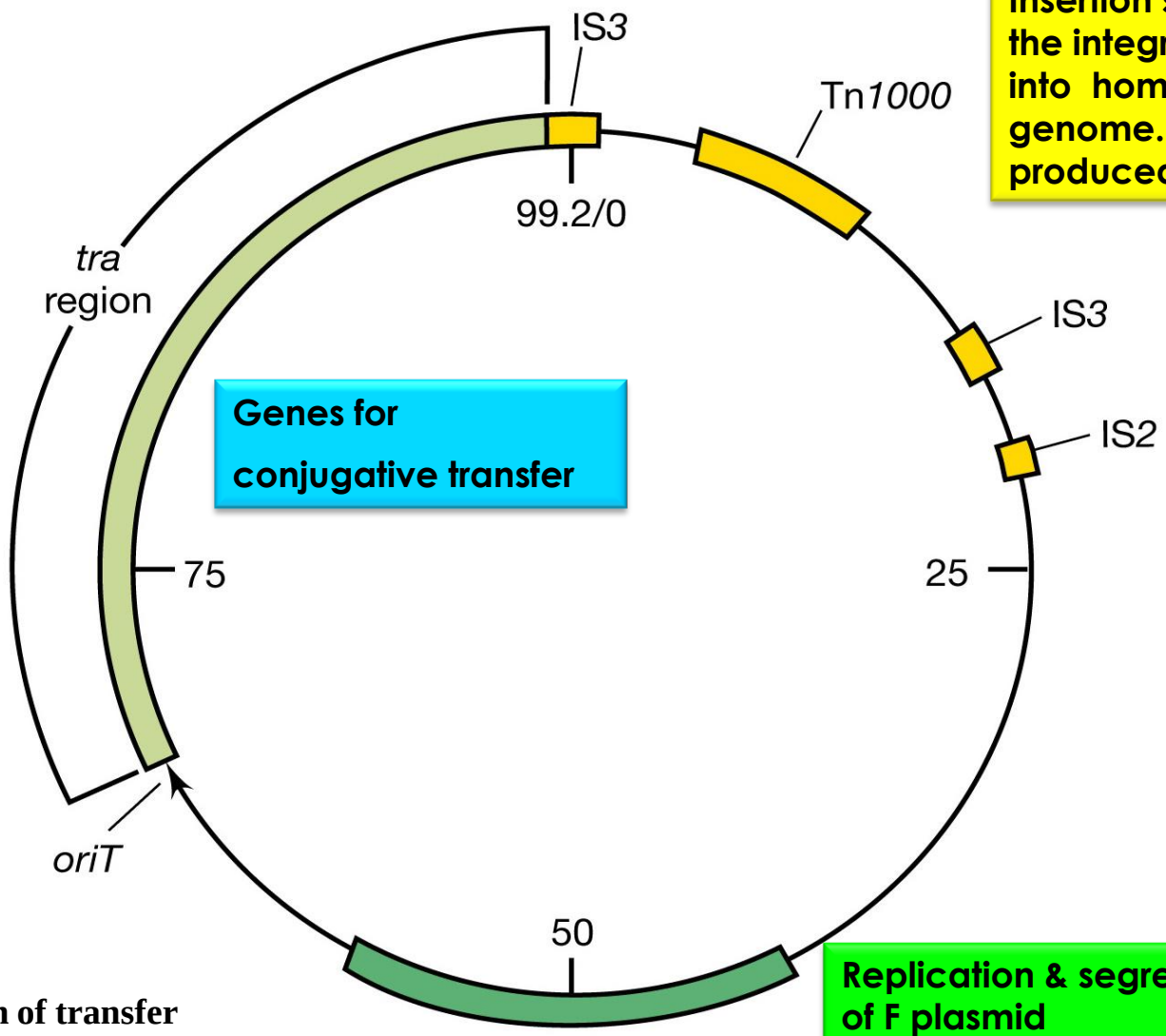
- This, in turn, **allows transfer of chromosomal genes by the F-plasmid** as explained. The chromosome of *E. coli* has seven copies of IS1, 13 copies of IS2 and six copies of IS3 scattered around it more or less at random.

- The **F-plasmid**, which is roughly one-fiftieth as big, **has three insertion sequences** : zero copies of IS1, one copy of IS2, and two copies of IS3.

Insertion Sequences on F-plasmid



Insertion sequences (IS) assist in the integration of transposons (Tn) into homologus sites of recipients genome. Different Hfr strains are produced as a result



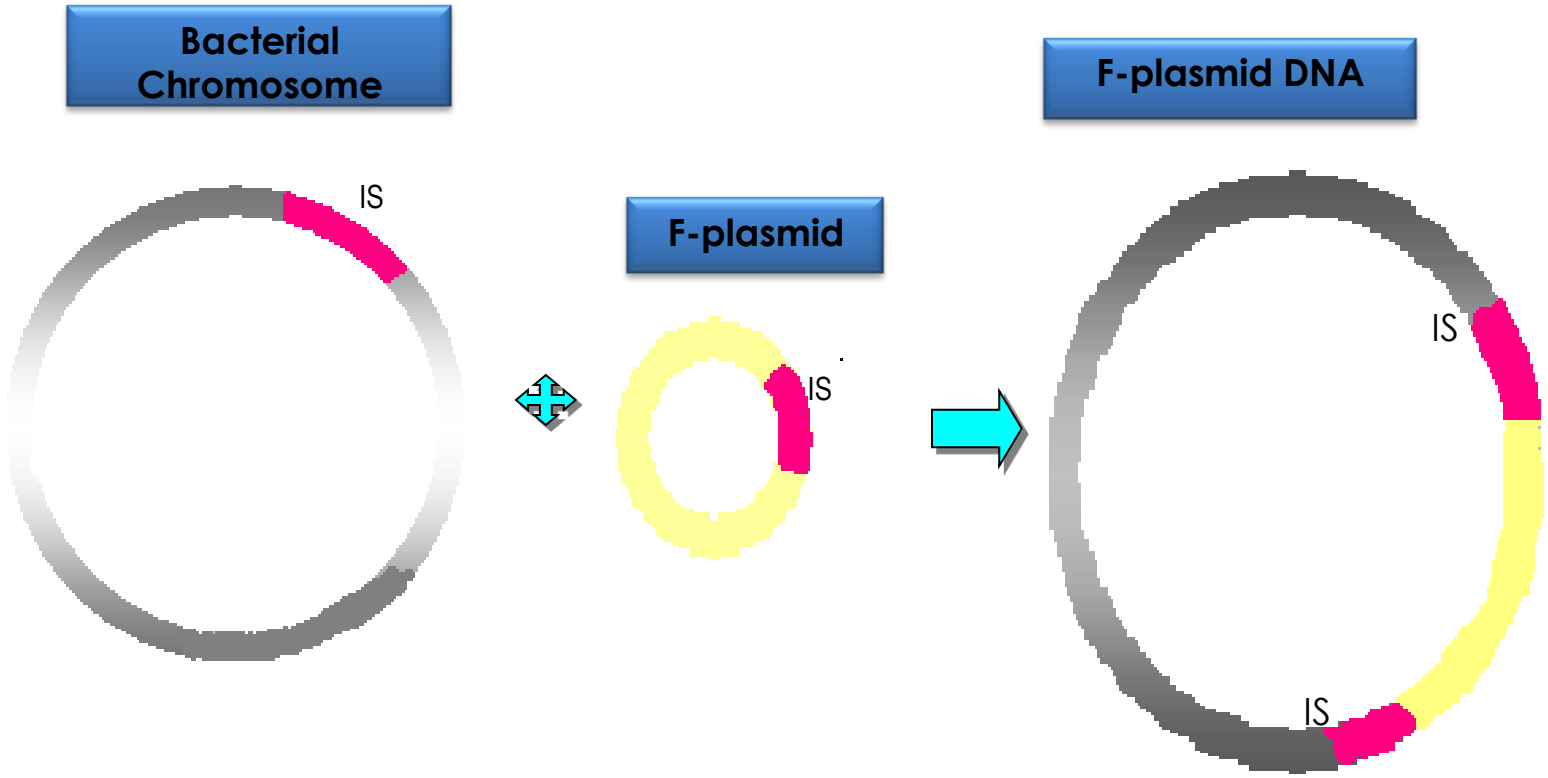
99kb F (Fertility) Plasmid Genetic Map (*E. coli*)

- Consequently, **integration** of F-plasmid can occur at the IS2 or IS3 sites, a total of 19 sites scattered around the chromosome. Integration of the F-plasmid may occur in **either orientation**.

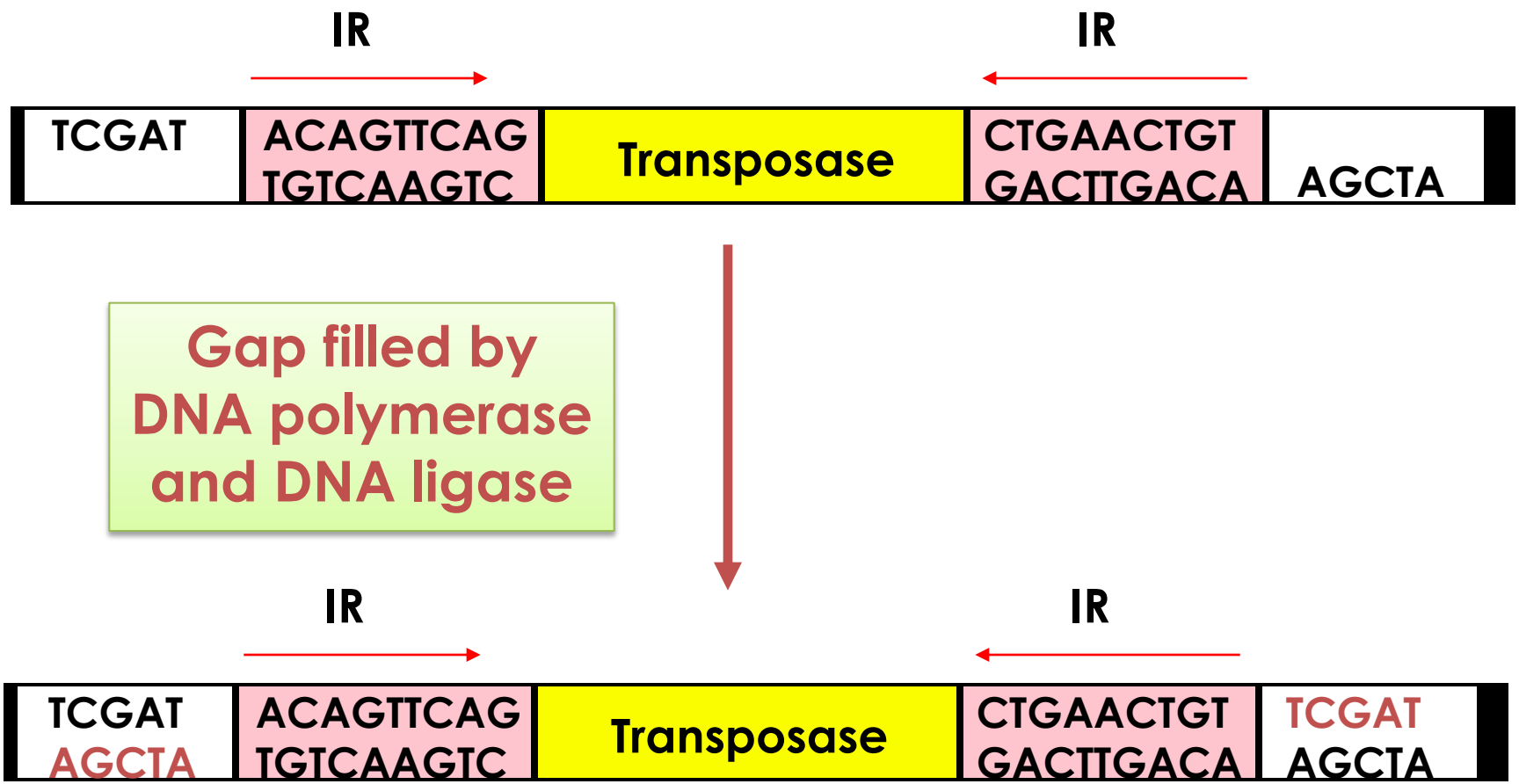
- When an F-plasmid that is integrated into the chromosome is **transferred by conjugation**, it drags along the chromosomal genes to which it is attached.

- Just as before, only a single strand of the DNA moves and the recipient cell as to make the complementary strand itself.

F-plasmid Inserts into Bacterial Chromosome



Transposition Mechanism



- Consequently, bacterial strains with an F-plasmid integrated into the chromosome are known as **Hfr-strains** because they **transfer chromosomal genes at high frequency**.

- A prolonged mating of 90 minutes or so is needed to transfer the whole chromosome. More often, bacteria break off after a shorter period of, say, 15 to 30 minutes, and **only part of the chromosome is transferred**.

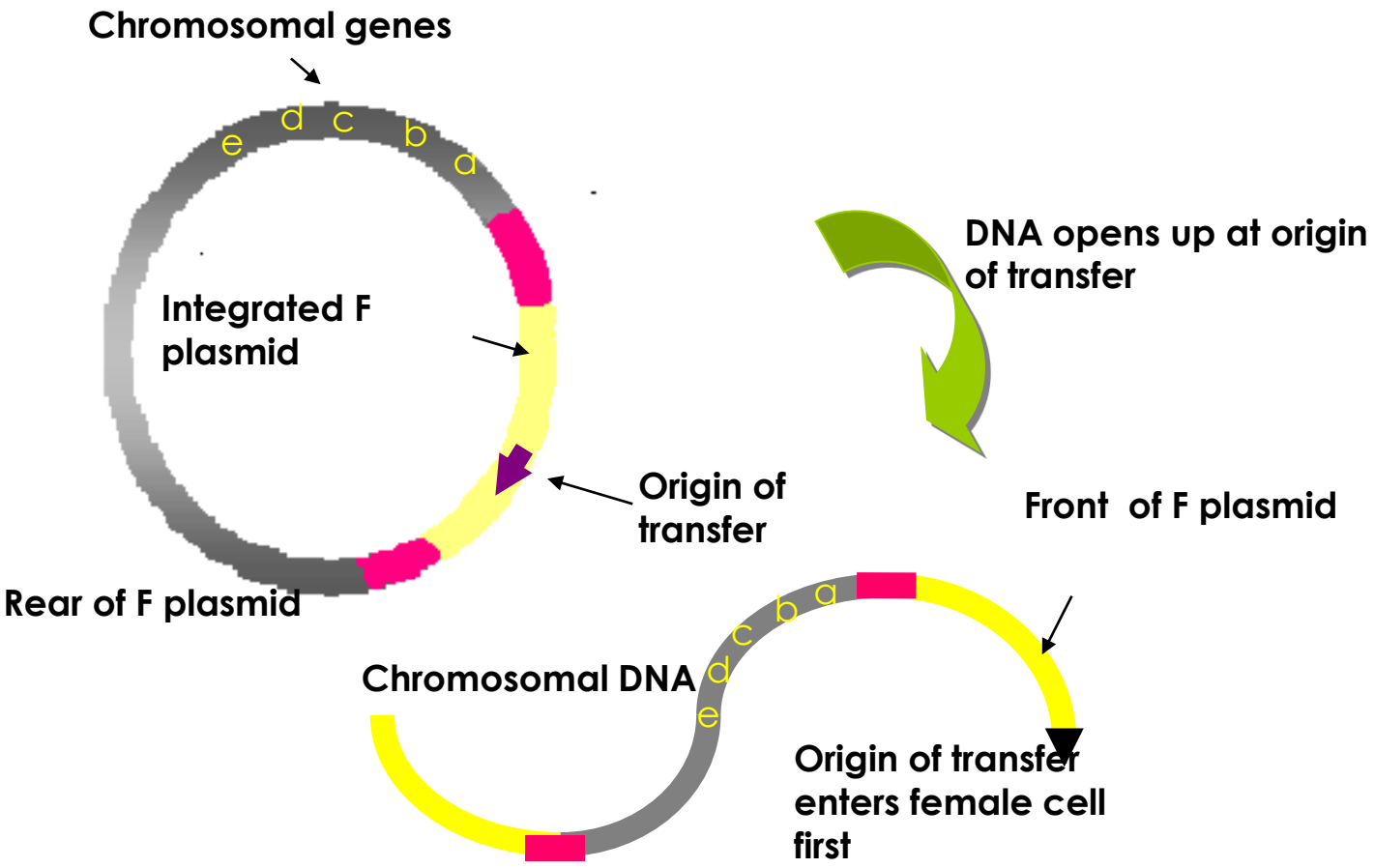
- Since different Hfr-strains have their F-plasmids **inserted at different sites** on the bacterial chromosome, they **start their transfer** of chromosomal genes **at different points**.

Hfr cells can transfer chromosomal genes

- Sometimes the F-factor will integrate into the chromosome of the donor cell creating an **Hfr** (**high frequency recombination**) cell

- The recipient cell can receive new chromosomal genes from a donor Hfr cell

Conjugational Transfer of Chromosomal DNA



How were Plasmids Discovered?

- Plasmids were discovered in Japanese bacteria just after World War II. They were responsible for the problem known as **transmissible antibiotic resistance**. Dysentery due to bacteria was originally treated with sulfonamides, the earliest type of antibiotic.

- However, it wasn't long before human were attacked again as bacteria resistant to these antibiotics began appear! What was far, far, worse was that once resistance had arisen, it was transferred from one strain of bacteria to another at a high frequency.

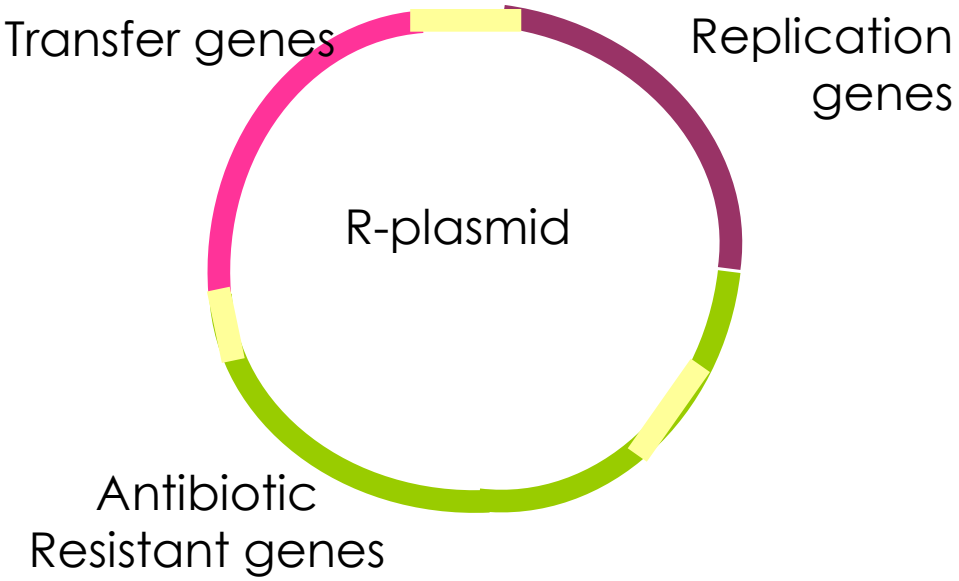
- It turned out that the genes for antibiotic resistance is carried from one bacterium to another on plasmids. Plasmids that confer antibiotic resistance are called **R-plasmids** or **R factors**.

- When the cell in which a plasmid is in divides, the plasmid must divide too. The **plasmid replicates itself in along with the host chromosome** so, at cell division, each daughter cell gets a copy of the plasmid as well as its own chromosome.

- This **vegetative replication** is quite distinct from type of replication that happens during plasmid transfer. Vegetative replication starts at the ***oriV***, **origin of vegetative replication**, which is at a different site on the plasmid from ***oriT***, **the origin used during transfer**.

- All plasmids **must have a vegetative origin** since they must all divide to survive. But only those **plasmids which can transfer** themselves have a **special transfer origin**.

A Typical R-plasmid



- Since 1953, the year Watson and Crick discovered the double helix, 80 percent of the dysentery-causing bacterium found in Japan had become resistant to sulfonamides. A single plasmid may carry genes for **resistance to more than one antibiotic**.

- By 1969, a third of the *Shigella* strains in Japan were resistant to four antibiotics: sulfonamides, chloramphenicol, tetracycline and streptomycin.

- Today, the transfer of plasmids between bacteria has become a major clinical problem. Patients with infections after surgery or with severe burns that have become infected are most at risk.

General Properties of Plasmids

- Plasmids are **circular DNA molecules** that can **replicate independently** of the bacterial chromosome. They have their own life cycles and also, usually, genes that affect the properties of the host cell. These properties vary greatly from plasmid to plasmid, the best known being **resistance to various antibiotics**.

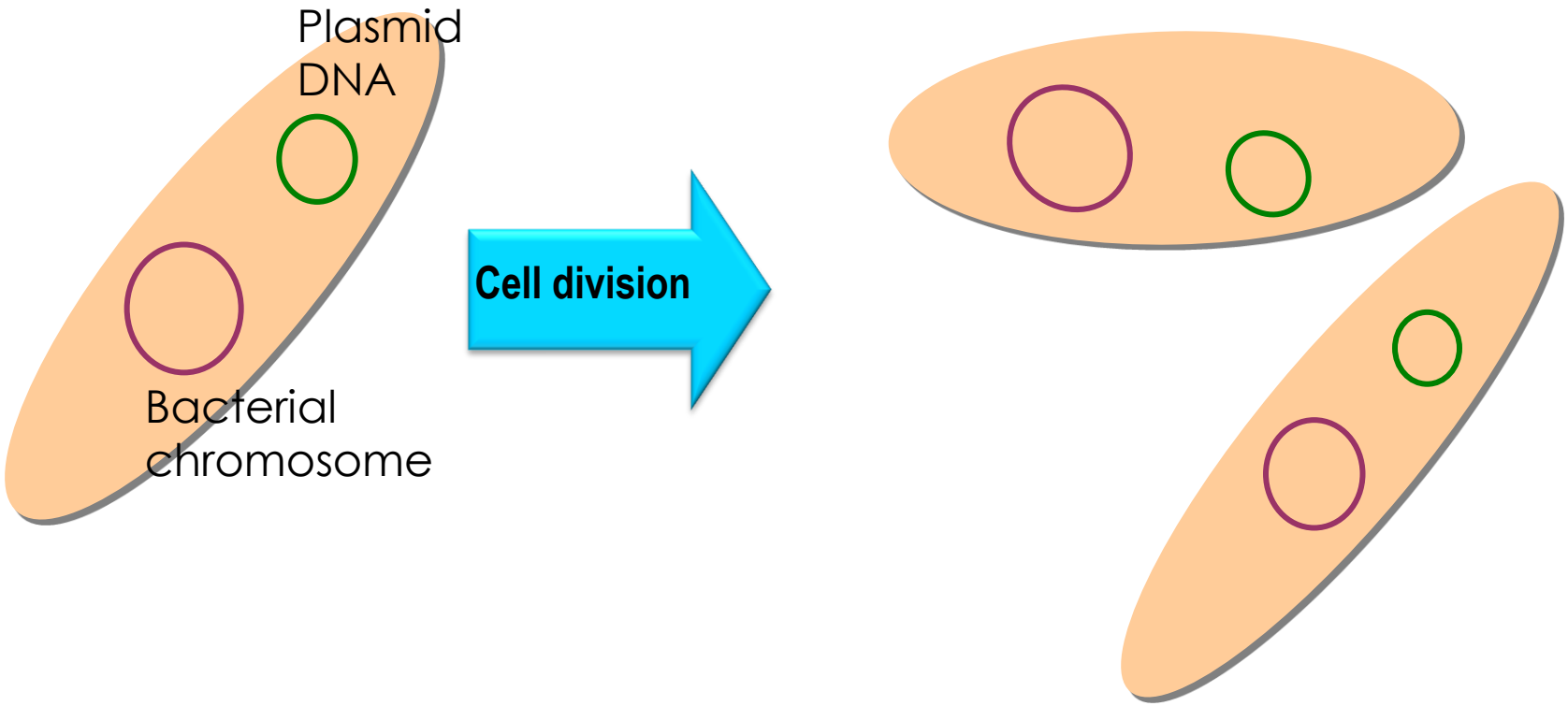
- Because of their unique properties, plasmids are invaluable to the molecular biologist and are used to **carry genes for genetic engineering**. A variety of plasmids, modified for different purposes are widely used in all molecular biology labs.

- When **the cell** which contains plasmid **divides**, the plasmid must divide too. The **plasmid replicates itself in along with the host chromosome.** So, at cell division, each daughter cell gets a copy of the plasmid as well as its own chromosome.

- This **vegetative replication** is quite distinct from type of replication that happens during plasmid transfer. Vegetative replication starts at the *oriV*, **origin of vegetative replication**, which is at a different site on the plasmid from *oriT*, the origin used during transfer.

- All plasmids **must have a vegetative origin** since they must all divide to survive. But only those **plasmids which can transfer** themselves have a **special transfer origin**.

Plasmid Replicates in Step With Cell Division



- The **host range of plasmids varies widely**. Some plasmids are **restricted to a few closely related bacteria**; for example, the **F-plasmid** only inhabits *E. coli* and related enteric bacteria like *Shigella* and *Salmonella*.

- **Others have a wide host range**; for example, plasmids of the **P-family can live in hundreds of different types of bacteria.** P-type plasmids were originally discovered in bacteria called *Pseudomonas*, which sometimes infect patients with severe burns.

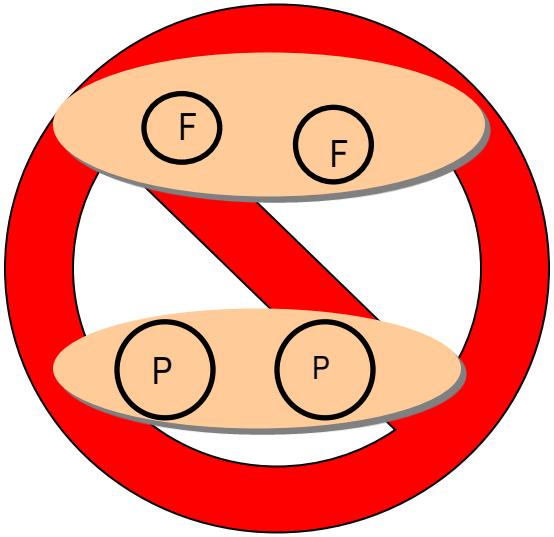
- They are often responsible for resistance to multiple antibiotics including penicillins.

- When a plasmid settles down to live in a bacterial cell, it becomes very possessive of its home. The resident plasmid **keeps out other closely related plasmids**. Thus, two plasmids belonging to the same family cannot coexist peacefully in the same bacterial cell.

- This is called **incompatibility** and the families are known as **incompatibility groups** and are designated by letters of the alphabet. E.g., F-type plasmids include the F -plasmid and its relatives.

- Plasmids of the same incompatibility group have **almost identical DNA sequences in their genes for replication**, although the genes they carry for optional characteristics may be very different.

Plasmid Incompatibility



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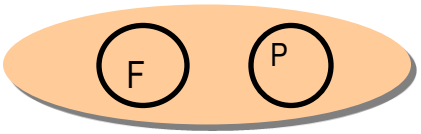

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- It is perfectly **possible to have two, or even more**, plasmids in the same cell **as long as they belong to different families**. So, a P-type plasmid will happily share the same cell with an F-type.

- The **copy number** is just what it sounds like, the number of copies of the plasmid in each bacterial cell.

- It is usually one or two plasmids per chromosome (as with for F- and P-plasmids) but may be as many as 50 or more in certain cases (such as **ColE1 plasmids**).

- The number of copies has a major effect on the **strength of plasmid-borne characters**, especially on their antibiotic resistance. The more copies of the plasmid per cell, the more copies there will be of the antibiotic resistance genes and the higher the resulting level of antibiotic resistance.

- The size of plasmids varies enormously. The F-plasmid is fairly average in this respect and is about **1 percent the size** of the *E coli* chromosome. Most **high copy plasmids** are **much smaller** (*ColE1*-plasmids are about 10 percent the size of the F-plasmid).

- **Very large plasmids**, up to **10 percent of the size of a chromosome**, are sometimes found but they are difficult to work with and few have been properly characterized.

Movement of Plasmids

- **Transferability** is the ability of certain plasmids to transfer themselves from one bacterial cell to another. To do this, they need to manufacture a sex-pilus and form a conjugation bridge with a suitable recipient cell. Many **medium size plasmids** such as the **F-type** and **P-type plasmids**, can do this and are referred to as *Tra*+ (transfer positive).

- Since **plasmid transfer requires the operation of a large number of genes**, only **medium or large plasmids** possess this ability. **Very small plasmids** such as the *ColE*-plasmids, simply **do not have enough** DNA to carry the genes needed.

- Although **small plasmids cannot transfer themselves**, they can sometimes **hitch a ride** with larger plasmids, a property known as **mobilizability** example, the ***ColE*1 plasmid** can be **mobilized by the F-plasmid**. Some but not all, non-self-transferable plasmids can be mobilized.

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- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009). Brock Biology of Microorganisms: Pearson Education, USA.

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