Gene transfer mechanism: Conjugation, Transformation and Transduction

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Bacteria were known to reproduce only asexually by binary fission until the discovery of genetic exchange by parasexual processes (conjugation, transformation and transduction). These processes play an important role in bacterial evolution as they allow them to acquire and efficiently spread new characteristics such antibiotic resistance which confers adaptive advantages in changing environmental conditions.

There are three ways of gene transfer namely transformation, conjugation and transduction known in bacteria. They are parasexual processes, as recombination occurs in absence of meiosis. In bacteria recombination usually occurs between the main chromosome of the recipient and a DNA fragment from another strain (donor). To initiate recombination a transfer of DNA from donor to recipient is a prerequisite condition as they are haploid organisms. The two products of genetic exchange may or may not be recovered. In order to get recombinants in an exchange involving a circular chromosome and linear DNA fragment, an even number of cross overs are required (Fig. 5.1a) while a single exchange between two circular DNAs leads to integration of donor DNA into the recipient chromosome (Fig. 5.1b). The diploid state in bacteria is partial (merodiploid) and generally transient.

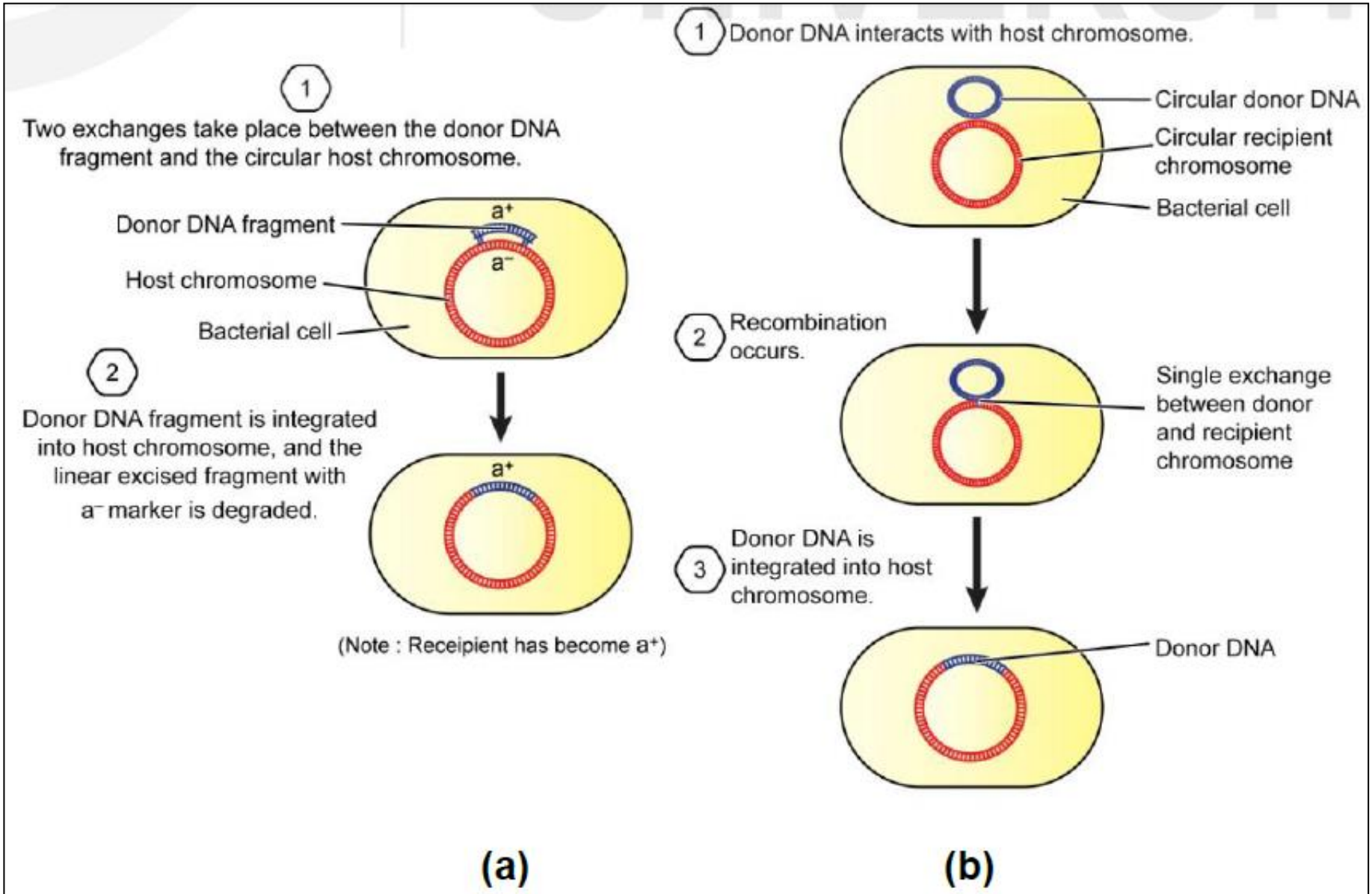

Fig. 5.1: Recombination in bacteria (Adapted from Genetics: Snustad & Simmons).

The three processes can be easily distinguished by testing whether cell-cell contact is required or not and nuclease sensitivity. Conjugation is nuclease resistant and requires cell-cell contact; transformation is nuclease sensitive and occurs without the need for cell-cell contact and finally transduction is neither nuclease sensitive nor requires contact between donor and recipient cells.

TRANSFORMATION

Transformation is the uptake of free extracellular DNA fragment(s) from surrounding medium by competent recipient cells. The donor DNA must recombine with the homologous region of recipient's main chromosome to bring about heritable transformation. Bacterial species can even be induced to take up naked DNA if they lack the ability to do so.

Griffith's Experiment

Transformation was discovered by **Frederick Griffith** in *Streptococcus pneumoniae* (*Diplococcus pneumoniae*) in 1928. It was the first kind of genetic exchange described in bacteria. The wild type strains of this bacterium are pathogenic in mammals and they form smooth (S strain) glistening colonies on agar plates due to the presence of capsular polysaccharide. Mutations that affect polysaccharide synthesis produce bacterial colonies with rough morphology (R strains) and these strains are avirulent. In addition there were biochemically distinct variants of S type known (type II, type III, etc) which could be easily distinguished immunologically. The conversion of smooth to rough is type specific.

Fredrick Griffith
(1879-1941)

F.Griffith was a British bacteriologist who studied medicine and later worked at the pathological lab of the ministry of health. He is known for his discovery of pneumococcal transformation. His work later led to the discovery of DNA as the genetic material.

He died in 1941 during a German bombing raid on London.

Griffith injected these bacteria into mouse and observed their effect after few days. He used two strains; a type IIIS and a type IIR and injected live and / heat killed bacteria. When he injected live type IIIS, heat killed type IIIS or type IIR the results were as expected. The mouse that received live type IIIS died and type IIIS bacteria could be isolated from its viscera. Those that were injected live type II R or heat killed type IIIS survived and no living bacteria could be isolated from the mouse.

Interestingly in one of his experiments Griffith found that mice were killed when they were injected with a mixture of live type IIR and heat killed type III S. On further analysis of dead mouse he could isolate live type IIIS bacteria. He called this process **transformation**. Later similar results were reported by *in vitro* studies and with partially purified extracts from heat killed S bacteria. Finally after many years, **the transforming principle was shown to be DNA by Ostwald T. Avery, Colin M. Macleod and Maclyn McCarty in 1944**.

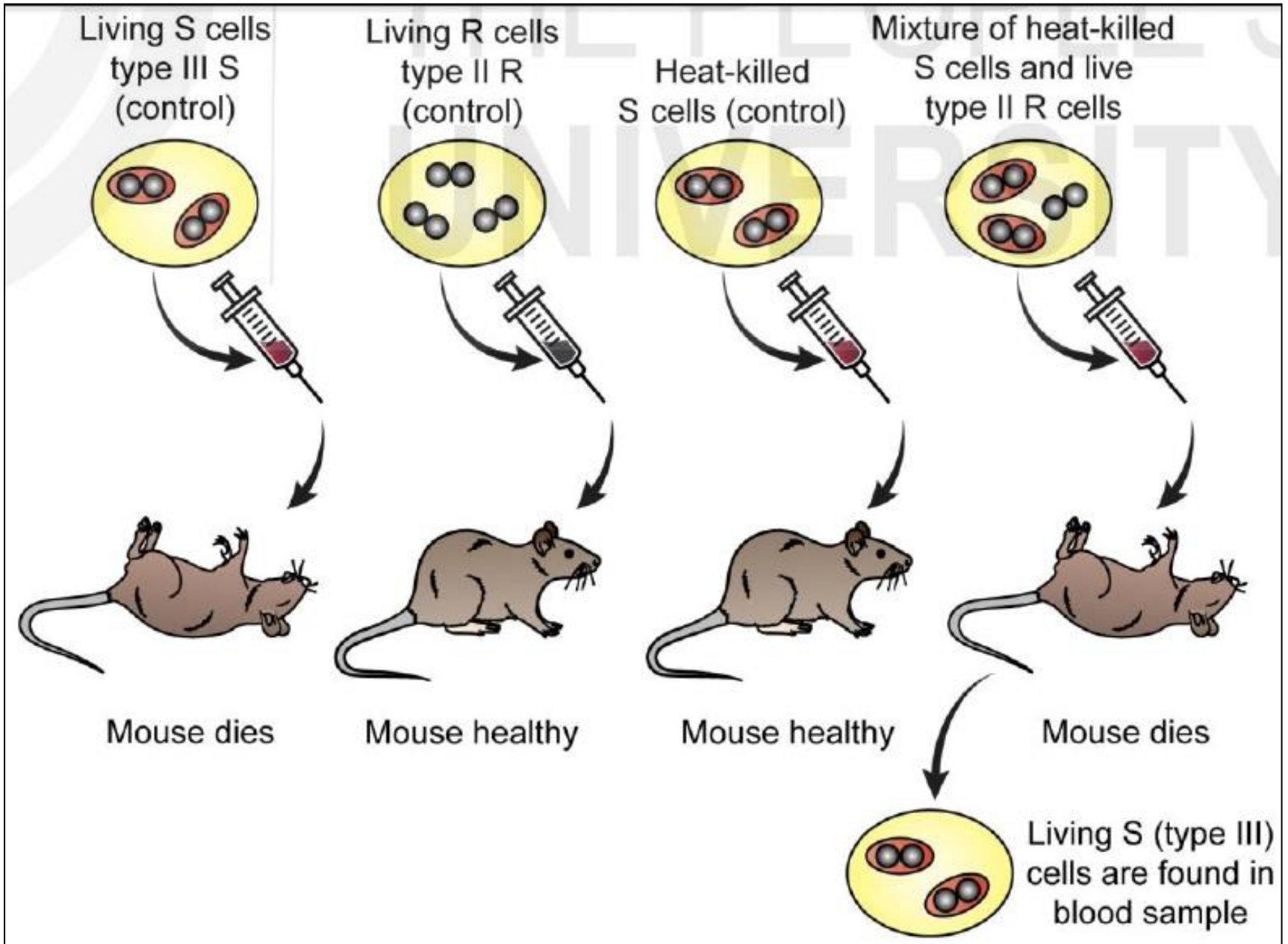

Fig. 5.2: Griffith's experiment on transformation.

Mechanism of Transformation

Naturally transformable bacteria possess an innate ability to develop a state of "**competence**" such that they become capable of internalizing DNA. Here we shall take the example of *a* Gram positive spore forming bacteria, *Bacillus subtilis* to describe the mechanism of transformation (Fig. 5.3). In this species competence is established towards the end of log phase when the cells reach high density.

A number of bacterial species are known to communicate via secreted factors or pheromones. They trigger various responses such as production of virulence factors, competence, sporulation and secondary metabolism.

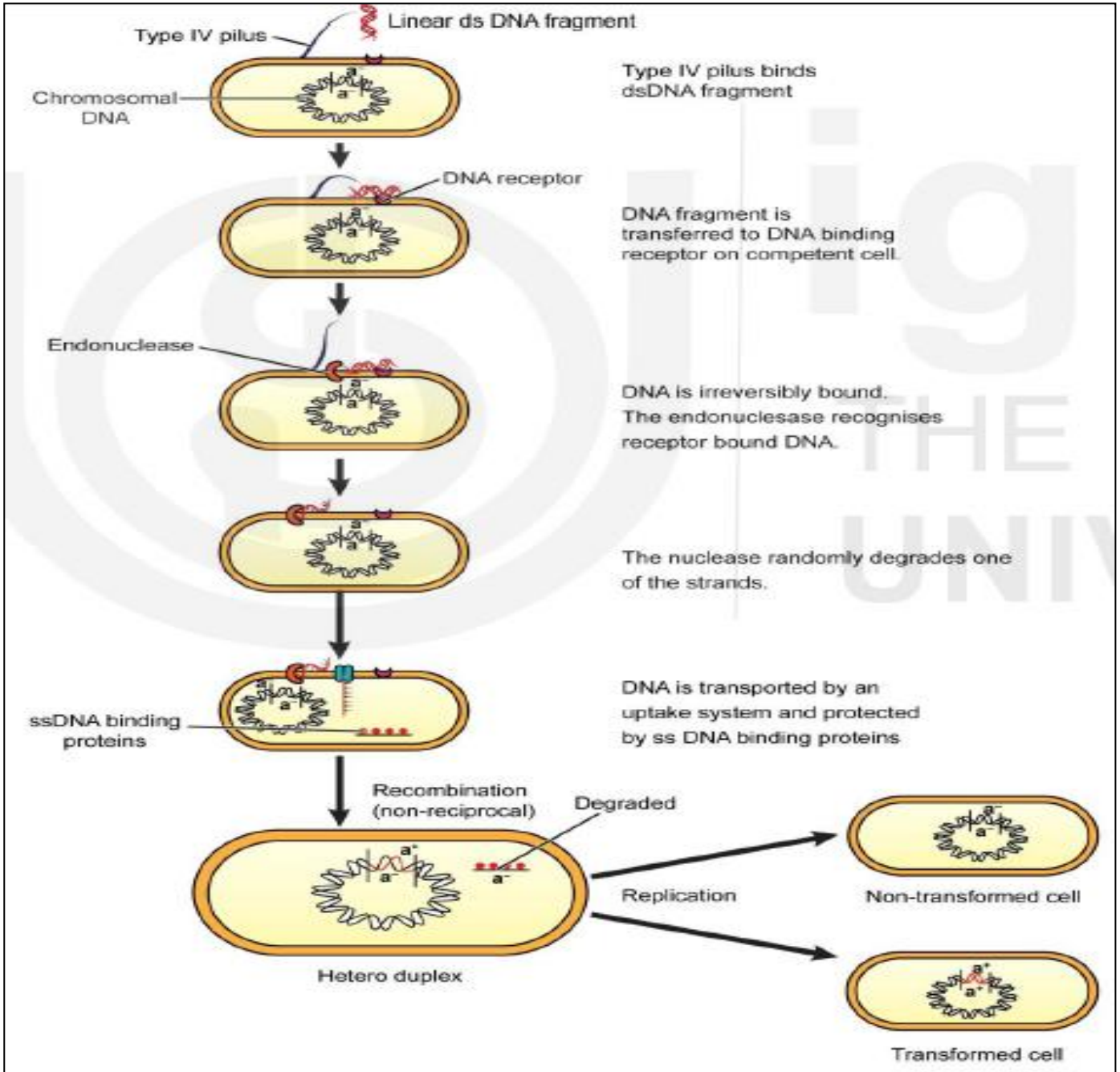

Fig. 5.3: Transformation in *Bacillus subtilis*.

Generally about 20% of cells become competent and remain in this state for several hours. Actively growing cells secrete **competence factor** (pheromone) which at high concentration induces the synthesis of a number of proteins (Com proteins) needed for establishment of competent state. In a competent culture some cells function as donors and release randomly cut DNA fragments while the other cells are equipped to take up DNA and assimilate it. Let us now elaborate the key steps of transformation:

- The recipient cell surface binds naked double stranded DNA, initially via type IV pili.
- The bound DNA is then transferred to a DNA binding receptor which lacks sequence specificity. Therefore DNA can be taken up even from heterologous sources. The DNA – receptor complex changes slowly from reversible to an irreversibly bound state.
- Before DNA enters the cell one of its strands is randomly degraded by a membrane bound endonuclease.
- Single stranded DNA finally enters assisted by the receptor and an uptake system. It is protected from nuclease action by coating with single strand DNA binding proteins.
- The donor DNA fragment undergoes homologous recombination with the recipient's chromosomal DNA. The exchange is **non reciprocal** as the recipient is genetically altered and the other product of recombination is degraded.

CONJUGATION

The process of conjugal transfer was discovered by **Joshua Lederberg and Edward L. Tatum** in 1946. They used auxotrophic strains (nutritional mutants) of *E.coli* each with multiple nutritional defects (mutations) and demonstrated the appearance of prototrophs in mixed cultures at a relatively higher frequency ($1x\ 10^{-7}$) than expected from simultaneous reversion of multiple markers (Fig.5.5). Strain A or B alone did not yield prototrophs.

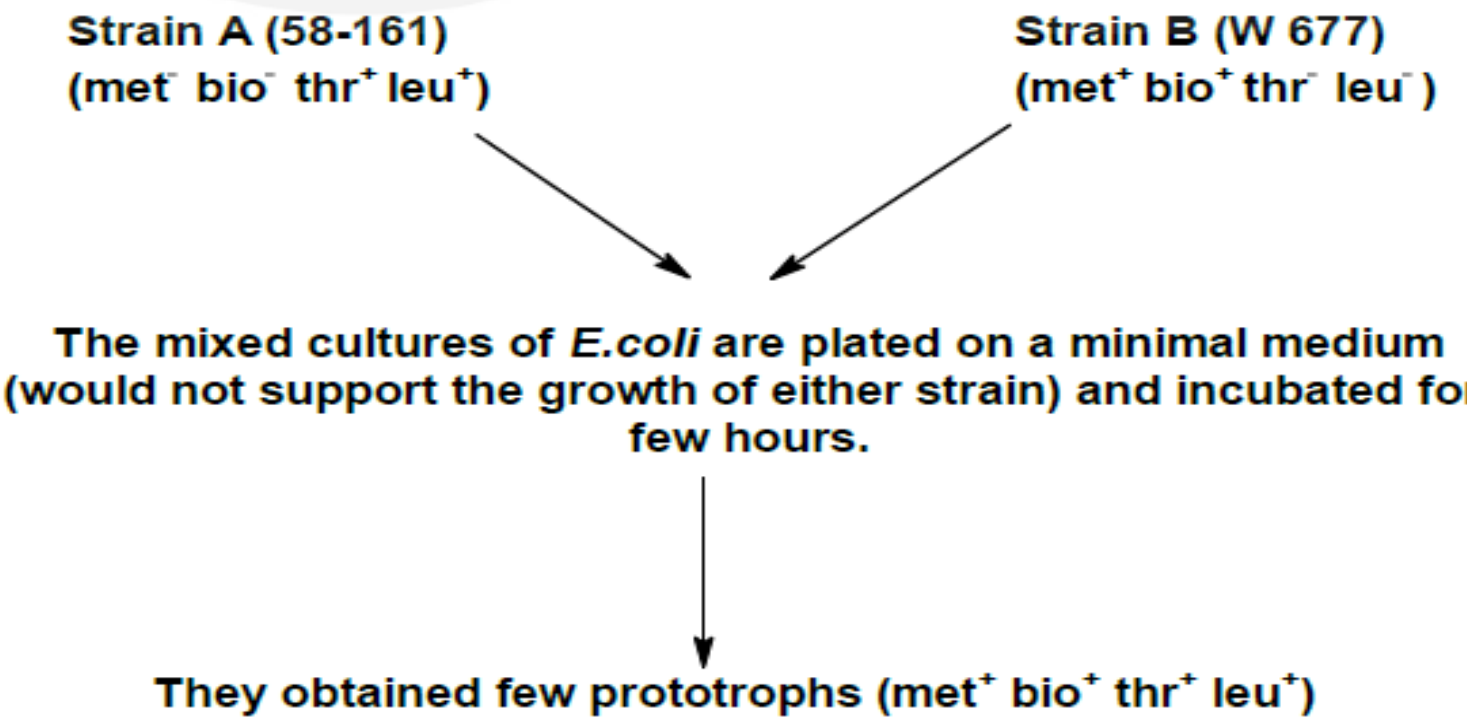

Fig. 5.5: Discovery of conjugation in *E.coli*.

The prototrophs were genetically stable as they could be further sub-cultured on minimal medium. This led them to suggest that some sort of assortment of genetic material between the two strains has occurred and named it **conjugation**. They eliminated the possibility of transformation by showing that extracted DNA from either strain failed to transform the other strain.

The proof for cell-cell contact in conjugation came in 1950 when **Bernard Davis** demonstrated it with the **U-tube experiment** (refer to Fig. 5.10). Later **William Hayes** discovered that gene transfer was **unidirectional** (from donor to recipient). The donor strain harbors the sex (fertility) factor which is almost always transferred to the recipient in an $F^+ \times F^-$ mating (infectious fertility).

In 1958 Joshua Lederberg shared with George Beadle and Edward L. Tatum the Nobel Prize in Physiology or Medicine for his contributions on genetic recombination in bacteria.

Fertility (F) Factor

The fertility factor is a self transmissible, relatively large (100 kb), low copy number (1-2 copies /cell) plasmid that encodes all functions needed for its transfer during conjugation (Fig.5. 6). Many of these genes (*tra genes*) are clustered on one side of the F factor (not shown). The tra region has genes for pilus biogenesis & assembly; mating pair stabilization; nickase, DNA helicase, coupling factor and to control the entry of multiple F factors.

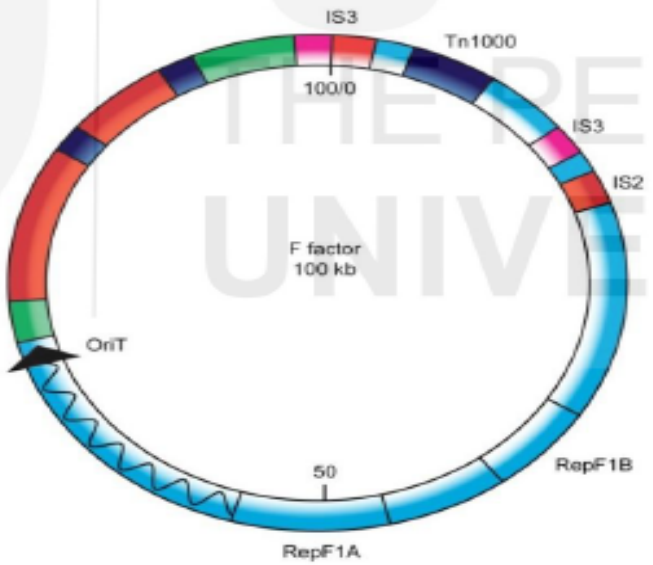

Fig. 5.6: A simplified map of the fertility factor.

A donor strain may have the F factor integrated into the main chromosome by recombination. The IS (insertion) sequences (IS2 and IS3) and Tn1000 serve as portable regions of homology. The term '**episome**' applies to such DNA elements that have a dual existence; maintained either as free autonomously replicating molecules or integrated into the main chromosome. F factor has multiple origins of replication but only **OriT** is the origin of conjugal transfer. It replicates by rolling circle mode and transfers the displaced single strand to the recipient.

Mating using different donor strains (F^+; Hfr and F')

In this subsection we shall learn about the outcome of mating with three variants of donor strains, beginning with F^+ (Fig. 5.7). The process is initiated by cell-cell contact via sex pilus expressed on the surface of donor. DNA replication precedes transfer of F factor. It is initiated by introducing a nick at OriT and as DNA replicates the displaced single strand is simultaneously transferred to the recipient. In the recipient the single strand is circularized and converted to duplex DNA. The transfer of F factor is very efficient under lab conditions and therefore after mating the recipient becomes a donor and the donor remains F^+. In this type of mating the transfer of chromosomal markers is very inefficient. You may recall Lederberg and Tatum's experiment in which they reported prototrophs for chromosomal genes. Can you think how this could have happened?

Occasionally an F plasmid integrates into the main chromosomes generally by utilizing portable regions of homology such as IS sequences. Such as donor strain is called **Hfr** (high frequency recombination). As the name suggest an Hfr strain transfers chromosomal markers at high frequency. There are multiple potential sites on the *E.coli* chromosome where integration can occur thereby generating different Hfr donors (Hfr H, Hfr C, Hfr b, etc).The Hfr strains also differ in the orientation of the F factor integration which determines whether the direction of transfer will be clockwise or counterclockwise.

A mating between Hfr strain and F^- results in the transfer of part of the F factor and chromosomal markers close to the point of its integration in the main bacterial chromosome. In this case also replication is initiated by nicking at OriT and as it proceeds the displaced 5′ end enters the recipient cell. All steps of conjugation remain the same as described earlier. A significant feature of this mating is that the recipient remains a recipient because it requires around 100 min to transfer the entire bacterial chromosome and then the remaining part of the F factor will enter the recipient. Generally the matings are spontaneously broken and therefore recipient does not become a donor.

The fate of the linear DNA fragment transferred depends on whether it recombines with the homologous chromosomal markers and survives with new characteristics for example, gal^- becomes gal^+ in a selection medium or is degraded as it is not autonomously replicating.

The F factor can also excise from an Hfr strain by recombination. Sometimes excision is not precise and carries along with it chromosomal markers and may at times leave behind few F factor genes. The modified F^- factors are called **F′** (F-prime) **factors** and they also serve as donors in conjugation. In an F′ x F^- mating, the bacterial genes incorporated in the F′ plasmid are transferred at high frequency. The transfer of bacterial genes by a sex factor is called **sexduction**. These genes may not always recombine with the host chromosome for their survival as the F′ factor is autonomously replicating. In such a situation stable partial diploidy for these genes is created. Following mating the recipient becomes a donor. F′ factors are extremely useful tools to determine dominance relationships, gene mapping and complementation analysis.

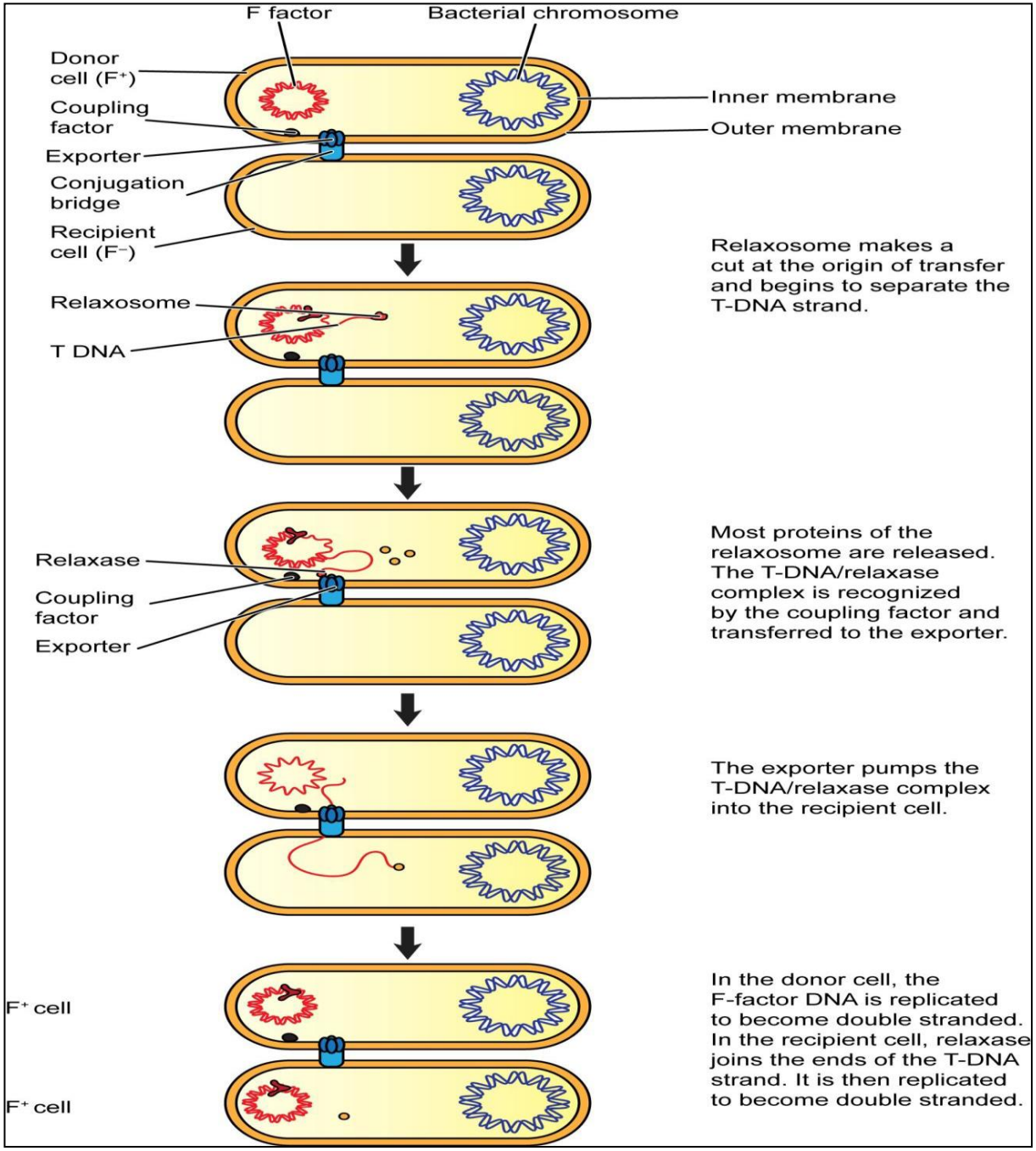

Fig. 5.7: Conjugation in *E.coli* (F_+ x F_-) (Adapted from Brooker).

TRANSDUCTION

Transduction is the process of moving bacterial genes from one bacterial cell (donor) to another bacterium (recipient) by a bacteriophage. It was discovered accidently by **Norton D. Zinder and J. Lederberg** (1952) while attempting to induce sexual mating in *Salmonella typhimurium*. They used two auxotrophic strains for their experiment; strain LA-2 was met^- his^- phe^+ trp^+ and LA-22 was met^+ his^+ phe^- trp^-. The two strains were mixed and incubated in an amino acid free medium. Few colonies appeared at a frequency of 1 in 100, 000 cells suggesting some kind of genetic exchange.

To test for conjugation or transformation they grew the two strains in the two arms of the Davis U tube separated by a sintered glass filter in a medium containing DNase (Fig.5.10).

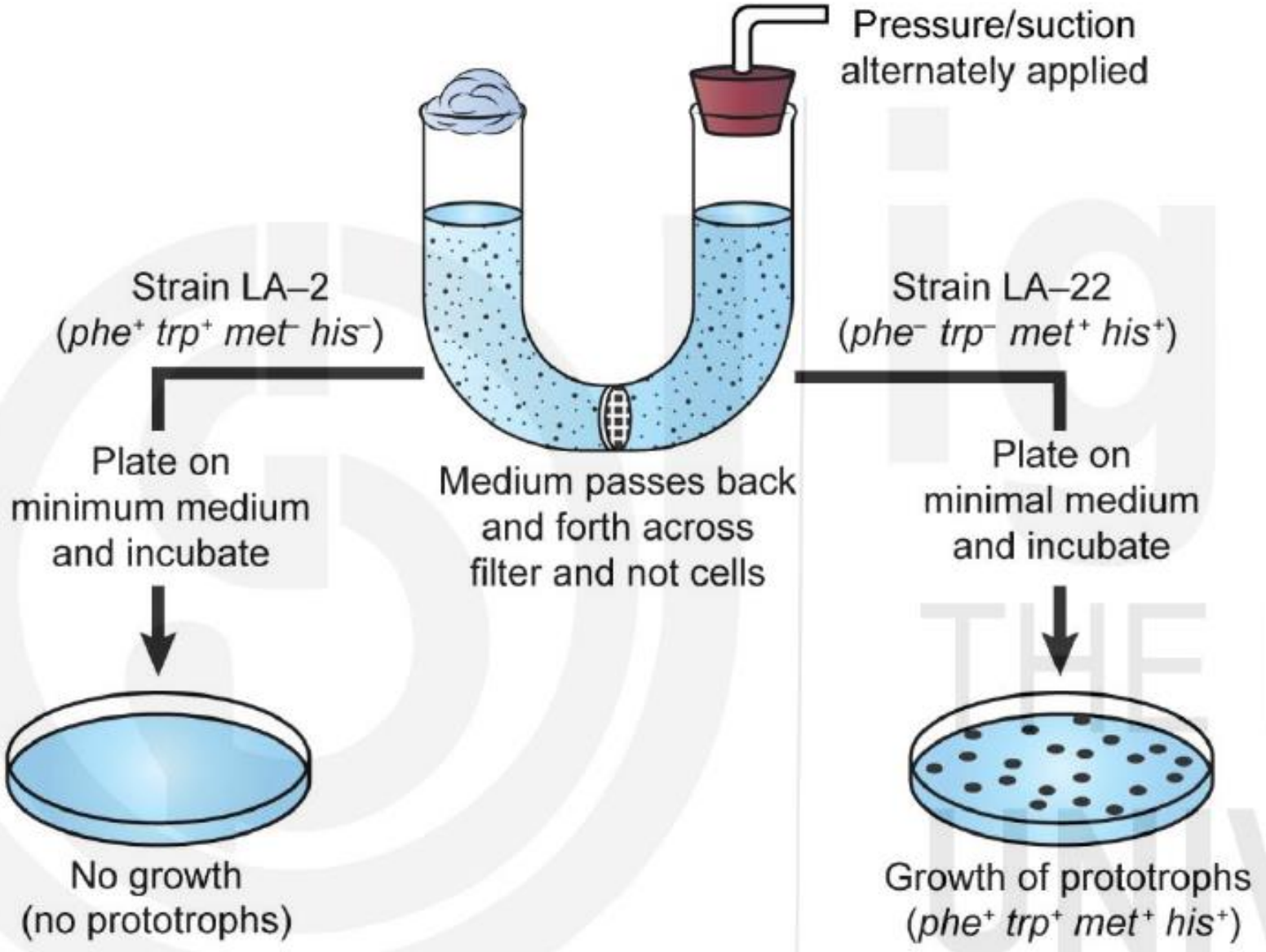

Fig. 5.10: Zinder and Lederberg's experiment.

The filter allows the exchange of media, DNA and virus but not bacterial cells. Surprisingly prototrophs still appeared but only in one arm of the U-tube. The results could not be explained by transformation (DNase sensitive) or conjugation (requires cell-cell contact).

Further work revealed that one of the bacterial strains used carried a temperate virus (P22) in a latent form. Upon induction it released **transducing particles** that could pass through the filter and infect the other strain. The bacterial genes are then assimilated into the main chromosome by recombination.

Phage particles that carry bacterial DNA are transducing phages. They are produced due to errors in the phage life cycle and can transfer bacterial genes to a recipient. Depending on whether a phage carries only specific or any part of the bacterial genome they are classified into **specialized and generalized transducing phages,** respectively.

Generalized Transduction

Bacteriophage P1 is one of the best studied generalised transducing phage. It has double stranded DNA genome (90 Kb) packaged into phage head. When it infects an *E. coli* cell the phage DNA is released into the host cell where it replicates. The replicated DNA is packaged into phage head by introducing cuts at specific sites (pac sites) on DNA. Most phage particles carry only phage DNA.

Occasionally phage particles package fragments of bacterial chromosomal DNA by making cuts at sites that resemble the phage pac sites (pseudo pac). This results in transducing phage particles that carry 90 Kb of host DNA and no phage DNA. Such **packaging errors** are rare (1 in 500). A P1 lysate generally has 10^9 infectious phages / ml which means it has 10^6-10^7 transducing particles per ml.

The bacterial DNA fragment (with a alarge number of close by genes) can be derived from any part of the chromosome (hence the name generalized transduction). It takes 52 transducing phages to cover the entire bacterial chromosome (4369/ 90). Therefore the lysate has enough transducing particles covering the entire genome.

When a transducing phage infects new host cell it transfers only chromosomal DNA which may either recombine with the recipient's chromosomal DNA or is degraded. Such an infection produces no phage particles. The frequency of transduction for any given bacterial gene is about 1 per 10^6 phage particles.

Specialized Transduction

Specialized transduction is characterised by transfer of only certain regions of bacterial chromosome between bacteria through a phage. The markers transferred by a given virus depend on specific point of its integration in the host chromosome which varies from one virus to another. Thus different phages carry different parts of the bacterial chromosome. The specialized transducing phages are produced due to **imprecise excision** (illegitimate recombination) of the viral DNA upon induction. The phage head packages both phage as well as bacterial DNA fragments as a hybrid chromosome.

Bacteriophage lambda (λ) is the best understood specialized transducing phage of *E.coli*. It has linear double stranded DNA genome with 12 base pair cohesive / sticky ends that facilitate circularization and play a role in packaging of DNA into phage head. Phage λ is a **temperate phage** which can enter either a lytic or lysogenic cycle. In the latter decision (lysogenic cycle) it first circularizes and then integrates into host DNA by site-specific recombination using homologous attachment sites (*att*B on *E.coli* and *att*P on phage DNA).

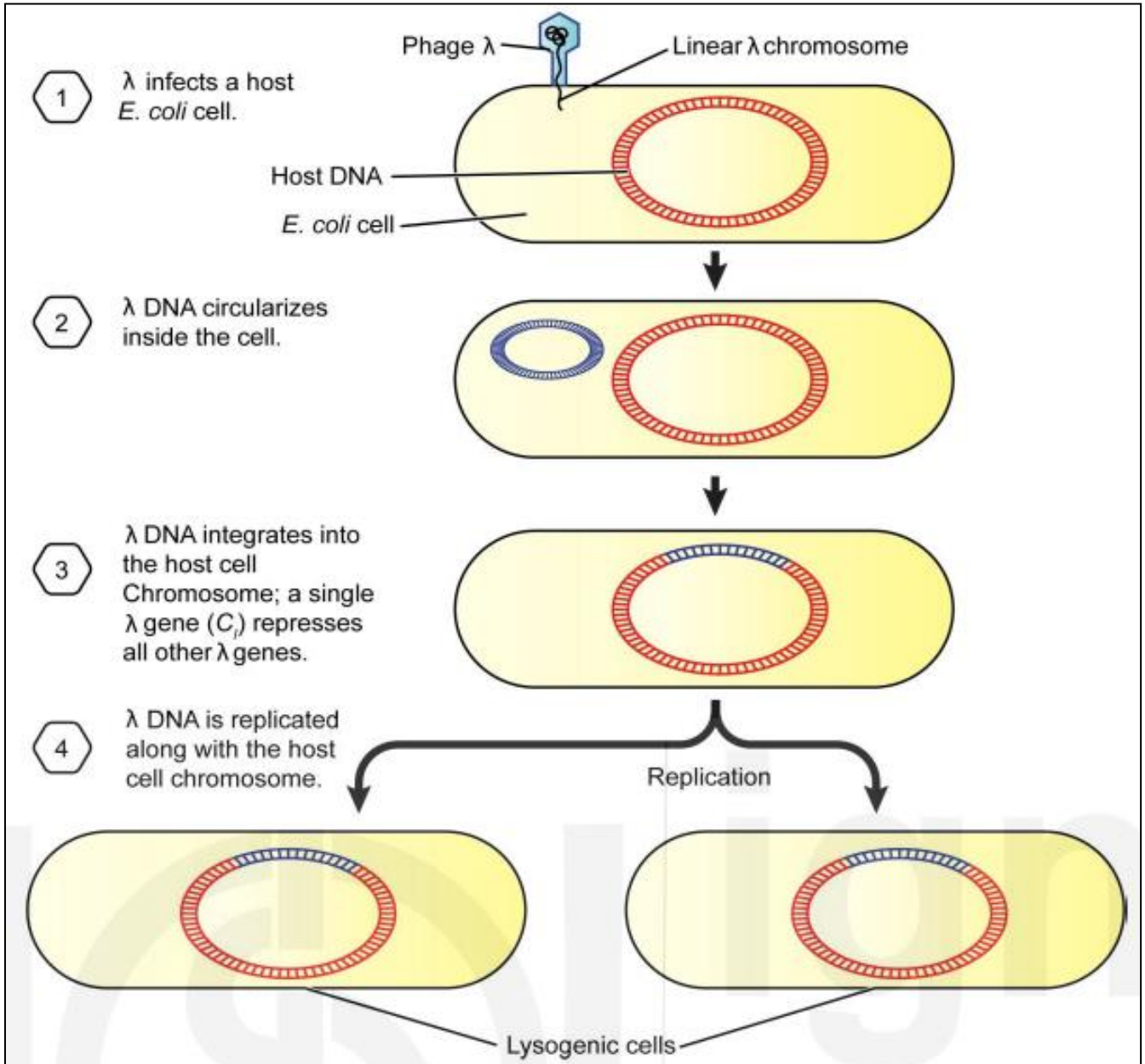

Fig. 5.11: Lysogenic cycle of phage lambda (Adapted from Genetics: Snustad & Simmons).

During lysogeny very few phage genes are expressed (**prophage**) and it multiplies with the host chromosome and does not produce new virus particles (Fig.5.11). This may continue for several generations until conditions favor induction such as exposure to UV or change in nutrient status. Then the virus excises and enters the lytic cycle. Phage DNA excision is essentially the reverse of the site-specific integration process and generally yields intact circular phage and bacterial chromosome. At times excision is imprecise (recombination occurs at a site other than the original attachment site) and results in transducing phages carrying chromosomal markers present on either side of the integration site (gal or bio locus) and it may leave few phage genes. Such a phage is defective (λ dgal or λ dbio) and requires a wild type helper virus for infection.

The excised phages express their genes in a defined sequence to finally assemble mature particles which are released by lysis of the host cell. Most of the virus particles in the lysate have normal λ and only few transducing particles (1in 10^5 or 10^6 particles). Such a lysate is a **low frequency transduction (LFT) lysate**.

The infection of the defective transducing phage and helper phage initiates another cycle. The defective phage (λ dgal) may either recombine with the homologous chromosomal marker on the bacterial chromosome (gal^-) or

integrate along with the helper DNA forming an unstable double lysogen. In the former case a stable gal$^+$ transductant is generated while in Fig.5.12 the latter the double lysogen excises to enter the lytic cycle and produces a lysate that has both normal and defective phages at almost equal frequency (**high frequency transduction or HFT lysate**).

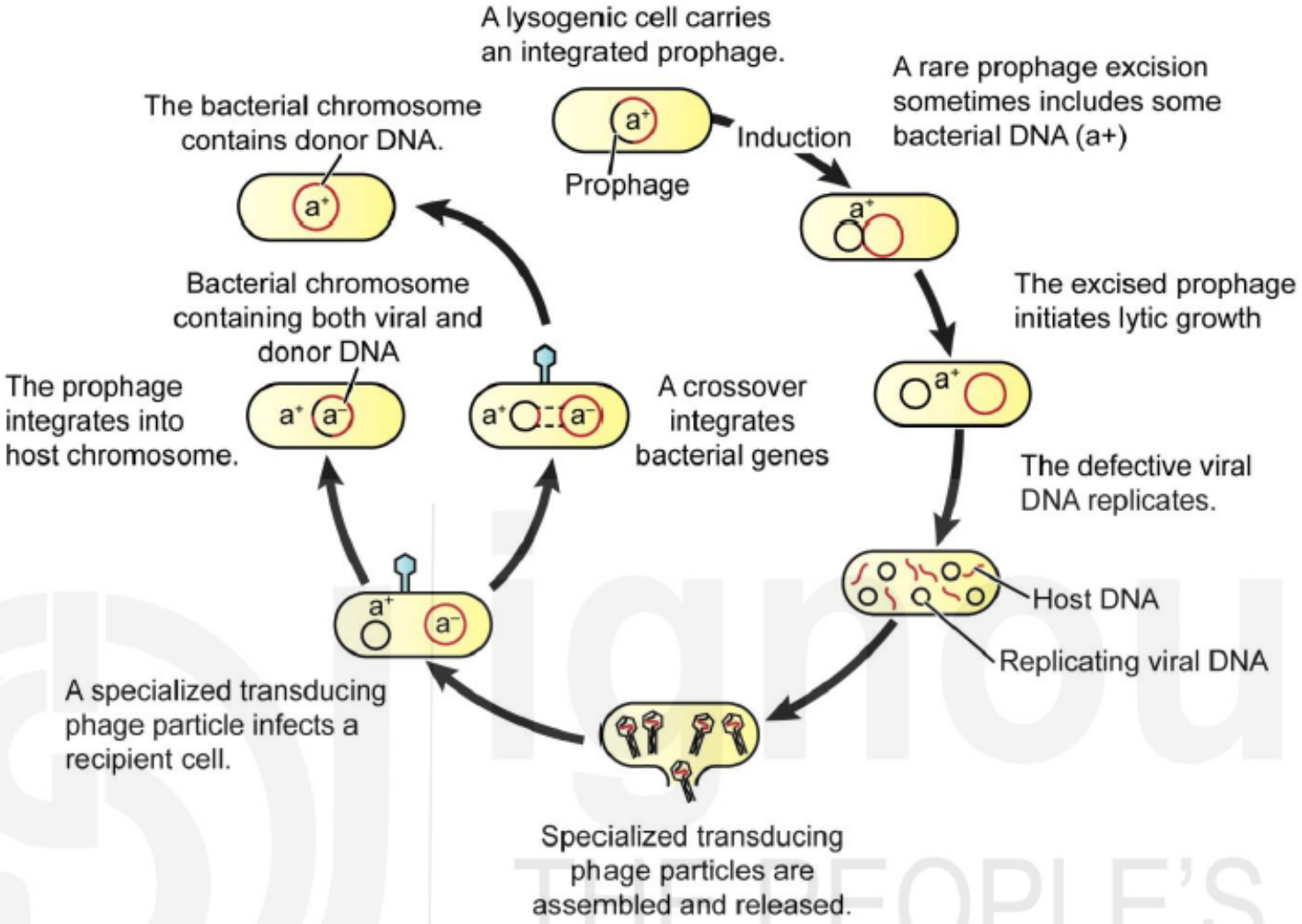

Fig. 5.12: Specialised transduction (Adapted from Genetics: Snustad & Simmons).