# Microbial Genetics

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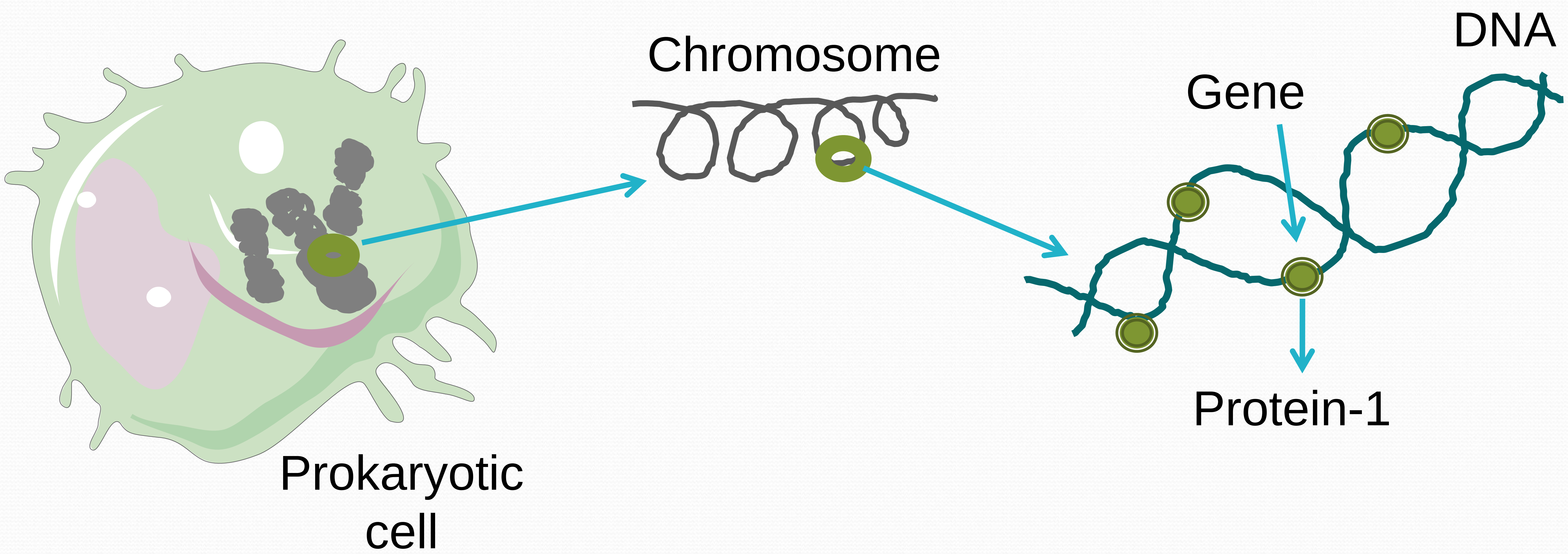
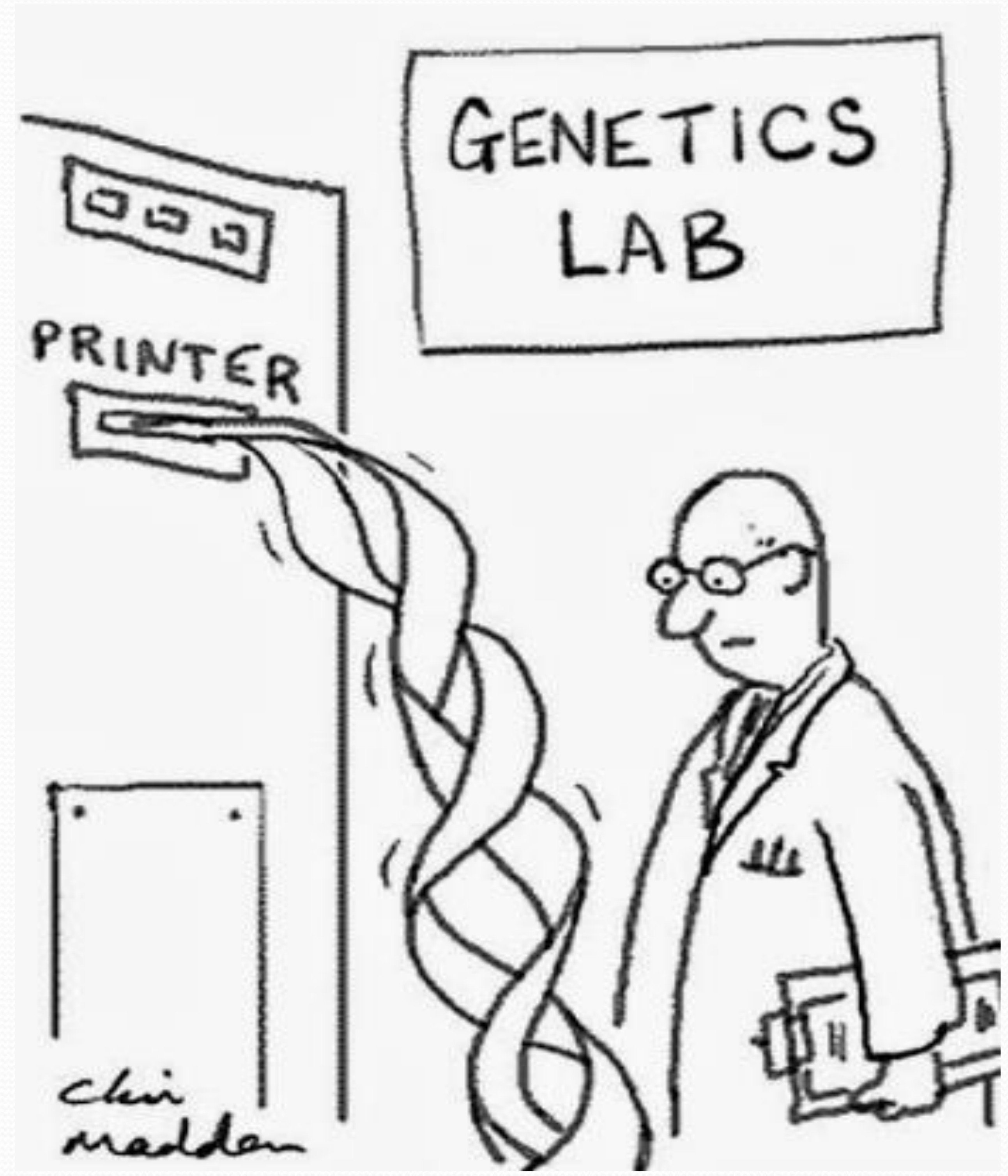
# Genes: an overview

- A gene is the functional unit of heredity
- Each chromosome carry a linear array of multiple genes
- Each gene represents segment of DNA responsible for synthesis of RNA or protein product
- A gene is considered to be unit of genetic information that controls specific aspect of phenotype




Courtesy: Team Shrub
https://twitter.com/realscientists/status/927667237145767937

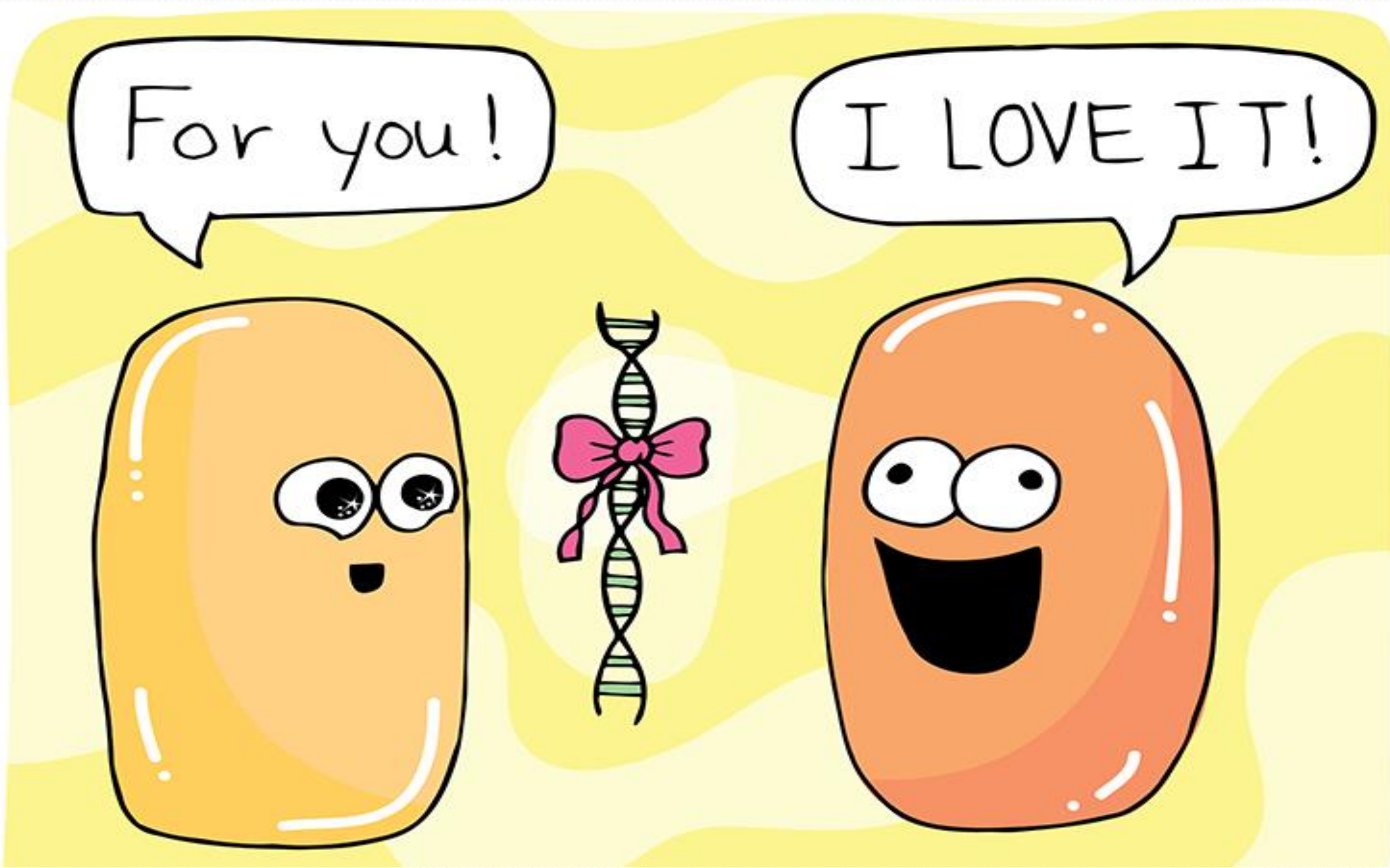
# Genetic exchange within Prokaryotes

The genetic exchange occurring in bacteria involve transfers of genes from one bacterium to another. The gene transfer in prokaryotic cells is thus unidirectional and the recombination events usually occur between a fragment of one chromosome (from a donor cell) and a complete chromosome (in a recipient cell)

# Mechanisms for genetic exchange

Bacteria exchange genetic material through three different *parasexual processes** namely **transformation, conjugation and transduction.**

*Parasexual process involves recombination of genes from genetically distinct cells occurring without involvement of meiosis and fertilization




Courtesy: Beatrice the Biologist.com
(http://www.beatricebiologist.com/2014/08/bacterial-gifts/)

*Principles of Genetics-sixth edition*
*by D. Peter Snustad & Michael J. Simmons*

# Transformation: an introduction

Transformation involves the uptake of free DNA molecules released from one bacterium (the donor cell) by another bacterium (the recipient cell). Frederick Griffith discovered transformation in *Streptococcus pneumoniae* (pneumococcus) in 1928. In his experiments, Griffith used two related strains of bacteria, known as R and S.

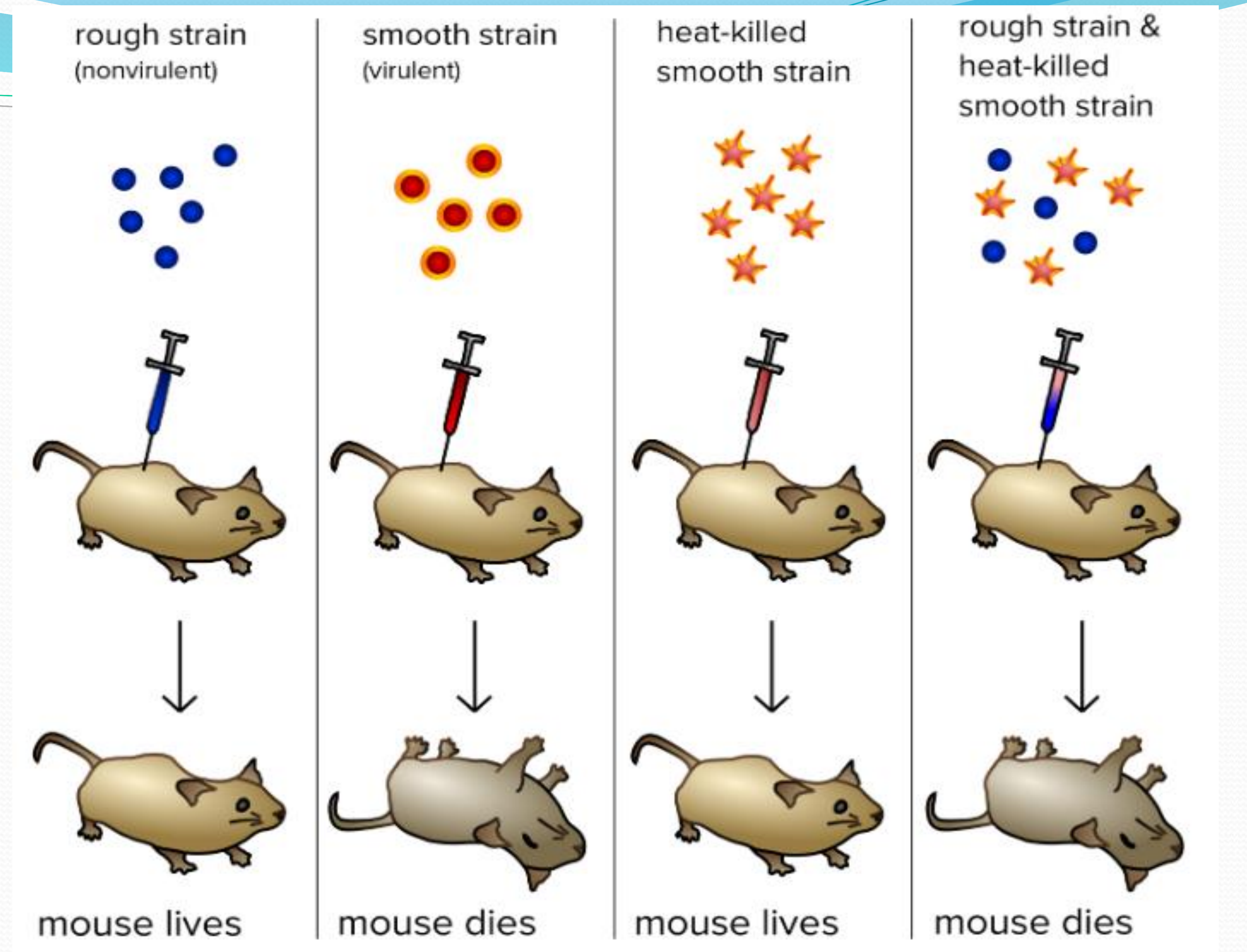
Frederick Griffith
1877-1941

The R bacteria (nonvirulent) formed colonies, or clumps of related bacteria, that had a rough appearance (hence the abbreviation "R").

The S bacteria (virulent) formed colonies that were rounded and smooth (hence the abbreviation "S"). The smooth appearance and virulence was due to a polysaccharide, or sugar-based coat produced by the bacteria. Mice injected with live S bacteria developed pneumonia and died.

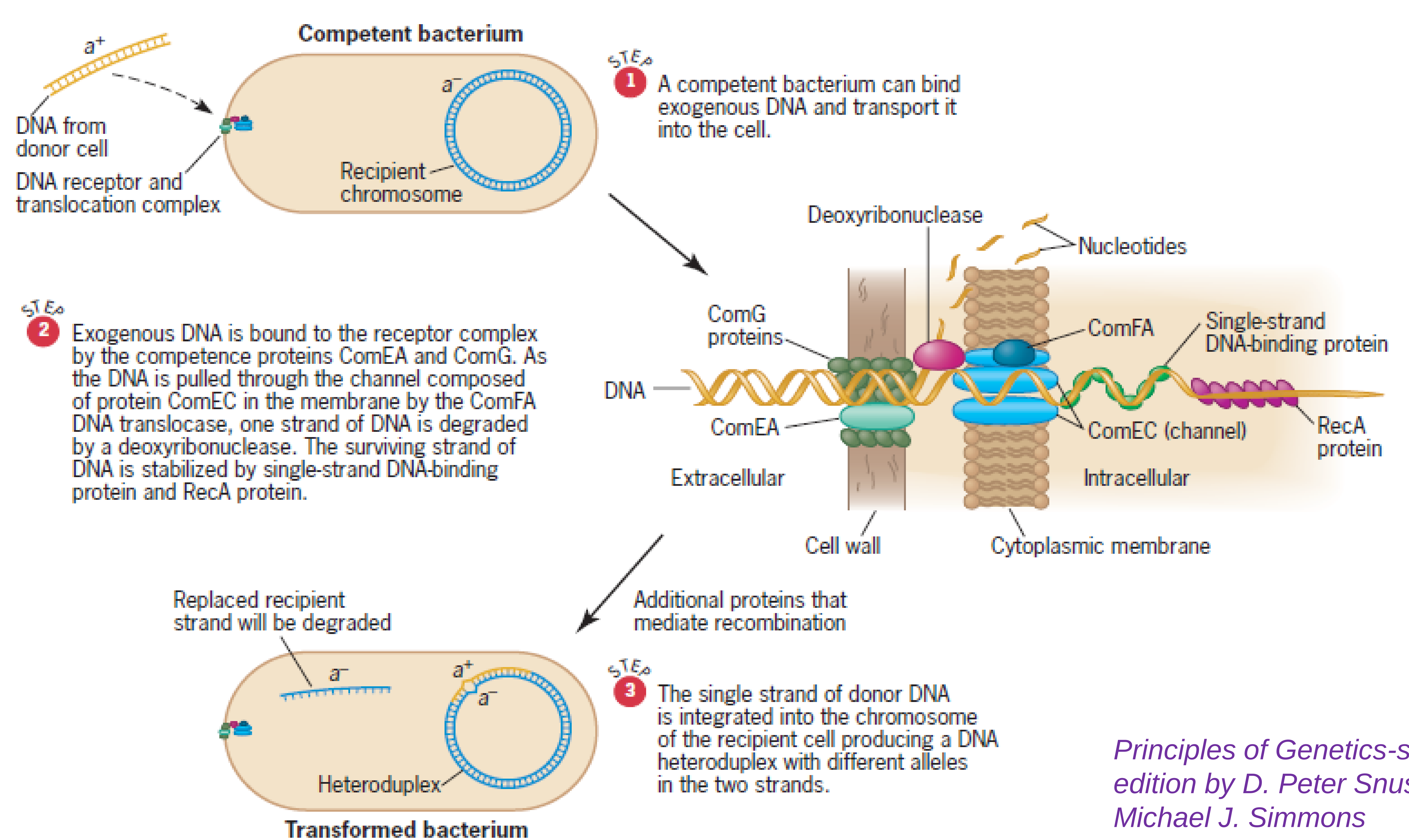
As part of his experiments, Griffith tried injecting mice with heat-killed S bacteria  and unsurprisingly, the heat-killed S bacteria did not cause disease in mice.

However, when harmless R bacteria were combined with harmless heat-killed S bacteria and injected into a mouse. Not only did the mouse develop pnenumonia and die, but when Griffith took a blood sample from the dead mouse, he found that it contained living S bacteria.




# Inference

Griffith concluded that the R-strain bacteria must have taken up what he called a  "transforming principle" from the heat-killed S bacteria, which allowed them to "transform" into smooth-coated bacteria and become virulent.




*Principles of Genetics-sixth edition by D. Peter Snustad & Michael J. Simmons*

The mechanism of transformation in *Bacillus subtilis*. A competent bacterium contains a DNA receptor/translocation complex that can bind exogenous DNA and transport it into the cell, where it can recombine with chromosomal DNA of the recipient cell. ComEA, EC, FA, and G are competence proteins; they are synthesized only in competent cells.

Types of Recombination

1. Homologous recombination- The incorporating DNA strand has a sequence that is similar to a region of DNA in the bacterial chromosome.

2. Non-homologous or illegitimate recombination- The incorporating DNA strand may not be homologous to any gene of the recipient bacterial chromosome

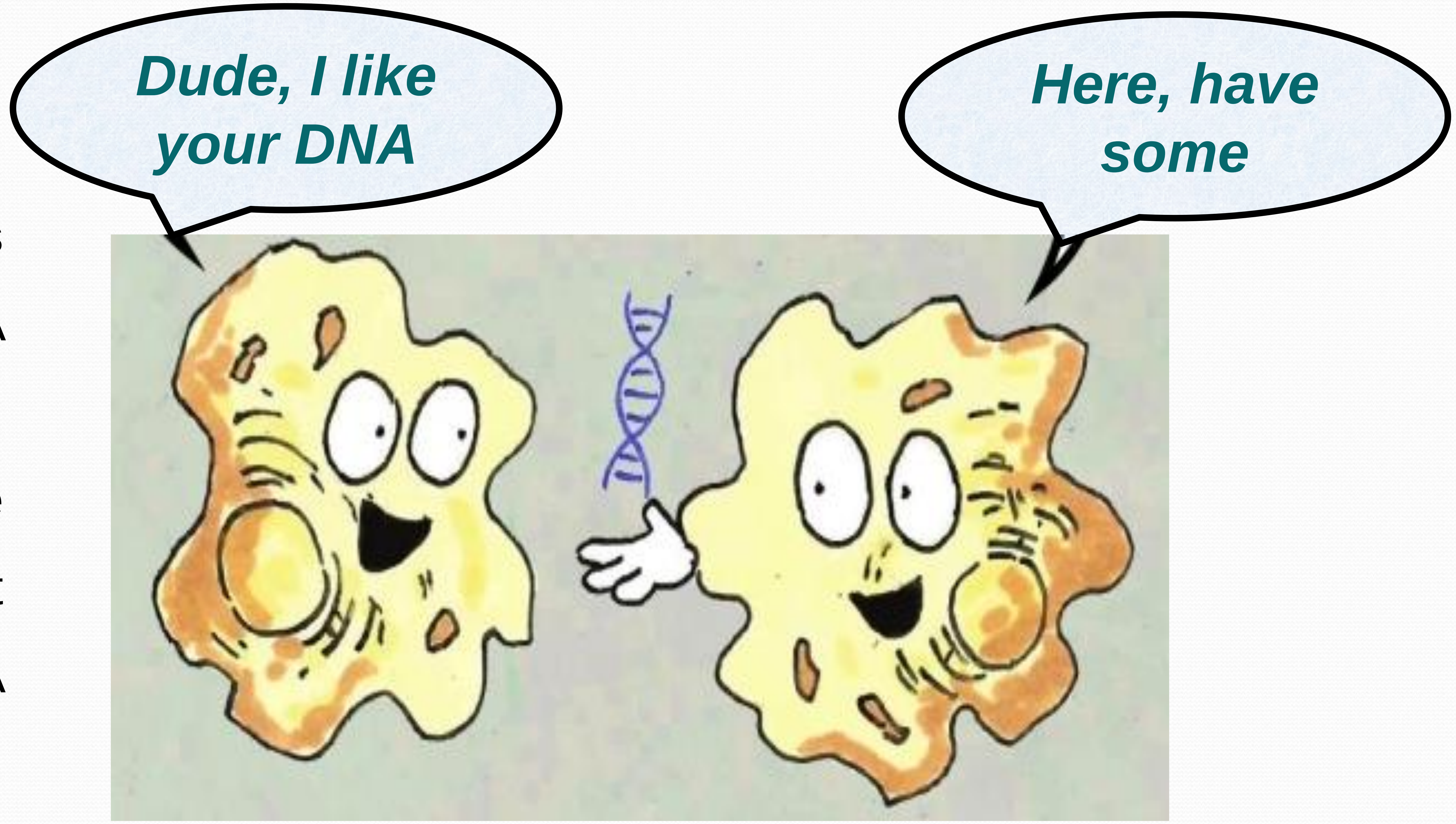
3. Competence Stimulating Peptide

4. DNA uptake signal sequences

# Conjugation: an introduction

During conjugation , DNA is transferred from one bacterium to another. After the donor cell pulls itself close to the recipient using a structure called a pilus, DNA is transferred between cells. In most cases, this DNA is in the form of a plasmid.

Donor cells typically act as donors because they have a chunk of DNA called the fertility factor (or F factor). This chunk of DNA codes for the proteins that make up the sex pilus. It also contains a special site where DNA transfer during conjugation begins.

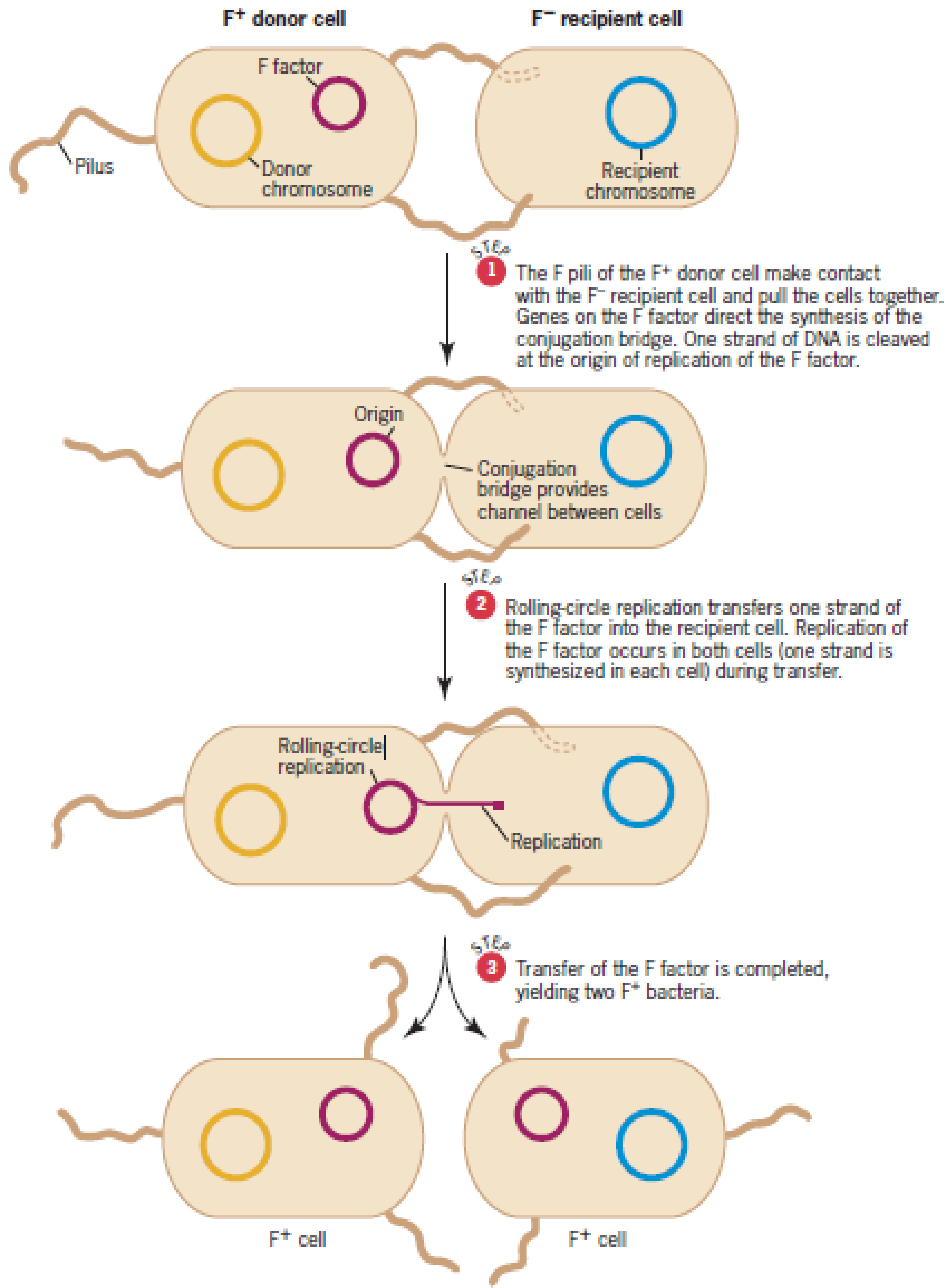



Courtesy: Planetpilly.com
https://planetpailly.com/2016/03/04/sciency-words-bacterial-conjugation/

Step 1:The pilus enables direct contact between the donor and the recipient cells.

Step 2: Because the F-plasmid consists of a double-stranded DNA molecule forming a circular structure, i.e., it is attached on both ends, an enzyme (relaxase, or relaxosome when it forms a complex with other proteins) nicks one of the two DNA strands of the F-plasmid and this strand (also called T-strand) is transferred to the recipient cell.

Step 3: Donor cell and the recipient cell, both containing single-stranded DNA, replicate it and thus end up forming a double-stranded F-plasmid identical to the original F-plasmid. (see below), the old recipient cell is now a donor cell with the F-plasmid and the ability to form pili, just as the original donor cell was. Now both cells are donors or F+.

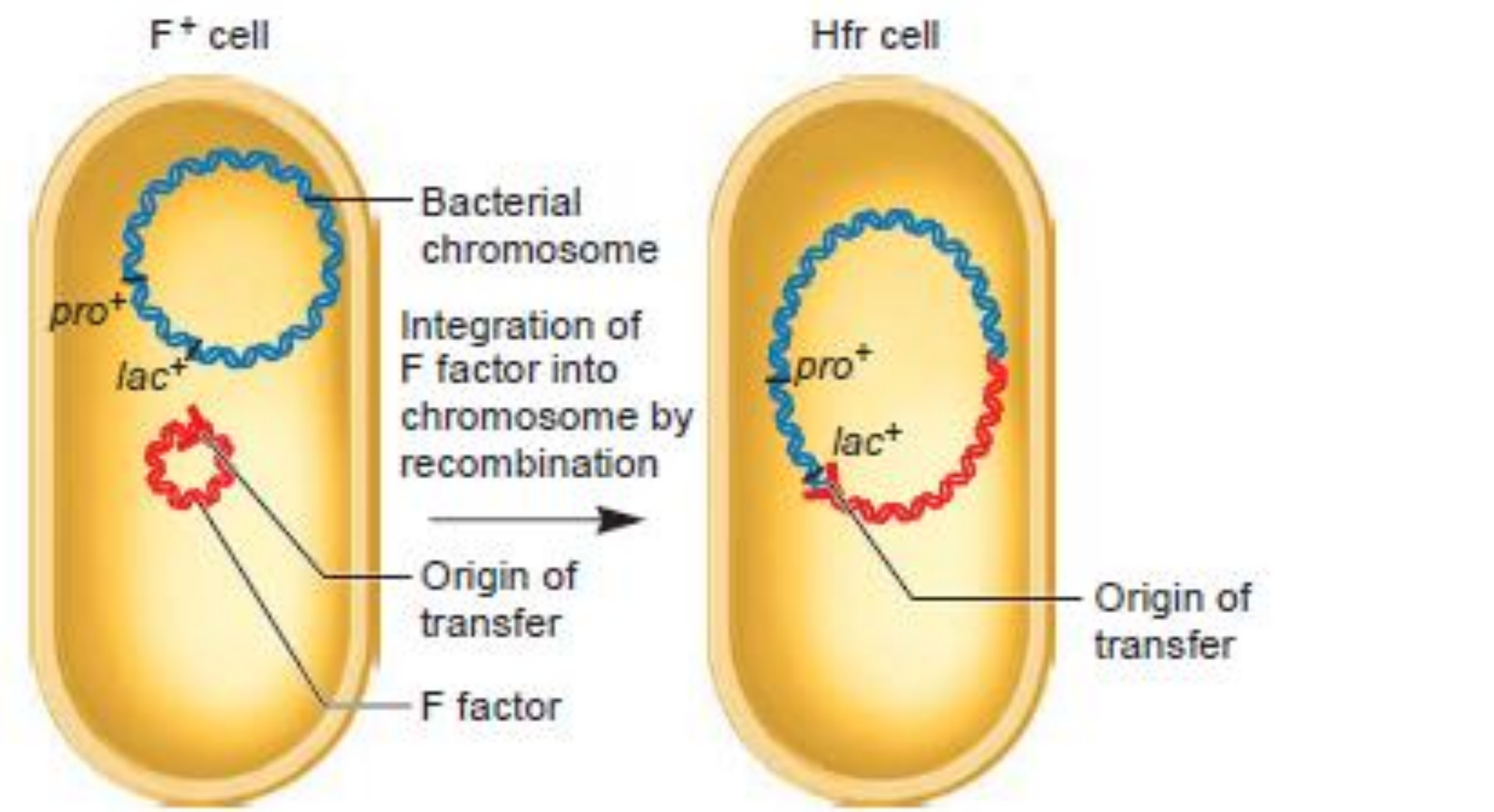


## Integration of an F factor to form an *Hfr cell* and its subsequent excision to form an F′ factor

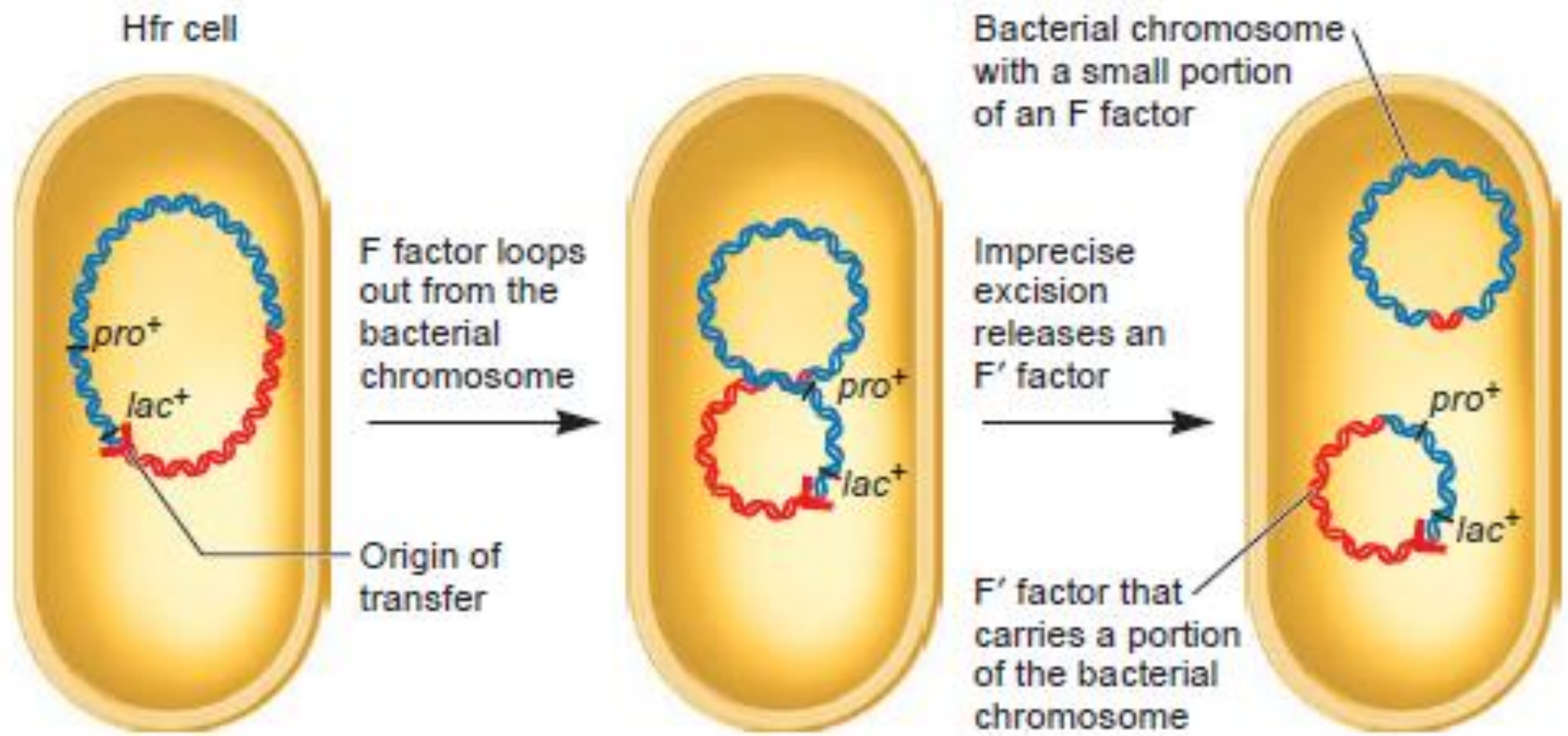
Luca Cavalli-Sforza discovered a strain of *E. coli that was very* efficient at transferring many chromosomal genes to recipient *F – strains* and named these strains as **Hfr strains**

***Hfr Strains Can Transfer a Portion of the Bacterial* Chromosome to Recipient Cells**

**The mating time decides the length of fragment transfered**




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



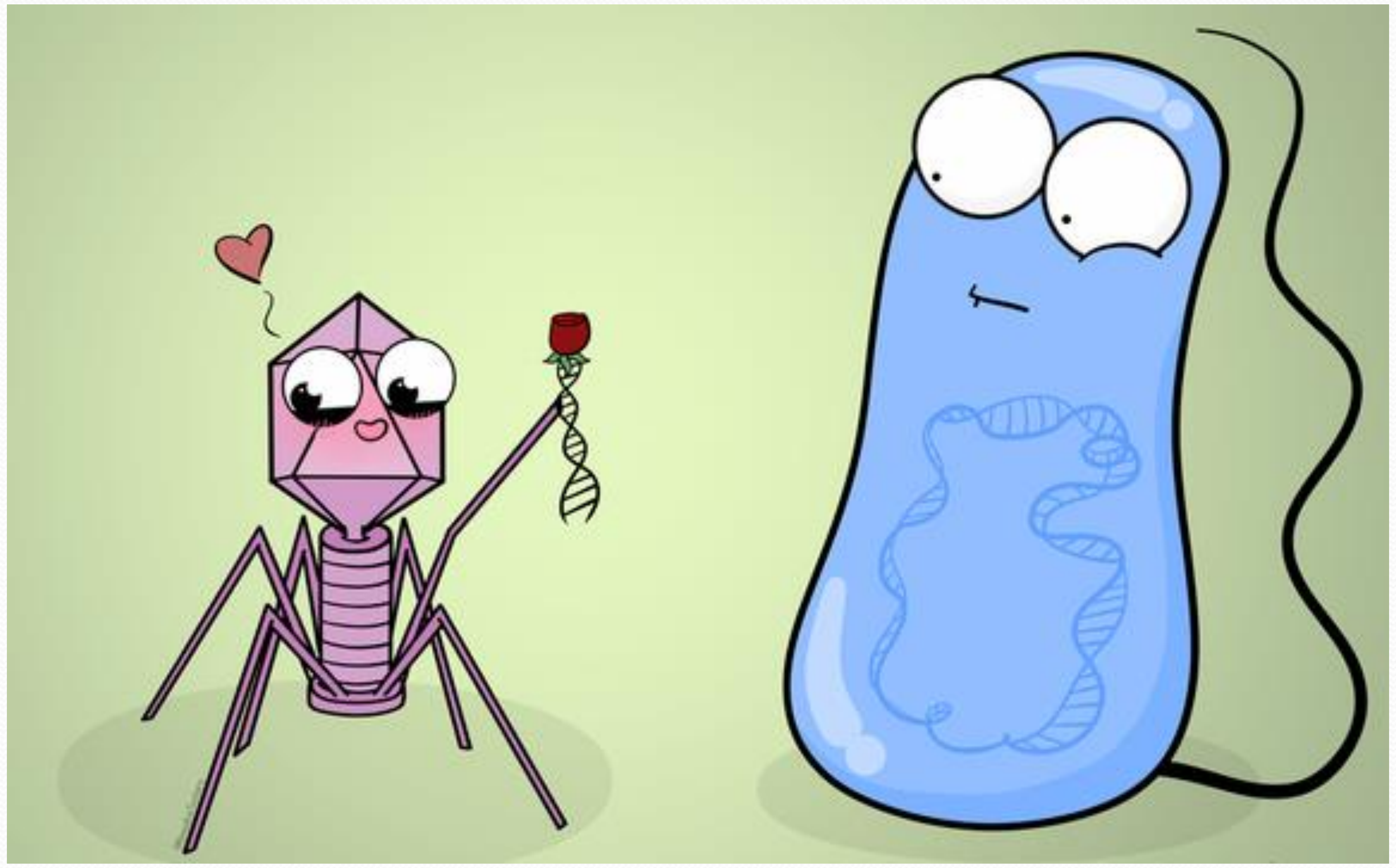

(b) When an F factor excises imprecisely, an F′ factor is created.

Source: Genetic Analysis and Principles, $4^{th}$ Edition

# Transduction

Transduction is the process by through which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector.

When bacteriophages (viruses that infect bacteria) infect a bacterial cell, their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.



Courtesy: The Amoeba sisters
https://www.amoebasisters.com/parameciumparlorcomi
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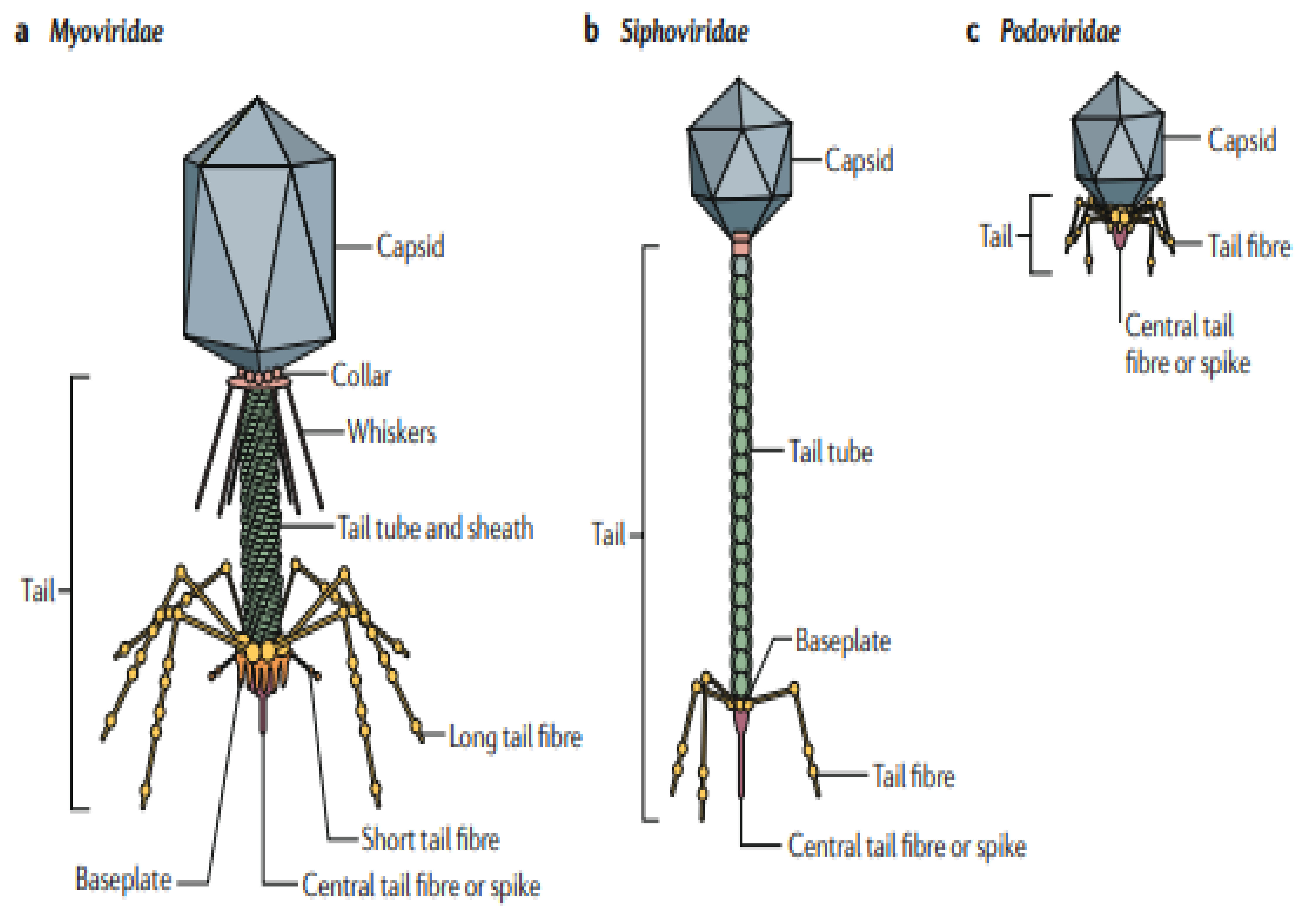
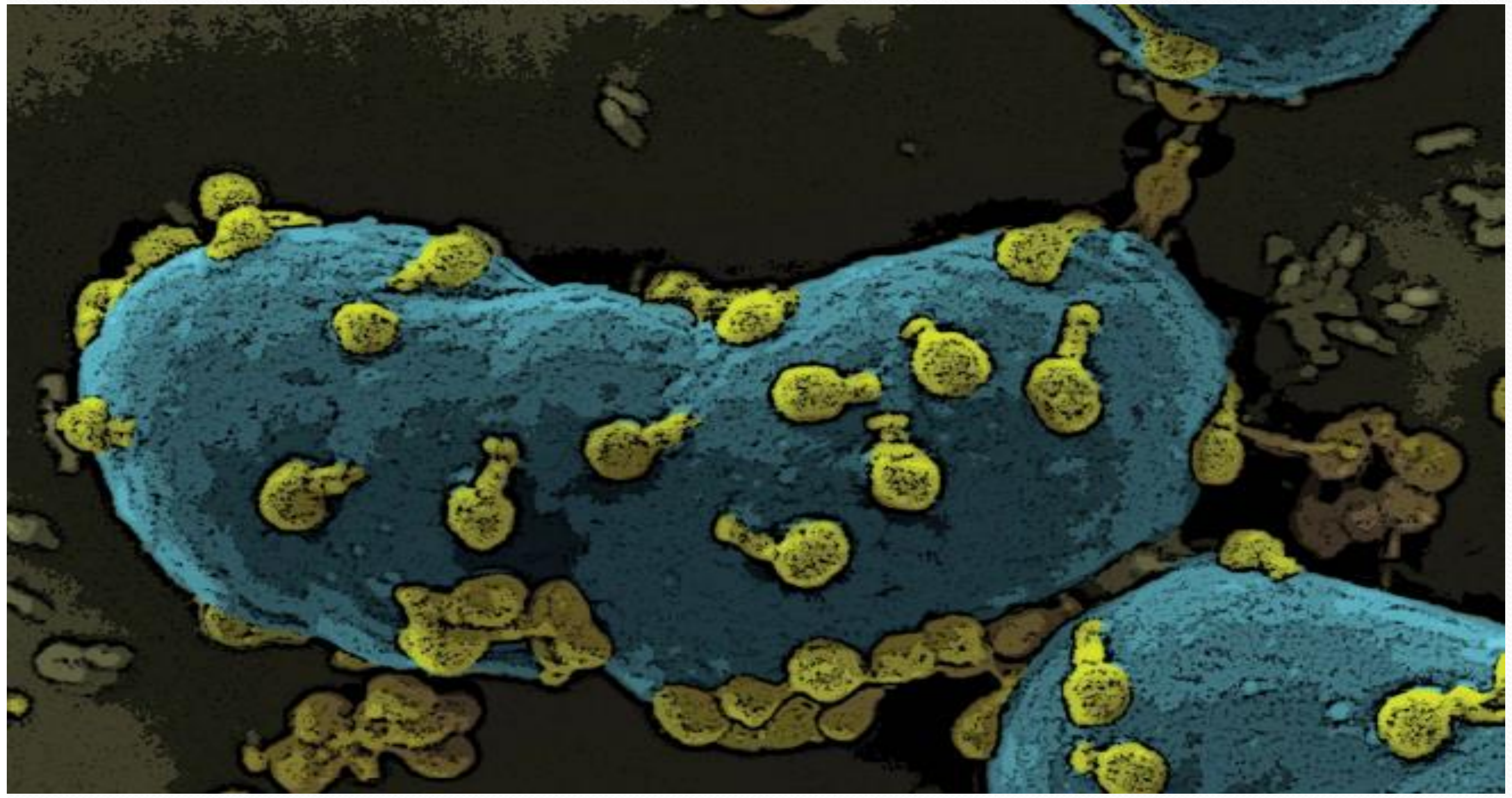
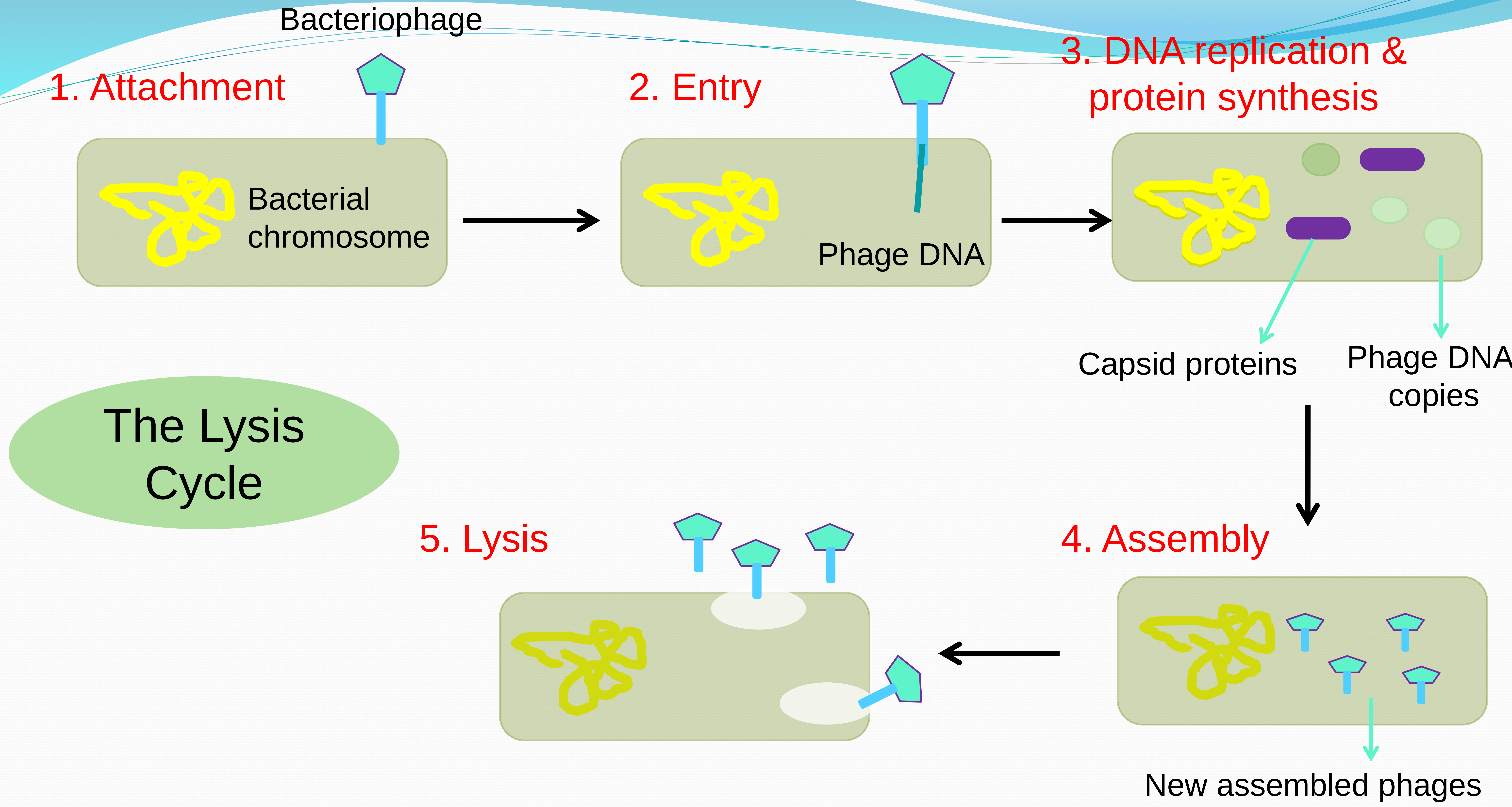
# Bacteriophage: viruses thriving on bacteria



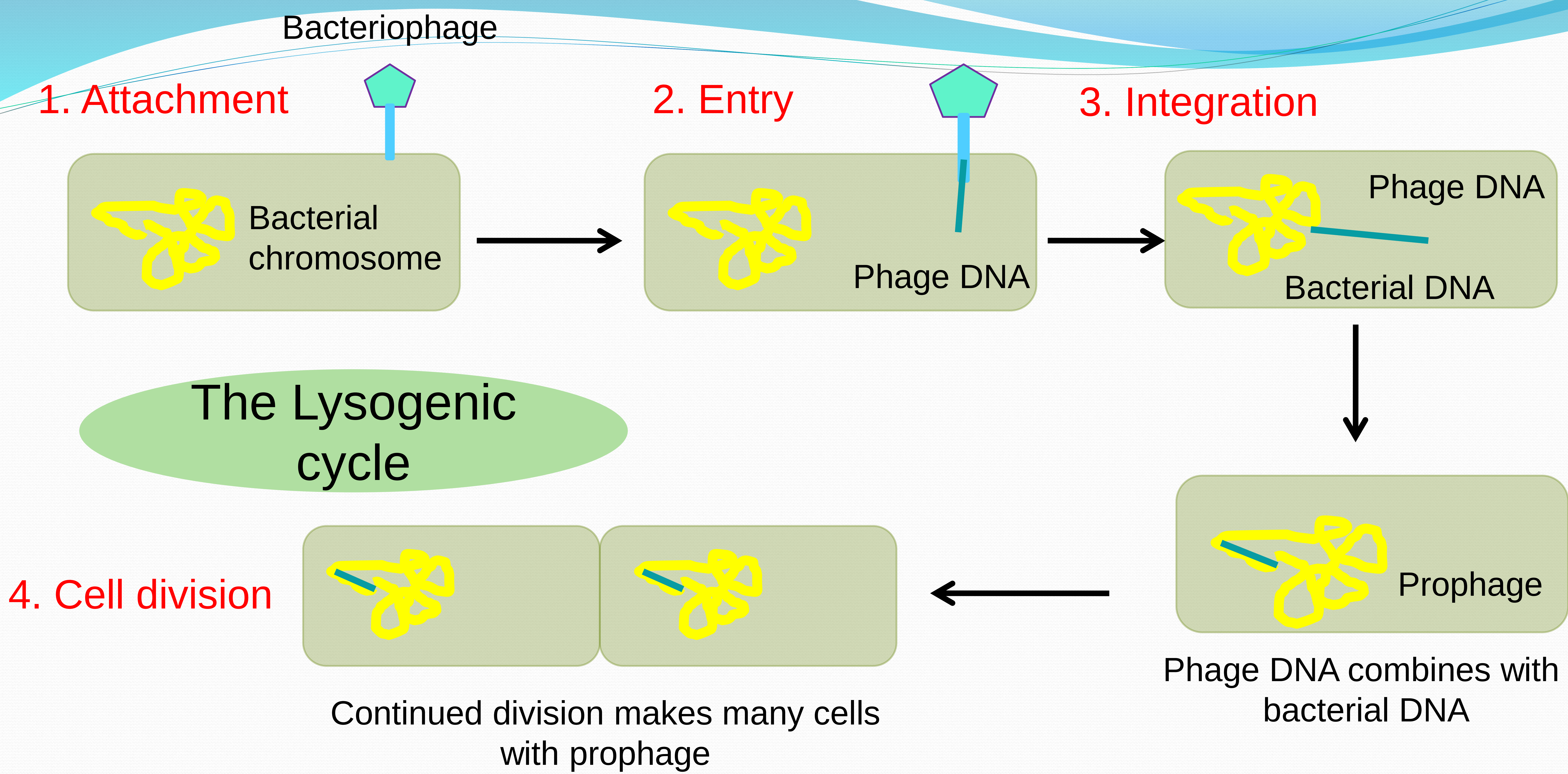

Nobrega, F.L., Vlot, M., de Jonge, P.A. et al. Targeting mechanisms of tailed bacteriophages. Nat Rev Microbiol 16, 760–773 (2018). https://doi.org/10.1038/s41579-018-0070-8



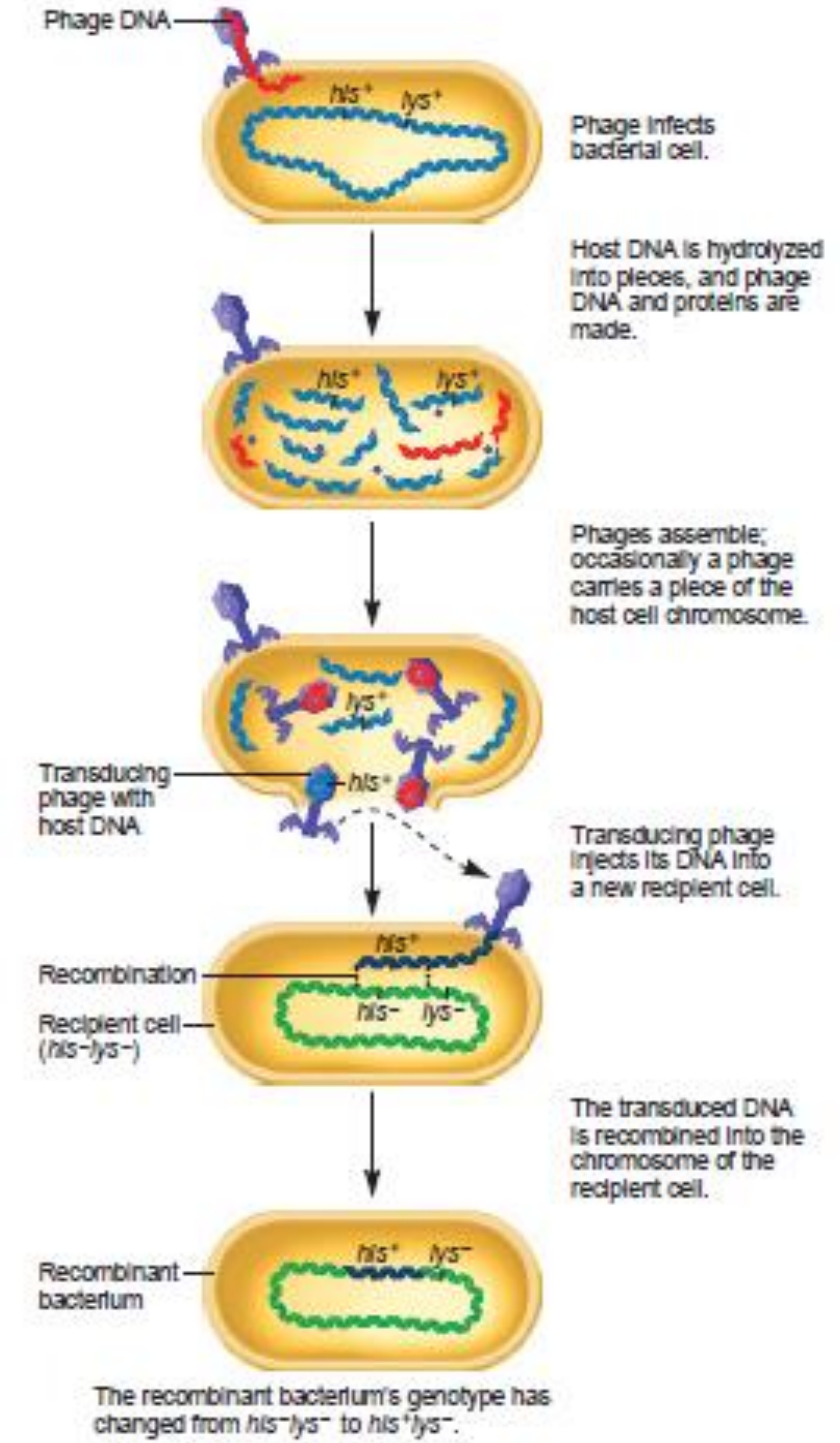
Schmidt, C. Phage therapy's latest makeover. Nat Biotechnol 37, 581–586 (2019). https://doi.org/10.1038/s41587-019-0133-z



Bacteriophage
1. Attachment
Bacterial chromosome
2. Entry
Phage DNA
3. DNA replication & protein synthesis
Capsid proteins
Phage DNA copies
The Lysis Cycle
5. Lysis
4. Assembly
New assembled phages


Bacteriophage
1. Attachment
2. Entry
3. Integration
Bacterial
chromosome
Phage DNA
Phage DNA
Bacterial DNA
The Lysogenic cycle
Prophage
4. Cell division
Phage DNA combines with bacterial DNA
Continued division makes many cells with prophage

The transducing phage (P22 and P1 phages infecting the bacterial species *Salmonella typhimurium and E. Coli)* introduce DNA into a new recipient cell that was originally *his*$^-$ *lys*$^-$ (unable to synthesize histidine and lysine). During transduction, it received a segment of bacterial chromosomal DNA that carried *his*$^+$. *Following recombination, the recipient* cell's genotype was changed to *his*$^+$, so it now could synthesize histidine



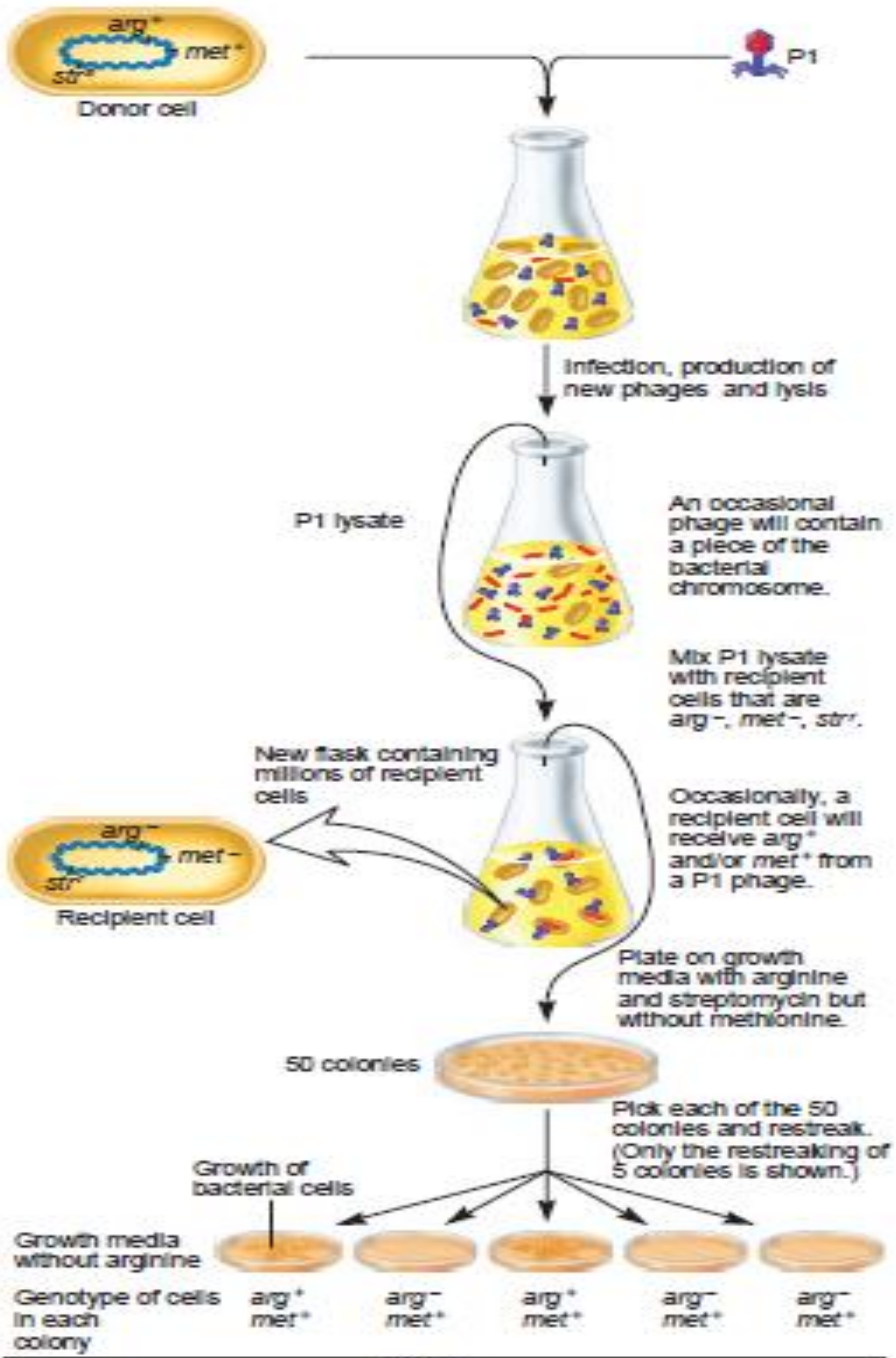

Source: Genetic Analysis and Principles, 4th Edition

# Cotransduction

Cotransduction is a phenomenon when two genes are close together along the chromosome, and a bacteriophage may package a single piece of the chromosome that carries both genes and transfer that piece to another bacterium.

The likelihood that two genes will be cotransduced depends on how close together they lie.

**Cotransduction can be used to map genes that are within 2 minutes of each other**




**Results**

| Selected gene | Nonselected gene | Number of colonies that grew on media + arginine | Number of colonies that grew on media − arginine | Cotransduction frequency |
|---|---|---|---|---|
| $met^+$ | $arg^+$ | 50 | 21 | 0.42 |

# Co transduction experiment to map the genes

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

*d = distance between two genes in minutes*
*L = the size of the chromosomal pieces (in minutes)*
that the phage carries during transduction (For P1 transduction, this size is approximately 2% of the bacterial chromosome, which equals about 2 minutes.)

Source: Genetic Analysis and Principles, 4th Edition by Brooker

THANK YOU