iGEM Microbiology Intro

Prof. Sebastian Maerkl

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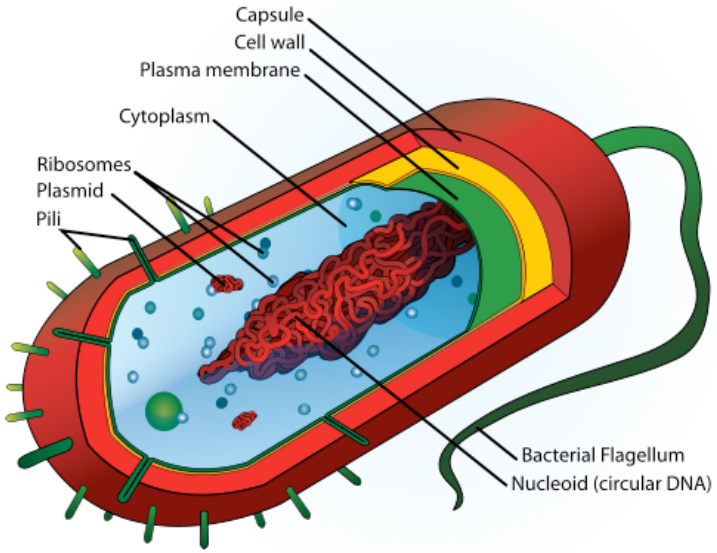
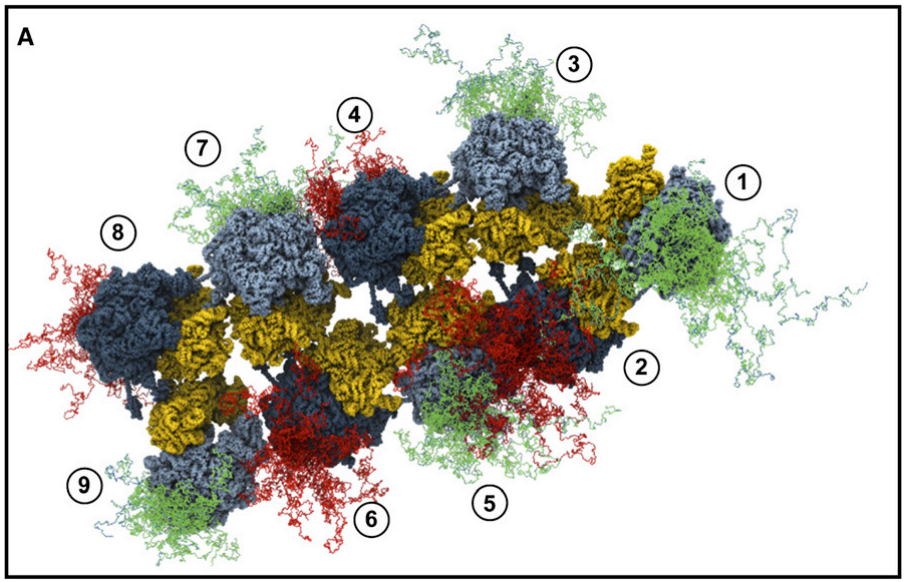
Prokaryotic Cell Structure

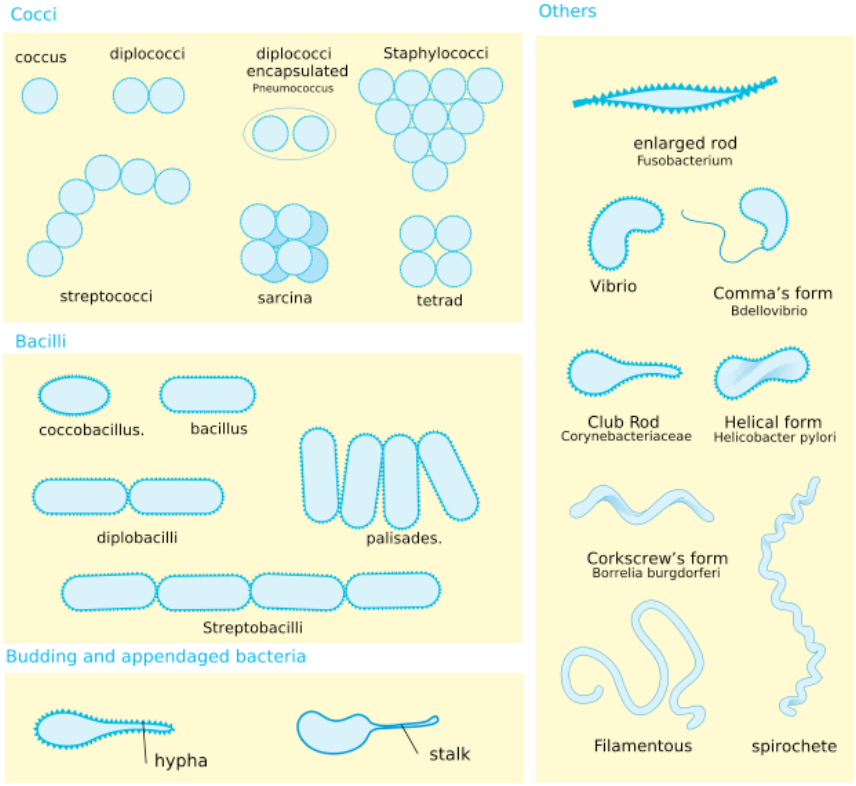




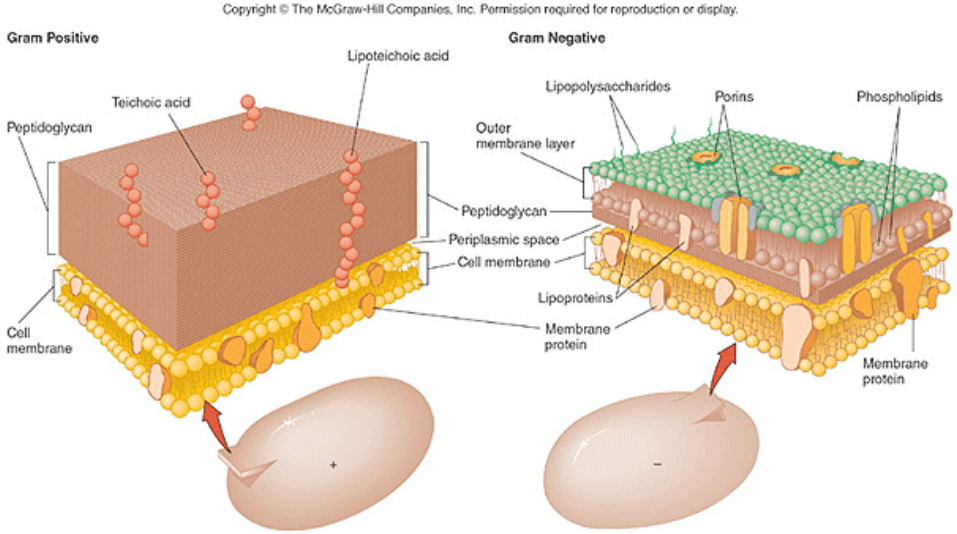
- **Plasma Membrane**
- **Gas vacuole:** buoyancy
- **Ribosomes:** protein synthesis
- **Inclusion Bodies:** storage of C, P, etc
- **Nucleoid:** chromosomal DNA
- **Periplasmic space:** hydrolytic and binding proteins
- **Cell Wall:** mechanical rigidity
- **Capsules:** resistance to phagocytosis; adhesion
- **Fimbriae and pili:** attachments and mating
- **Flagella:** movement
- **Endospore:** survival mechanism

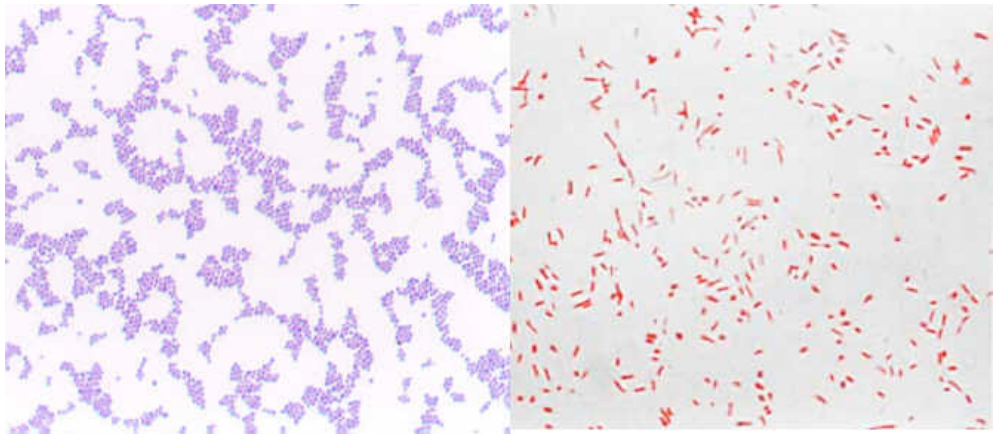
10.1016/j.cell.2008.11.016

Morphology and Classification

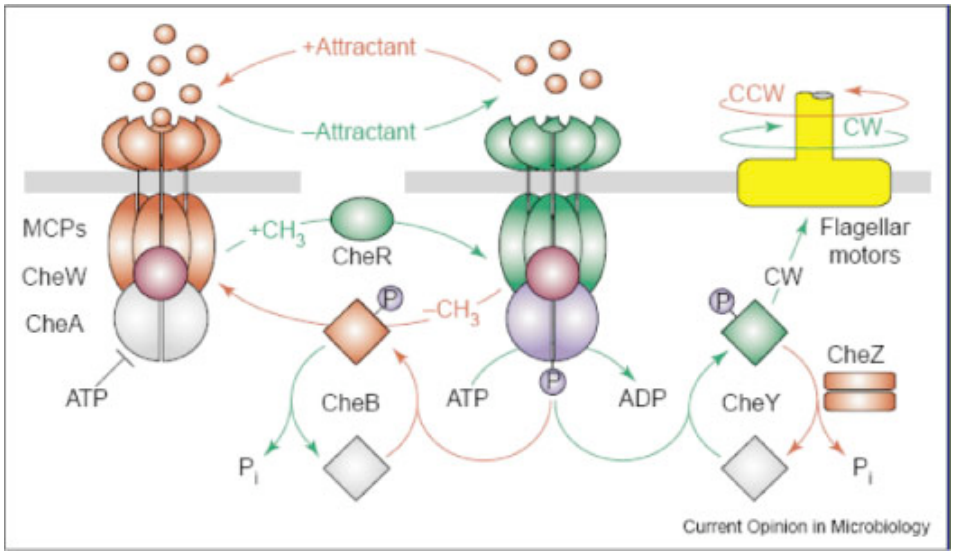


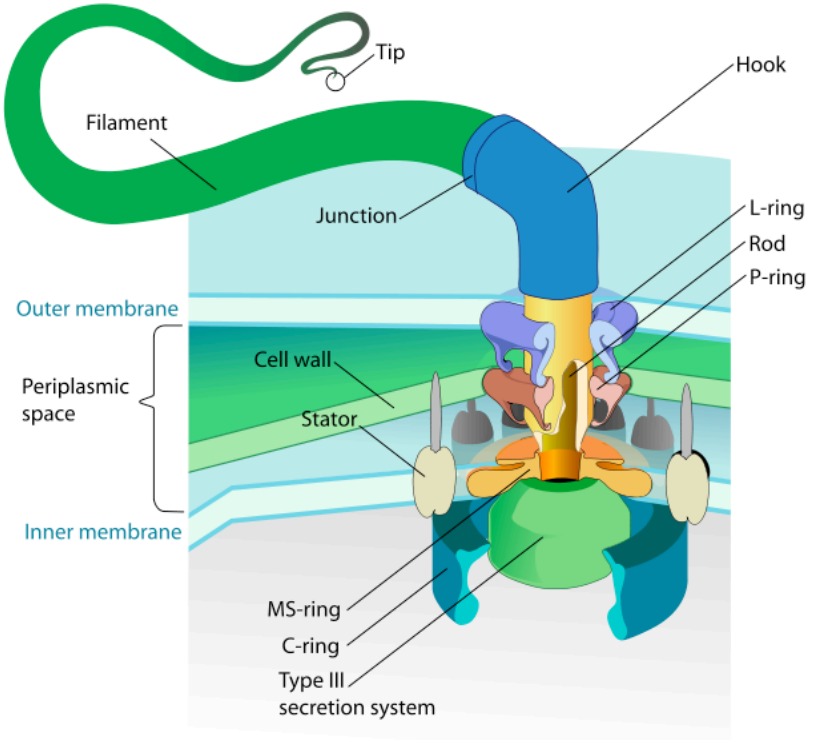
Gram-Positive **Gram-Negative**



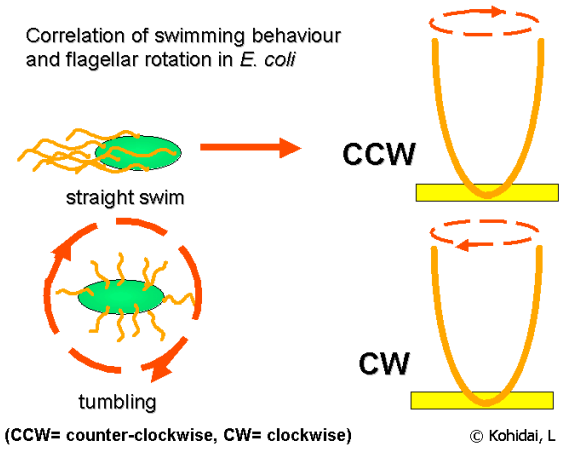


Flagella and Chemotaxis

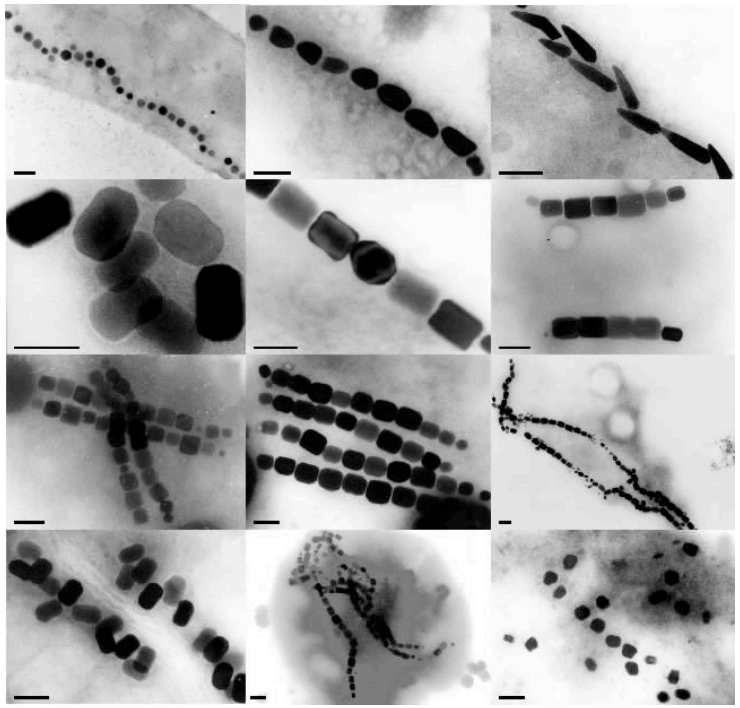




- Tumble:
  - CheA autophosphorylates if MCP is not bound
  - CheA then phosphorylates CheY, which causes a tumble (CheYp is rapidly degraded to CheY by CheZ
- Run:
  - Attractant binds to MCP causing a stearic shift, limiting autophosphorylation of CheA
  - CheYp levels drop, causing CCW rotation
- Resetting:
  - CheR methylates MCPs causing increased CheA autophosphorylation
  - This causes increases in CheYp and CheBp, which lead to CW rotation and de-methylation of MCPs



Living Magnets (Magnetotactic Bacteria)



- Magnetosomes consist of magnetic iron mineral crystals made from: Fe_3O_4, greigite, or Fe_3S_4
- Magnetosomes align the bacteria in the geomagnetic field, allowing for directed taxis

Microbial Metabolism

- Prototrophs: can thrive on minimal medium
- Auxotrophs: lack the ability to synthesize a particular organic compound (commonly used in yeast for selection)

Carbon Sources

- Autotrophs: CO_2 principal carbon source
- Heterotrophs: reduced, preformed organic molecules

Energy Sources

- Phototrophs: light
- Chemotrophs: oxidation of organic or inorganic compounds

Hydrogen or Electron Sources

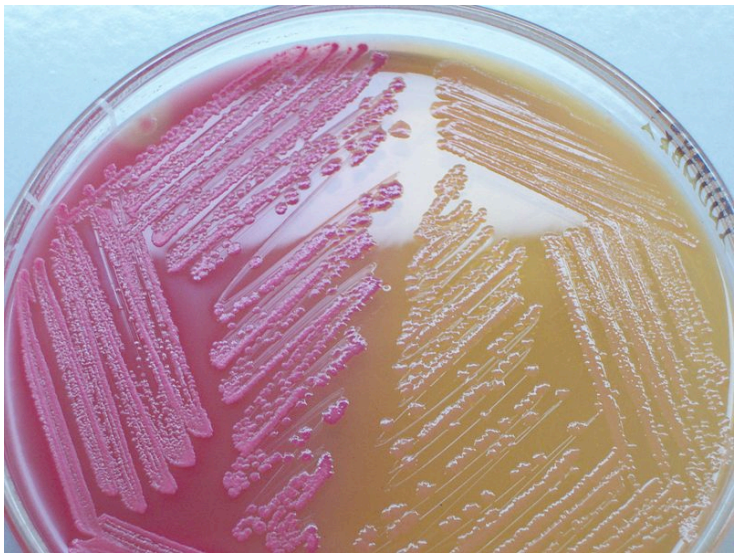
- Lithotrophs: reduced inorganic molecules
- Organotrophs: organic molecules

Algea: photolithotrophic autotrophs

E.coli: chemoorganotrophic heterotroph

Media

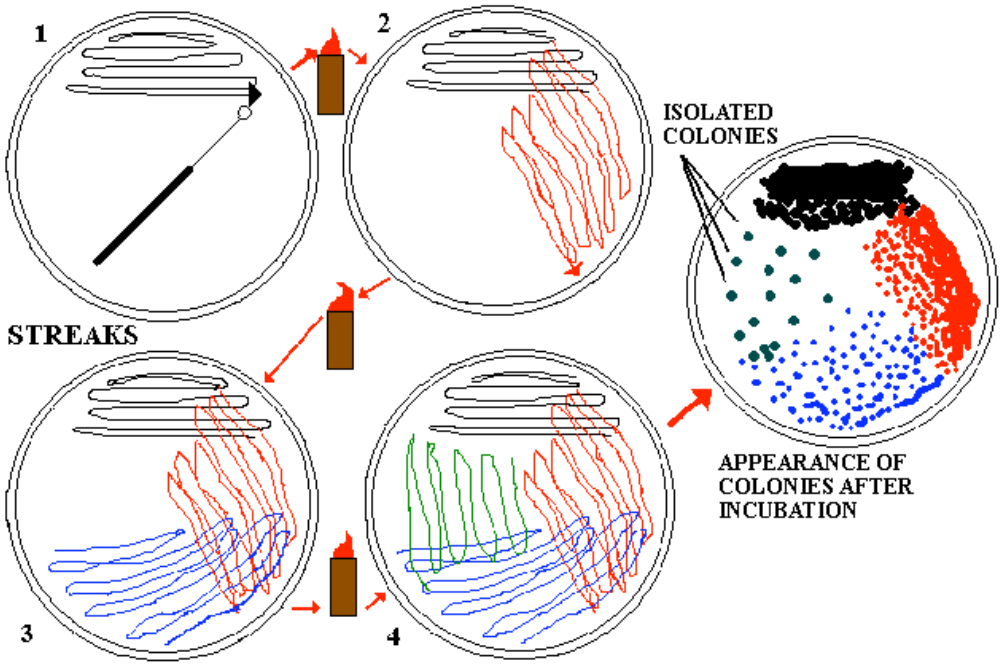
- Synthetic or Defined Media: all components are known. Chemoorganotrophic heterotrophs can be grown on media containing glucose, ammonium salts and other salts.
- Complex Media: contain undefined components such as peptones (protein hydrolysates), meat extract or yeast extract
- Selective Media: favor particular microorganisms
- Differential Media: permit the identification of bacteria based on biological characteristics



MacConkey agar is both selective and differential: it selects for gram-negative bacteria and stains for lactose fermentation

Culturing

- Generation of Pure Cultures is extremely important in general microbiology, but also in cloning!
- Two approaches: streak plating and spread plating
- Spread plating is also used to obtain growth curves by counting colony forming units (CFUs)
- Each colony is **clonal !** They arise from a single bacterium and thus are genotypically the same.



OK

BAD

Spread Plating

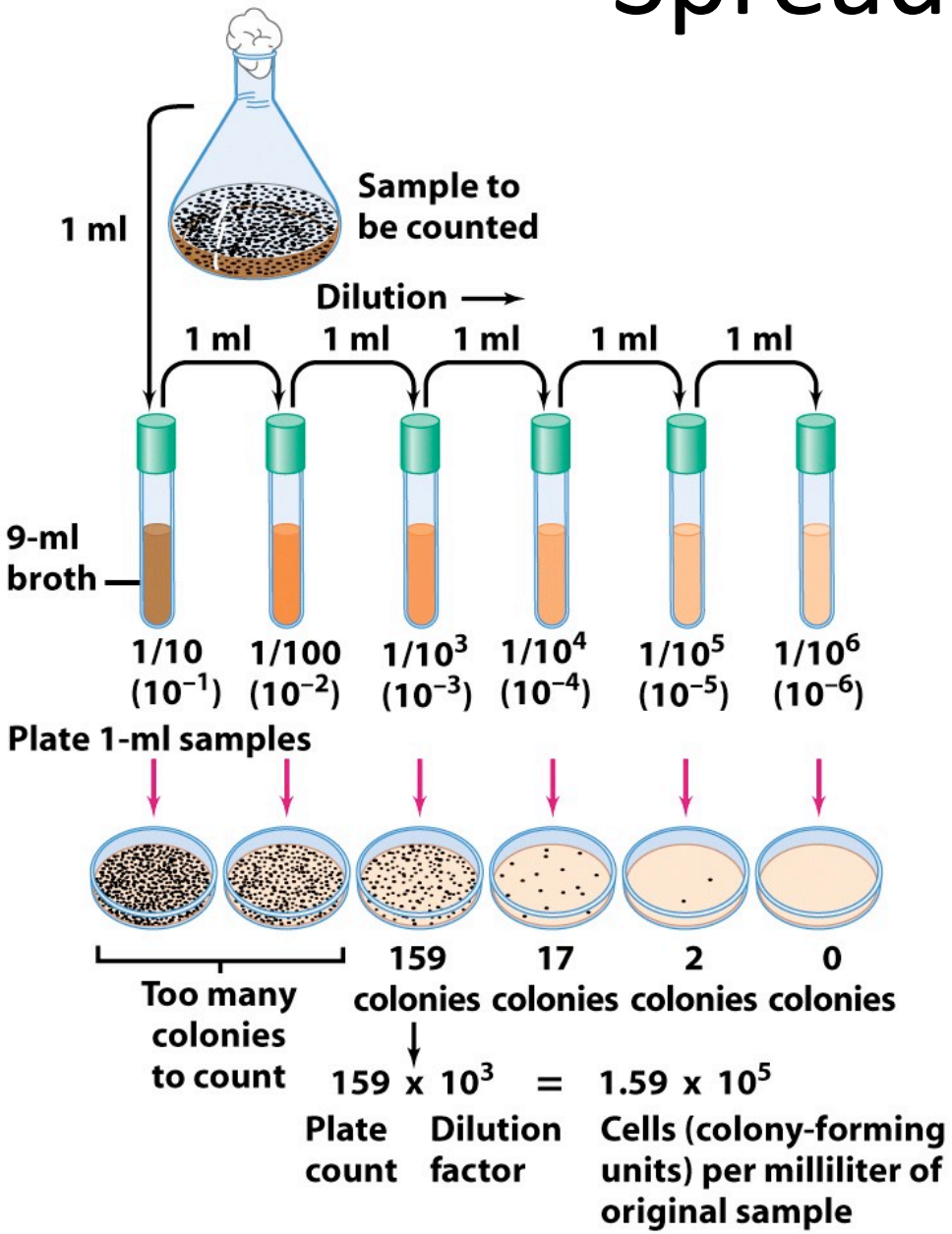
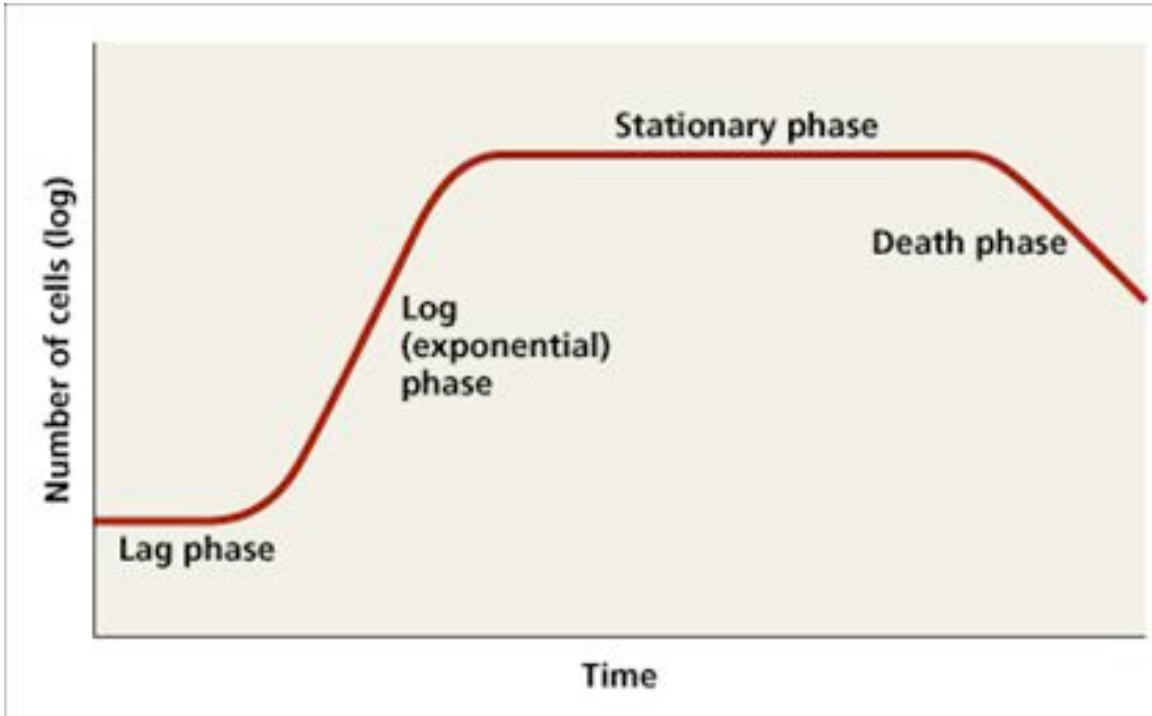


Figure 6-11 Brock Biology of Microorganisms 11/e

- Spread plating is also used to obtain growth curves by counting colony forming units (CFUs).
- The most accurate count of live cells. Of course dead or dormant cells are not counted, as they do not form a colony.
- Faster: optical **turbidity measurements**
- Bacterial lawns are generated by spread plating.
- Transformed bacteria are generally spread plated.

Batch Growth



N_t = population at time t

N_0 = initial population

n = number of generations in time t

$N_t = N_0 \times 2^n$

Solve for n:

$n = \log N_t - \log N_0 / \log 2$

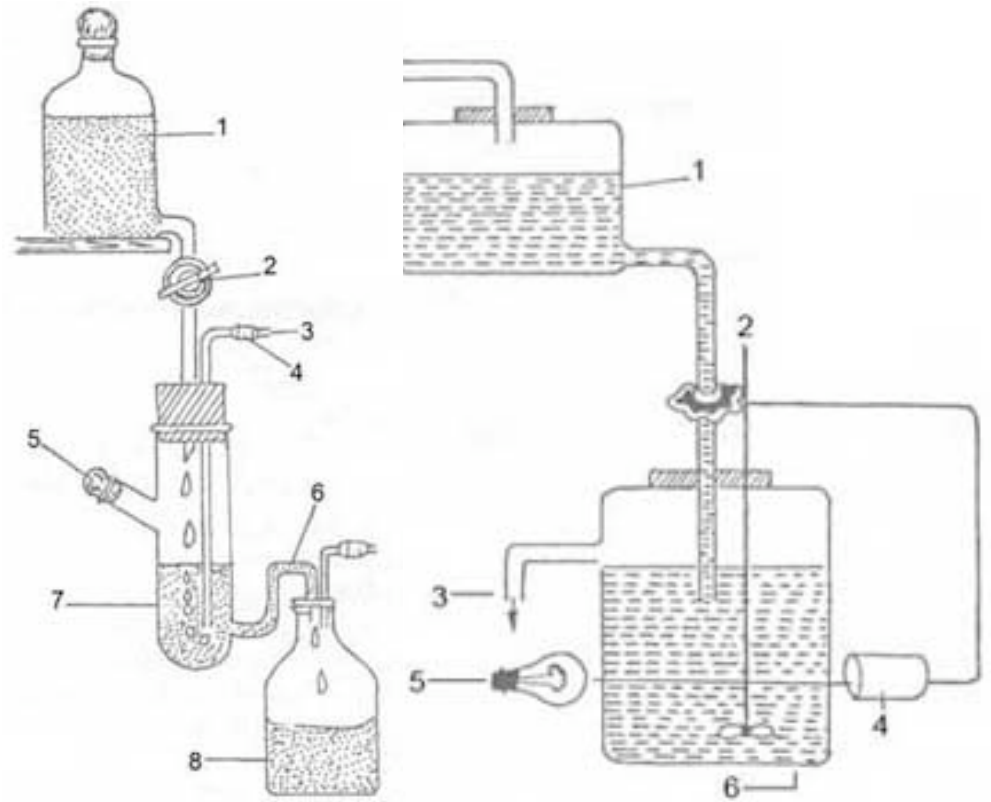
Mean growth rate constant (k): $k = n/t$

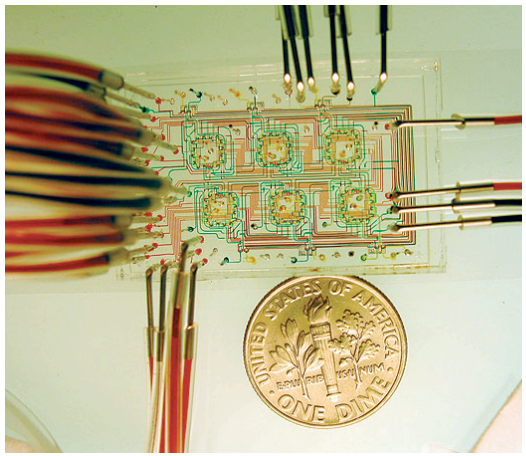
Mean generation time (g): $g = 1/k$

Some generation times:

- E.coli: 21 minutes
- B.subtilis: 26 minutes
- S.cerevisiae: 120 minutes
- M.tuberculosis: 720 minutes (12 hours)

Chemostats / Turbidostats





- **Chemostat:**
  - Constant dilution rate
  - D=flow rate/Volume
  - At steady state the specific growth rate (μ) is equal to D
  - If μ_{max} is < than D, culture will wash out
- **Turbidostat:**
  - Cell density determines dilution rate
  - More stable at high-dilution rates and low-cell densities
  - μ_{max} is more easily achieved

Recombination

Genetic recombination is the process by which a strand of genetic material (usually DNA; but can also be RNA) is broken and then joined to a different DNA molecule.

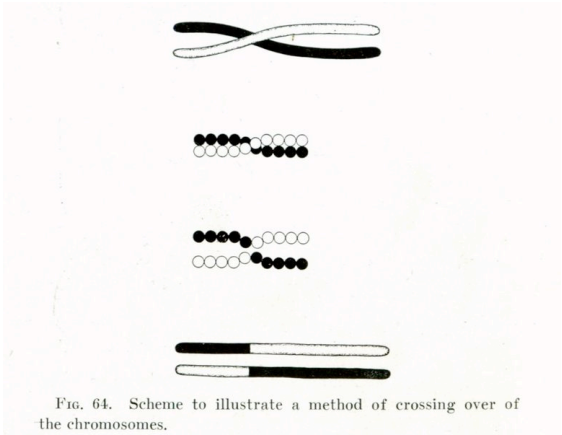


FIG. 64. Scheme to illustrate a method of crossing over of the chromosomes.

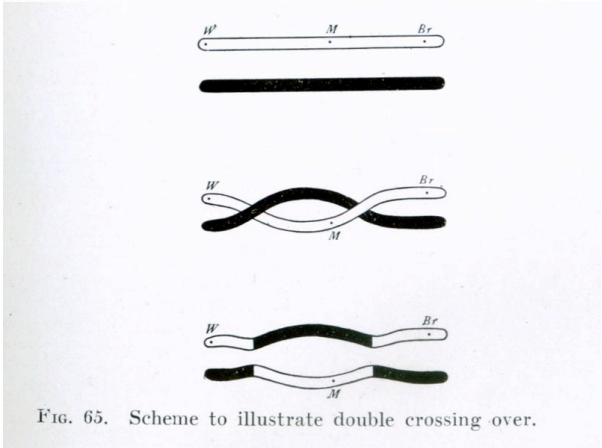
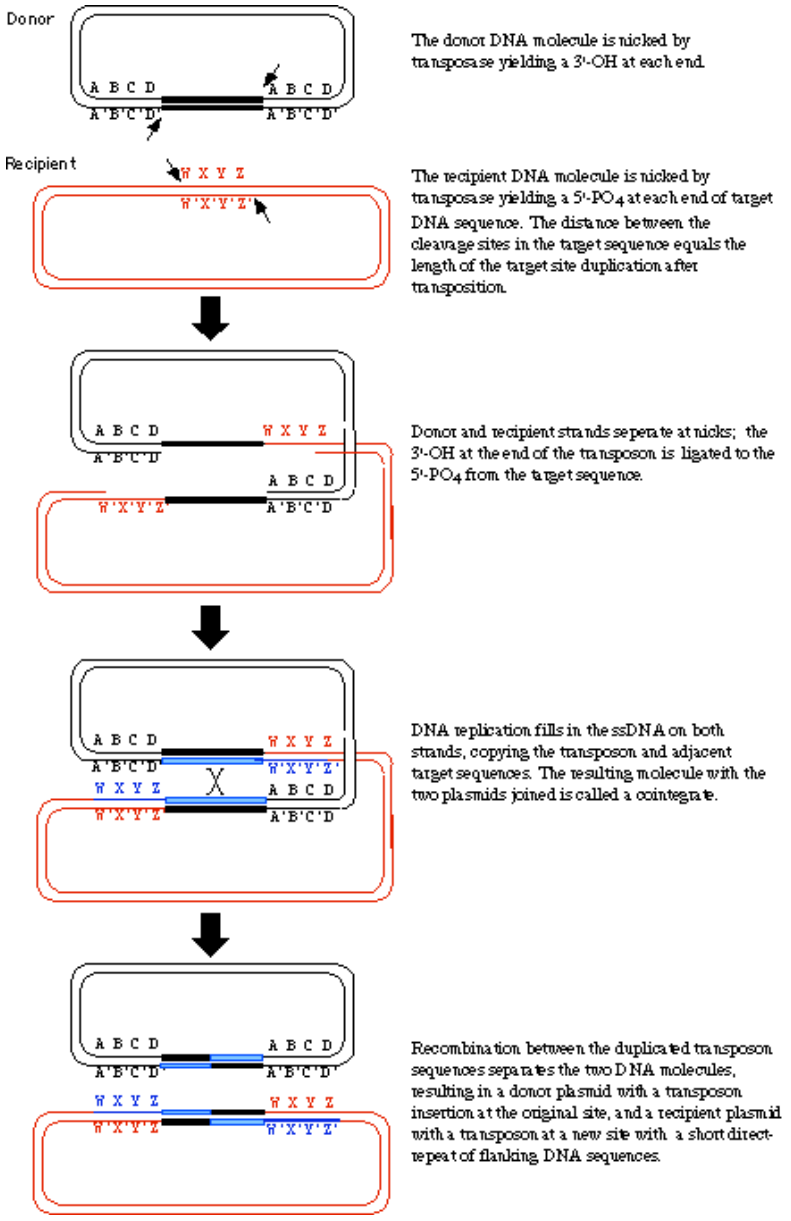


FIG. 65. Scheme to illustrate double crossing over.

- Prokaryotic recombination takes place after **horizontal gene transfer** (as opposed to during meiosis in eukaryotes)
- Three possible ways for the horizontal transfer of DNA:
  - Conjugation
  - Transformation
  - Transduction

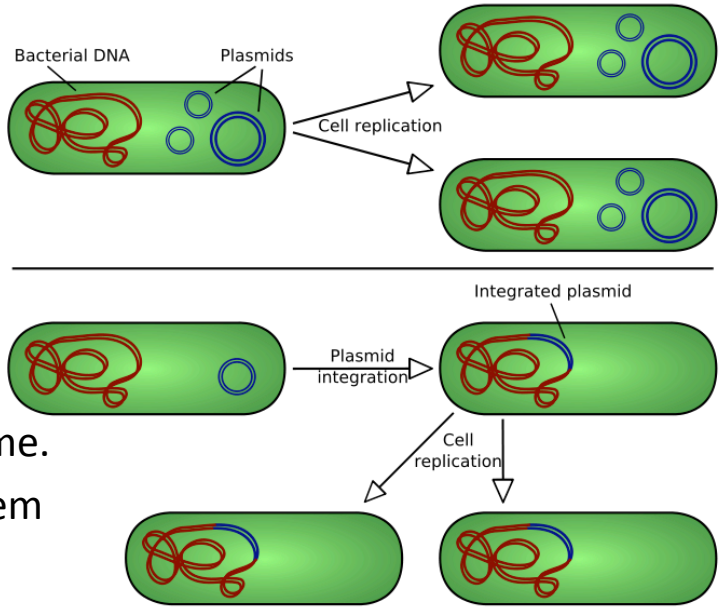
Transposition

- **Transposons** are pieces of DNA that can “hop” in and out of target DNA sequences.
- They code for a transposase which recognizes inverted repeats flanking the transposon (such a simple transposon is called **insertion sequence**, **IS**s containing other genes are called **composite transposons**)
- Transposons play an important role in evolution!

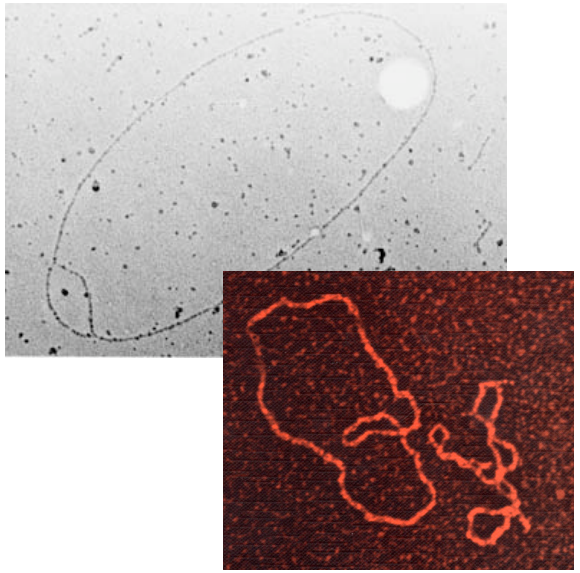


Bacterial Plasmids

- **Plasmids** are circular DNA molecules that can exists independently of host chromosomes and they:
  - Have their own replication sites (they are a **replicon).**
  - Contain a relatively small number of genes that are non-essential to the host.
  - Can exists either as a single copy or multiple copies in a cell.
  - **Episomes** are plasmid that can integrate into the host genome.
  - **Conjugative** plasmids carry genes coding for pili allowing them to undergo horizontal gene transfer through conjugation.



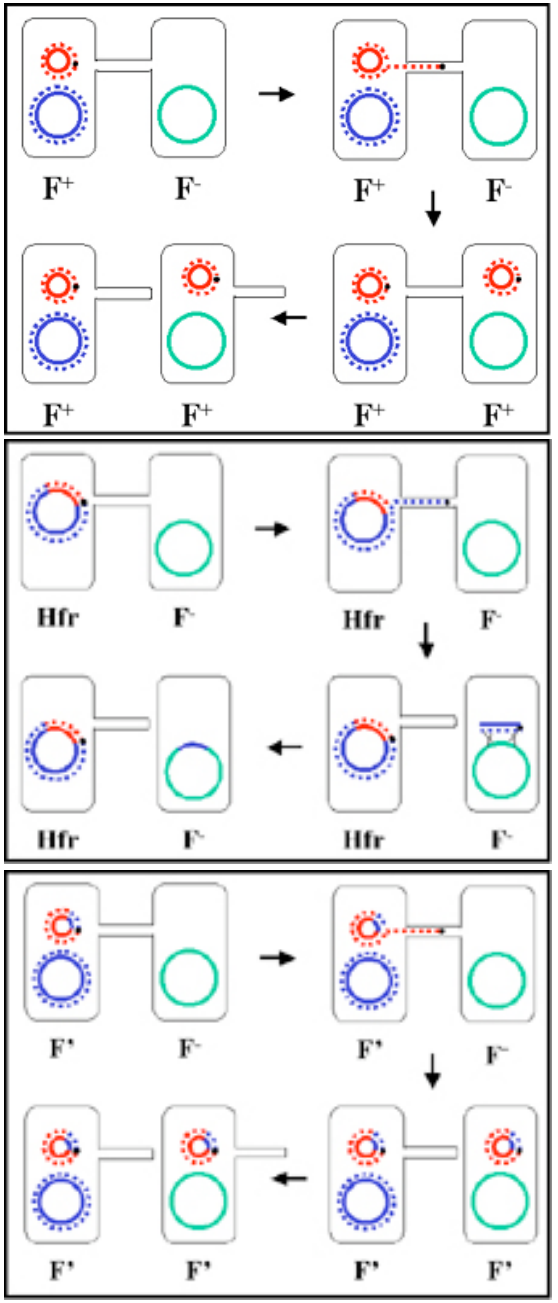
- **Types of Plasmids:**
  - **Fertility Factors:** allows for conjugation; carries genes for sex pili formation, DNA transfer, etc. Is an episome.
  - **R Plasmids:** resistance factors; carry genes capable of neutralizing antibiotics
  - **Col Plasmids:** code for bacteriocins, which are bacterial toxins
  - **Virulence Plasmids:** make their host more pathogenic
  - **Metabolic Plasmids:** as the name implies



- **Vectors** are plasmids used in genetic engineering.

Conjugation

- **Bacterial conjugation** is the transfer of genetic material between bacteria through direct cell-to-cell contact.
- Lederberg and Tatum showed in 1946 that DNA could be transferred between two auxotrophic (triple auxotrophs) strains of bacteria
- Bernard Davis then showed in 1950 that this DNA transfer requires direct physical contact.
- **F^+ x F^- conjugation:** transfers a f factor plasmid without transferring bacterial genes
- **Hfr conjugation:** transfer of an integrated f factor, causes transfer of chromosomal material, but as only part of the f factor is transferred recipient remains F^-
- **F′ conjugation:** a Hfr host de-integrates the f factor including part of the bacterial chromosome. The entire F′ plasmid is transferred.



Transformation

- **Transformation** is the uptake of naked DNA from the environment in an inheritable form.
- The natural transformation efficiency of **competent cells** is on the order of 10^{-3} (1 in 1000 cells takes up DNA).
- Laboratory generated competent cells have efficiencies on the order of $10^{6} – 10^{9}$ transformants per µg of DNA.
- Reversal of auxotrophies or antibiotic resistance is often used to select for transformants in the lab.

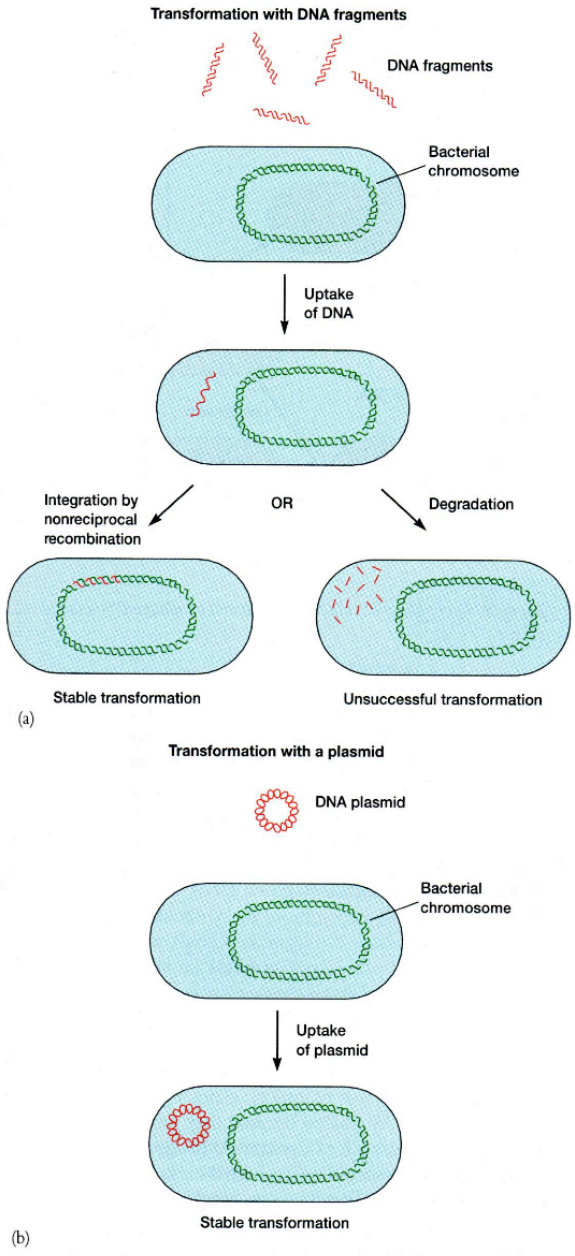
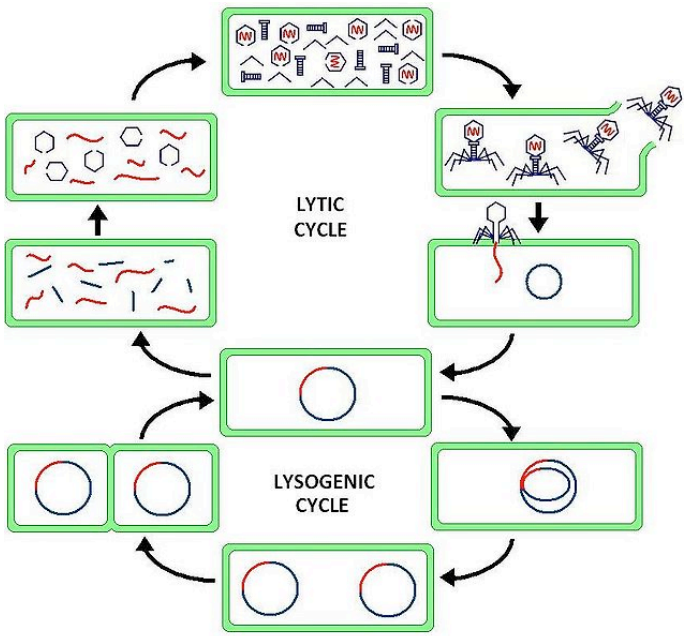
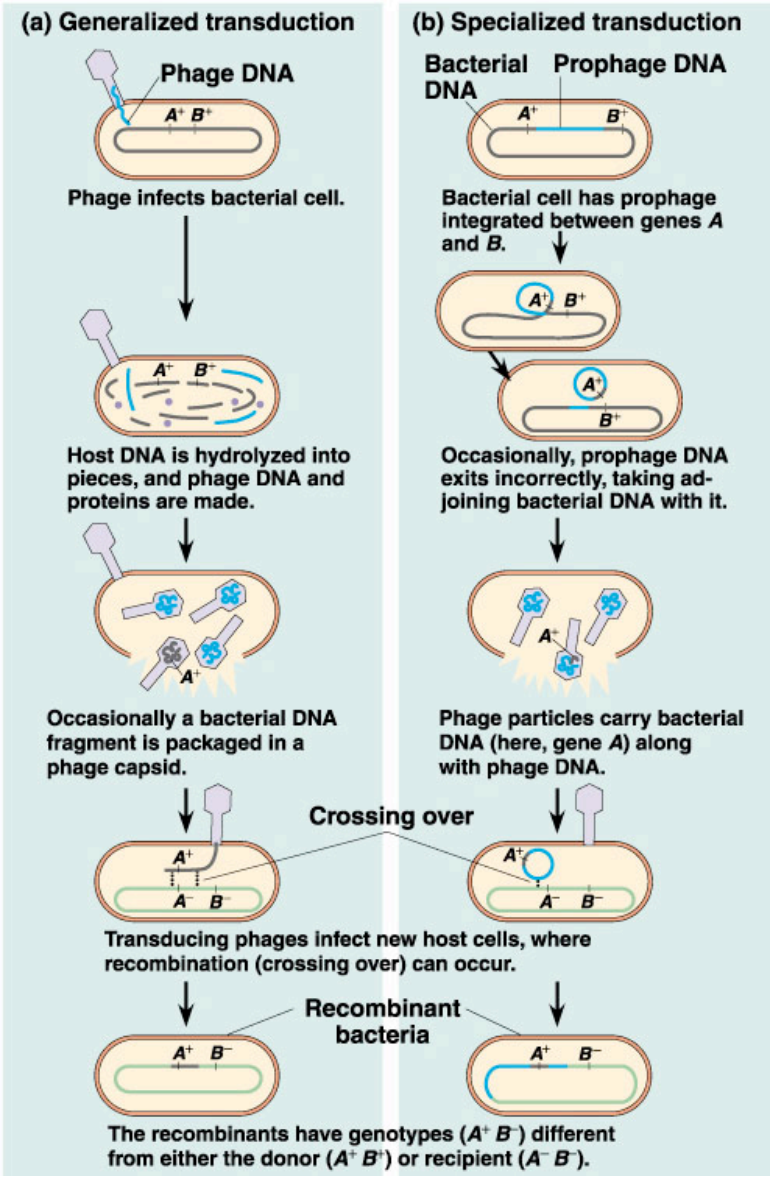


Figure 14.16 Bacterial Transformation. Transformation with (*a*) DNA fragments and (*b*) plasmids. Transformation with a plasmid often is induced artificially in the laboratory. See text for details. The transforming DNA is in red.

Transduction



- **Transduction** is the process by which DNA is transferred from one bacterium to another by a virus.
- Phage have two life cycles:
  - **Lysogenic** and **lytic**
- **Generalized transduction:** consequence of the lytic cycle, bacterial DNA may be randomly packaged
- **Specialized transduction:** if a prophage excises incorrectly and takes along a piece of chromosomal DNA



Homework

- Take a look at my favorite iGEM projects from 2008:
  - UC Berkeley: Clonebots http://2008.igem.org/Team:UC_Berkeley
  - Imperial College: Biofabricator subtilis http://2008.igem.org/Team:Imperial_College
  - Caltech: multi-functional probiotic bacteria http://2008.igem.org/Team:Caltech
  - Harvard: Bactricity http://2008.igem.org/Team:Harvard
- Read up on some common/interesting bugs:
  - E.coli, S.cerevisiae, H.pylori, B.subtilis, S.oneidensis, Magnetotactic bacteria

Seminars

- GHI Seminar every Thursday at 12:15 in AI 1153
- Friday, March 13th 2009 - 15:30pm
  Location: AI 1 153

Life without a wall or division machine in Bacillus subtilis