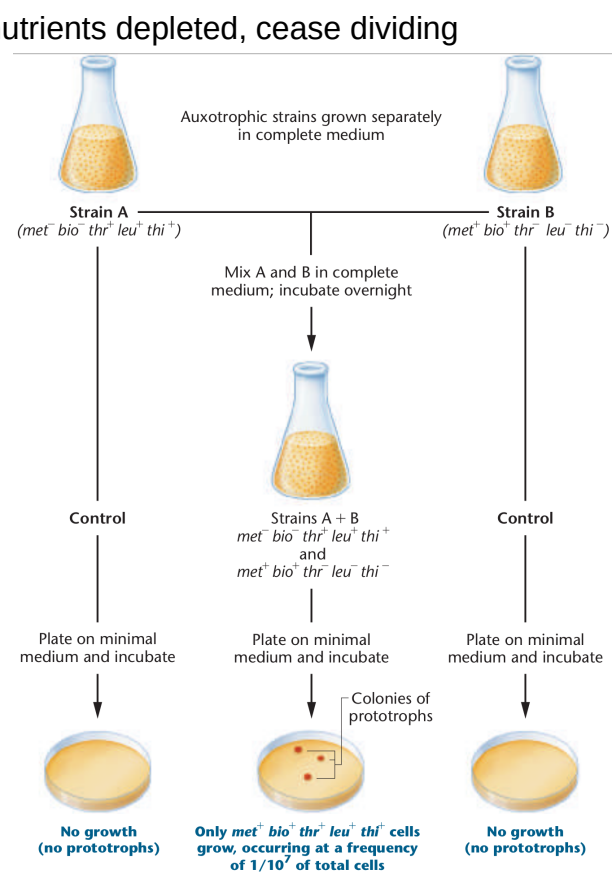
Genetic Analysis and Mapping in Bacteria and Bacteriophages

6.1 Bacteria Mutate Spontaneously and Grow at an Exponential Rate

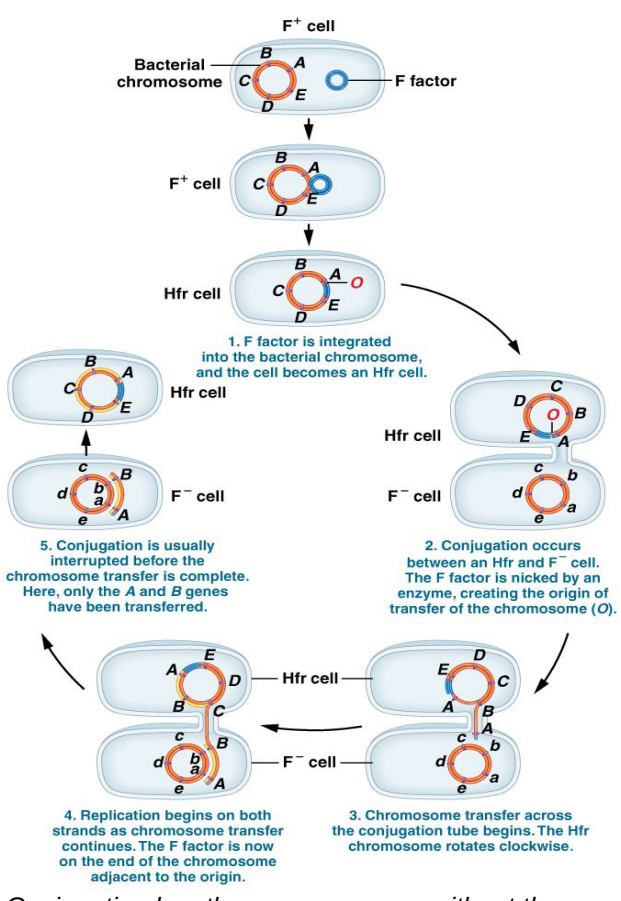
- **Adaptation hypothesis**: Exposure to bacteriophage can induce resistance in the host bacteria (T1 and E. coli).
- *Spontaneous mutant* cells in rather pure cultures can be isolated and established with the use of selection techniques:
  - Growth of organism under conditions where only desired mutant does well. Any wild types fall of.
  - Bacteria and viruses usually carry only one copy of a single chromosome, all mutations are expressed directly in descendants.
  - **Minimal medium**: Simple nutritional components, organic carbons, inorganic ions.
  - **Complete medium**: Adding amino acid supplements.
- **Prototroph**: Bacterium, can grow in minimal medium and synthesize all essential organic compounds.
- **Auxotroph**: Bacterium, needs complete medium, lost ability to synthesize via mutation.
- Growth phases:
  1. **Lag phase**: Slow growth
  2. **Log phase**: Rapid growth (logarithmic)
  3. **Stationary phase**: Culture medium reached, nutrients depleted, cease dividing

6.2 Genetic Recombination Occurs in Bacteria

- **Genetic recombination**: Exchange of genetic material where offspring carries traits that differ from those found in parents.
  - Basis for development of chromosome mapping methodology
- **Vertical gene transfer**: Transfer of genetic information between SAME species.
- **Horizontal gene transfer**: Transfer of genetic information between related but distinct species.
  - Significant role in evolution of bacteria.
- CONJUGATION = BACTERIAL SEX:
  - Genetic information is transferred to another bacterium.
  - Recombines at independent locations to become wild-type cells
  - See figure



- Fertility factor (F factor): unidirectional transfer of genetic material
  - F^- cells are recipients
  - F^+ cells donate DNA and contain **fertility factor** (ability to donate part of chromosome)
  - Conjugation is mediated by **F pilus**.
  - Copy of F factor is transferred, converting the recipient to F^+ state
- High-frequency recombination (Hfr) is a special type of F^+ strains, where the F factor is integrated into the bacterial chromosome.
  - $F^+ \times F^- \rightarrow$ recipient becomes F^+ (low rate of recombination)
  - Hfr X $F^- \rightarrow$ recipient stays F^- (high rate of recombination)
  - **Interrupted mating technique**: Interrupting the conjugation process shows that some genes transfer faster or earlier than others.
- **Time mapping** is the first genetic map of the E. coli chromosome.
  - Chromosome of Hfr was transferred linearly
  - Gene order and distance between genes could be predicted.
- F factor can lose integration and reverts to F^+ state, now F' cell
  - **Merozygotes** are F^- cells that replicated the F factor of F' cell and are now also F' cells.



Conjugation has the same process, without the integration

6.3 Rec Proteins Are Essential to Bacterial Recombination

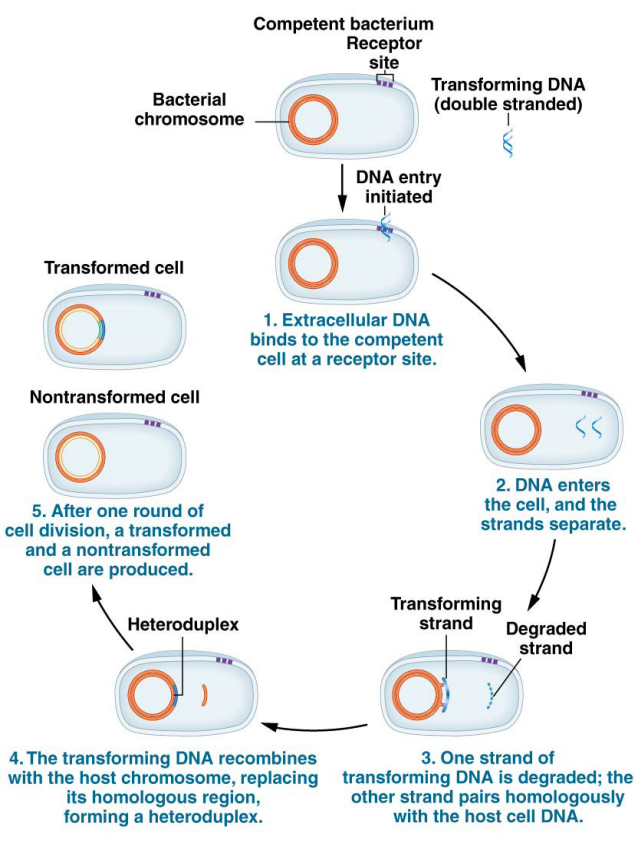
- *RecA*, *recB, recC* and *recD* are mutant *rec*ombination genes.
- First mutant gene *recA* diminshed genetic recombination.
- RecA protein plays important role in recombination involving either ssDNA or lineair end of dsDNA.
- The RecBCD protein complex unwinds dsDNA so RecA can facilitate recombination.

6.4 The F Factor is an Example of a Plasmid

- **Plasmids** are closed circle dsDNA molecules:
  - Multiple copies in cytoplasm
  - Can contain multiple genes
  - Can integrate into host chromosome (**episomes**)
- R plasmid:
  - **RTF** (resistance transfer factor) encodes genetic information for transferring.
  - **r-determinants** are genes, grants resistance against antibodies.
- Col plasmid:
  - Encode **colicins** which are toxic to bacteria that don't carry the plasmid, killing neighboring bacteria.
  - *Colicinogenic*
  - Not transmissible

6.5 Transformation is a Second Process Leading to Genetic Recombination in Bacteria

- With **transformation**, small pieces of extracellular DNA are taken in and integrated into chromosome.
- Only one cell after division contains the foreign DNA sequence
- **Heteroduplex**: Recombinant region holds one host and one mutant strand, which contain mismatches of base pairs.
- **Cotransformation** happens when genes are close enough and get transferred simultaneously.

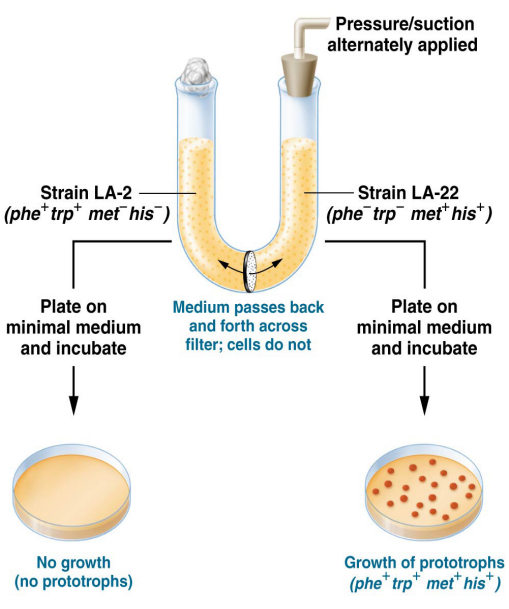


6.6 Bacteriophages are Bacterial Viruses

- **Phages** take bacteria as host and reproduction can lead to **transduction** (recombination by phages/viruses).
- **T4:**
  - The moment absorbed, all bacterial synthesis is inhibited and synthesis of viral molecules start.
  - After assembly, lysis of cell (new virus molecules kill cell to escape)
  - *Virulent*: can only lyse cell
- **Plaque assay** determines number of phages produced after infecting bacteria.
- **Lysogenic** bacteria (or **prophage**) have viral DNA integrated in host chromosome
  - Viral DNA can be replicated and passed on to daughter cells
  - *Temperate* can remain dormant

6.7 Transduction is Virus-Mediated Bacterial DNA Transfer

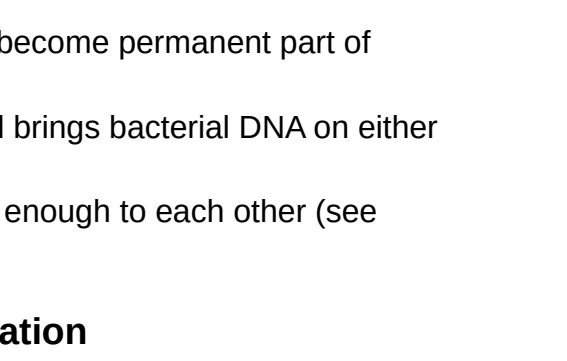
- **The Lederberg-Zinder Experiment**:
  - Salmonella strains were mixed and grown in minimal medium to create 2 prototrophs, but showed only growth in one strain. Unknown source of growth was called **filterable agent** (FA).
  - LA-2 only grew with LA-22, but no recombination if LA-2 culture medium was later added to LA-22: They had to share a common medium.
  - DNase was added, but FA was still active, ruling out transformation.
  - When filter pores where reduced to smaller than phages, FA could not pass through.



- **Generalized transduction** involve random parts of the DNA. Is used in linkage and chromosomal mapping.
  - **Abortive transduction** is when bacterial DNA is injected into the host and only partially diploid.
  - **Complete transduction** is when transduced genes become permanent part of chromosome and is passed onto daughter cells.
- **Specialized transduction** are strain-specific genes and brings bacterial DNA on either ends.
- **Cotransduction** only occurs when two genes are close enough to each other (see **cotransformation**).

6.8 Bacteriophages Undergo Intergenic Recombination

- Phage mutations affect the morphology of the plaques; mutations can be detected by looking at changes in the phenotype of the plaque.
- **Mixed infection experiment**: Letting two distinct mutant strains infect the same bacterial culture.
  - Simultaneous and more viral particles than bacterial cells.
  - **Intergenic**: When two loci are involved



6.9 Intragenic Recombination Occurs in Phage T4

- Seymour Benzer created experiments that recover rare genetic recombinants from **intragenic** exchange in the *rII* locus.
  - **Intragenic recombination** occurs in phage T4 when two strains infect the host simultaneously. It's the equivalent to eukaryotic crossing over, but within a gene.
- By infecting the host with two mutant strains, recombination could restore the wild-type gene.
  - The wild-type could lyse *E. coli* B and *E. coli* K12, while the other strain could not lyse *E. coli* K12.
- This phenomenon was called **complementation** and would only work if the genes were complemented (on different **cistrons/complementation groups**
- **Deletion testing** showed that recombination cannot occur when parts are missing.
  - This could localize mutations if in deleted area.