Genetic Exchange in Bacteria

- Homologous recombination
- Transformation
- Plasmids and conjugation
- Transposable elements
- Transduction (virus mediated exchange)
Gene exchange in bacteria

• Transfer of DNA from one bacterium to another is a common means of gene dispersal. It has a big effect on bacterial evolution, and tremendous practical implications. For example, lateral transfer is responsible for the spread drug resistance determinants between bacterial species.

• Three common mechanisms of lateral gene exchange:
  - Transformation (extracellular DNA uptake)
  - Conjugation (bacterial mating systems)
  - Transduction (viral mediated gene exchange)
RecA mediated
Homologous recombination

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Gene exchange in bacteria

**Transformation**

- Discovered by Griffith in 1928 during the course of his studies of virulence in *Streptococcus pneumoniae*.

- **S**=smooth colony morphotype

- **R**=rough colony morphotype

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**The Griffith Experiment**

1. **S** = Injection
   - Live "smooth" (encapsulated) type 1 pneumococci (S₁)
   - Heat-killed S₁

2. **Dead S** = Injection
   - Live "rough" (unencapsulated) pneumococci (R₁ or R₂) derived by subculture from S₁ or S₂, respectively

3. **R₁ + Dead S**
   - Live R₁ + Killed S₁
   - Dead mouse; yields S₁ cells

4. **R₂ + Dead S**
   - Live R₂ + Killed S₂
   - Dead mouse; yields S₁ cells

Figure by MIT OCW.
Avery, MacLeod, and McCarthy (1944) fractionation studies led to conclusion that transformation principle is DNA.

The Griffith Experiment

- **S** (Live "smooth" (encapsulated) type 1 pneumococci (S₁))
  - Injection
  - Dead mouse; yields S₁ cells

- **Dead S** (Heat-killed S₁)
  - Live mouse

- **R₁ + Dead S**
  - Live R₁
  - Killed S₁
  - Dead mouse; yields S₁ cells

- **R₂ + Dead S**
  - Live R₂
  - Killed S₂
  - Dead mouse; yields S₁ cells
Gene exchange mechanisms in bacteria
Transformation (uptake of exogenous DNA)

- Physiological transformation occurs in nature in a wide variety of genera which include:
  - 1) *Streptococcus*
  - 2) *Staphylococcus*
  - 3) *Bacillus*
  - 4) *Acinetobacter*
  - 5) *Hemophilus*
  - 6) *Neisseria*

Diagram showing the genetic interconnections demonstrated between bacterial groups removed due to copyright restrictions.
Natural Bacterial Transformation

Closely Linked Genes will Tend to Transform Together More Frequently than More Distal Genes
Gene exchange mechanisms in bacteria

Transformation

- Competence. The ability to take up DNA varies regularly during the cell cycle. In Streptococcus competence is highest shortly after cell division.
- Heteroduplex formation with homologous recipient DNA.

Image removed due to copyright restrictions.
Artificial Transformation

Double-stranded DNA forced through cytoplasmic membrane

Integration of linear fragments by homologous recombination or replication of plasmid

Figure by MIT OCW.
Bacterial Conjugation

Image of bacterial conjugation, showing the donor (F+), pilus, and recipient (F-), removed due to copyright restrictions.
PLASMIDS

- Extrachromosomal DNA, usually circular
- Usually encode ancillary functions for in vitro growth
- Can be essential for specific environments: virulence, antibiotics resistance, use of unusual nutrients, production of bacteriocins (colicins)
- Must be a replicon - self-replicating genetic unit
Plasmid Replication

- Plasmid DNA must replicate each time cell divides or it will be lost.
- Host cells do not “spit out” plasmid DNA.
- Two functions required in replication:
  - DNA replication
  - Partitioning (distributing plasmid to progeny cells)
- High copy (>20) and low copy (<5) plasmids.
Plasmid Replication

- High copy plasmids are usually small; low copy plasmids can be large
- Partitioning is strictly controlled for low copy, but loose for high copy
- Plasmid replication requires host cell functions (DNA polymerase, etc.)
- Copy number is regulated by initiation of plasmid replication
- Plasmids are incompatible when they cannot be stably maintained in the same cell because they interfere with each other’s replication.
Confers resistance:
sulfonamide
chloramphenicol
mercury ions
streptomycin
tetracycline

concatemers

ROLLING-CIRCLE MODEL OF BACTERIOPHAGE λ DNA REPLICATION FOR THE SYNTHESIS OF DOUBLE-STRANDED DNA daughters
ColEI plasmid

- small (6.6 kb)
- medium copy #/cell (20 copies/cell)
- non-self-transmissible
- does not require de novo protein synthesis for replication (chloramphenicol amplifiable)
- RNA-II is transcribed through the origin of replication, gets cut by RNaseH and serves as the primer for DNA replication
- RNA-I is transcribed in the opposite orientation and is complementary to RNA-II.
- binding of RNA-II and RNA-I prevents initiation of replication (RNA-I is a negative regulator)
- the Rom/Rop protein made by the rom/rop gene stabilize the binding of RNA-I and RNA-II (also negative regulator)
**F plasmid**

- large (100 kb)
- low copy #/cell (1-2 copies/cell)
- self transmissible (tra genes)
- requires protein synthesis (chloramphenicol-sensitive)
- *repE* gene encodes RepE protein
  - RepE protein binds to origin of replication (*oriS*) and initiates DNA replication
  - RepE binds to the *repE* promoter and activates transcription
  - RepE binds to the *copA/incC* locus and is titrated away from *oriS* and *repE* (negative regulation of replication)
Image removed due to copyright restrictions.
Image removed due to copyright restrictions.
Creation of an F’ Strain

E. coli chromosome

Will transfer Lac+ frequently

Figure by MIT OCW.
Creation of an F’ Strain

Lac merozygote (can assess dominance)
Hfr Strains

• The F plasmid can integrate into the chromosome (many sites – directed by transposon homology). This creates a high frequency of recombination (Hfr) strain.

• The integrated F plasmid directs transfer of the chromosome, starting from the origin. Genes close to the site of integration will be transferred first.

• Transfer continues, with the order of transfer matching the order of genes along the chromosome, until it is interrupted.

(interrupted mating experiments for chromosomal mapping...
Creation of an Hfr Strains

F is integrated into the host chromosome

Hfr strain
DNA Transfer in an Hfr Strain

Diagram removed due to copyright restrictions.
Image removed due to copyright restrictions.
Order of Gene Transfer in an Hfr Strain

Order: Hfr – Azi – Ton – Lac – Gal

Figure by MIT OCW.
Different Hfr Strains

Order of transfer

1st

last

thi gly his gal pur lac pro thr
High Resolution Mapping Using Hfr Strain

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Temperate Phage and Lysogeny

Virus particles
Attachment
Injection
Lytic pathway
Lysogenic pathway
Viral DNA replicates
Viral DNA is integrated into host DNA
Lysogenized cell
Cell division
Lysis
Coat proteins synthesized; virus particles assembled
Normal cell growth
Cell (host)

Microscopic photograph removed due to copyright restrictions.

Figure by MIT OCW.
Phage conversion

Dormant prophage – integrated bacteriophage – carries genes that alter the phenotype of the microbe

- best examples are pathogens and toxin production

*Corynebacterium diptheriaeae*

Phage produces diptheria toxin

This is what makes people sick

*C. diptheriaeae*

without phage strain produces no toxin

Does not cause diptheria
The lysogenic pathway of bacteriophage infection

- **Virus particles** attach to the host cell.
- **Viral DNA** replicates within the host cell.
- Viral DNA becomes integrated into the host DNA, forming a lysogenized cell.
- During cell division, the viral DNA is passed on to new host cells.
- In the lytic pathway, viral DNA is replicated, coat proteins are synthesized, and virus particles are assembled.
- Lysis occurs, releasing new virus particles.
- In the lysogenic pathway, normal cell growth occurs without lysis.

Figure by MIT OCW.
Site-specific integration of $\lambda$

- $\text{attP}$ binds Integrase, IntHostFactor
- Complex binds $\text{attB}$
- Int recombines the two molecules using the match “O” sequence
- Xis removes “lysogenic” phage in response to environmental stress

Figure by MIT OCW.
Invitrogen image of Phage lambda recombination in *E. coli* removed due to copyright restrictions.
Mechanism of Integrase action

Diagram showing the mechanism of integrase action removed due to copyright restrictions.

• ATP independent process
• 5’ OH and 3’ phosphates
• Covalent enzyme-tyrosine-integrase attachment - akin to topoisomerases
Specialized transduction (in phage lambda)

Specialized transduction is site specific, and so results in transfer only of specific genes.

Eg, genes next to the attB site for lambda Infecting E. coli

Diagram removed due to copyright restrictions.
Visible effects on DNA during viral infection

Images showing DNA and T4 phage in a pre-infection and post-infection cell removed due to copyright restrictions.
The “headfull” mechanism utilized by some bacteriophage lends itself to mispackaging – there are no specific sequences recognized by the packaging machinery.

Molecular Genetics of Pathogenic Bacteria, 1996, Stewart and Maloy.

Packaging mechanism of Phage P22 of *Salmonella typhimurium*
Generalized transduction
This happens when host DNA, instead of phage DNA is accidentally packaged.

Generalized transduction is more or less random, and so can result in the transfer of almost any gene.

Image removed due to copyright restrictions.
An example – P22 phage transduction of *Salmonella typhimurium*

P22 HT is a efficient generalized transducer
- its sloppy – 50% of the viral particles contain host cell DNA (ie are transducing particles or TPs)

Each transducing particle (TP) carries 44 kb of DNA – the *Salmonella* genome is app. 4400 kb in size

Therefore, if the process is random 100 different transducing particles should represent the entire genome.

\[
(0.5)(10^{11} \text{ viruses/ml})/(100 \text{ TP [1 genome]}) = 5 \times 10^8 \text{ copies of the genome/ml of lysate}
\]
Generalized transduction is a useful way to exchange genes between bacteria.

Also extremely useful for mapping of genetic markers relative to each other.
Mobile genetic elements
DNA transposition

- Movement of DNA sequences from a “donor site” to a new “target site” within the genome
- Discovered by Barbara McClintock “jumping genes”
- Takes place in virtually all organisms
- Potentially mutagenic (transposon mutagenesis)
- Rare infrequent events (tightly regulated)

- Donor site
  contains a transposable element (transposon)

- Target site
  in general is random
  hot spots: preferred sequences that are targeted
Mobile genetic elements

Insertion sequences (I.S. elements, Class I transposons). Small discrete segments of DNA ranging in size from 750 bp to 1600 bp.
Bacterial transposable elements
Class I transposons (insertion sequences)

- Relatively small (~ 750 - 1600 bp)
- Flanked by terminal inverted repeats (IRs)
- Generally only 1 gene
- transposase (tnpA) = ~ 37 Kda
- “Hop” from one part of the genome to another.
- Sometimes have an outward facing promoter!
Mobile genetic elements

I.S. elements can act in pairs to mobilize intervening DNA.

I.S. elements can mobilize important determinants such as antibiotic resistance genes, genes for lactose utilization, or genes for bacterial enterotoxins.

In *E. coli* the ST enterotoxin gene is encoded by a transposon and is sometimes found on plasmids and sometimes on temperate phages.
Mobile genetic elements
Transposon formation.

Figure by MIT OCW.
Mobile genetic elements

Class II transposon structure.

Transposon (2600 bp)

750 bp

1100 bp

IS1

Chloramphenicol-resistance gene

5-bp direct repeat

Figure by MIT OCW.
Image removed due to copyright restrictions.
Bacterial transposable elements

Class II transposons (complex transposons)
- \(tnpA\) (transposase) \(\sim 120\) Kda
- \(tnpR\) (site-specific recombinase) \(\sim 21\) Kda
- TnpR acts on resolutions site (\(res\))
- long terminal inverted repeats (35 - 40 bp) (LTRs)
- duplicate a \(\sim 5\)- bp target site upon transposition
- often carry genetic markers (antibiotic resistance genes)
- Families: \(Tn3\) & \(Tn501\)
Bacterial transposable elements

Class III transposons (Mu and others)

Bacteriophage Mu
- ~ 38 Kb linear DNA molecule
- transposition results in duplication of target site
- lacks terminal inverted repeats
- A-gene (transposase)
- B-gene (replication and transposition)
Image removed due to copyright restrictions.
Direct transposition (conservative)

Transposon at old location

encodes transposase

excision

New target sequence

makes staggered cuts at new target site

DNA repair mechanisms result in duplication of the target site
Replication-dependent transposition

Image removed due to copyright restrictions.
Strategy for transposon mutagenesis

Diagram showing the process of transposon mutagenesis removed due to copyright restrictions.
In vivo Tn mutation

Mutagenesis of bacteria

Generating mutants templates in vitro

Diagrams removed due to copyright restrictions.

Epicentre Biotechnologies Website
Gene expression on cloned operons from environmental libraries

Proteorhodopsin/retinal + β-carotene/retinal/proteorhodopsin operon

Images removed due to copyright restrictions.