

Mechanisms of Infectious Disease • Fall 2006

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Genetic Basis of Variation in Bacteria

- I. Organization of genetic material in bacteria
 - a. chromosomes
 - b. plasmids
- II. Genetic variation: Source
 - a. point mutation
 - b. DNA rearrangements
- III. Genetic variation: Transmission
 - a. transformation
 - b. transduction
 - c. conjugation
- IV. Genetic variation: Implications for pathogenesis

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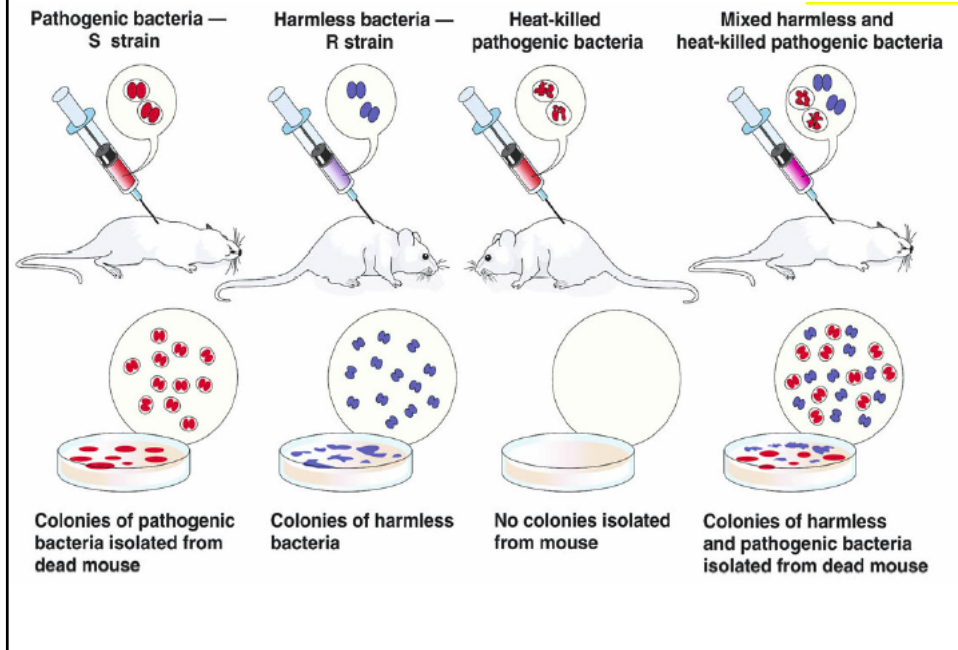
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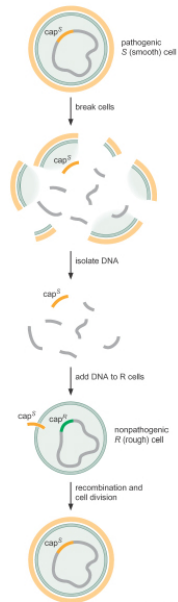
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Genetic basis of variation: **Griffiths (1928)**

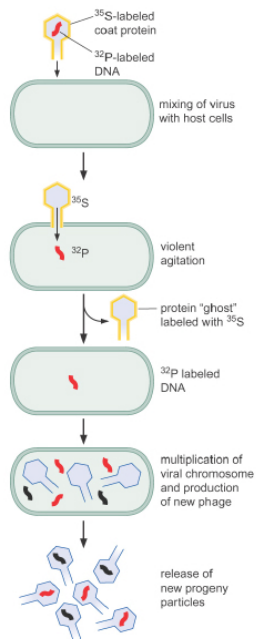


Genetic basis of variation: **Avery et.al (1944)**



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Genetic basis of variation: Hershey and Chase (1952)



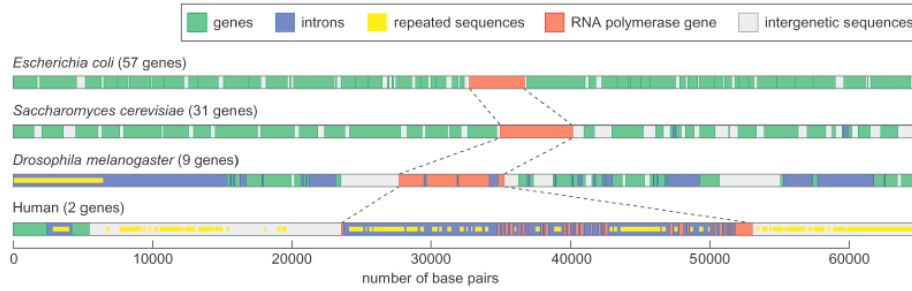
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Organization of genetic material in bacteria: chromosomes

- **Most** bacteria contain a single chromosome (+ extrachromosomal elements)
- **Some** bacteria have been found also to contain 2-3 replicons which can be considered either megaplasmids or minichromosomes e.g. 3.0 Mb and 0.9 Mb replicons in *Rhodobacter sphaeroides*
- A **few** bacterial genera contain >1 chromosome e.g. 2.1 Mb and 1.2 Mb chromosomes in *Brucella*
- **Some** bacteria harbour large replicons essential for survival in a specific ecological niche but not under laboratory conditions e.g. 1.4 Mb and 1.7 Mb replicons in *Rhizobium meliloti* are required for plant symbiosis

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Organization of genetic material in bacteria: **chromosomes**

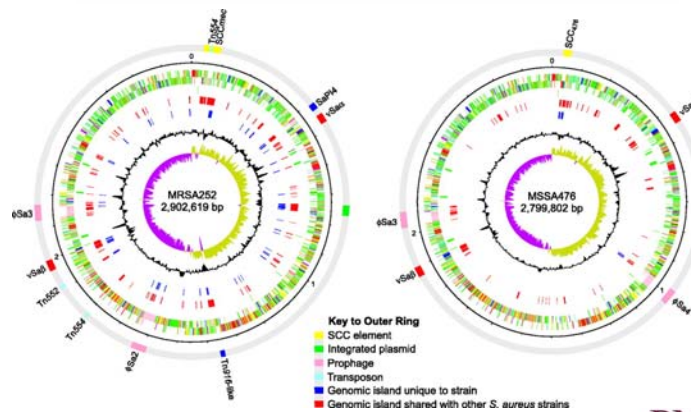


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Organization of genetic material in bacteria: **chromosomes**

Complete genomes of two clinical *Staphylococcus aureus* strains: Evidence for the rapid evolution of virulence and drug resistance

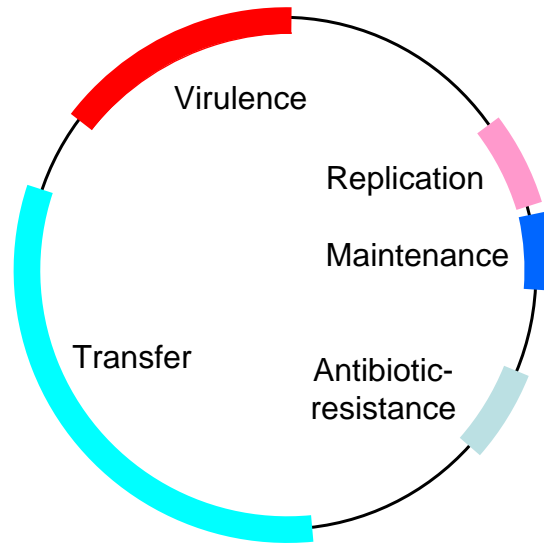
Matthew T. G. Holden*, Edward J. Feil*, Jodi A. Lindsay*, Sharon J. Peacock[†], Nicholas P. J. Day[¶], Mark C. Enright*, Tim J. Foster[§], Catrin E. Moore[¶], Laurence Hurst*, Rebecca Aktin*, Andrew Barron*, Nathalie Bason*, Stephen D. Bentley*, Carol Chillingworth*, Tracey Chillingworth*, Carol Churcher*, Louise Clark*, Craig Corton*, Ann Cronin*, Jon Doggett*, Linda Dowd*, Theresa Felwell*, Zahra Hance*, Barbara Harries*, Heidi Hauser*, Simon Holroyd*, Kay Jagels*, Keith D. James*, Nicola Lennard*, Alexandra Line*, Rebecca Mayes*, Sharon Moule*, Karen Mungall*, Douglas Ormond*, Michael A. Quail*, Ester Rabinowitz*, Kim Rutherford*, Mandy Sanders*, Sarah Sharp*, Mark Simmonds*, Kim Stevens*, Sally Whitehead*, Bart G. Barrell*, Brian G. Spratt**, and Julian Parkhill**



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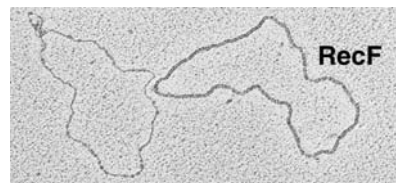
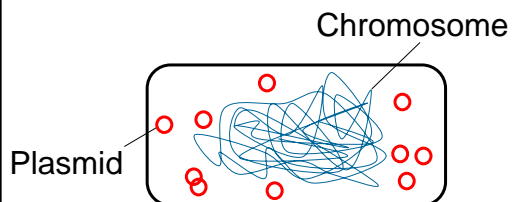
PNAS

Organization of genetic material in bacteria: **plasmids**



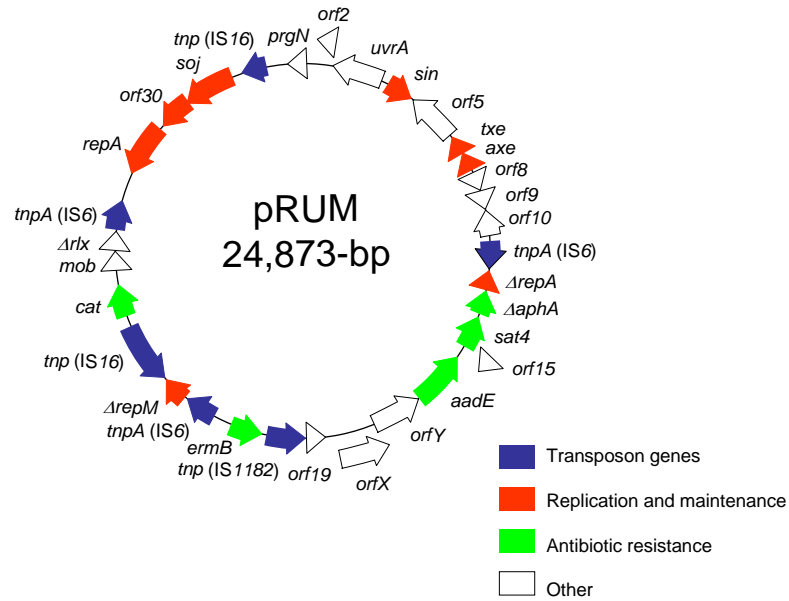
Organization of genetic material in bacteria: **plasmids**

- Extrachromosomal
- Circular or linear
- 2 kb to hundreds of kb in size
- Non-essential
- May carry 'supplemental' genetic information or may be cryptic
- Employ host functions for most of DNA metabolism



<http://www.biochem.wisc.edu/inman/empics/dna-prot.htm>

Organization of genetic material in bacteria: plasmids



Organization of genetic material in bacteria: plasmids

Examples of naturally-occurring plasmids and relevant features

Plasmid	Host	Plasmid size (kb)	Relevant feature
pT181	<i>Staphylococcus aureus</i>	4.4	Tetracycline resistance
ColE1	<i>Escherichia coli</i>	6.6	Colicin production and immunity
pGKL2	<i>Kluyveromyces lactis</i> ^b	13.5	Killer plasmid
pAMP β 1	<i>Enterococcus faecalis</i>	26.0	Erythromycin resistance
pSK41	<i>Staphylococcus aureus</i>	46.4	Multidrug resistance
pBM4000	<i>Bacillus megaterium</i>	53.0	rRNA operon
pl258	<i>Staphylococcus aureus</i>	28.0	Metal ion resistance
pSLT	<i>Salmonella enterica</i> subsp. <i>typhimurium</i>	93.9	Virulence determinants
pMT1	<i>Yersinia pestis</i>	101.0	Virulence determinants
pADP-1	<i>Pseudomonas</i> sp.	108.8	Atrazine (herbicide) catabolism
pWW0	<i>Pseudomonas putida</i>	117.0	Aromatic hydrocarbon degradation
pBtoxis	<i>Bacillus thuringiensis</i> subsp. <i>israelensis</i>	137.0	Mosquito larval toxicity
pX01	<i>Bacillus anthracis</i>	181.7	Exotoxin production
pSOL1	<i>Clostridium acetobutylicum</i>	192.0	Solvent production
pSymB	<i>Sinorhizobium meliloti</i>	1683.3	Functions associated with plant symbiosis

Sources of genetic variation: point mutations

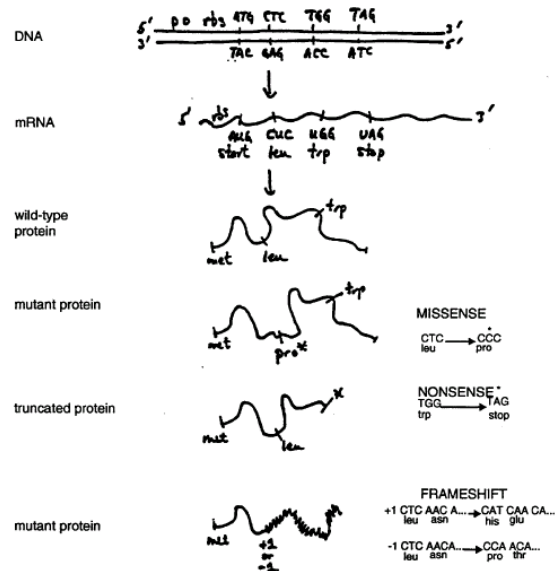
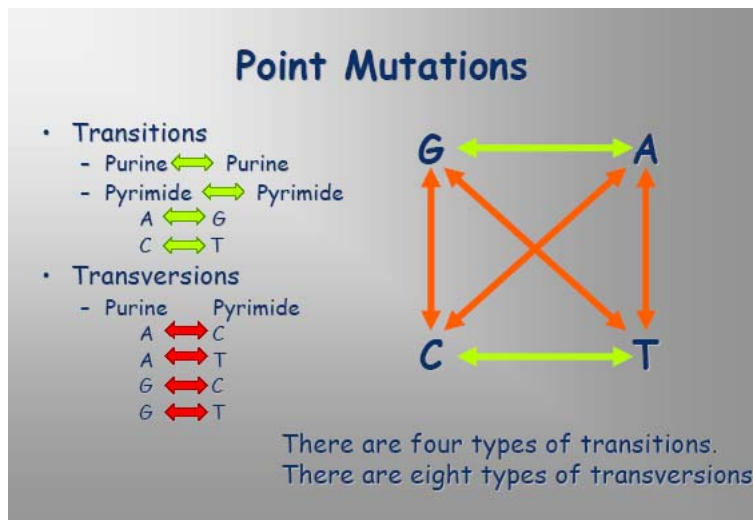


Fig. 3. Point mutations.

Sources of genetic variation: point mutations



Sources of genetic variation: point mutations

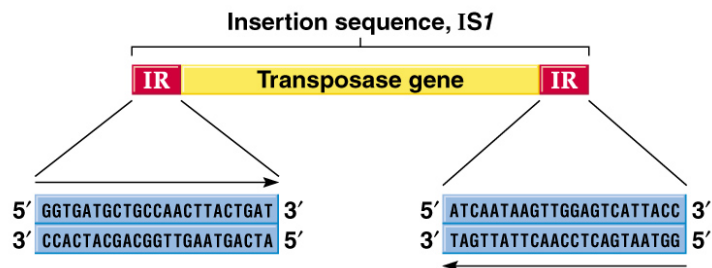
Mutation phenotypes

- Silent mutation (synonymous), no change in amino acid
AGG > AGA, both codons specify Arginine
- Missense mutation (replacement; nonsynonymous), change in amino acid
 - Nonsynonymous missense (or radical replacement)
UUU (Phe) > UCU (Ser); Phe is hydrophobic and Ser is polar
- Nonsense mutation, premature termination of translation
CAG (Gln) > UAG (Stop)
- Frameshift, addition or deletion of base pairs, not in a multiple of three, within the coding region of a gene.

Sources of genetic variation: DNA rearrangements

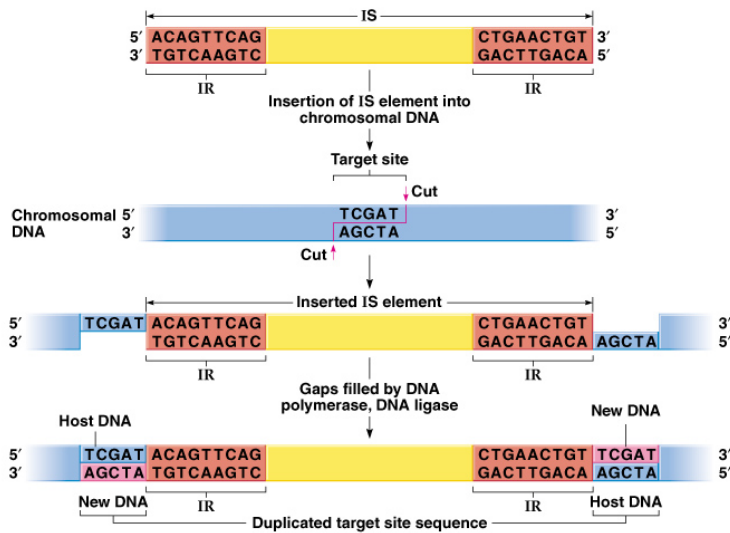
Insertion sequence (IS) elements:

1. Simplest type of transposable element found in bacterial chromosomes and plasmids.
2. Encode only genes for mobilization and insertion.
3. Range in size from 768 bp to 5 kb.
4. IS1 first identified in *E. coli*'s lactose operon is 768 bp long and is present with 4-19 copies in the *E. coli* chromosome.
5. Ends of all known IS elements show inverted terminal repeats (ITRs).



Sources of genetic variation: DNA rearrangements

Integration of IS element in chromosomal DNA.



Sources of genetic variation: DNA rearrangements

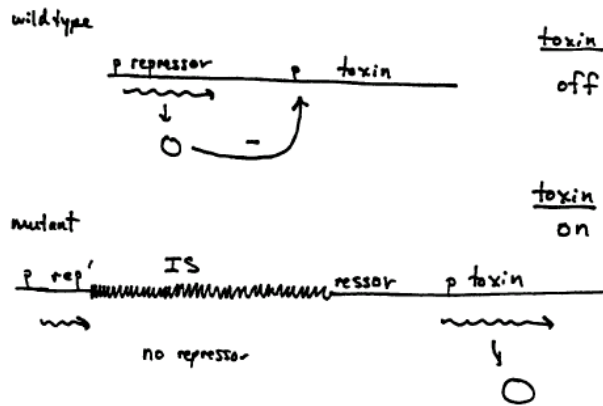


Fig. 5 (at left).
Disruption of a
gene by IS ele-
ment transposi-

Sources of genetic variation: DNA rearrangements

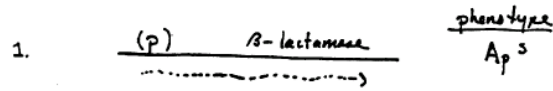


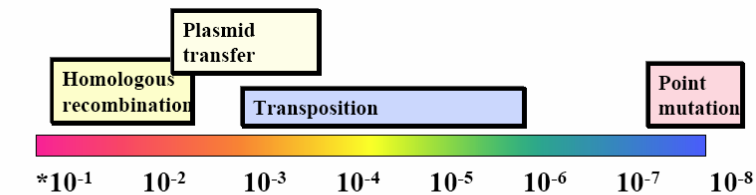
Fig. 6 (at left). Activation of a gene by IS element transposition.

poor promoter
weak transcription of β -lactamase gene
no protection against ampicillin



insertion of IS element brings strong promoter
strong expression of β -lactamase gene
protects cells against ampicillin

Sources of genetic variation: frequency of occurrence

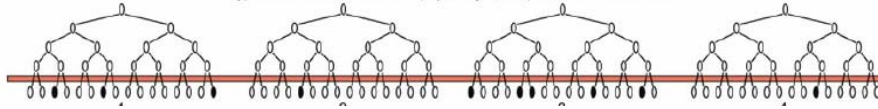


High frequency \longrightarrow Low frequency
Low diversity \longrightarrow High diversity

* As frequency per cell per generation

Transmission of genetic variation: Luria-Delbruck test

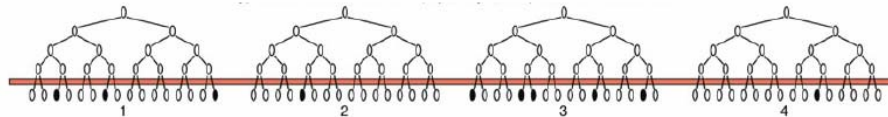
1. Resistance by mutation is a physiological response



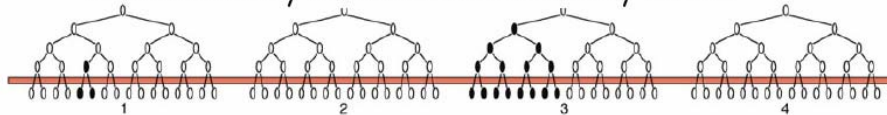
Time of exposure to selective agent

Transmission of genetic variation: Luria-Delbruck test

1. Resistance by mutation is a physiological response



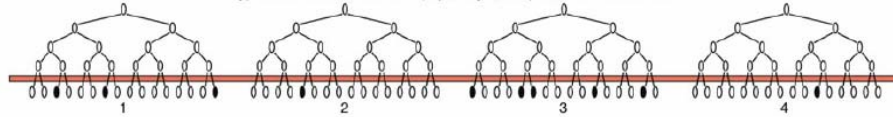
2. Resistance by mutation arises randomly in time



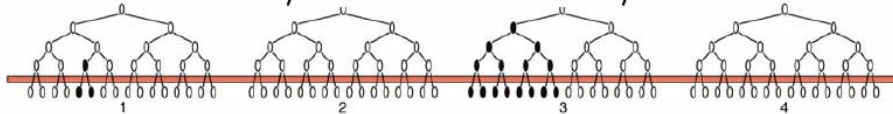
Time of exposure to selective agent

Transmission of genetic variation: Luria-Delbruck test

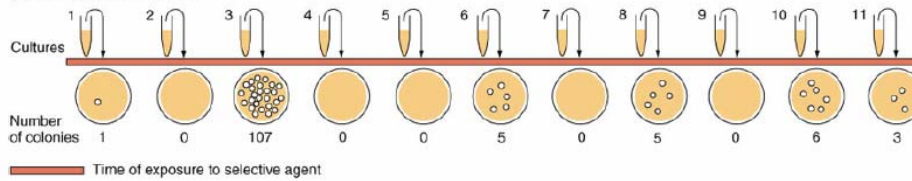
1. Resistance by mutation is a physiological response



2. Resistance by mutation arises randomly in time



(b) Fluctuation test results



Results fit with expectations if random mutation occur at random.

Linear transmission of genetic variation

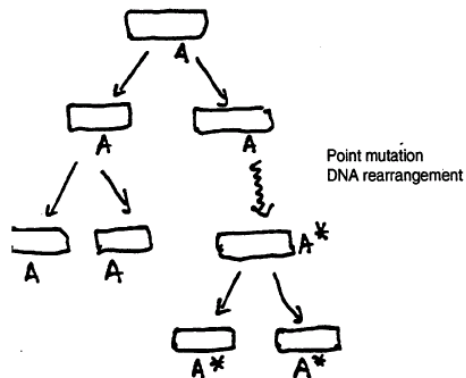


Fig. 1. Clonal variation.

Horizontal transmission of genetic variation

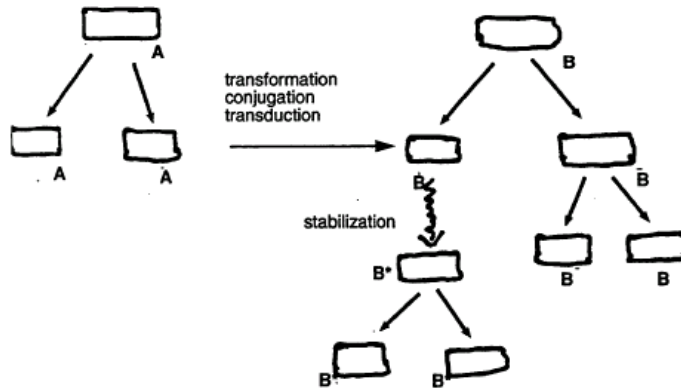
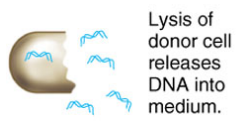


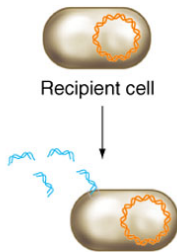
Fig. 2 (at left). Variation by transfer of genetic information.

Transmission of genetic variation: mechanisms

Transformation



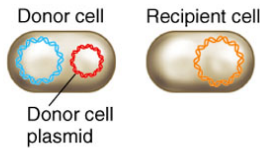
Lysis of donor cell releases DNA into medium.



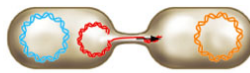
Recipient cell

Donor DNA is taken up by recipient.

Conjugation

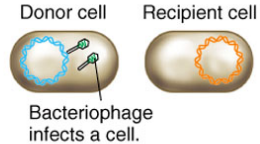


Donor cell
Donor cell plasmid

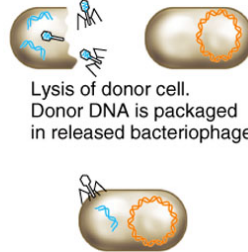


Donor DNA is transferred directly to recipient through a connecting tube. Contact and transfer are promoted by a specialized plasmid in the donor cell.

Transduction



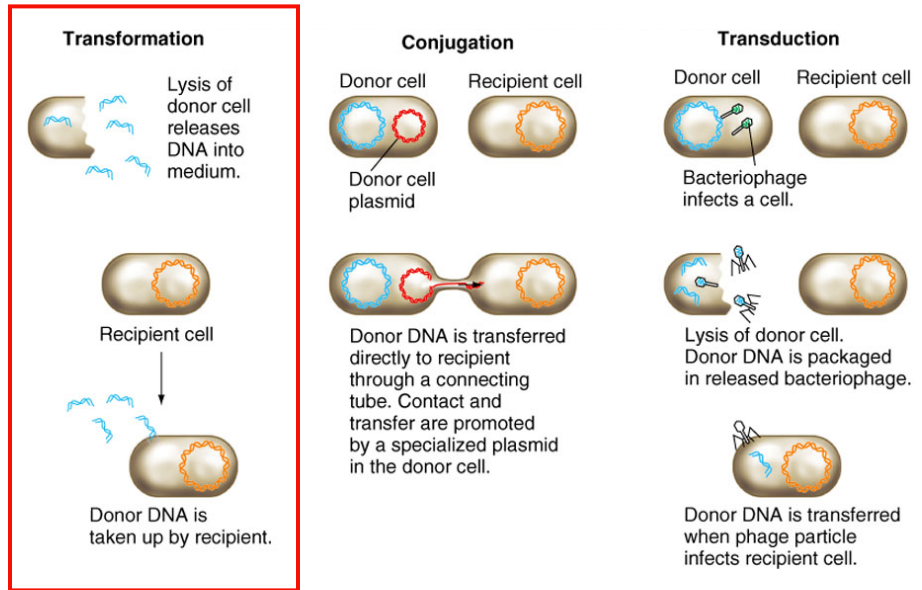
Donor cell
Recipient cell
Bacteriophage infects a cell.



Lysis of donor cell. Donor DNA is packaged in released bacteriophage.

Donor DNA is transferred when phage particle infects recipient cell.

Transmission of genetic variation: transformation



Transmission of genetic variation: transformation

- Gene transfer resulting from the uptake of DNA from a donor.
- Factors affecting transformation
 - DNA size and state
 - Sensitive to nucleases
 - Competence of the recipient (*Bacillus*, *Haemophilus*, *Neisseria*, *Streptococcus*)
 - Competence factor
 - Induced competence

Transmission of genetic variation: transformation

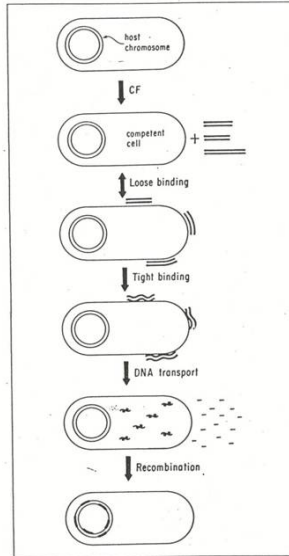
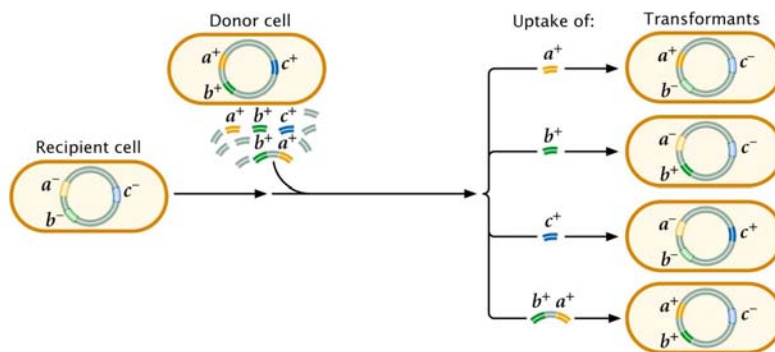
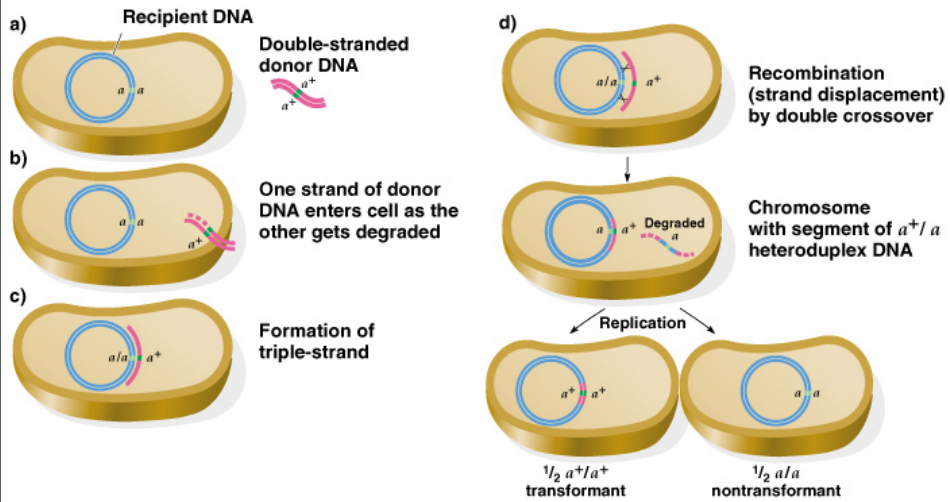


Fig. 8-11. Model for the transformation of *Streptococcus*. Competence factor (CF) is required for the induction of the competent state. Duplex DNA binds to competent cells in at least two distinct steps, referred to as loose and tight binding. Transport of single-strand fragments results in intracellular protein-DNA complexes, and subsequent recombination of the fragments into the host chromosome.

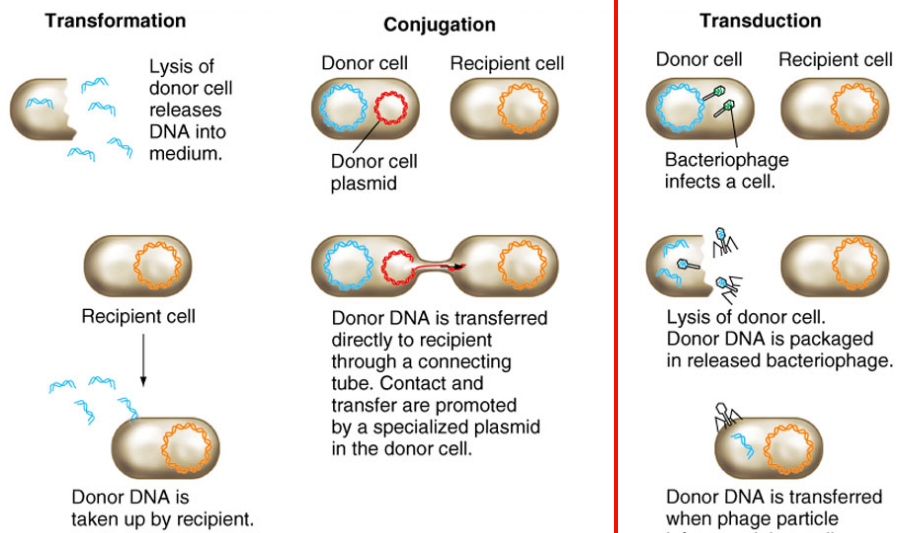
Transmission of genetic variation: transformation



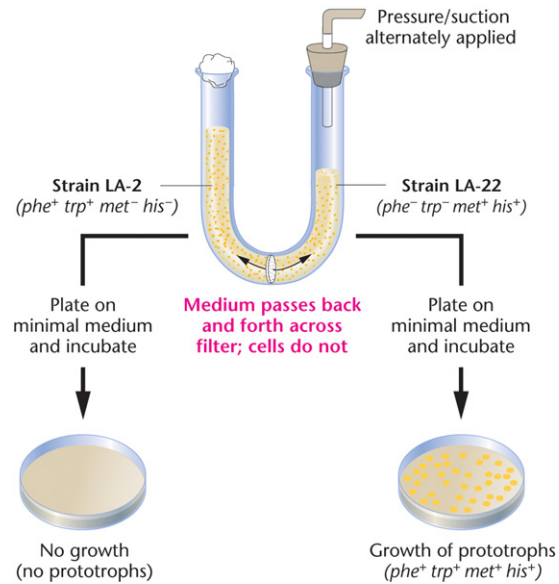
Transmission of genetic variation: transformation



Transmission of genetic variation: transduction



Transmission of genetic variation: **transduction**

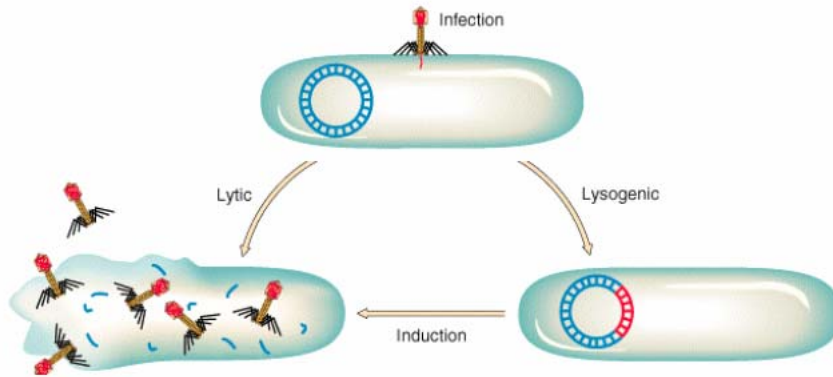


Transmission of genetic variation: **transduction**

How did Zinder and Lederberg prove that the phenotype was the result of transduction?

- presence of DNAase rules out transformation
- filter prevented contact so no conjugation
- reducing filter pore size to below size of phage inhibited

Transmission of genetic variation: **transduction**



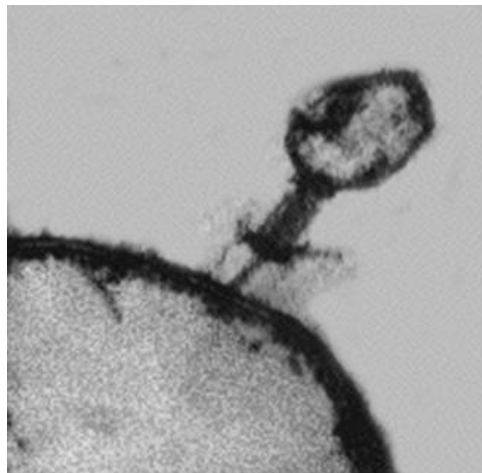
Transmission of genetic variation: **transduction**

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Transmission of genetic variation: **transduction**



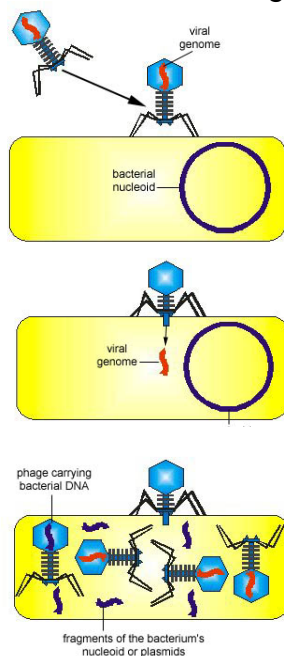
Transmission of genetic variation: **transduction**



Transmission of genetic variation: **transduction**

- There are two types of transduction:
 - **generalized transduction**: A DNA fragment is transferred from one bacterium to another by a **lytic bacteriophage** that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.
 - **specialized transduction**: A DNA fragment is transferred from one bacterium to another by a **temperate bacteriophage** that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle

Transmission of genetic variation: **generalized transduction**

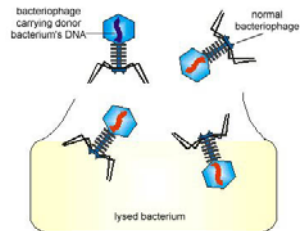


1. A **lytic bacteriophage** adsorbs to a susceptible bacterium.

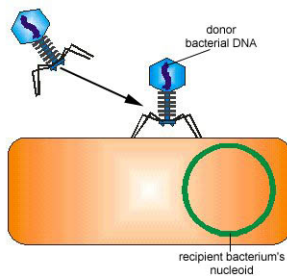
2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes

3. Occasionally, a bacteriophage head or capsid assembles around a fragment of donor bacterium's nucleoid or around a plasmid instead of a phage genome by mistake.

Transmission of genetic variation: **generalized transduction**

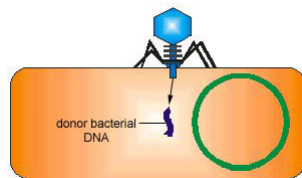


4. The bacteriophages are released.

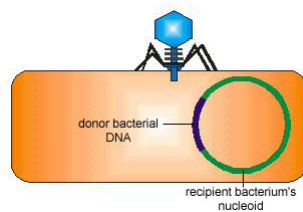


5. The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium

Transmission of genetic variation: **generalized transduction**



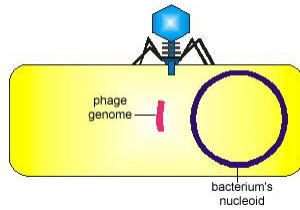
6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium .



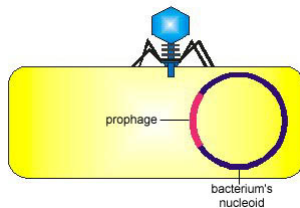
7. The donor bacterium's DNA is exchanged for some of the recipient's DNA.

<http://www.cat.cc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/transduction/transduction.html>

Transmission of genetic variation: **specialized transduction**

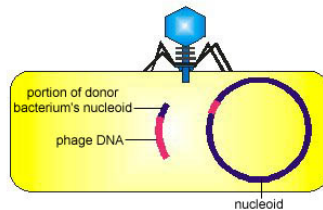


1. A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome .

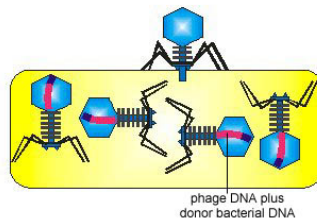


2. The bacteriophage inserts its genome into the bacterium's nucleoid to become a prophage.

Transmission of genetic variation: **specialized transduction**

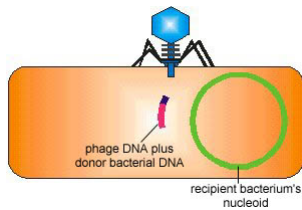


3. Occasionally during spontaneous induction, a small piece of the donor bacterium's DNA is picked up as part of the phage's genome in place of some of the phage DNA which remains in the bacterium's nucleoid.

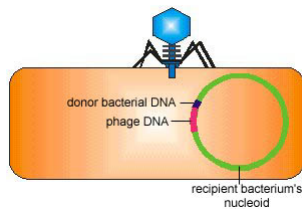


4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage's genome. Every phage now carries that segment of bacterial DNA.

Transmission of genetic variation: **specialized transduction**



5. The bacteriophage adsorbs to a recipient bacterium and injects its genome.

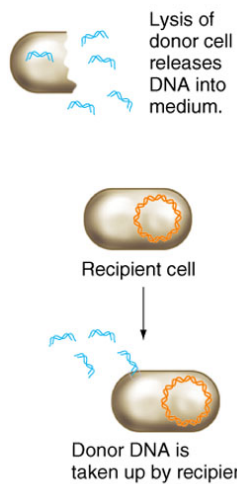


6. The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's nucleoid.

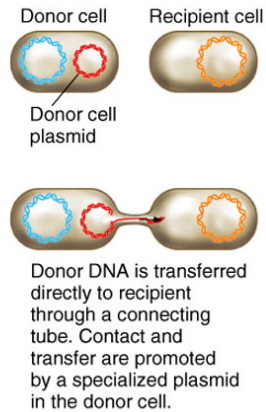
<http://www.cat.cc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/transduction/spectran.html>

Transmission of genetic variation: **mechanisms**

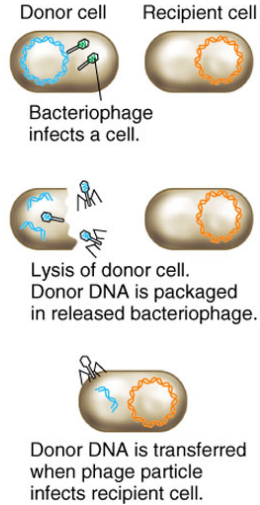
Transformation



Conjugation

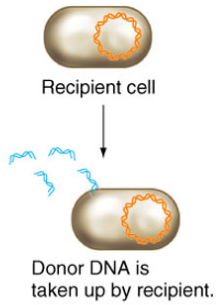


Transduction

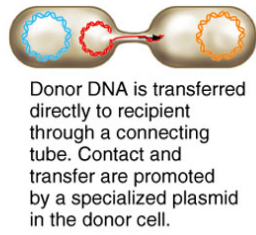
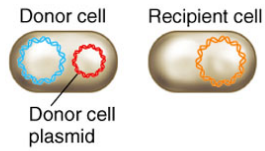


Transmission of genetic variation: conjugation

Transformation



Conjugation



Transduction

