

**Mechanisms of Infectious Disease • Fall 2009**

**Genetics I**

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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 mutations
  - b. DNA rearrangements
- III. Genetic variation: Transmission
  - a. transformation
  - b. transduction
  - c. conjugation
- IV. Genetic variation: Implications for pathogenesis

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

There are two strains of *Streptococcus pneumoniae*.

**ROUGH COLONY (R)**

**SMOOTH COLONY (S)**

**R strain is benign**  
(Lacking a protective capsule, it is recognized and destroyed by host's immune system)

**S strain is virulent**  
(Polysaccharide capsule prevents detection by host's immune system)

### Genetic basis of variation: Hershey and Chase (1952)

**Radioactive protein capsule (<sup>35</sup>S)**

Most <sup>35</sup>S found in supernatant

**Radioactive DNA (<sup>32</sup>P)**

Most <sup>32</sup>P found in pellet

Labels: Bacterial Cell, DNA, Protein capsule, Blender separates phages outside the bacteria from the cells and their contents, Cells and Phages are separated by centrifugation.

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

**The controls:**

Live encapsulated virulent bacteria → Injection → Mouse dies

Live nonencapsulated nonvirulent bacteria → Injection → Mouse lives

Live encapsulated virulent bacteria → heat → Heat-killed virulent bacteria → Injection → Mouse lives

**The experiment:**

Heat-killed virulent bacteria + Live nonencapsulated nonvirulent bacteria → Mixture → Injection → Mouse dies

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

**DNA as the transforming principle**

**DNA/RNA mix from S strain**

DNA/RNA mix from S strain → Treat w/ DNase → DNA only → Add to R strain → Plate for colonies → S transformants Recovered

**DNA/RNA mix from R strain**

DNA/RNA mix from R strain → Treat w/ DNase → RNA only → Add to R strain → Plate for colonies → NO S transformants

**Transformation:**

Live non-encapsulated nonvirulent bacteria + DNA isolated from heat-killed virulent bacteria → transformed → Encapsulated virulent bacteria → Mouse dies

### 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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## 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: point mutations

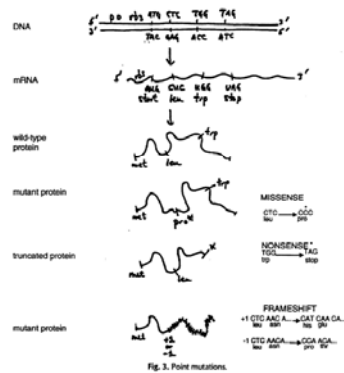
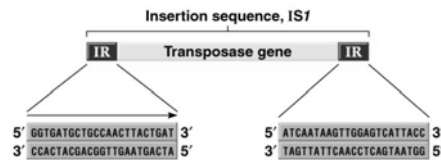


Fig. 3. Point mutations.

## Sources of genetic variation: DNA rearrangements

### Insertion sequence (IS) elements:

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



## Sources of genetic variation: point mutations

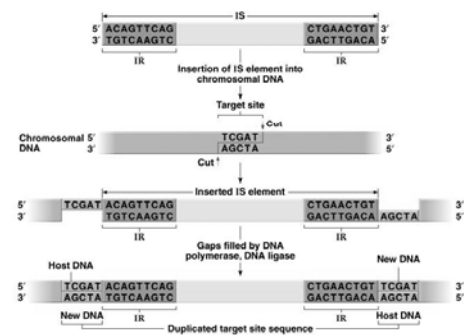
### Point Mutations

- Transitions**
  - Purine ↔ Purine (A ↔ G, G ↔ A)
  - Pyrimide ↔ Pyrimide (C ↔ T, T ↔ C)
- Transversions**
  - Purine ↔ Pyrimide (A ↔ C, C ↔ A, G ↔ T, T ↔ G)

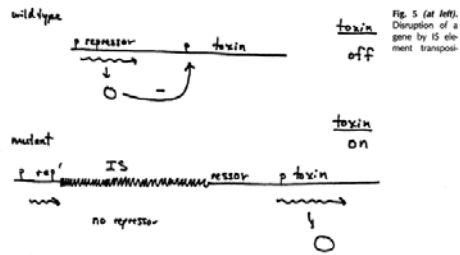
There are four types of transitions.  
There are eight types of transversions.

## Sources of genetic variation: DNA rearrangements

### Integration of IS element in chromosomal DNA.



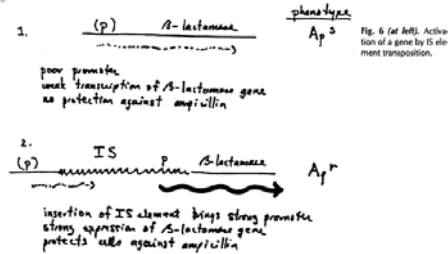
Sources of genetic variation: DNA rearrangements



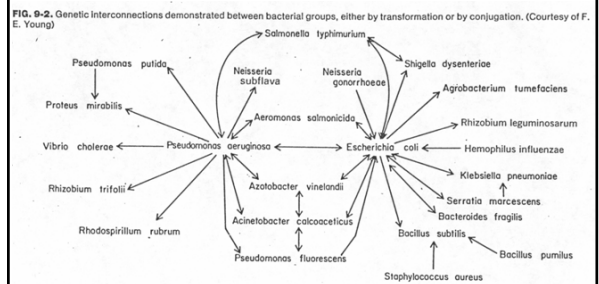
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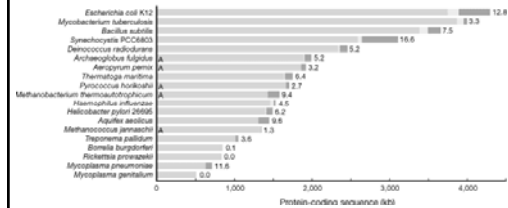
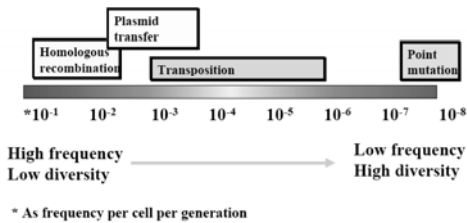
Sources of genetic variation: DNA rearrangements



Transmission of genetic variation



Sources of genetic variation: frequency of occurrence



Lengths of bars denote the amount of protein-coding DNA. For each bar, the native DNA is blue; foreign DNA identifiable as mobile elements, including transposons and bacteriophages, is yellow, and other foreign DNA is red. The percentage of foreign DNA is noted to the right of each bar. 'A' denotes an Archaeal genome.

Transmission of genetic variation: antibiotic resistance

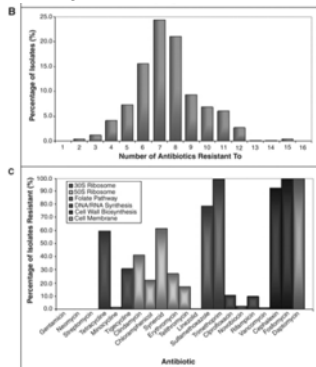


Fig. 1. Antibiotic resistance profiling of 480 soil-derived bacterial isolates

V. M. D’Costa et al. Science (2006)

Transmission of genetic variation: Luria-Delbruck test

1. Resistance by mutation is a physiological response

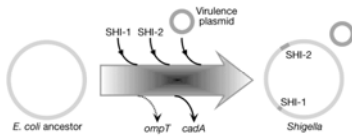


2. Resistance by mutation arises randomly in time



Time of exposure to selective agent

Transmission of genetic variation: pathogenesis



Transmission of genetic variation: Luria-Delbruck test

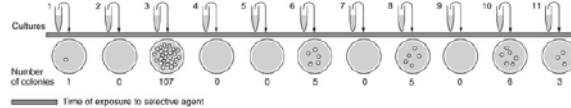
1. Resistance by mutation is a physiological response



2. Resistance by mutation arises randomly in time



(9) Fluctuation test results



Results fit with expectations if random mutation occur at random.

Transmission of genetic variation: Luria-Delbruck test

1. Resistance by mutation is a physiological response



Time of exposure to selective agent

Linear transmission of genetic variation

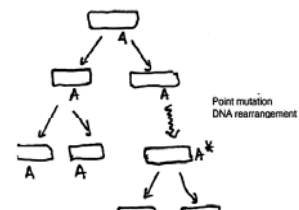
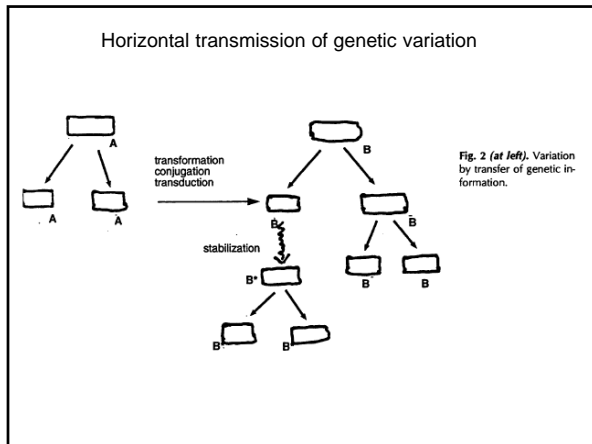
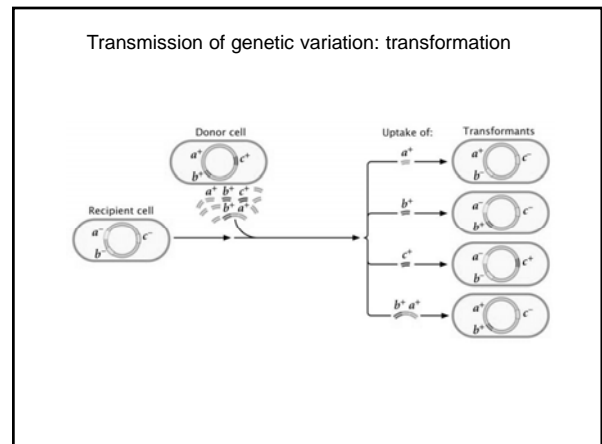
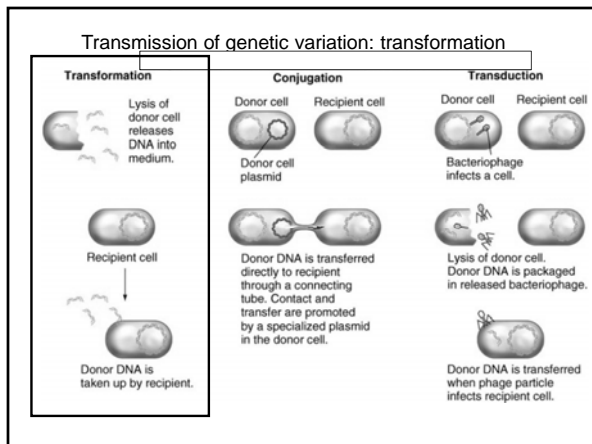
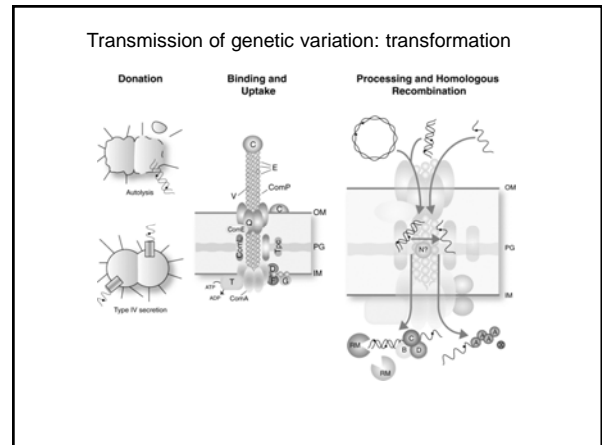
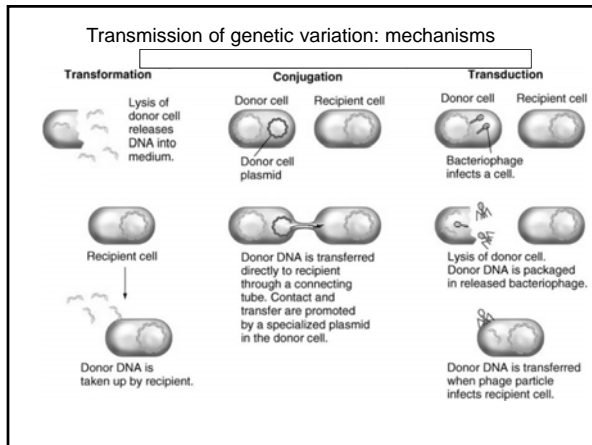
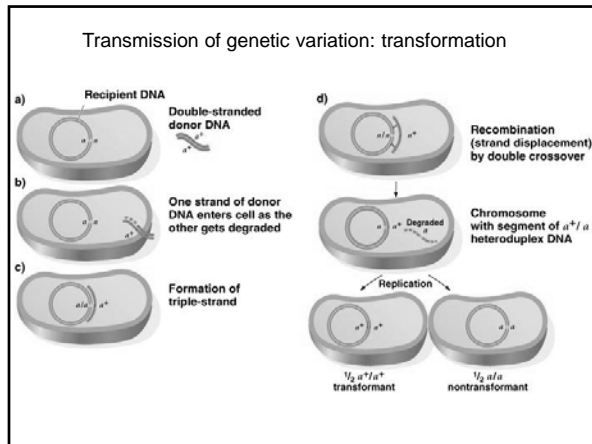


Fig. 1. Clonal variation.

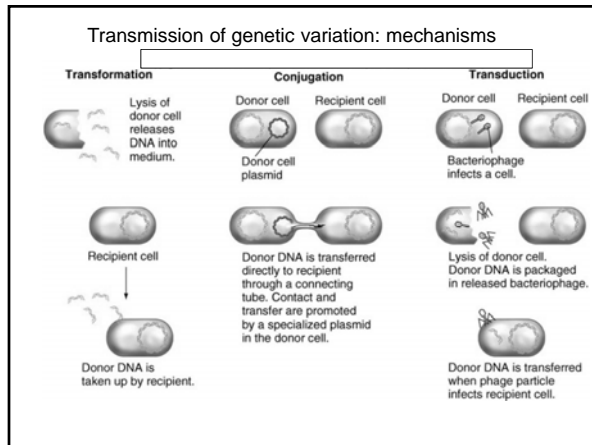


- ### Transmission of genetic variation: transformation
- Gene transfer resulting from the uptake of DNA from a donor.
  - Factors affecting transformation
    - DNA size and state
      - Sensitive to nucleases
    - Competence of the recipient (*Bacillus*, *Haemophilus*, *Neisseria*, *Streptococcus*)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





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