

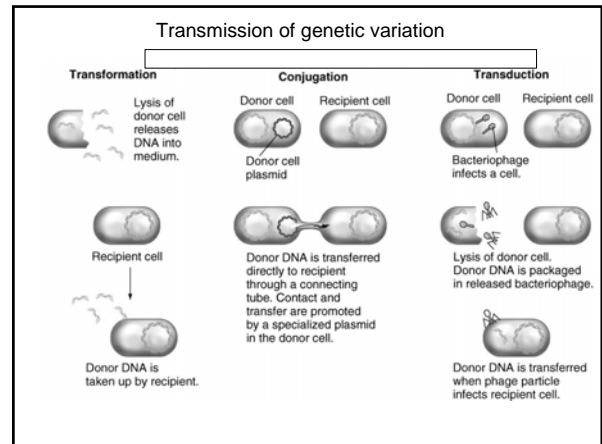
Mechanisms of Infectious Disease • Fall 2008

Lecture 2

Jonathan Dworkin, PhD

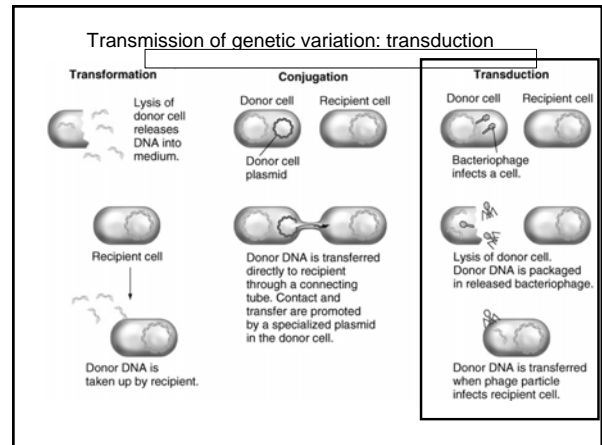
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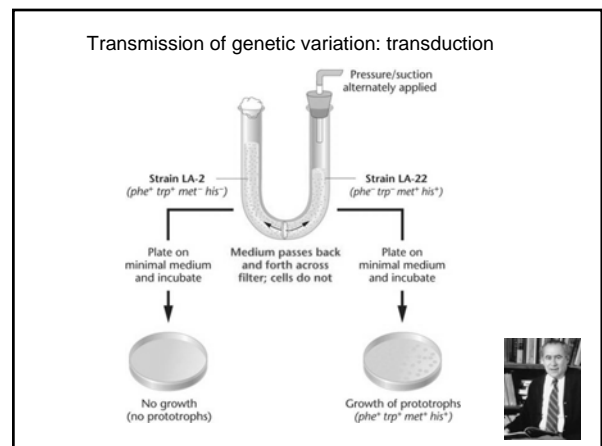
Genetic Basis of Variation in Bacteria

- 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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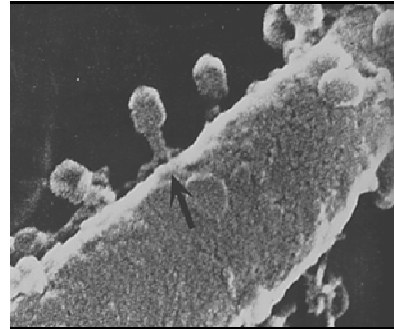


Transmission of genetic variation: transduction

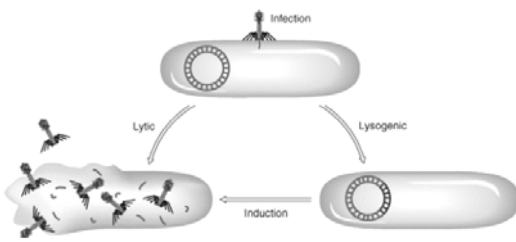
How did Zinder and Lederberg prove that the phenotype was the result of transduction?

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

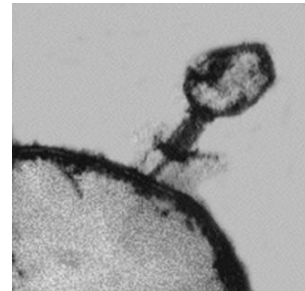
Transmission of genetic variation: transduction



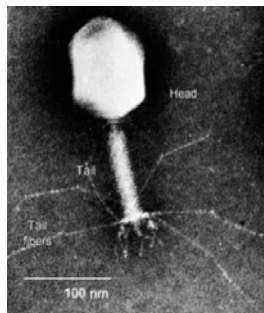
Transmission of genetic variation: transduction



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: 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: 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: 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: generalized transduction

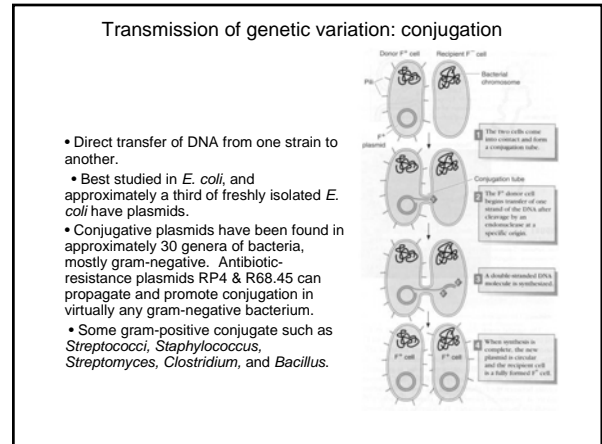
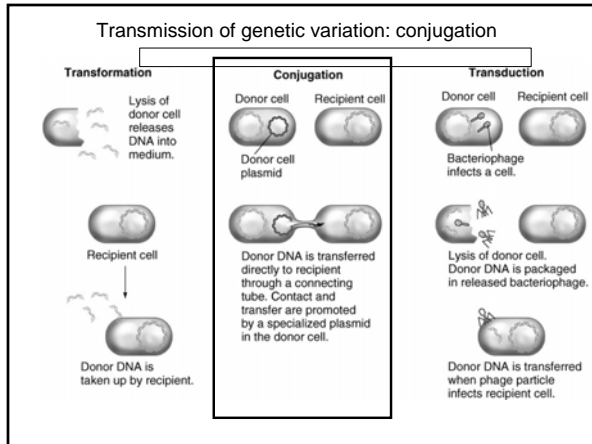
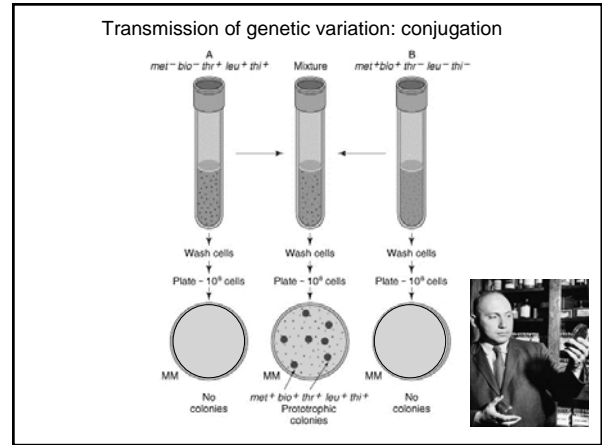
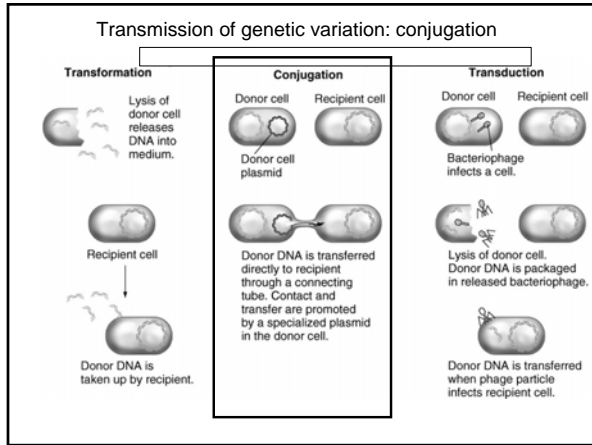
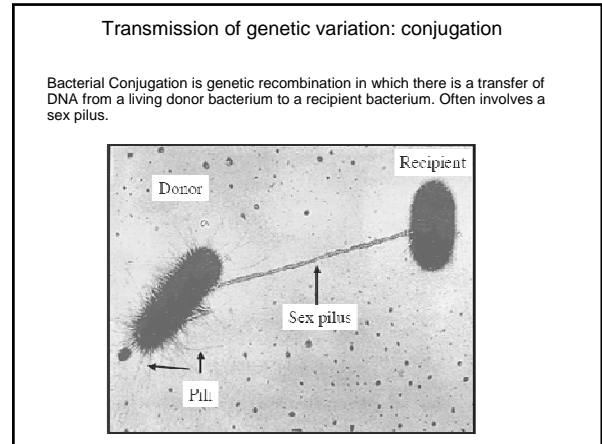
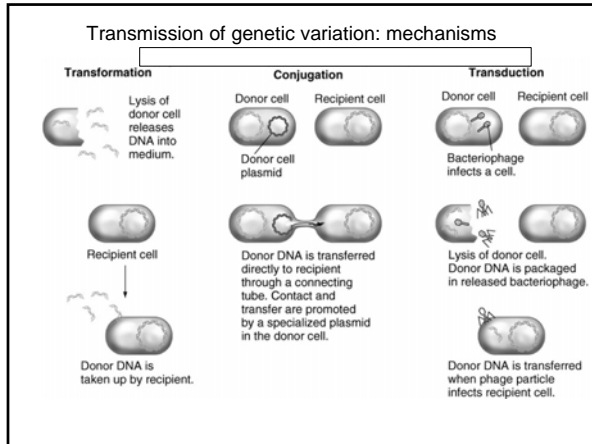
6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium .
7. The donor bacterium's DNA is exchanged for some of the recipient's DNA.

<http://www.cat.cs.mil.us/courses/bio141/reqguide/unit4/genetics/recombination/transduction/transduction.html>

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.cs.mil.us/courses/bio141/reqguide/unit4/genetics/recombination/transduction/special.html>

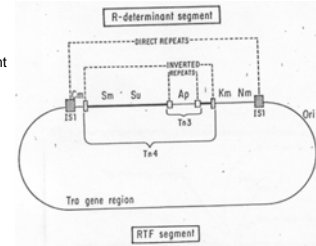


Transmission of genetic variation: F+ conjugation

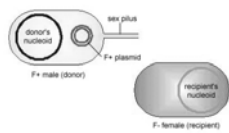
F+ Conjugation: Genetic recombination in which there is a transfer of a large (95kb) plasmid F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process. F can be transferred from *E. coli* to *Salmonella*, *Shigella*, and *Proteus*.

Transmission of genetic variation: R-plasmid conjugation

- R factors - Drug-resistance plasmids first isolated in late 1950's in *Shigella* during an outbreak of dysentery. The first plasmid isolated carried resistant determinants to four different antibiotics: chloramphenicol, tetracycline, streptomycin, and sulfonamides. Later the same plasmid was found in *E. coli*.
- In patients given oral tetracycline, the predominant fecal *E. coli* isolates carry tetracycline-resistance R plasmids within one week.



Transmission of genetic variation: F+ conjugation

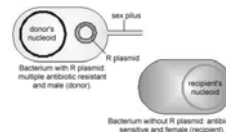


1. The F+ male has an F+ plasmid coding for a sex pilus and can serve as a genetic donor



2. The sex pilus adheres to an F- female (recipient). One strand of the F+ plasmid breaks

Transmission of genetic variation: R-plasmid conjugation

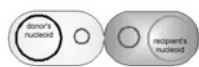


1. The bacterium with an R-plasmid is multiple antibiotic resistant and can produce a sex pilus (serve as a genetic donor).

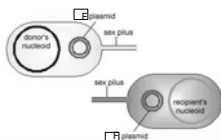


2. The sex pilus adheres to an F- female (recipient). One strand of the R-plasmid breaks.

Transmission of genetic variation: F+ conjugation



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the F+ plasmid enters the recipient bacterium



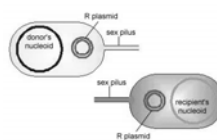
4. Both bacteria make a complementary strand of the F+ plasmid and both are now F+ males capable of producing a sex pilus. There was no transfer of donor chromosomal DNA although other plasmids the donor bacterium carries may also be transferred during F+ conjugation.

<http://www.csl.cmc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/conjugation7.htm>

Transmission of genetic variation: R-plasmid conjugation



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the R-plasmid enters the recipient bacterium.



4. Both bacteria make a complementary strand of the R-plasmid and both are now multiple antibiotic resistant and capable of producing a sex pilus.

<http://www.csl.cmc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/conjugation7.htm>

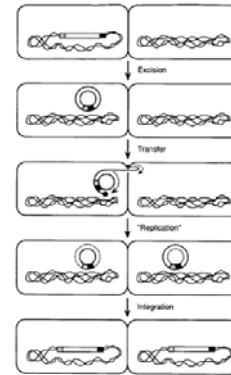
Transmission of genetic variation: R-plasmid conjugation

Properties of some R plasmids

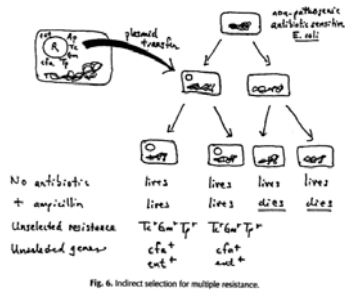
| Plasmid | Origin | Resistances | Size (kb) |
|---------|--------------|-----------------------|-----------|
| RP1 | England | CbKmTc | 36 |
| R527 | Spain | CbCmGmKmSmSuTcHg | 49 |
| pMG5 | Japan | AKKmSuTmBorHgPmrTer | 280 |
| pMG90 | France | CbCmGmKmSmSuTcTmBorHg | 150 |
| Rms149 | Germany | CbGmSmSuTra | 36 |
| pMG38 | USA | CbGmKmSuTcTmHg | 53 |
| FP110 | Australia | CmaPaeFp110 | 60 |
| pMG25 | South Africa | CbCmGmKmSmSuTmBor | 66 |
| pMG69 | Ireland | CbGmKmSmSuTcTmTra | 47 |

Transmission of genetic variation: conjugative transposition

B. Intercellular Transposition



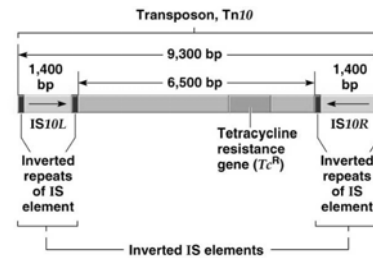
Transmission of genetic variation: R-plasmid conjugation



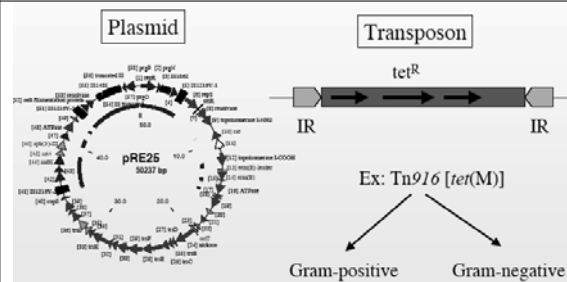
Transmission of genetic variation: conjugative transposition

Composite transposons (Tn):

- Carry genes (e.g., a gene for antibiotic resistance) flanked on both sides by IS elements.
- Tn10** is 9.3 kb and includes 6.5 kb of central DNA (includes a gene for tetracycline resistance) and 1.4 kb inverted IS elements.
- IS elements supply transposase and ITR recognition signals.



Transmission of genetic variation: conjugation



Genetic variation: Implications for pathogenesis and antibiotic resistance

- I. Transduction
 - a. *Vibrio cholera*
 - b. *Corynebacterium diphtheriae*
 - c. *Neisseria meningitidis*
- II. Transformation
 - a. *Neisseria gonorrhoeae* pilin variation
- III. Conjugation
 - a. *Bacillus spp.*

Genetic variation: Implications for pathogenesis and antibiotic resistance

- I. Transduction
 - a. *Vibrio cholera*
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- II. Transformation
 - a. *Neisseria gonorrhoeae* pilin variation
- III. Conjugation
 - a. *Bacillus spp.*

Transduction: Examples of Virulence Factors Carried by Phage

| Bacterium | Phage | Gene Product | Phenotype |
|------------------------------------|--------------------|---------------------|---------------------------|
| <i>Vibrio cholerae</i> | CTX phage | cholerae toxin | cholera |
| <i>Escherichia coli</i> | lambda phage | shigalike toxin | hemorrhagic diarrhea |
| <i>Clostridium botulinum</i> | clostridial phages | botulinum toxin | botulism (food poisoning) |
| <i>Corynebacterium diphtheriae</i> | corynephage beta | diphtheria toxin | diphtheria |
| <i>Streptococcus pyogenes</i> | T12 | erythrogenic toxins | scarlet fever |

Genetic variation: Implications for pathogenesis and antibiotic resistance

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Transduction: *Corynebacterium diphtheriae*

STUDIES ON THE VIRULENCE OF BACTERIOPHAGE-INFECTED STRAINS OF CORYNEBACTERIUM DIPHTHERIAE¹

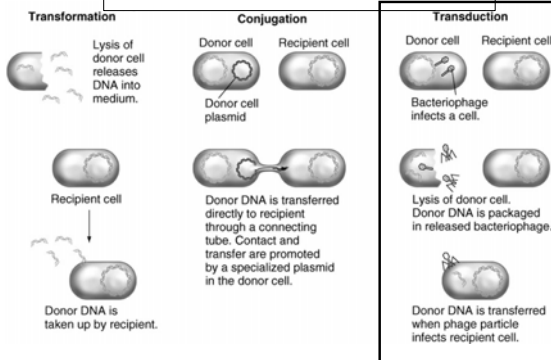
VICTOR J. FREEMAN

Department of Public Health and Preventive Medicine, University of Washington, School of Medicine, Seattle, Washington

Received for publication February 26, 1951

The relationship of naturally occurring avirulent strains to virulent strains of *Corynebacterium diphtheriae* is an unanswered question in the epidemiology of diphtheria and in the evolution of the diphtheria bacillus. The detailed investigations reported here have revealed that avirulent strains of *C. diphtheriae* infected with bacteriophage have yielded virulent *C. diphtheriae* strains.

Transmission of genetic variation



Transduction: *Corynebacterium diphtheriae*

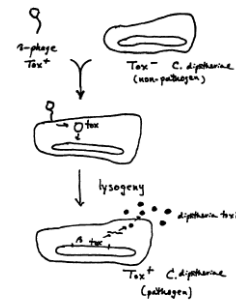


Figure 8. Lysogenic conversion in *C. diphtheriae*

Transduction: *Corynebacterium diphtheriae*

TABLE 3
Subcutaneous tests of bacteriophage lysates* in guinea pigs

| STRAIN NO. | CULTURE PLUS SALINE | CULTURE PLUS PHAGE A | CULTURE PLUS PHAGE B | CULTURE PLUS PHAGE B AND ANTITOXIN |
|------------|---------------------|----------------------|----------------------|------------------------------------|
| 444 | 0/3† | 0/1 | 4/4 | 0/2 |
| 1174 | 0/1 | 0/1 | 2/2 | 0/1 |
| 1180 | 0/1 | 0/1 | 2/2 | 0/1 |
| 770 | 0/1 | 0/1 | 2/2 | 0/1 |
| 411 | 0/1 | 0/1 | 0/1 | 0/1 |
| Total | 0/7 | 0/5 | 10/11 | 0/6 |

* All cultures and culture lysates were washed off agar media with 0.85 per cent saline and inoculated in 1.0-ml doses.
† The numerator represents the number of guinea pigs that died; the denominator, the total number tested.

Transduction: *Neisseria meningitidis*

A chromosomally integrated bacteriophage in invasive meningococci

Emmanuelle Bille,¹ Jean-Ralph Zahar,¹ Agnes Perrin,¹ Sandrine Morelle,¹ Paula Kriz,² Keith A. Jolley,² Martin C.J. Maiden,² Catherine Derrien,² Xavier Nassif,¹ and Colin R. Tinsley^{1,3}

¹ Institut National de la Santé et de la Recherche Médicale (INSERM), Faculté de Médecine Necker, 75014 Paris, France
² National Reference Laboratory for Meningococcal Infections, National Institute of Public Health, 160 43 Prague, Czech Republic
³ The Hill Institute Building for Pathogen Research and Department of Zoology, Oxford, OX1 3PS, England, UK
⁴ Institut National Agronomique Paris-Grignon, 91120 Paris, France

Chromosomal meningitis is a feared disease that can cause the death of a previously healthy individual within hours. Paradoxically, the causative agent, *Neisseria meningitidis*, is a common inhabitant of the human nasopharynx, and as such, may be considered a normal, commensal organism. Only in a small proportion of colonized people do the bacteria invade the bloodstream, from where they can cross the blood-brain barrier to cause meningitis. Furthermore, most meningococcal disease is caused by bacteria belonging to only a few of the phylogenetic groups among the large number that constitute the population structure of this genetically variable organism. However, the genetic basis for the differences in pathogenic potential remains elusive. By performing whole genome comparisons of a large collection of meningococcal isolates of defined pathogenic potential we brought to light a meningococcal phage present in disease-causing bacteria. The phage, of the filamentous family, excises from the chromosome and is secreted from the bacteria via the type IV pilus secretion. Therefore, this element, by spreading among the population, may promote the development of new epidemic clones of *N. meningitidis* that are capable of breaking the normal commensal relationship with humans and causing invasive disease.

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Vol. 201, No. 12, June 20, 2005 1905–1913 www.jem.org/cgi/doi/10.1084/jem.20050112

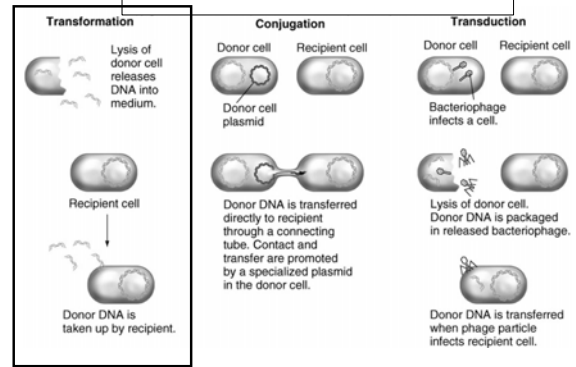
Transduction: *Vibrio cholerae*

Lysogenic Conversion by a Filamentous Phage Encoding Cholera Toxin

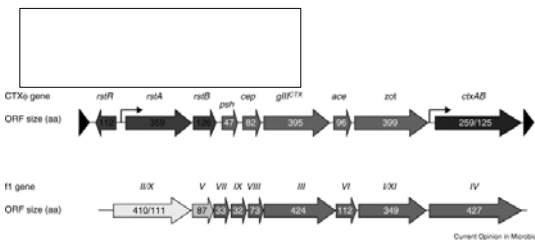
Matthew K. Waldor* and John J. Mekalanos

Vibrio cholerae, the causative agent of cholera, requires two coordinately regulated factors for full virulence: cholera toxin (CT), a potent enterotoxin, and toxin-coregulated pili (TCP), surface organelles required for intestinal colonization. The structural genes for CT are shown here to be encoded by a filamentous bacteriophage (designated CTXφ), which is related to coliphage M13. The CTXφ genome chromosomally integrated or replicated as a plasmid. CTXφ used TCP as its receptor and infected *V. cholerae* cells within the gastrointestinal tracts of mice more efficiently than under laboratory conditions. Thus, the emergence of toxigenic *V. cholerae* involves horizontal gene transfer that may depend on in vivo gene expression.

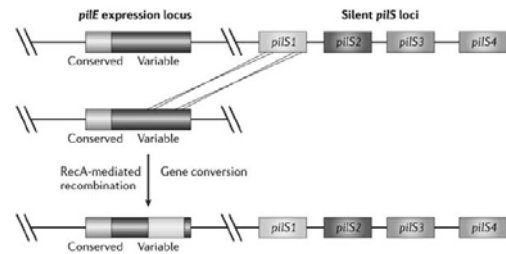
Transmission of genetic variation



Transduction: *Vibrio cholerae*

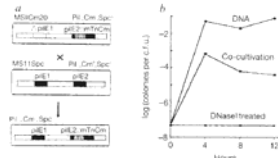


Transformation: *Neisseria gonorrhoeae* pilin variation

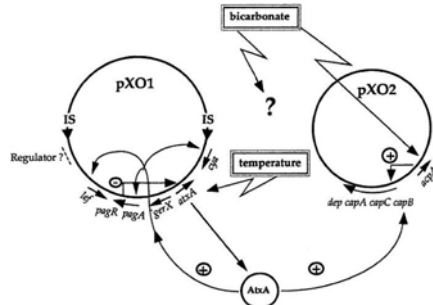


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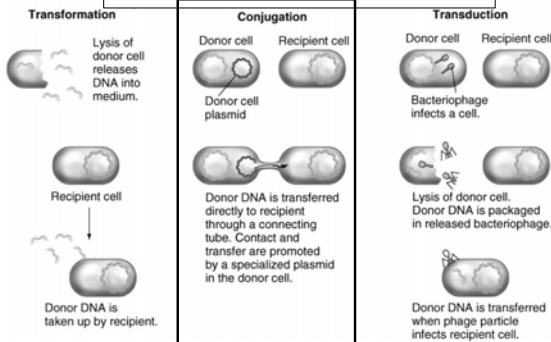
Transformation: *Neisseria gonorrhoeae* pilin variation



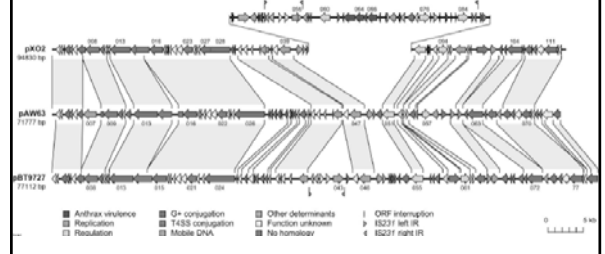
Conjugation: *Bacillus* spp.



Transmission of genetic variation



Conjugation: *Bacillus* spp.



Conjugation: *Bacillus* spp.

Bacillus anthracis, *Bacillus cereus*, and *Bacillus thuringiensis*—One Species on the Basis of Genetic Evidence

ERLENDUR HELGASON,^{1,2} OLE ANDREAS ØSTAD,^{1,2} DOMINIQUE A. CAIGANT,¹ HENNING A. JOHANSEN,¹ AGNÈS FOUET,¹ MICHEL F. MOCK,¹ IDA HEGNA,^{1,3} AND ANNE-BRIT KOLSTØY^{1,4}

¹The Biotechnology Centre of Oslo, University of Oslo, and Department of Microbiology, Institute of Pharmacy, Blindern, 0403 Oslo, and Department of Biotechnology, National Institute of Public Health, Torshov, 6030 Oslo, ²Norway, and ³Institut de Pathologie Bactérienne, URA 2172 CNRS, Institut Pasteur, 75724 Paris Cedex 12, France

Received 28 December 1999; Accepted 19 March 2000

Bacillus anthracis, *Bacillus cereus*, and *Bacillus thuringiensis* are members of the *Bacillus cereus* group of bacteria, demonstrating widely different phenotypes and pathologies. *B. anthracis* causes the acute fatal disease anthrax and is a potential biological weapon due to its high virulence. *B. thuringiensis* produces insecticidal protein crystals toxic to a wide number of insect larvae and is the most commonly used biological pesticide worldwide. *B. cereus* is a probably ubiquitous soil bacterium and an opportunistic pathogen that is a common cause of food poisoning. In contrast to the differences in phenotypes, we show by multilocus sequence electrophoresis and by sequence analysis of nine chromosomal genes that *B. anthracis* should be considered a lineage of *B. cereus*. This determination is not only a formal matter of taxonomy but may also have consequences with respect to virulence and the potential of horizontal gene transfer within the *B. cereus* group.

Conjugation: *Bacillus* spp.

Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax

Alex R. Hoffmaster^{1*}, Jacques Revel^{2*}, David A. Rasko^{3*}, Gail D. Chapman⁴, Michael D. Chrost⁵, Chung K. Marston⁶, Barun K. Das⁷, Claudio T. Sacchi⁸, Collette Fitzgerald⁹, Leonard W. Mayer¹⁰, Martin C. J. Maiden¹¹, Fergus G. Priest¹², Margaret Barker¹³, Lingxia Jiang¹⁴, Hagita Z. Car¹⁵, Jennifer Birkstone¹⁶, Scott H. Patterson¹⁷, Robin S. Weyant¹⁸, Daniel B. Galloway¹⁹, Timothy D. Besser²⁰, Tania Popovic²¹, and Claire M. Fraser^{1,2*}

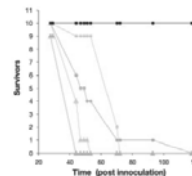


Fig. 4. Survival of *A. baumannii* sp. challenged with *B. cereus* DSM 9841 (green), *B. anthracis* (blue), and *B. cereus* ATCC 10987 (red) High-dose (1:10⁷) and low-dose (1:10⁵) are represented by triangles and circles, respectively. The experiment terminated after 14 days, after which the mice inoculated with *B. cereus* ATCC10987 were still alive.

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