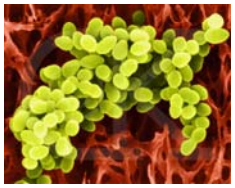
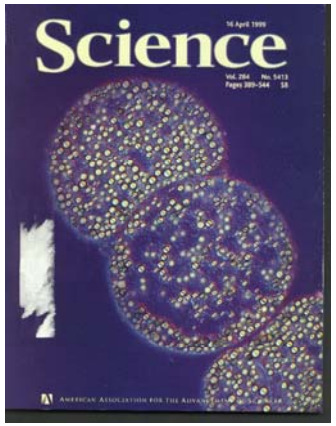
Bacteria are Diverse!!

- What they look like (morphology)
- What they can do (physiology)

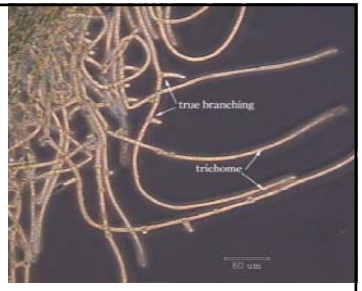




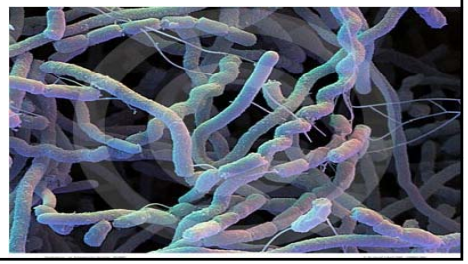
← 200 µm (0.2 mm) "giant" *Thiomargharita*

Tiny 0.1 µm nanobacteria

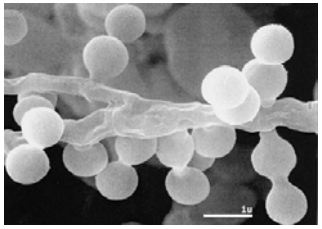
E. coli ≅ **2 µm is fairly "typical"**

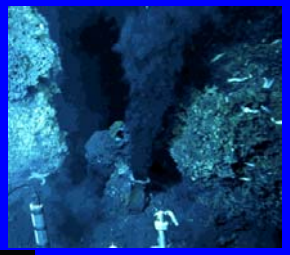
Widely Diverse Morphologies



More Diverse morphology, for example Actinoplanes

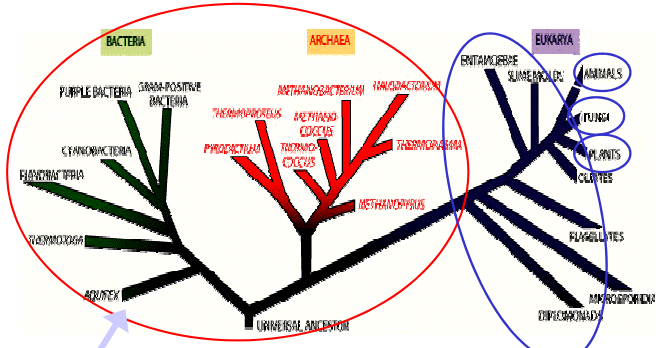

Temperature, Acidic, Basic… Extremophiles



Pyrococcus furiosis

World's Record Holder, 113°C

DETAILED TREE
Based on ribosomal RNA sequences



prokaryotes = "bacteria"

Kingdoms in old 5 kingdom system

How many bacteria are there?

LOTS!!! Each of you carries about 100 grams (1/4 pound) of live bacteria!!!! YUCK!! That's about 10^{14} individuals!

It's also at least 10-fold more than your own cell number. Yes, you are more bacterial than human (in numbers anyway)

Are they important?

- **Essential!!** Recycle carbon, oxygen, and most of the elements, e.g., fix nitrogen, decompose pollutants, photosynthesize
- **Dangerous!!!** TB, leprosy, Toxic shock syndrome, Strep, Syphilis, Lyme.....

What is advantage of using bacteria to study genetics?

- Large numbers, easy to grow
- Haploid: almost immediate expression of mutations
- Easy to select mutants

Bacteria as a model system in genetics

1) Simple growing requirements: water, carbon source and inorganic salts.

Many wild bacteria are **"prototrophic."** They can make all essential materials in the cell (amino acids, lipids, nucleotides, vitamins….).

2) Large numbers can be grown so that rare events (mutations) can be viewed.

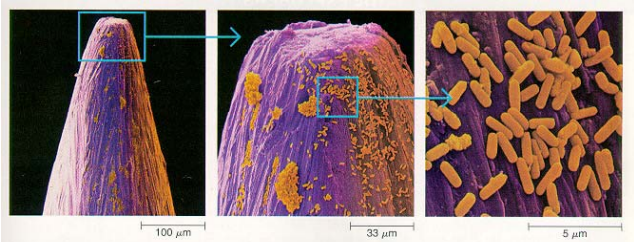


FIGURE 3-1 **A culture on the point of a pin.** The small size of bacteria can be appreciated when specimens on a pinpoint that has been dipped in *E. coli* are examined under greater and greater magnification.

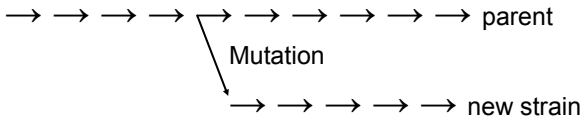
3) They are haploid and mutants are easily identified:

a) "Auxotrophic." Lacking the ability to make one or more essential components of a biosynthetic pathway.

b) Resistant to antibiotics or phage

c) Requiring different carbon sources.

- Bacteria are, in general CLONAL



The Boston Globe

Virtually untreatable' TB strain spreads

A deadly disease is said to lack cure

By Elisabeth Rosenthal, International Herald Tribune | September 6, 2006 PARIS –

The spread of a new, highly resistant form of tuberculosis that is ``virtually untreatable" is causing alarm among international health officials who say that it has now been identified in ``all regions of the world," according to the World Health Organization.

Why do these new strains of bacteria (antibiotic resistant "flesh eating") seem to evolve so quickly?

(9th Ed. Ch. 16 411-413 or 8th Ed: Ch. 15 pages 362-363)

Two Hypotheses were proposed

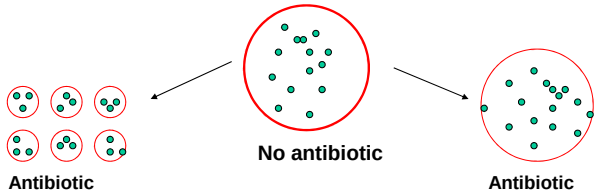
Adaptive Mutations : environment induces mutations

Spontaneous Mutations: because there are so many bacteria and they grow so fast, random mutations some of which may be adaptive, preexist in populations.

Luria and Delbruck 1943 Fluctuation Test. They used resistance to T1 phage, but the same is true for resistance to antibiotics.

Luria and Delbruck 1943 Fluctuation Test.

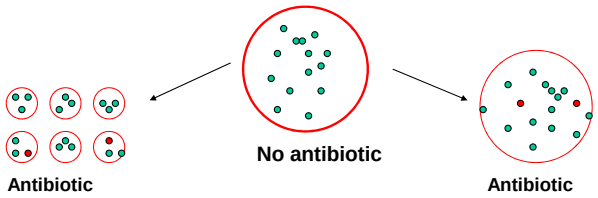
If Adaptive Mutation hypothesis is true:



Luria and Delbruck 1943 Fluctuation Test.

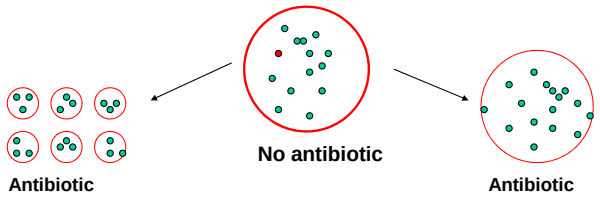
If Adaptive Mutation hypothesis is true:

Expect the mutants to be evenly distributed among the small populations.



Luria and Delbruck 1943 Fluctuation Test.

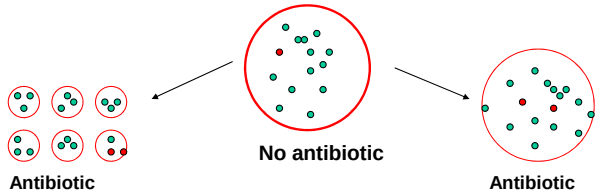
If Spontaneous Mutations hypothesis is true:



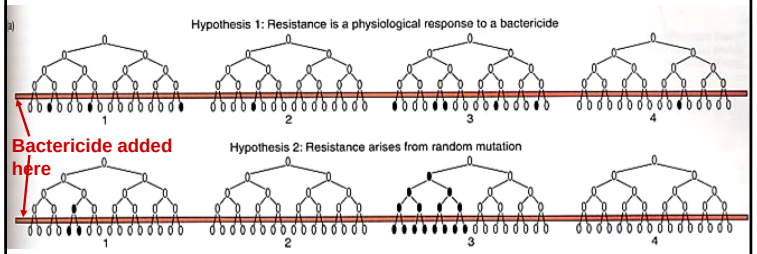
Luria and Delbruck 1943 Fluctuation Test.

<u>If Spontaneous Mutations</u> hypothesis is true:

Expect the mutants to be concentrated in just the small populations that received the pre-existing mutant.

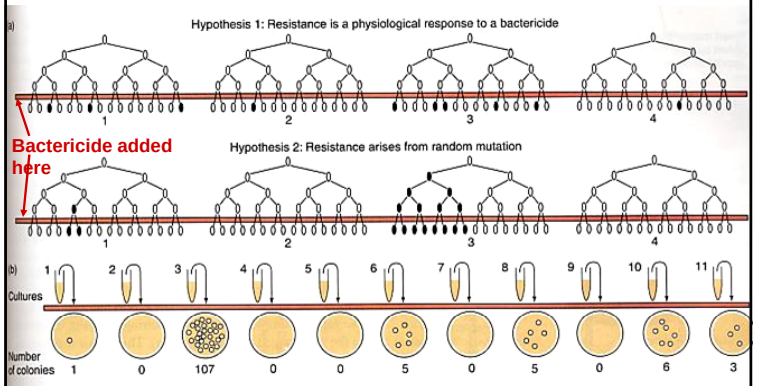


Adaptive: bacteria hit the bactericide (antibiotic or phage media) and develop resistance at a certain rate. Each plate would likely have a few induced resistant colonies.



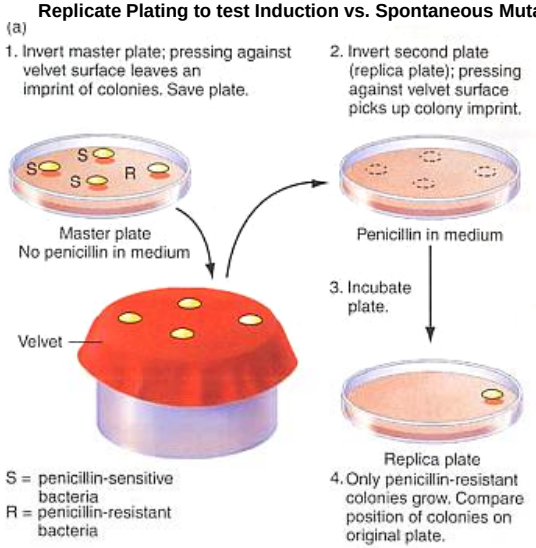
Spontaneous: resistance already present, only cultures that received a resistant cell will have resistant colonies. Some plates would have a lot of resistant colonies, others would have none.

Worth a Nobel Prize in 1969!!

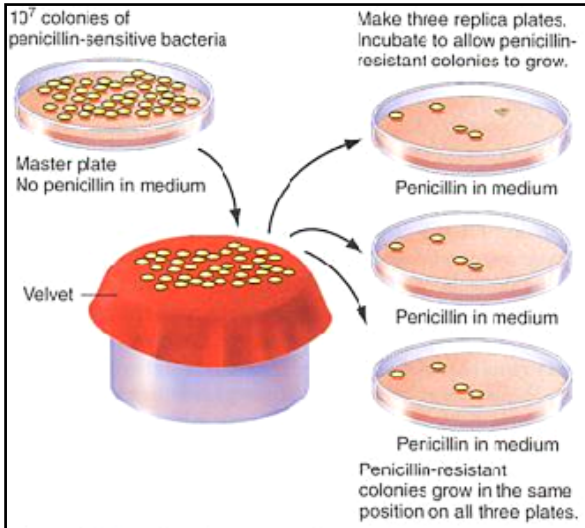


Is this support for Adaptive (A), Spontaneous Mutation (B) or neither (C) hypothesis?

Replicate Plating to test Induction vs. Spontaneous Mutation Hypotheses



J. Lederberg and E. Lederberg 1952



Is this support for Induction or Spontaneous Mutation Hypothesis??

Spontaneous Mutation!!!

Why are bacteria so diverse and why can they evolve so quickly?

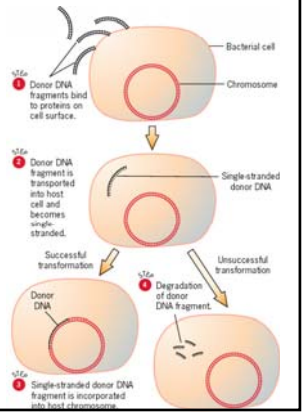
1) **Large numbers, short generation times and selection for rare mutants.**

Have we mentioned other mechanisms that might speed their rate of evolution?

2) **Transformation!! Bacteria can pick up genes from other bacteria!!**

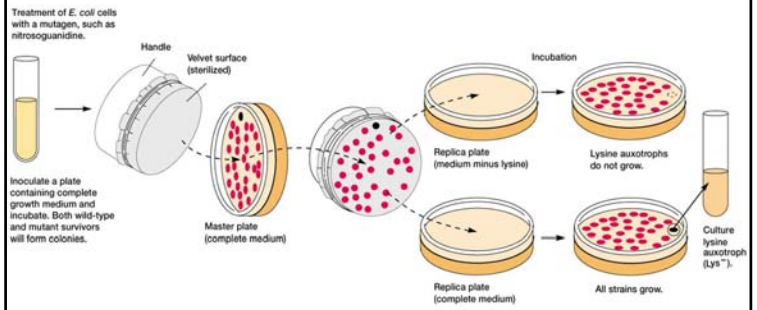
Griffith 1928, Avery et al. 1944

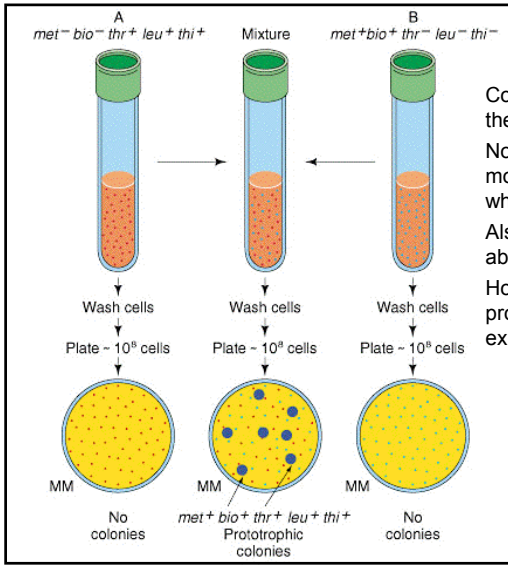
Frequency = 10^{-5} – 10^{-7}



Lederberg and colleagues looked further at mutations and their evolution by studying auxotrophs.
How to select for an auxotroph?

- REPLICA PLATING, e.g. for a lysine auxotroph





Lederberg and Tatum 1946

Could mutation account for the prototrophs?

No. You would not expect more prototrophs to appear when mixing strains.

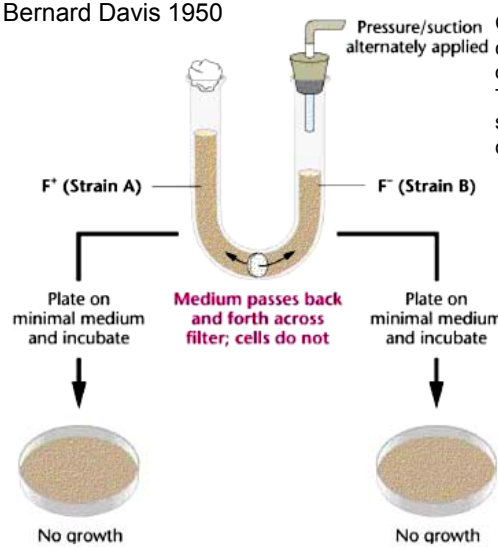
Also, mutation rates are about $1/10^8$ per gene.

How often would prototrophic mutants be expected?

$(1 \times 10^{-8})\ (1 \times 10^{-8}) =$

1×10^{-16}

Bernard Davis 1950

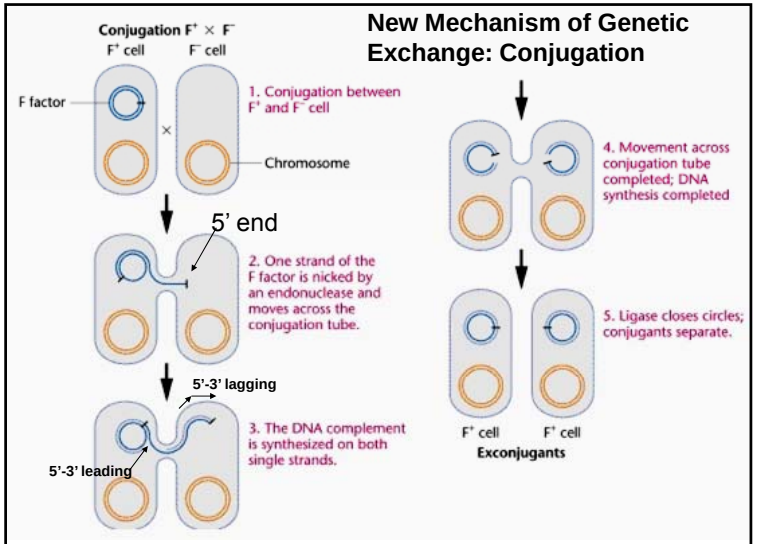


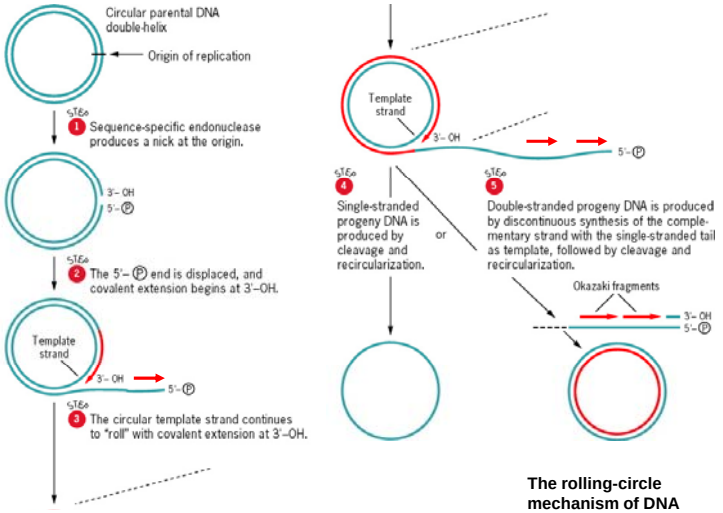
Conclusions: Mutation can not account for the observations. Transformation would seem to be a likely cause. But….

If transformation is the cause, what do you expect? Some colonies on both plates.

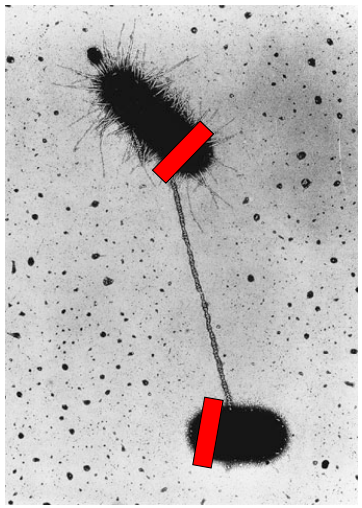
Conclusion: Transformation is not the cause!!!

New Mechanism of Genetic Exchange: Conjugation





The rolling-circle mechanism of DNA replication.



F factor plasmid

100 kb and 19 transfer genes plus other genes.

Cells carrying F produce pili, minute tubules.

F+ cells transfer a copy of plasmid and retain a copy.

F+ are inhibited from contacting other F+ cells

F factor plasmid can integrate into the bacterial genome!

Cavalli-Sforza 1950 and Hayes 1953 discovered High Frequency Recombination (Hfr) lines.

Recipient Cell gains genes of the donor

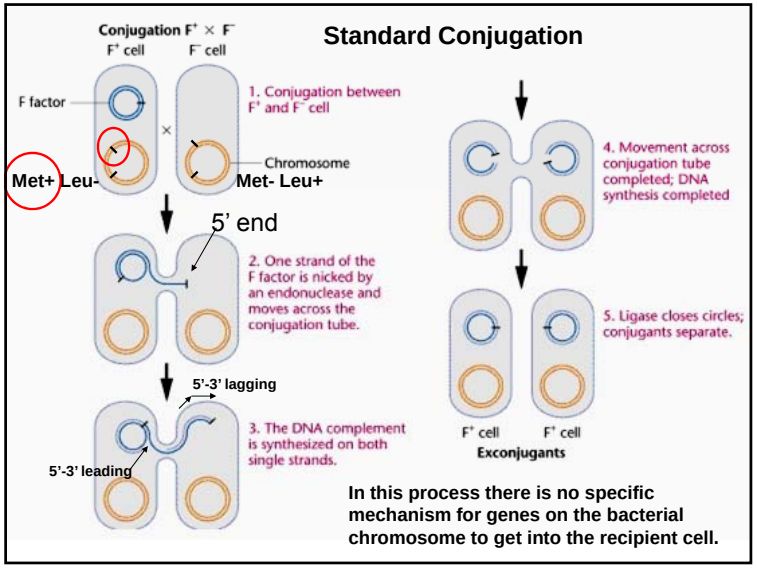
Normal Conjugations Lines

F^+_7 x F^- ⟶ F^+ F^+ Recombinants at 10^-

Hfr Lines

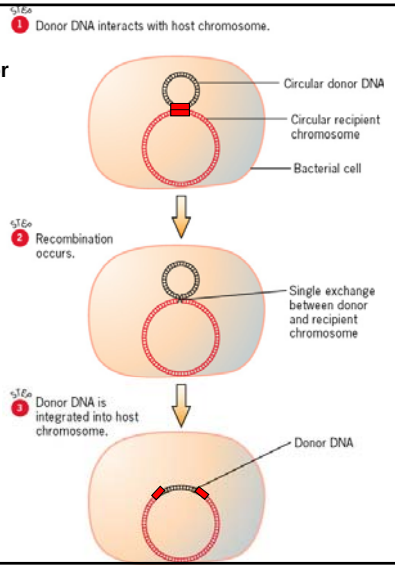
Hfr x F^- ⟶ Hfr F^- Recombinants at 10^{-4}

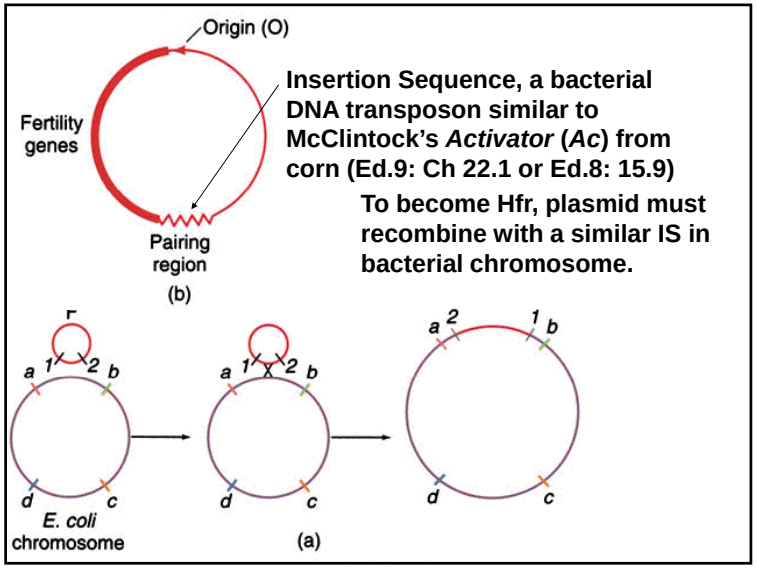
Recombination refers to the rate that prototrophs are produced in the recipient cell



In this process there is no specific mechanism for genes on the bacterial chromosome to get into the recipient cell.

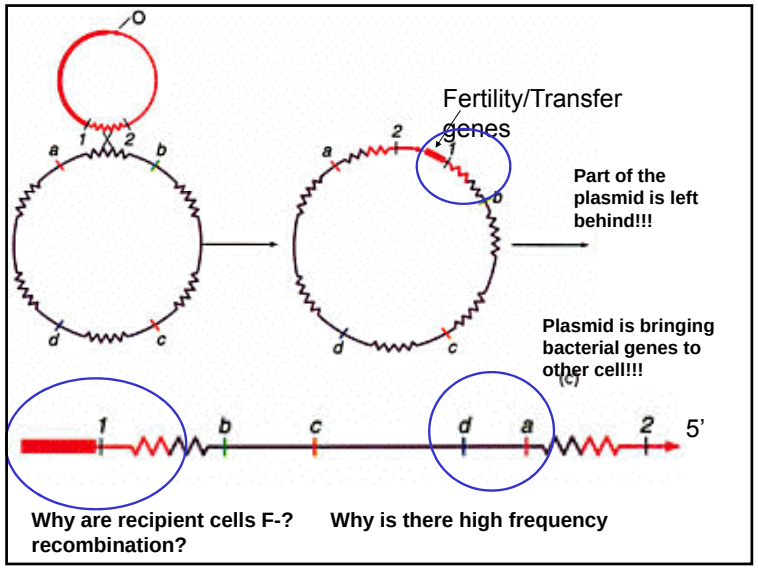
With Hfr there is a mechanism to move bacterial genes from donor to recipient.
How does it work?
Homologous Insertion Sequences (IS) pair
A single crossover will integrate plamid into bacterial chromosome.
The plasmid DNA will now be flanked by the 2 IS.

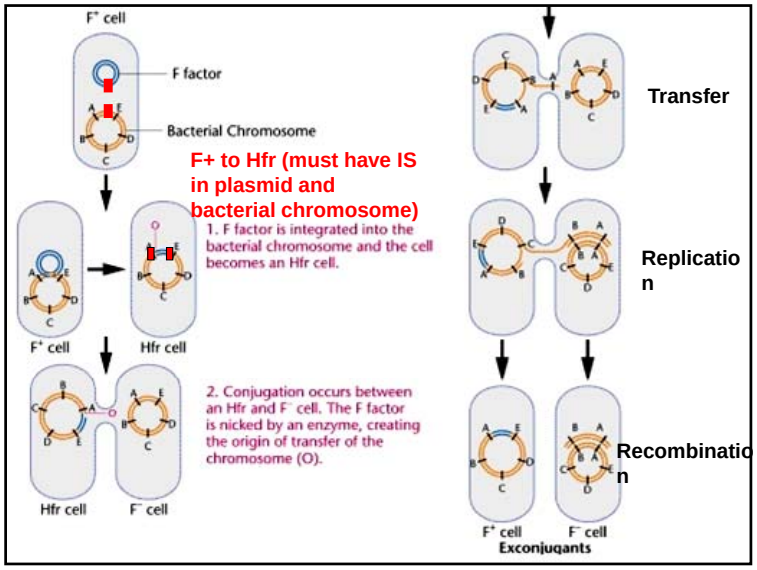


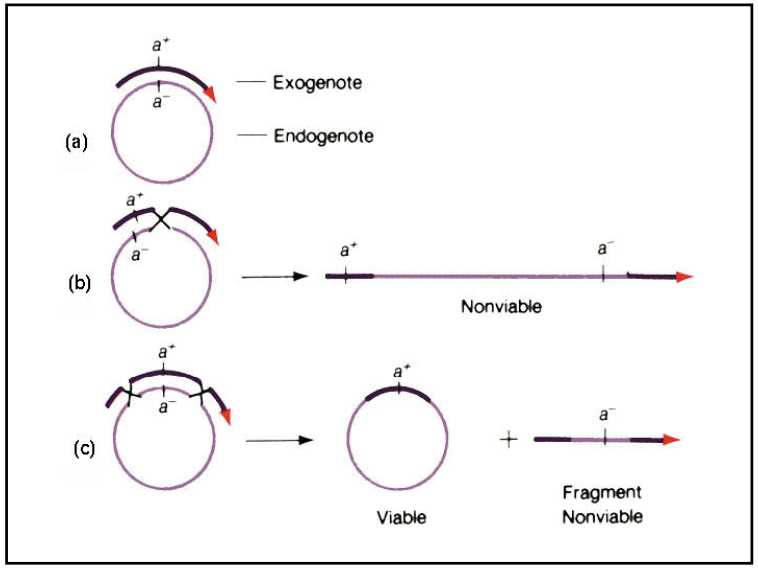


Insertion Sequence, a bacterial DNA transposon similar to McClintock's *Activator* (*Ac*) from corn (Ed.9: Ch 22.1 or Ed.8: 15.9)

To become Hfr, plasmid must recombine with a similar IS in bacterial chromosome.







Molecular Basis of Recombination in Prokaryotes (*E. coli*)

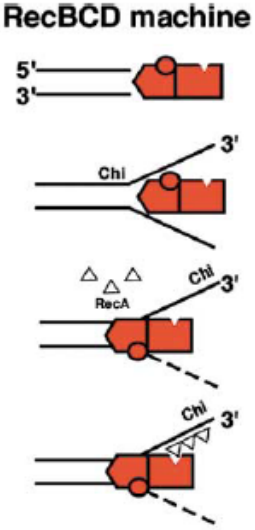
Three genes: *recB, recC, recD*

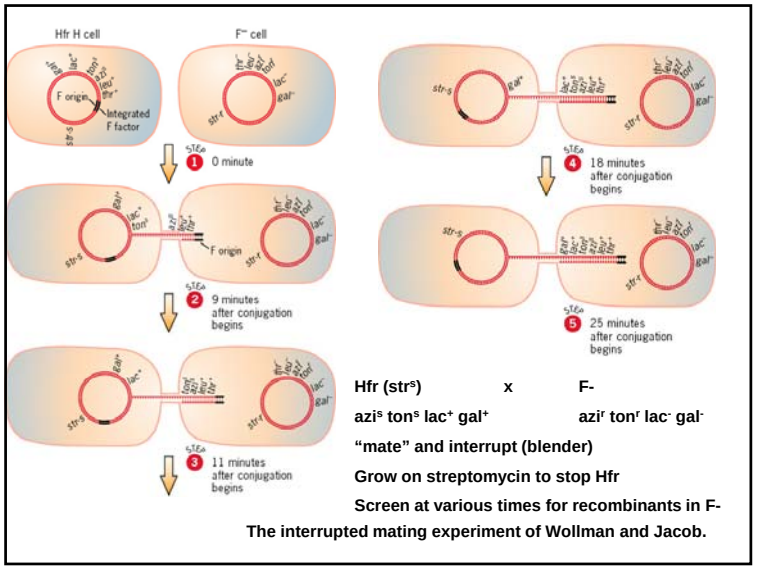
RecBCD protein has helicase and exonuclease activity

4th gene: *recA*

RecA (universal recombinase; = RadA in Archaea, Rad51/Dmc1 in Eukaryotes) binds to ssDNA and forms a highly recombinogenic 3'

Helicase	RecBCD
Nuclease	RecBCD
RecA-ss DNA filament formation	RecBCD





Hfr (str^s) x F-

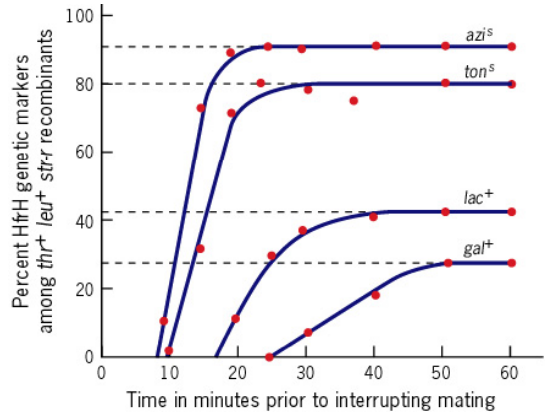
azi^s ton^s lac$^+$ gal$^+$ azi^r ton^r lac$^-$ gal$^-$

"mate" and interrupt (blender)

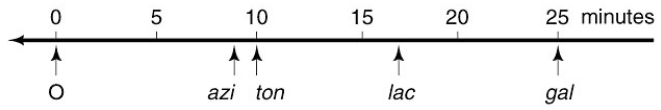
Grow on streptomycin to stop Hfr

Screen at various times for recombinants in F-

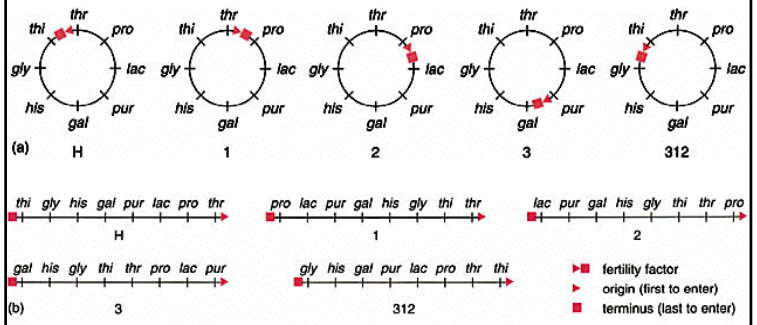
The interrupted mating experiment of Wollman and Jacob.



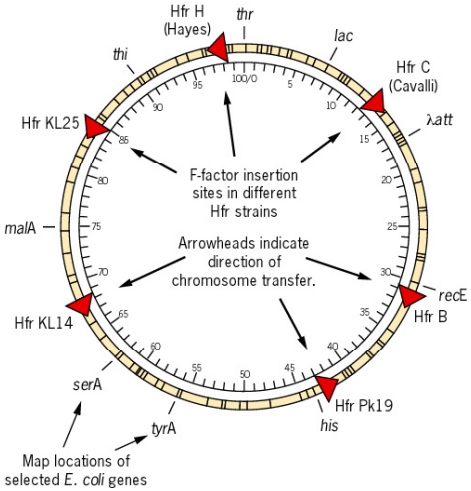
Data from the interrupted mating experiment.

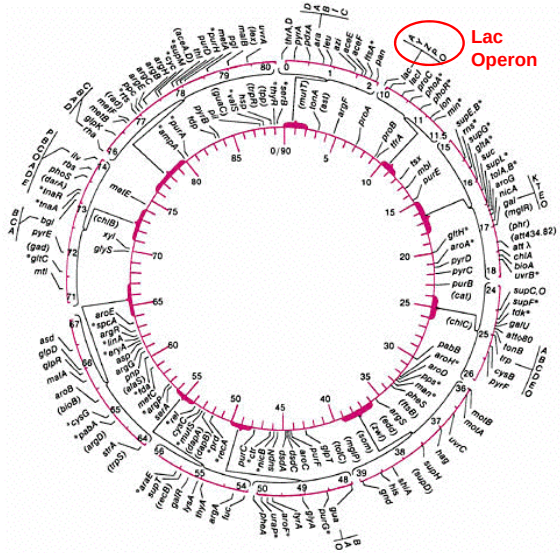


When different Hfr lines were used, they found different genes being transferred. Why?



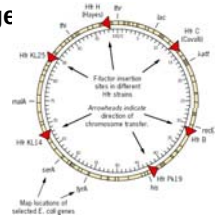
Why has the F plasmid integrated in different places?





Four Interrupted Mating Experiment with 4 different Hfr lines

Hfr	Order (letters represent different ge					
1)	E	R	I	U	M	B
2)	U	M	B	A	C	T
3)	R	E	T	C	A	B
4)	C	T	E	R	I	U



In which line(s) are the genes rearranged?

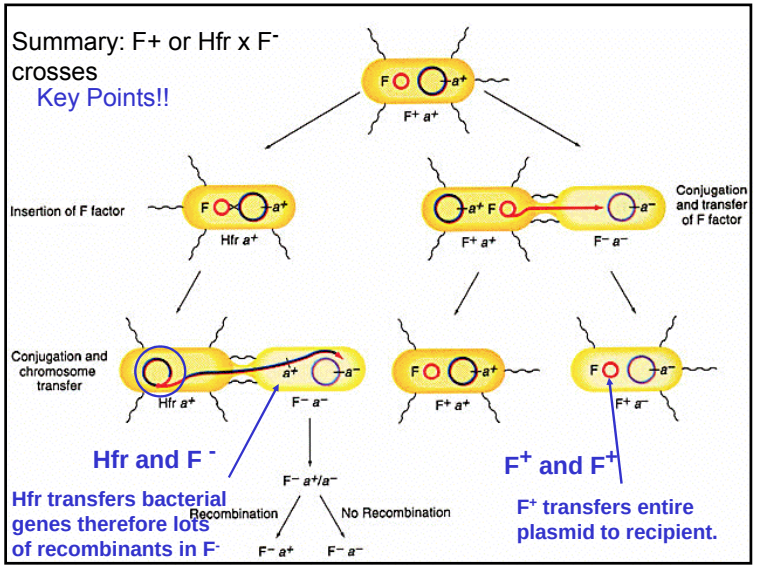
A) Hfr line 2 has a rearranged gene order

B) Hfr line 3 has a rearranged gene order

C) Hfr lines 2 and 4 have rearranged gene orders

D) All lines have rearranged gene orders

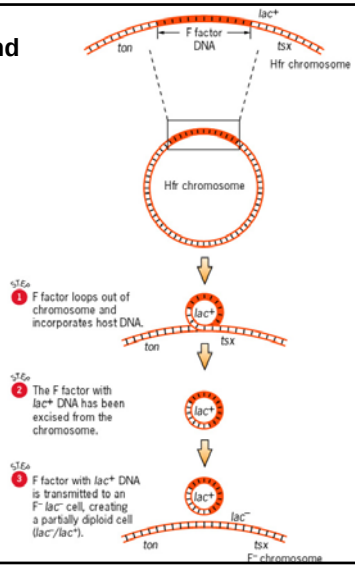
E) No line has rearranged gene orders

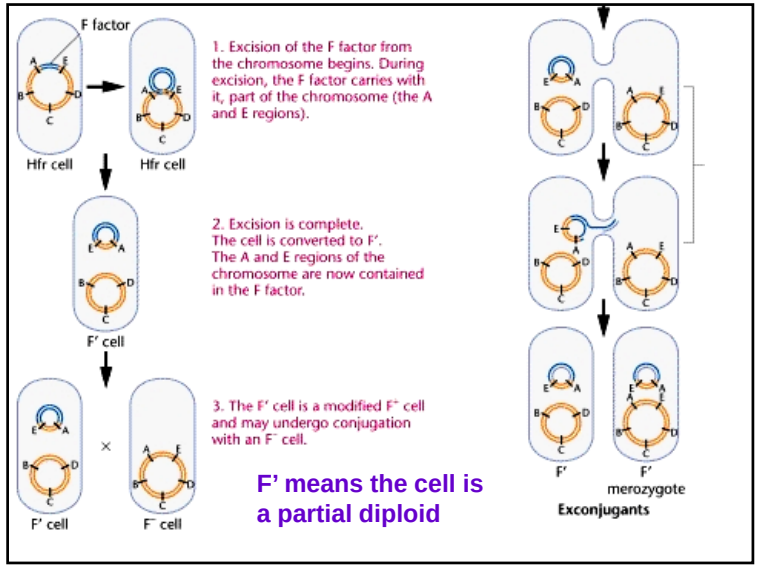


The plasmid can enter and excise from the bacterial chromosome (by recombination at the IS).

F+ ⇌ **Hfr**

Excision of the plasmid from the bacterial chromosome is not always perfect! (similar to unequal crossovers in Eukaryotes)



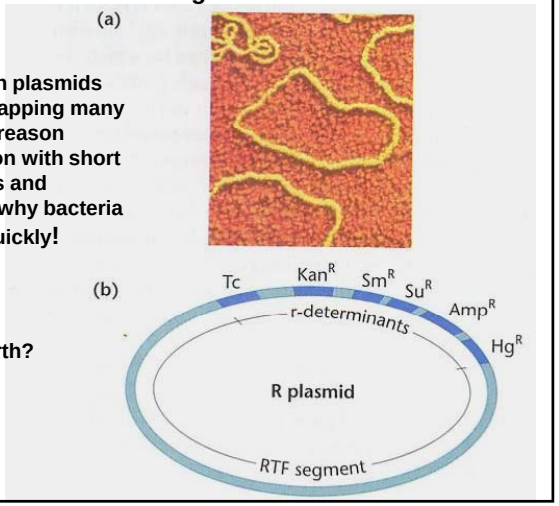


F' means the cell is a partial diploid

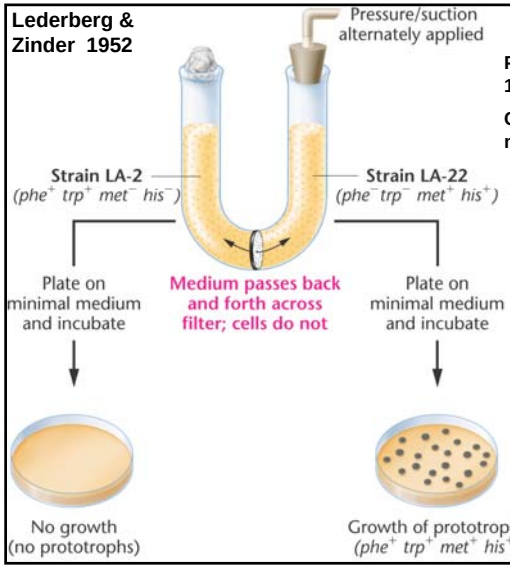
Plasmids, carriers of all sorts of bacterial genes including antibiotic resistance genes!!!

Conjugation with plasmids carrying and swapping many genes is a third reason (besides mutation with short generation times and transformation) why bacteria can evolve so quickly!

How about a fourth?



Lederberg & Zinder 1952



Prototrophs recovered at 1/100,000 (1 x 10^{-5}).

Could the cause be mutation?

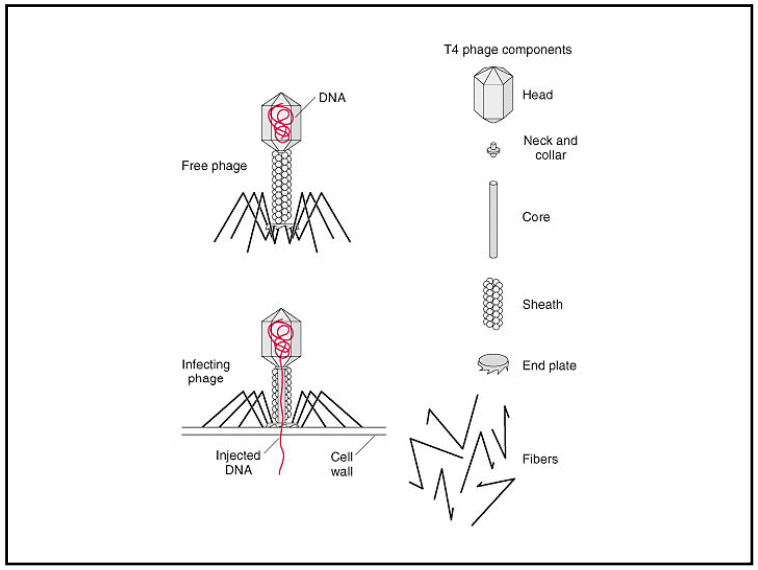
Could conjugation be the cause?

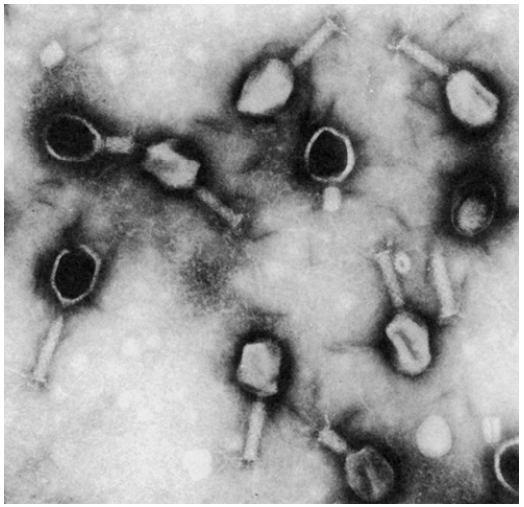
Could transformation be the cause?

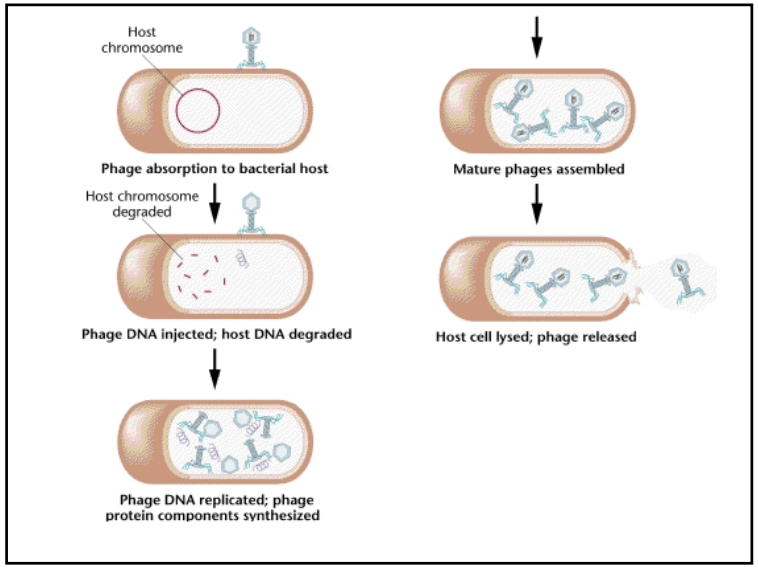
DNAse was added and prototrophs still recovered.

When pore size reduced to < 100 nm, no prototrophs recovered.

Conclusion: Another new mode for bacterial gene transfer!!!



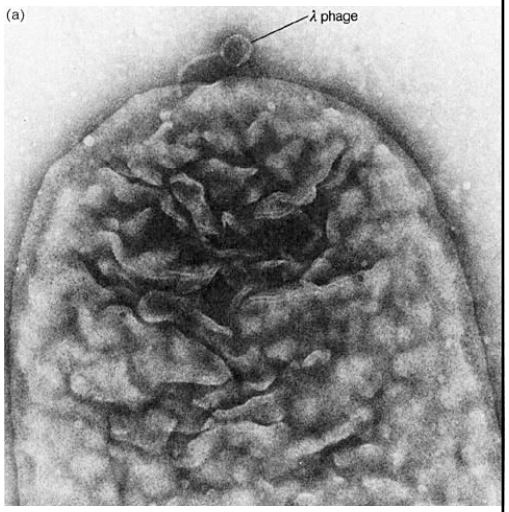




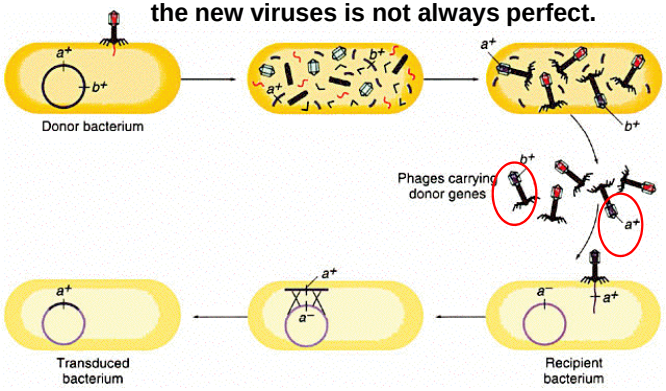
OK, so we can see that phage (bacterial viruses) can "hijack" the bacterial machinery to make more phage,

but how were the genes exchanged between the bacteria in the Lederberg and Zinder experiment?

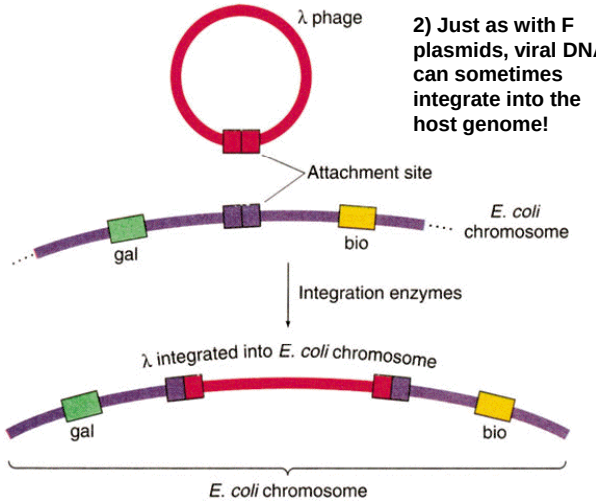
You need to know 2 more things!!



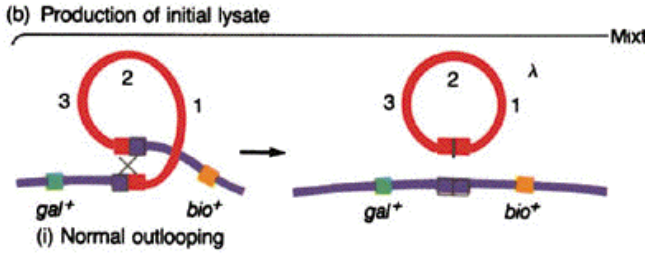
1) Generalized Transduction: assembly of the new viruses is not always perfect.



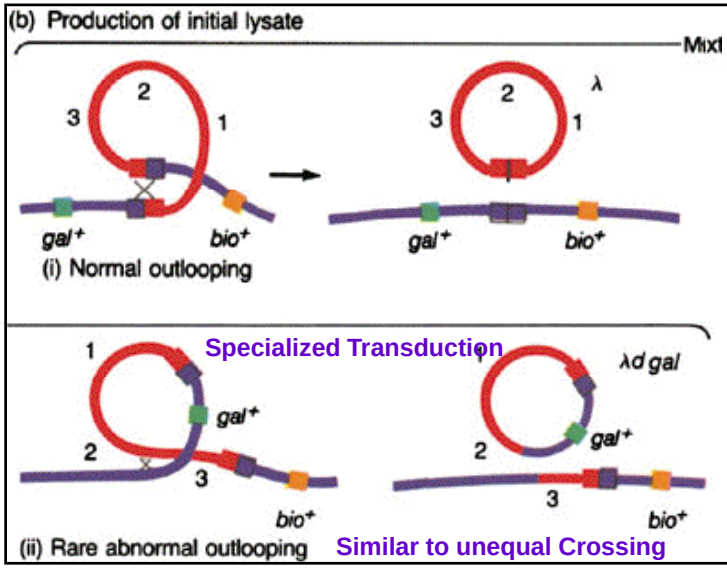
Again, need flanking recombination, otherwise fragment will be lost!!

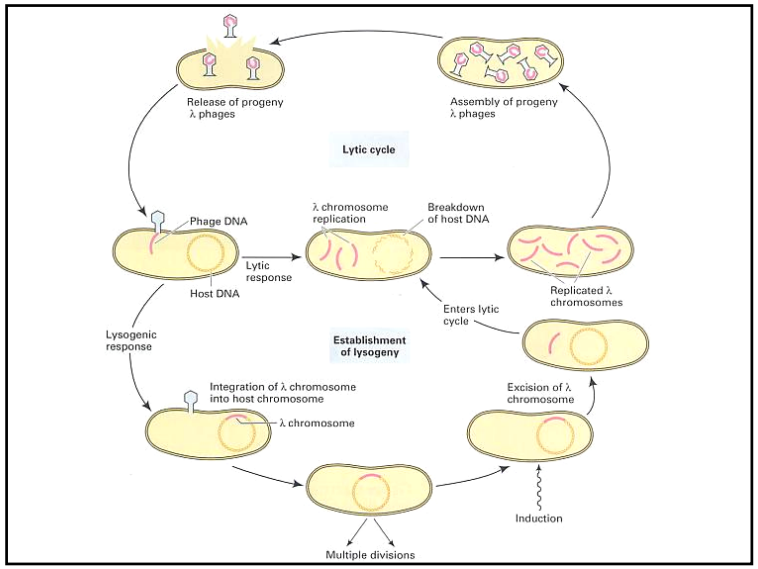


2) Just as with F plasmids, viral DNA can sometimes integrate into the host genome!

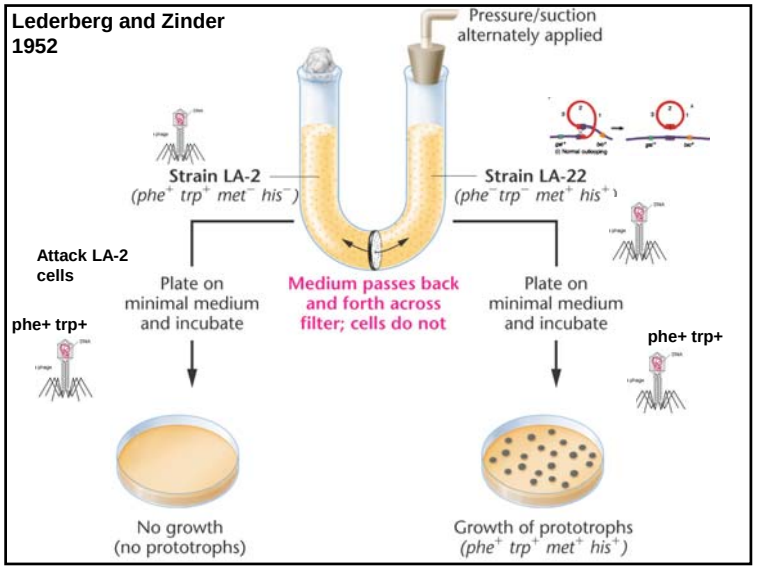


Lysogenic Stage → Lytic Stage

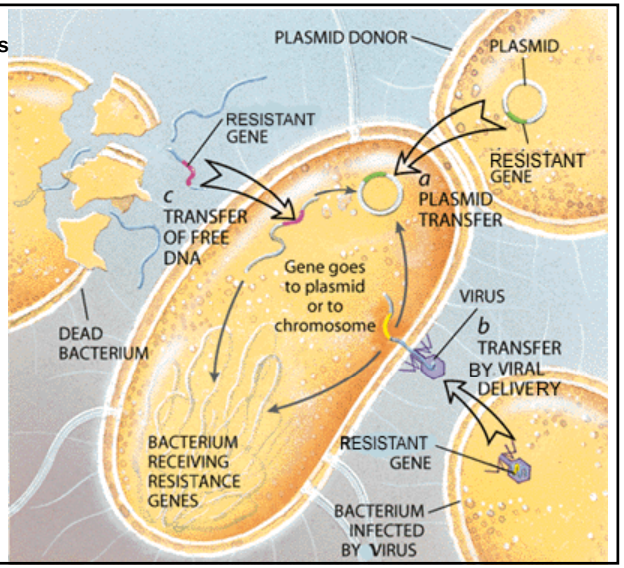




Lederberg and Zinder 1952



The promiscuous world of bacteria!



Levy 1997

Needed: New Drugs

Of the roughly 160 antibiotics now available, every one has some bacteria that are partially, or even fully, resistant to it. Here are the classes of the top selling antibiotics, along with some examples of common infections that have become resistant to them.

Class of antibiotic	*Worldwide sales* (in millions)*	*Some brand names in each class of antibiotic*	*Some of the infections that have shown resistance to treatment with this class of drugs*
Cephalosporins	$8,446	Cellacor, Cefuroxime	Bronchitis, pneumonia, meningitis
Penicillins**	4,413	Amoxicillin, Ampicillin	Pneumonia, septicemia, bronchitis
Flouro-quinolones	3,309	Ciprofloxacin, Ofloxacin	Toxic shock syndrome, meningitis
Macrolides	2,927	Clarithromycin, Erythromycin	Toxic shock syndrome, meningitis
Tetracyclines	744	Minocycline	Urinary tract infections, pelvic inflammatory disease
Aminoglycosides	729	Gentamicin	Intestinal infections, septicemia
Glycopeptides	462	Vancomycin	Intestinal infections
Carbapenems	443	Imipenem	Bronchitis, pneumonia
Trimethoprim combinations	381	TMP/SMX	Gastroenteritis, septicemia, bronchitis
All other systemic antibodies	1,049	Rifampin	Tuberculosis

*For 12-months ending September 1995. **Includes both broad-spectrum and medium/narrow spectrum penicillins.

These classes of antibiotics damage certain components of the bacterial cell. For example, Tetracycline attaches to bacterial ribosomes preventing protein systhesis. Vancomycin blocks cell wall synthesis.

Antibiotic	Percent of Strains Resistant
Gentamicin	92-98 %
Minocycline	92-98 %
Tetracycline	92-98 %
Erythromycin	92-98 %
Netilmycin	30 %
Sparfloxacin	40 %
Ciprofloxacin	42 %
Chloramphenicol	57 %
Trimethoprim	11-15 %
Fusidic acid	11-15 %
Rifampicin	11-15 %

Susceptibility of Methicillin (Multi) Resistant *Staphylococcus aureus* (MRSA) to Antibiotics Other Than Glycopeptides

Source: The Complete Guide to Anti-Infectives, *Scrips*, 1999.

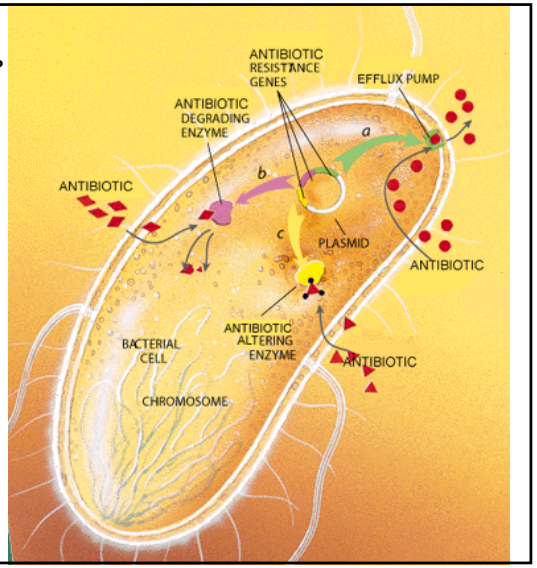
How do Antibiotic resistance genes work?

Degrade antibiotic

Alter antibiotic or its target

Block entrance of antibiotic into the cell

Pump antibiotic out of cell



Levy 1997

AGENT	MECHANISM OF ACTION	RESISTANCE MECHANISMS
Beta lactams: penicillins, cephalosporins	Block cell wall formation	Inactivation, mutation
Glycopeptides: vancomycin	Block cell wall formation	Mutation of binding molecules
Amino glycosides: gentamycin	Block protein synthesis	Inactivation
Tetracyclines	Block protein synthesis	Inactivation
Macrolides	Block protein synthesis	Ribosome protection
Quinolones	Inhibit DNA replication	Mutation of binding molecules
Rifampin	Inhibits bacterial RNA polymerase	Mutation of binding molecules
Trimethoprim Sulfonamides	Block formation of nucleic acids and f-met	Mutation of binding molecules

What do we do that accelerates the evolution of antibiotic resistance in bacteria?

Improper medicinal uses:

Prescriptions for viral infections (CDC estimates that 1/3 of outpatient prescriptions are unneeded!)

Patients fail to complete full prescriptions and stockpile leftovers making these less than therapeutic dosages.

In many countries little regulation and indiscriminant use antibiotics.

Non-medical uses:

"Anti-bacterial Fad" with proliferation of antibiotic disinfectants and antiseptics. (Use bleach, alcohol, ammonia and hydrogen peroxide instead!)

Agricultural Uses:

Massive amounts are mixed into animal feed.

Aerosol sprays on acres of fruit trees

Definitions: **Transformation**

Translocation

Transduction

Conjugation

A) Viral mediated DNA exchange; chromosome rearrangement; free DNA uptake; controlled DNA transfer

B) Controlled DNA transfer; chromosome rearrangement; viral mediated DNA exchange; free DNA uptake

C) Free DNA uptake; chromosome rearrangement; viral mediated DNA exchange; controlled DNA transfer

D) Controlled DNA transfer; chromosome rearrangement; viral mediated DNA exchange; free DNA uptake

E) None of the above is correct