Transposable Genetic Elements

Transposable genetic elements are DNA fragments containing genes that do not have a fixed location in a genome but can move from place to place within the genome

- "Jumping genes"
- Mobile genetics elements
- Controlling elements
- Transposons
- Exist in all organisms
- Changes the genetic architecture of organisms
- Tools for gene cloning and manipulation

- comprise 45% of human chromosomal DNA
- contribute to spontaneous mutation, genetic rearrangements, horizontal transfer of genetic material
- aid speciation and genomic change (in bacteria transposons are often associated with antibiotic resistance genes)
- cells must depress transposition to insure genetic stability

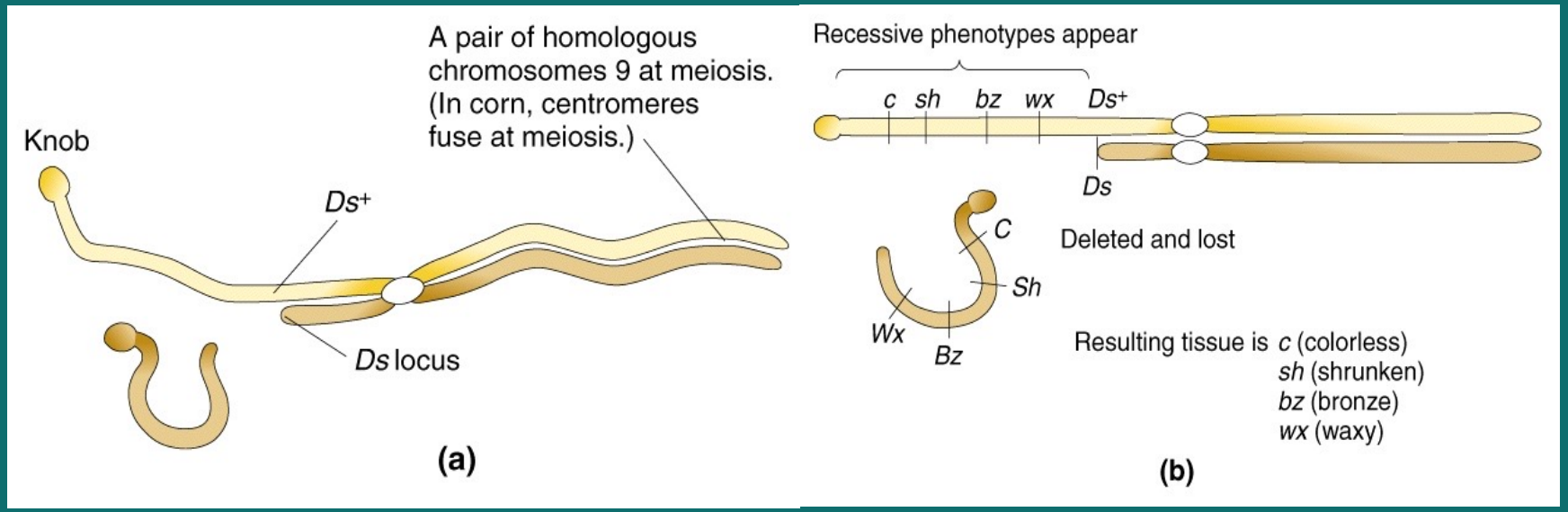
In 1950, Barbara McClintock was the first scientist to predict that transposable elements, mobile pieces of the genetic material (DNA), were present in eukaryotic genomes. She performed her work on corn and specifically followed seed color phenotypes

Ac/Ds Maize system

- Ac =activator
- An autonomous element

- Ds = dissociation
- A non-autonomous element
- Requires presence of Ac
- Causes chromosomal breakage

McClintock's experiments: the Ds element



- 1950's: McClintock reported the presence of a genetic factor **Ds** (for "Dissociation") whose presence caused a high degree of chromosome breakage wherever it appeared The action of Ds is another type of instability
- The instability of Ds turned out to depend on the presence of an unlinked gene **Ac** (for "Activator")
- Ac & Ds locus was constantly changing position

TRANSPOSABLE GENETIC ELEMENTS IN PROKARYOTES

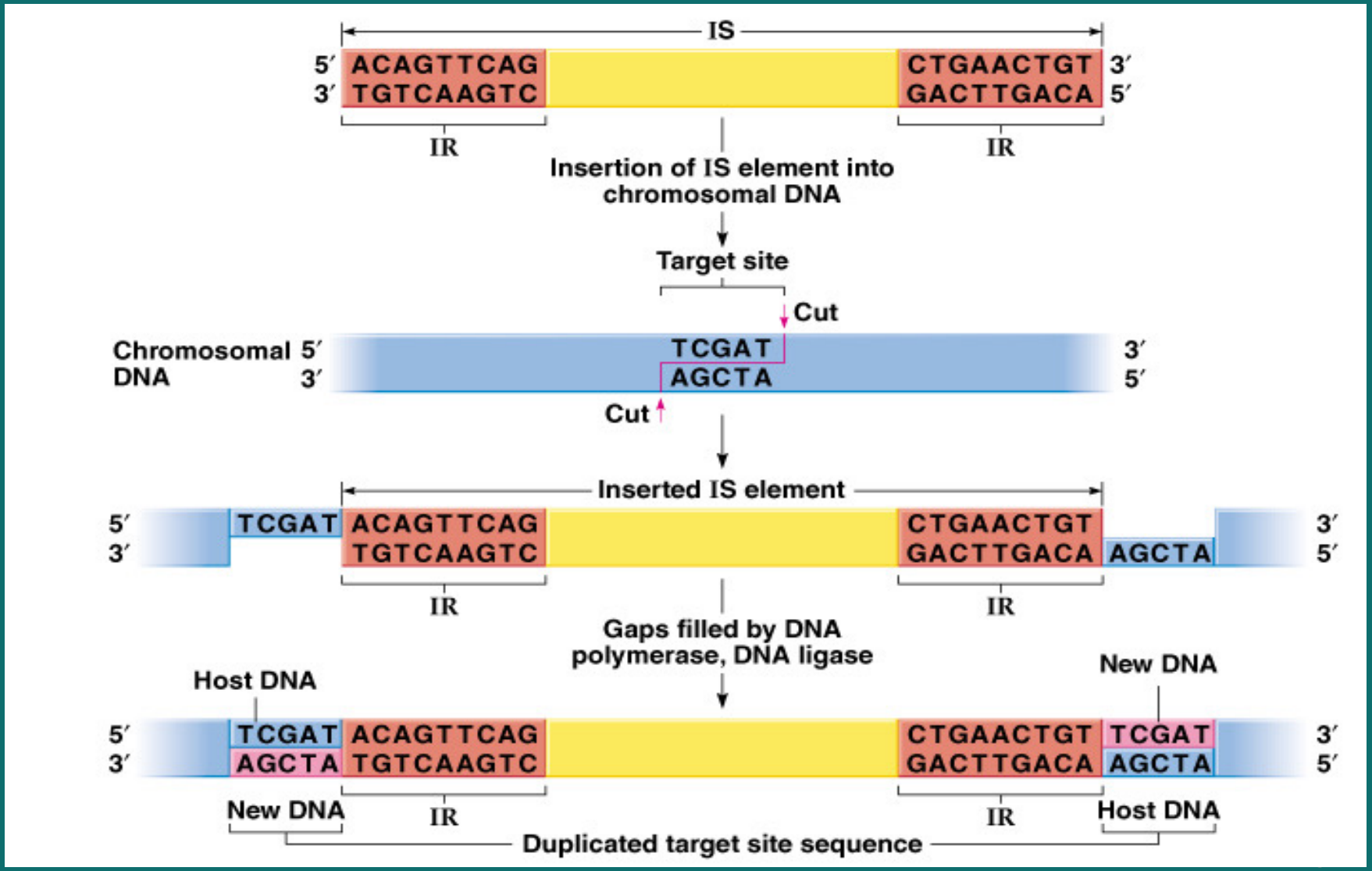
Insertion Sequences

- **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 glactose 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)**

- **Insertion sequences,** or **insertion-sequence (IS) elements,** are now known to be segments of DNA that can move from one position on a chromosome to a different position on the same chromosome or on a different chromosome.
- When IS elements appear in the middle of genes, they interrupt the coding sequence and inactivate the expression of that gene. Owing to their size, and in some cases the presence of transcription and translation termination signals, IS elements can also block the expression of other genes in the same operon if those genes are downstream from the promoter of the operon.

- IS were first discovered in the *gal* operon of *E. coli*
- Mutation by insertion is demonstrated with phage lambda particle carrying the bacterial gene for galactose utilization (gal+) or the mutant gene gal-

Integration of IS element in chromosomal DNA



20-1 TABLE Prokaryotic Insertion Elements

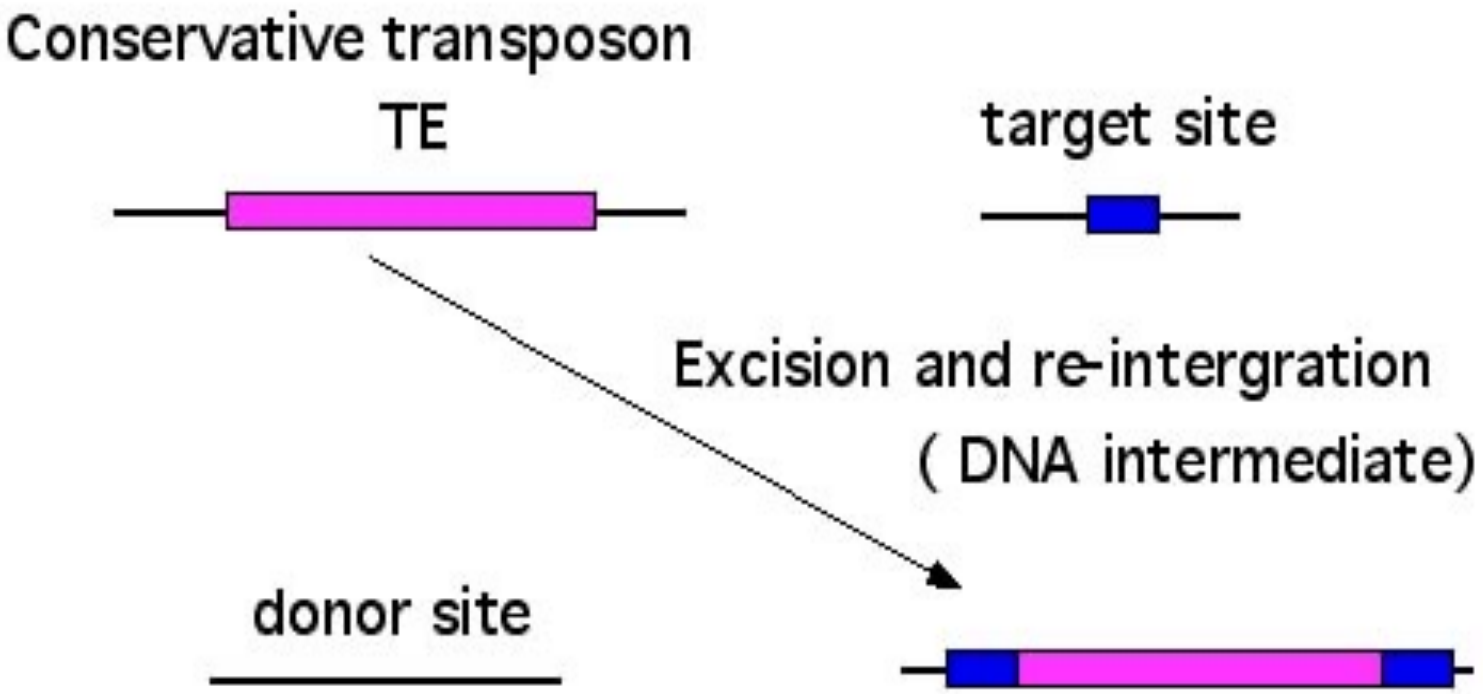
Insertion sequence	Normal occurrence In *E. coli*	Length (bp)	Inverted repeat* (bp)
IS1	5–8 copies on chromosome	768	18/23
IS2	5 copies on chromosome; 1 on F	1327	32/41
IS3	5 copies on chromosome; 2 on F	1400	32/38
IS4	1 or 2 copies on chromosome	1400	16/18
IS5	Unknown	1250	Short
γ-δ (TN1000)	1 or more copies on chromosome; 1 on F	5700	35
pSC101 segment	On plasmid pSC101	200	30/36

* Fraction of base pairs; for example, 18 of 23 bp, and so forth.
Source: M. P. Calos and J. H. Miller, *Cell* 20, 1980, 579–595.

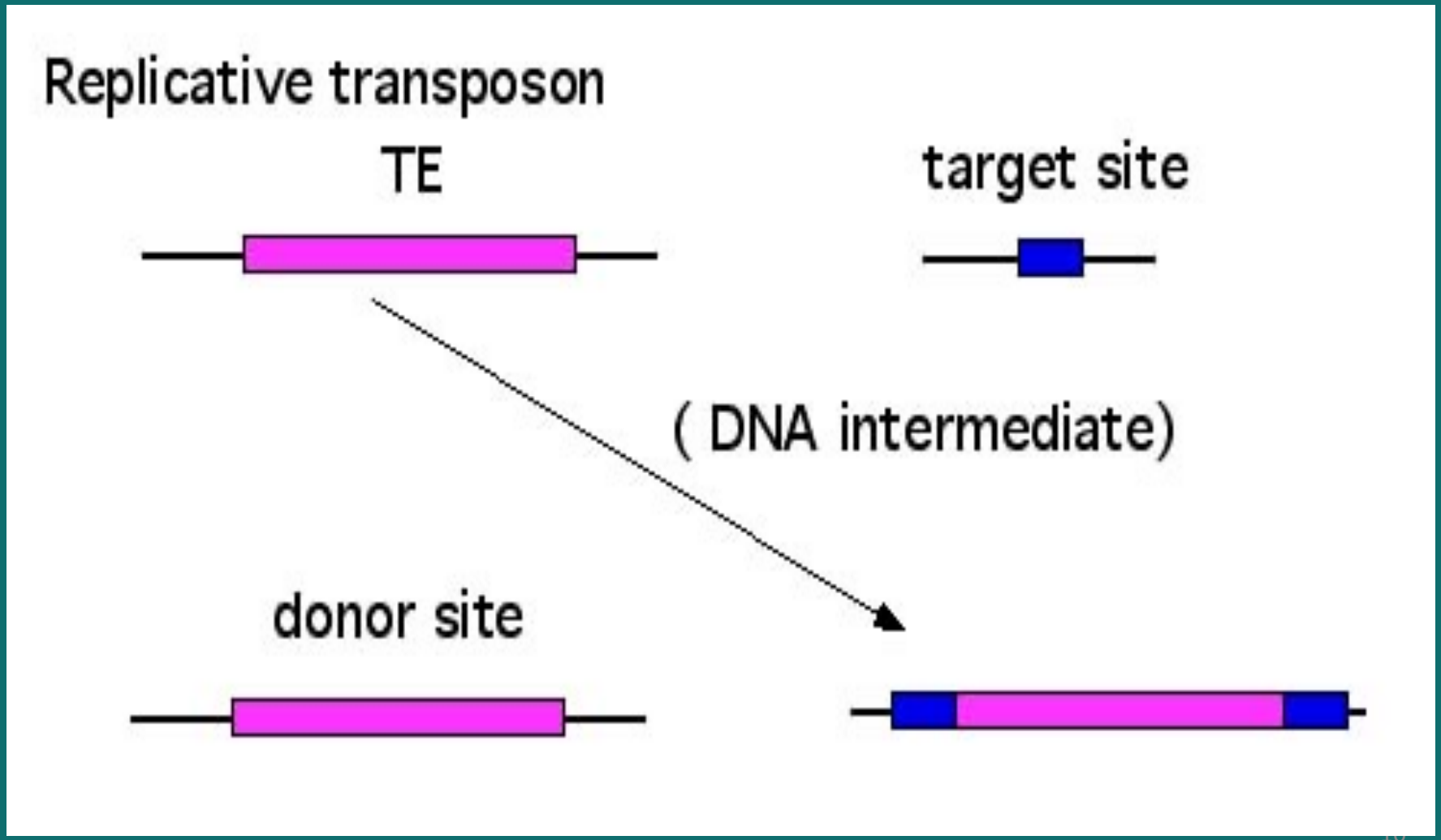
Three different mechanisms for transposition

- **Conservative transposition**: The element itself moves from the donor site into the target site
- examples: Tn5, Tn10, P elements
- **Replicative transposition**: The element moves a copy of itself to a new site via a DNA intermediate
- examples: Tn3, bacteriophage Mu
- **Retrotransposition**: The element makes an RNA copy of itself which is **reversed-transcribed** into a DNA copy which is then inserted (cDNA)

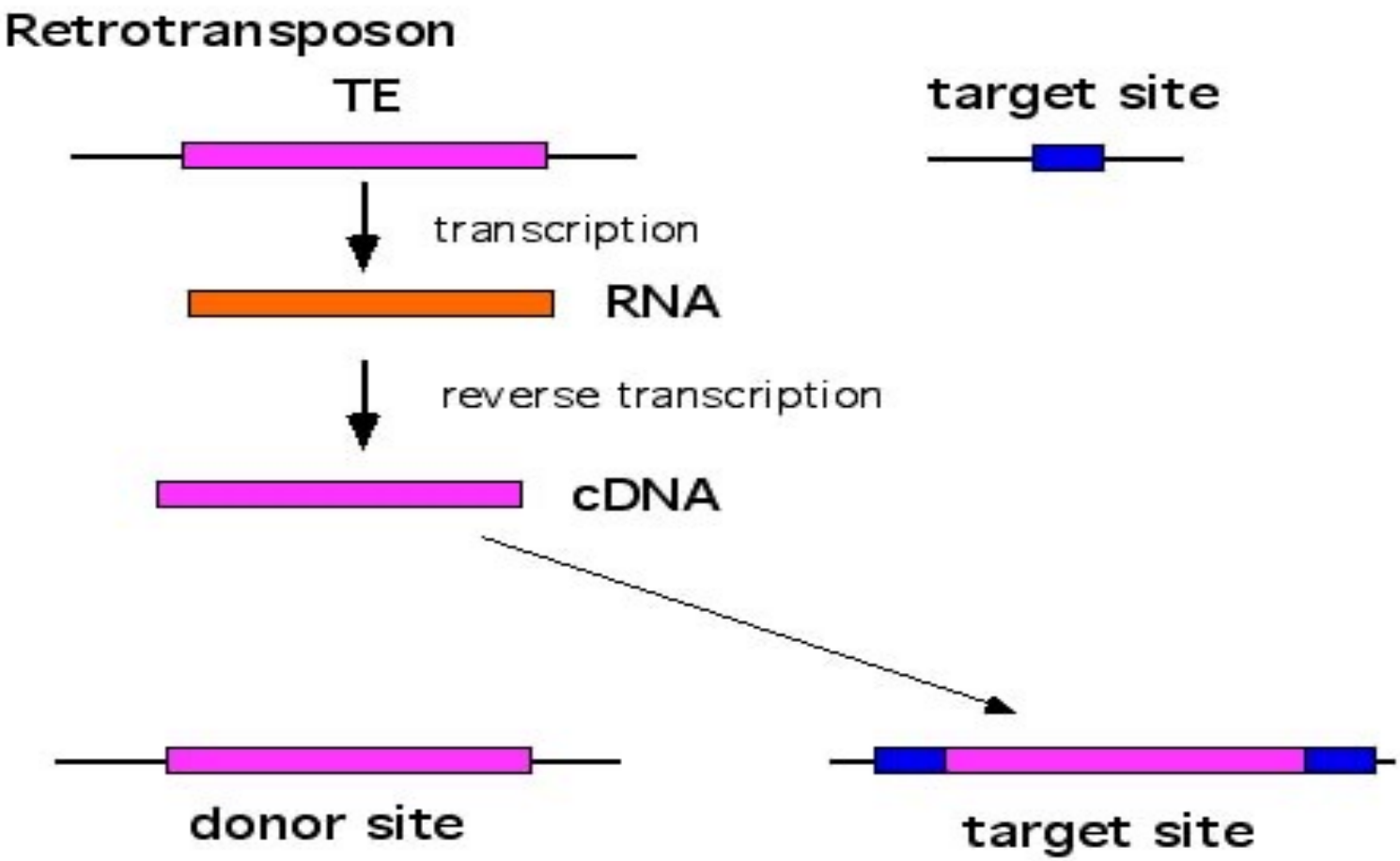
Conservative transposition



Replicative transposition



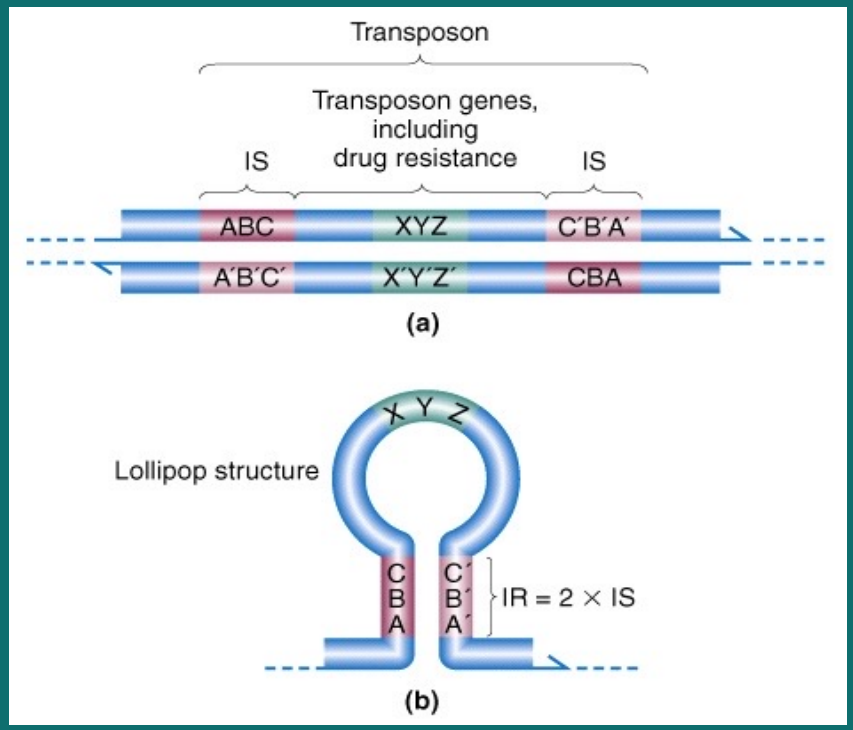
Retrotransposition

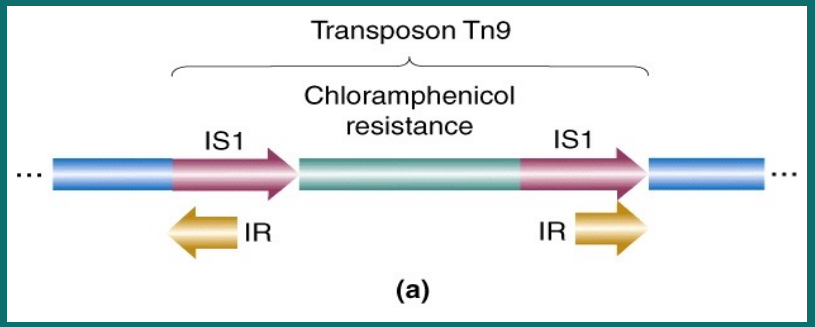


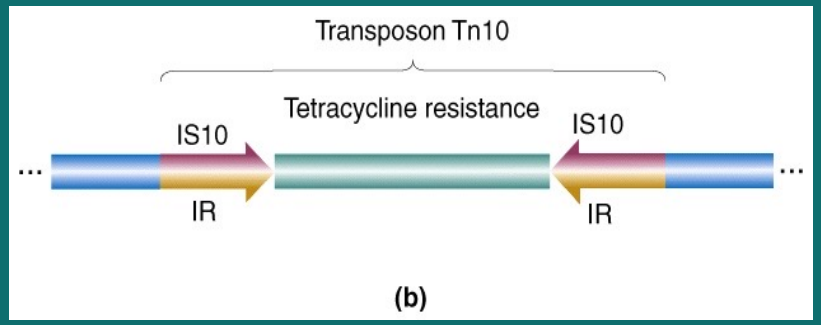
Prokaryotic transposons

Physical structure of transposons

-The IR sequences are a pair of IS elements in many cases
-The IR sequences together with their contained genes
→ transposon (Tn)
- Composite transposable genetics elements
- Consist of two IS flanking a gene
- Can mobilize the DNA between the two IS



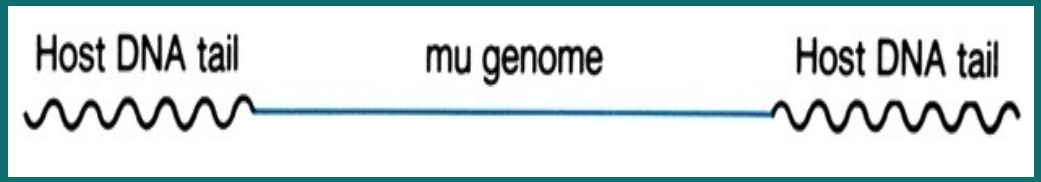




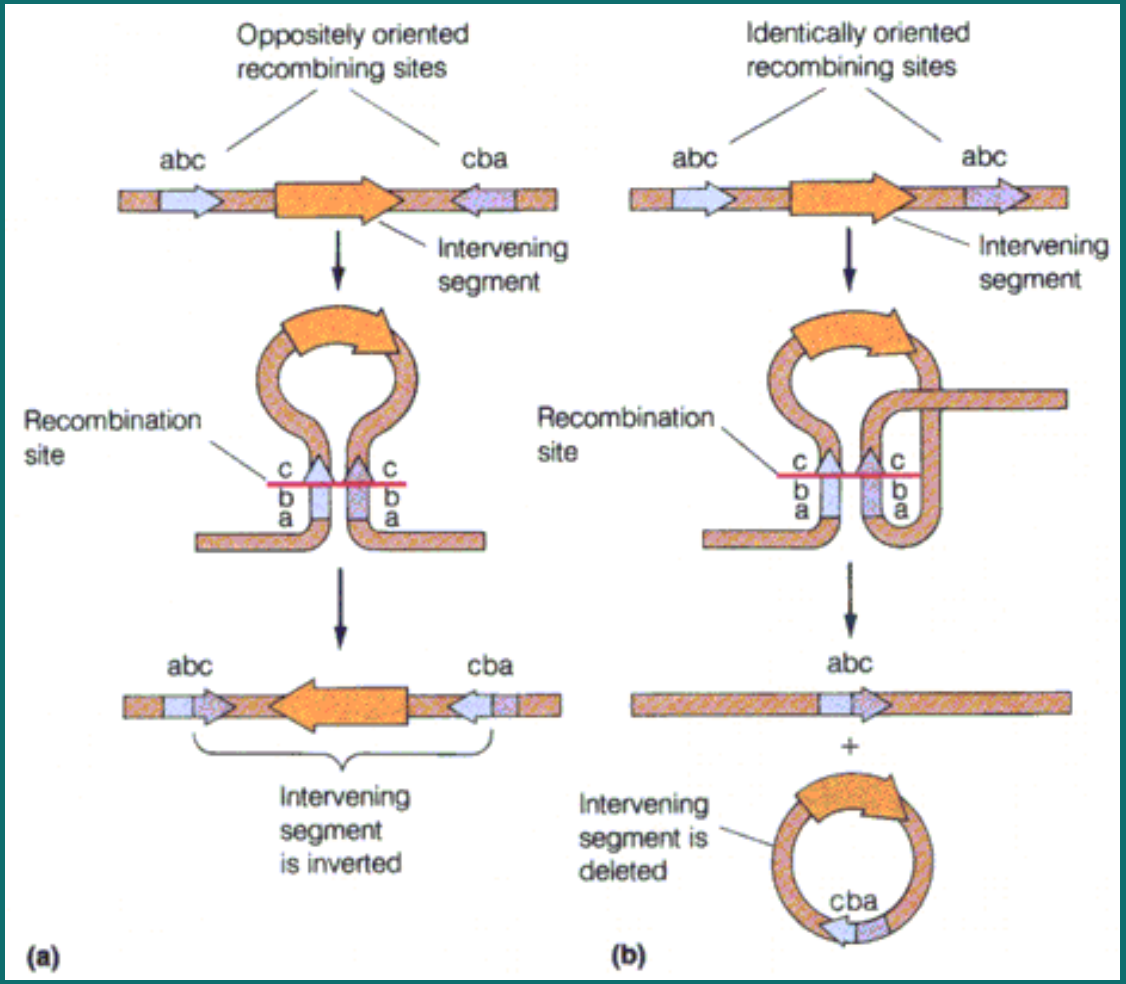
Prokaryotic transposons

Phage mu

- a normal-appearing phage
- 36,000 nucleotide long
- can insert itself anywhere in a bacterial or plasmid genome in either orientation; mutation in the genome like IS
- each mature phage particle has on each end a piece of flanking DNA from its previous host → no insert into genome in next generation
- contain its own IR sequences; but not in the chromosome ends



- genetic snap fastener: phage mu can mediate the insertion of phage λ or drug resistant gene into a bacterial chromosome using 2 mu genome



Genome rearrangements that can be promoted by homologous recombination between two copies of the same transposable element

Transposable elements can restructure a host chromosome. A **transposable element** can move from one site to another within the same chromosome, producing two homologous sequences resident in the same chromosome. Depending on whether these sequences are oriented identically or in reverse, homologous recombination between them can yield a deletion or an inversion

Retroviruses

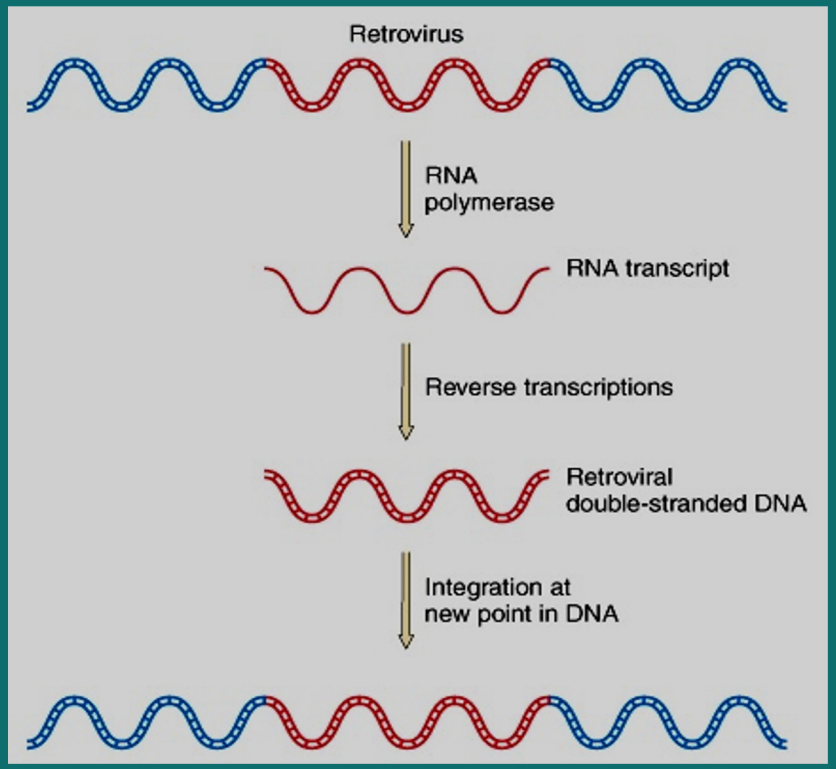
•**Retroviruses** are single-stranded RNA animal viruses that employ a double-stranded DNA intermediate for replication. The RNA is copied into DNA by the enzyme reverse transcriptase.. Some retroviruses, such as mouse mammary tumor virus (MMTV) and Rous sarcoma virus (RSV), are responsible for the induction of cancerous tumors. When integrated into host chromosomes as double-stranded DNA, these retroviruses are termed **provirus.** Proviruses, like the mu phage in bacteria, can be considered transposable elements, because they can, in effect, transpose from one location to another.

Retroviruses have structural features in common with some transposable elements from bacteria and other organisms. In particular, the ends of the proviruses have long terminal repeats (LTRs) reminiscent of the sequences of the Ty1 elements in yeast and the long terminal repeats of the *copia*-like elements in *Drosophila.* In addition, integration results in the duplication of a short target sequence in the host chromosome.

Molecular nature of transposable elements in eukaryotes

- Transposable elements are even more prevalent in eukaryotic chromosomes than in bacterial chromosomes

1. Retroviruses; ss RNA animal virus



Transposition by retrovirus

Some Important Studies in Genetics: Experimental Proof

- Fredrick Griffith: Transformation Experiment, 1928

- **Griffith used two strains of *Pneumococcus bacteria*, type III-S and type II-R.**

- **There is one major difference between these two types; the III-S strain has a smooth polysaccharide coat which makes it resistant to the immune system of mice, whereas the II-R strain lacks this coat and so will be destroyed by the immune system of the host.**

- **For the first stage of the transforming principle experiment, Griffith showed that mice injected with III-S died but when injected with II-R lived and showed few symptoms.**

- **The next stage showed that if the mice were injected with type III-S that had been killed by heat, the mice all lived, indicating that the bacteria had been rendered ineffective.**

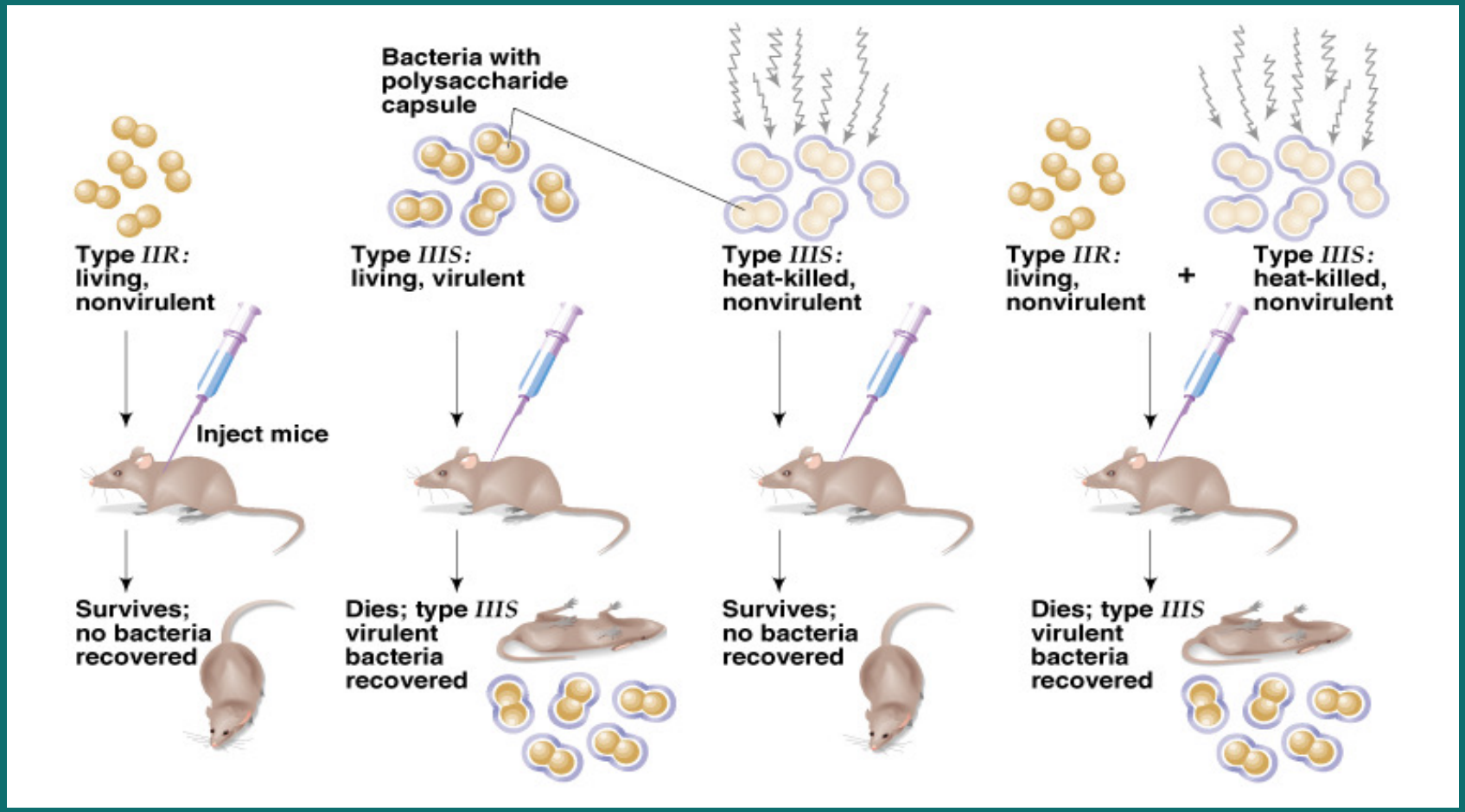
- **The interesting results came with the third part of the experiment, where mice were injected with a mixture of heat killed III-S and live II-R.**

- **Interestingly enough, the mice all died, indicating that some sort on information had been passed from the dead type III-S to the live type II-R. Blood sampling showed that the blood of the dead mice contained both live type III-S and live type II-R bacteria.**

- **Somehow the type III-S had been transformed into the type III-R strain, a process he christened the transforming principle**

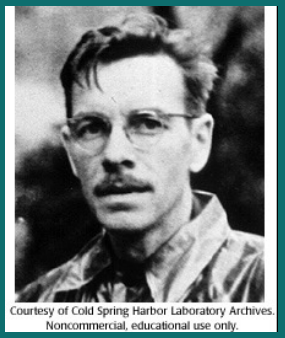
Frederick Griffith's Transformation Experiment - 1928

"transforming principle" demonstrated with *Streptococcus pneumoniae*



Hershey-Chase Bacteriophage Experiment - 1953

Evidence that DNA is the Genetic Material

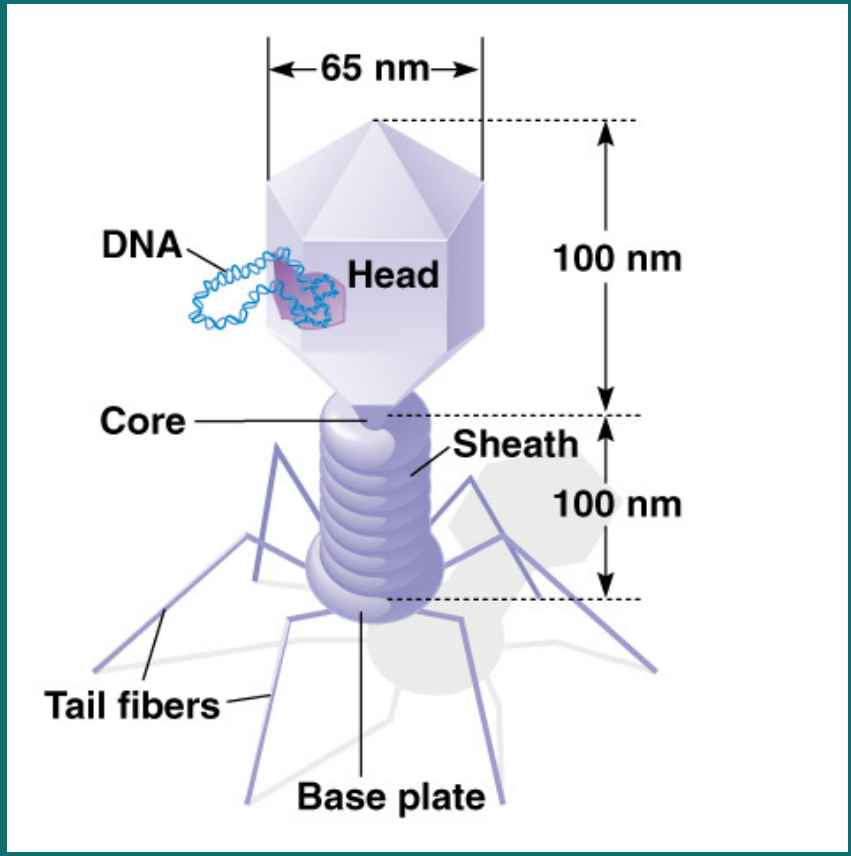


In 1952, American biologists Alfred Hershey and Martha Chase set out to determine what composed the genetic material of a bacteriophage

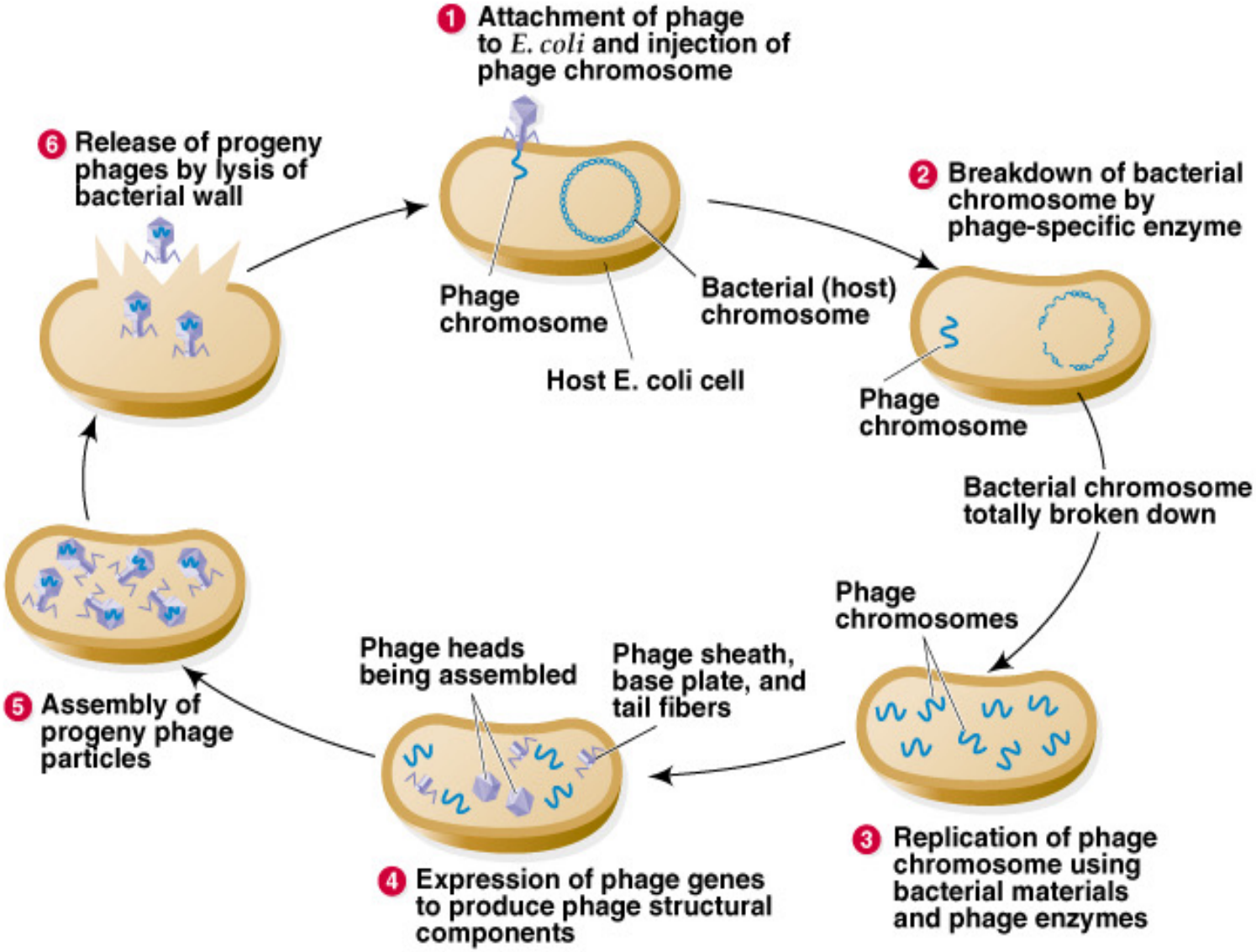
Bacteriophage = Virus that attacks bacteria and replicates by invading a living cell and using the cell's molecular machinery.

Structure of T2 phage

Bacteriophages are composed of DNA & protein



Life cycle of virulent T2 phage:



Hershey-Chase Bacteriophage Experiment - 1953

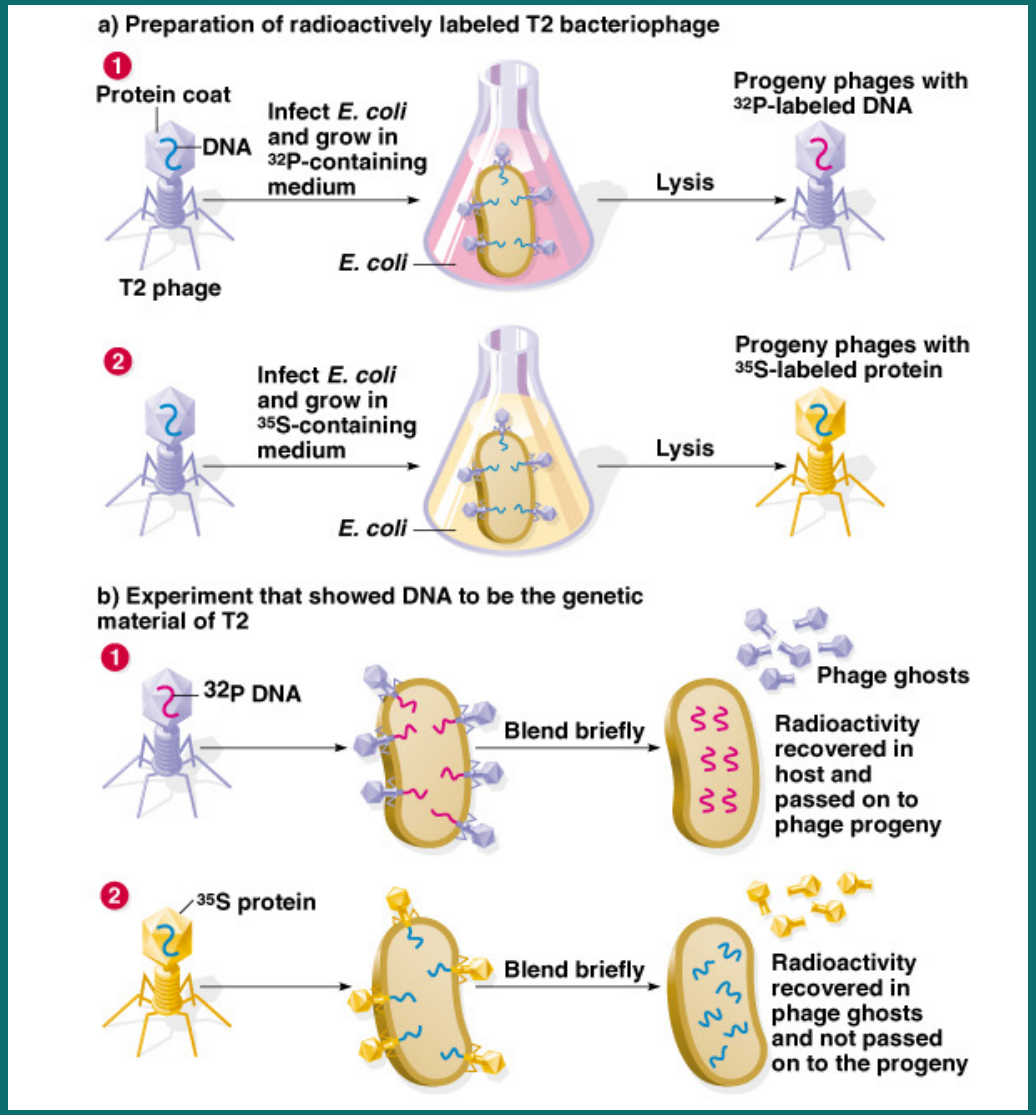
1. T2 bacteriophage is composed of DNA and proteins:

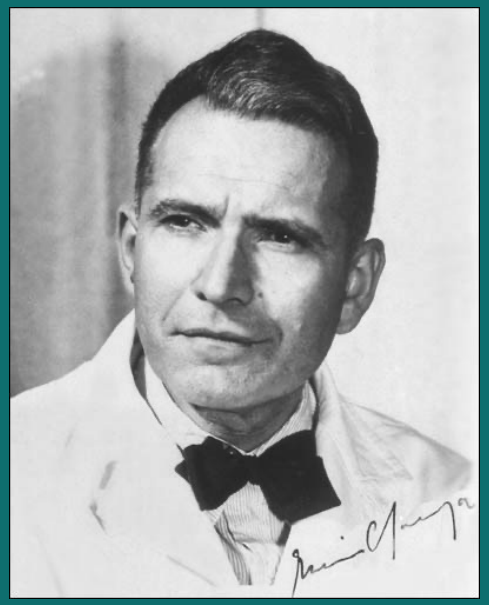
2. Set-up two replicates:

   - Label DNA with ^{32}P
   - Label Protein with ^{35}S

3. Infected *E. coli* bacteria with two types of labeled T2

4. ^{32}P is discovered within the bacteria and progeny phages, whereas ^{35}S is not found within the bacteria but released with phage ghosts.



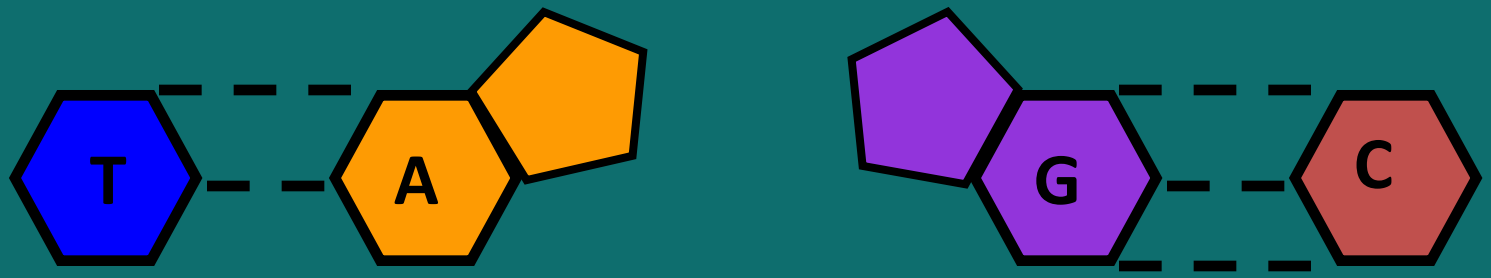


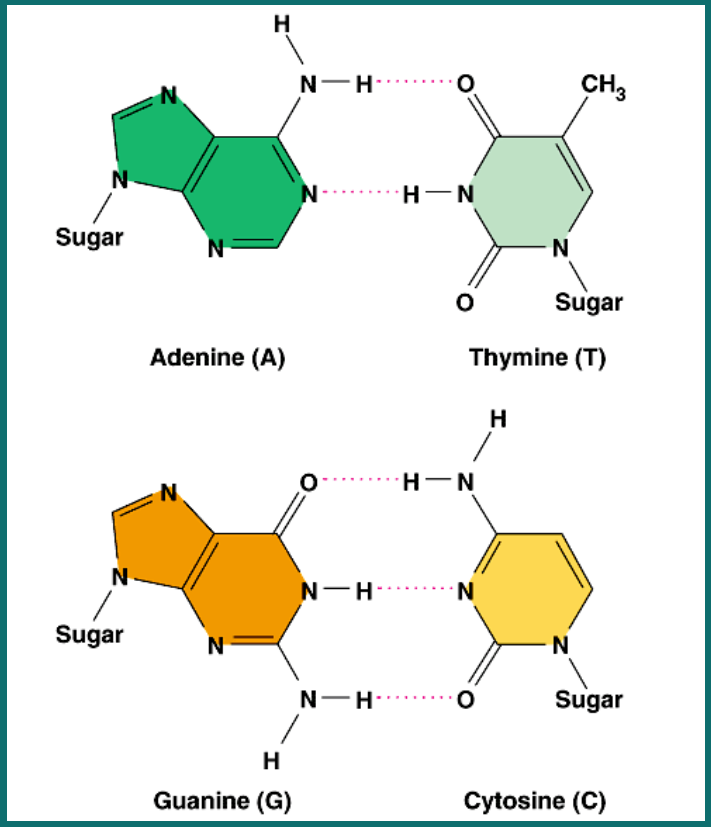
Erwin Chargaff

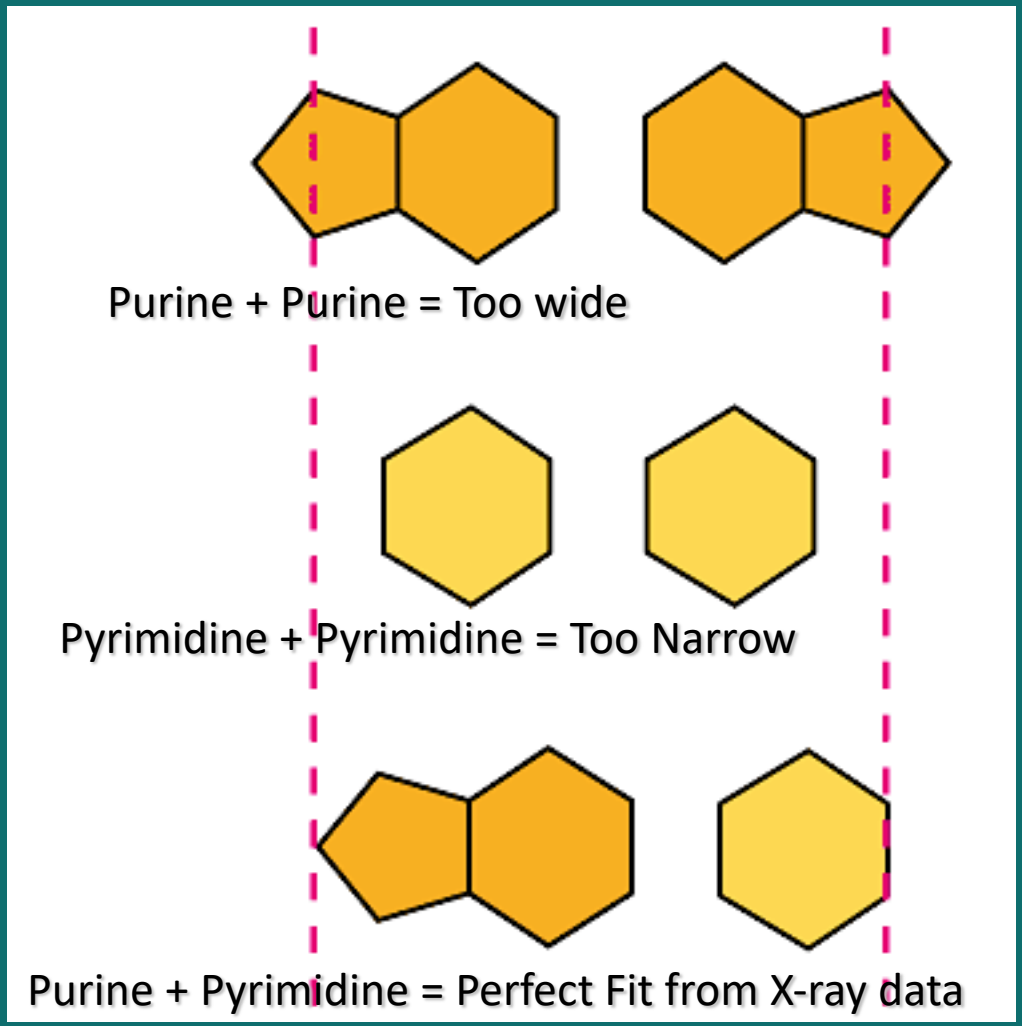
- **Relative quantities of the nitrogen bases found in DNA**
- **Chargaff's rules of DNA base pairing, 1950**

Chargaff's Rule

- **Adenine** must pair with **Thymine**
- **Guanine** must pair with **Cytosine**
- Their amounts in a given DNA molecule will be **about the same**.




H
N
H
O
CH3
N
N
N
H
N
Sugar
N
N
Sugar
O
Adenine (A)
Thymine (T)
H
O
H
N
N
N
H
N
Sugar
N
N
N
Sugar
H
H
Guanine (G)
Cytosine (C)


Purine + Purine = Too wide
Pyrimidine + Pyrimidine = Too Narrow
Purine + Pyrimidine = Perfect Fit from X-ray data

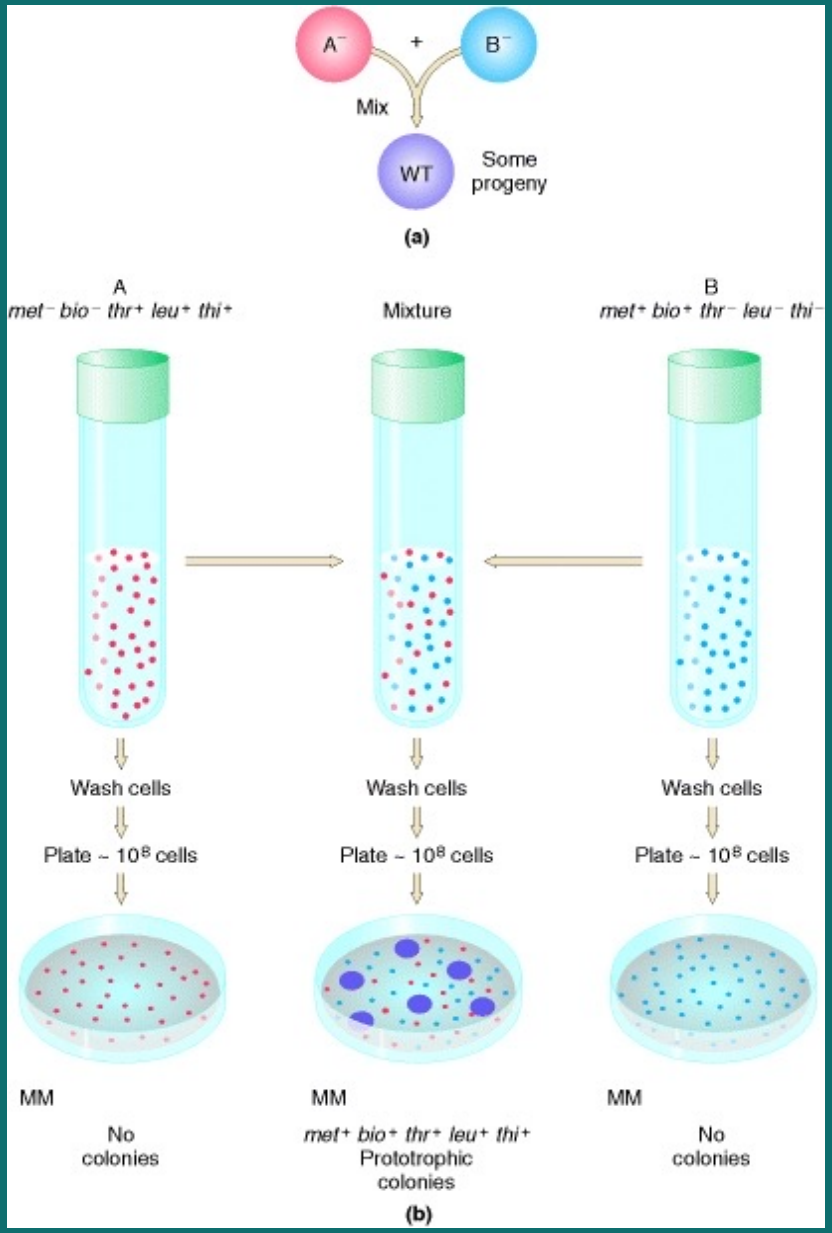
- The rules of base pairing (or nucleotide pairing) are:

A with **T**: the purine **adenine** (A) always pairs with the pyrimidine **thymine** (T)
C with **G**: the pyrimidine **cytosine** (C) always pairs with the purine **guanine** (G)

- only with A & T and with C & G are there opportunities to establish **hydrogen bonds** between them (**two between A & T**; **three between C & G**). The ability to form hydrogen bonds makes the base pairs **more stable** structurally.

- These base pair relationships are often called **Chargaff's rules of DNA base pairing**, named after the Columbia University scientists who observed that there are equal molar concentration of A & T, as well as G & C in most DNA molecules.
- The rules of base pairing tell us that if we can "read" the sequence of nucleotides on one strand of DNA, we can immediately deduce the complementary sequence on the other strand.
- The rules of base pairing explain the phenomenon that whatever the amount of adenine (A) in the DNA of an organism, the amount of thymine (T) is the same (**Chargaff's rule**). Similarly, whatever the amount of guanine (G), the amount of cytosine (C) is the same.

Lederberg and Tatum's Conjugation Experiment, 1946



Demonstration by Lederberg and Tatum of genetic recombination between bacterial cells. Cells of type A or type B cannot grow on an unsupplemented (minimal) medium (MM), because A and B each carry mutations that cause the inability to synthesize constituents needed for cell growth. When A and B are mixed for a few hours and then plated, however, a few colonies appear on the agar plate. These colonies derive from single cells in which an exchange of genetic material has occurred; they are therefore capable of synthesizing all the required constituents of metabolism.

Joshua Lederberg and Edward Tatum (1946)

- physical contact between the bacterial strains was required
  - Strains separated by a filter in a U-tube
  - Pressure and suction move liquid through filter
    - Pores allow passage of DNA, but not cells
  - No colonies grew when bacteria were plated on minimal media