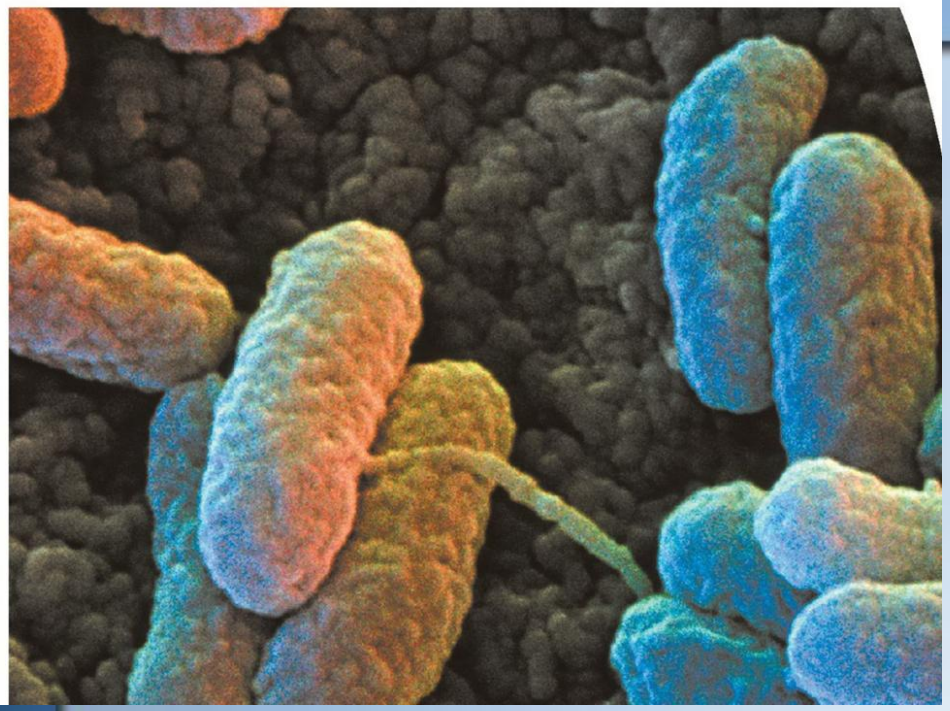
Analisi genetica e mappatura di batteri e batteriofagi



- Spostiamo ora la nostra attenzione sui batteri, e batteriofagi

Bacteria Mutate Spontaneously and Grow at an Exponential Rate. Useful for genetics studies, development of genetic engineering

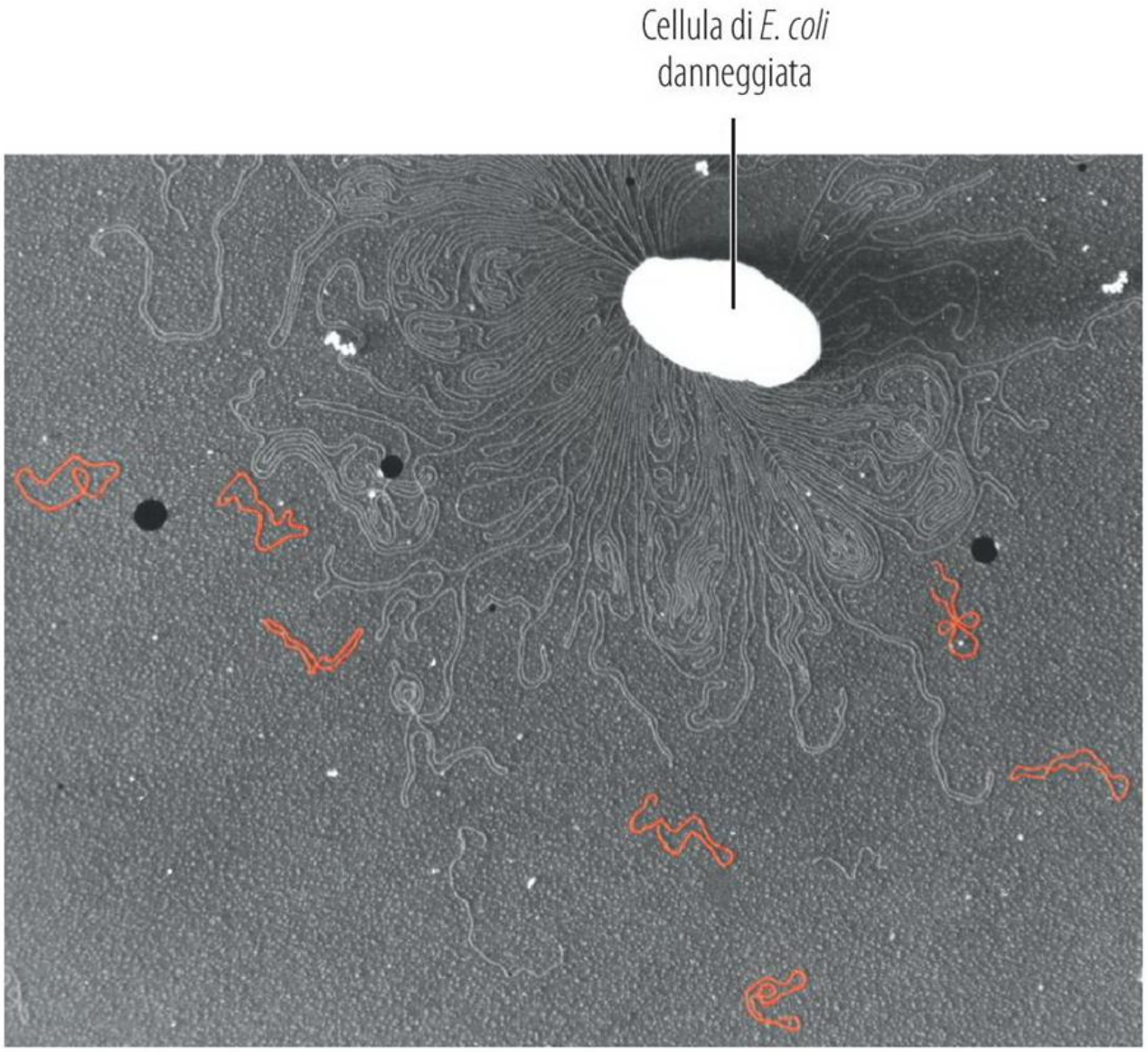
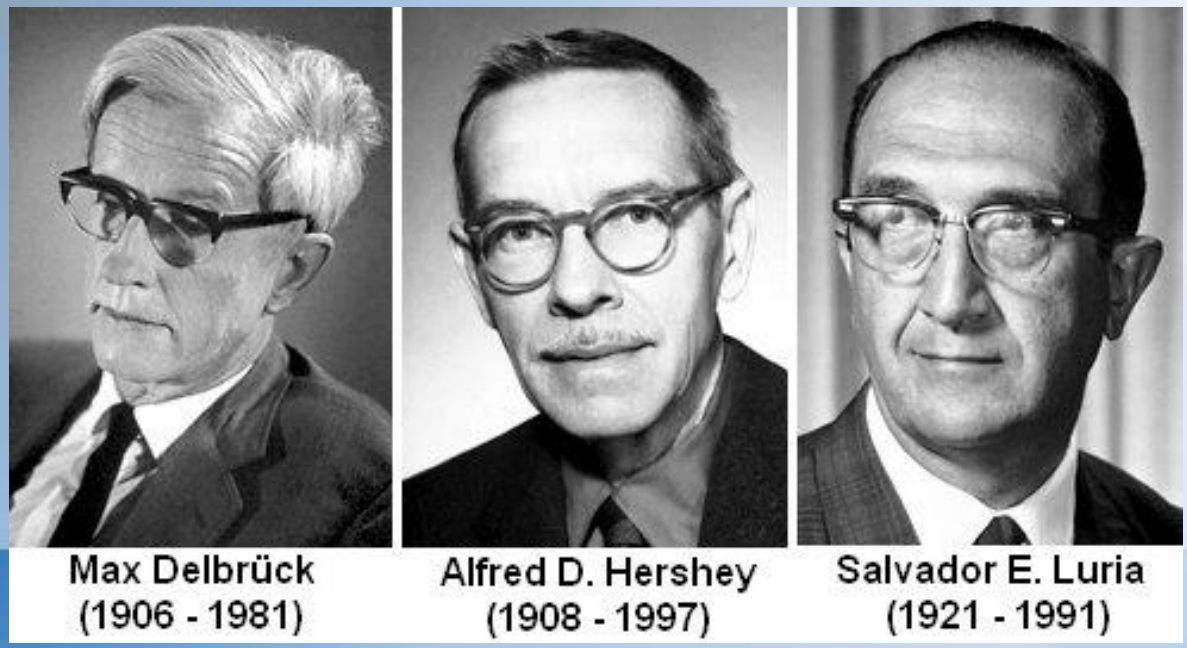


Figura 6.1 Cromosoma batterico e plasmidi. Una cellula di *E. coli* ha rilasciato il DNA cromosomico insieme a diversi plasmidi (rosso).

Teoria dell'adattamento

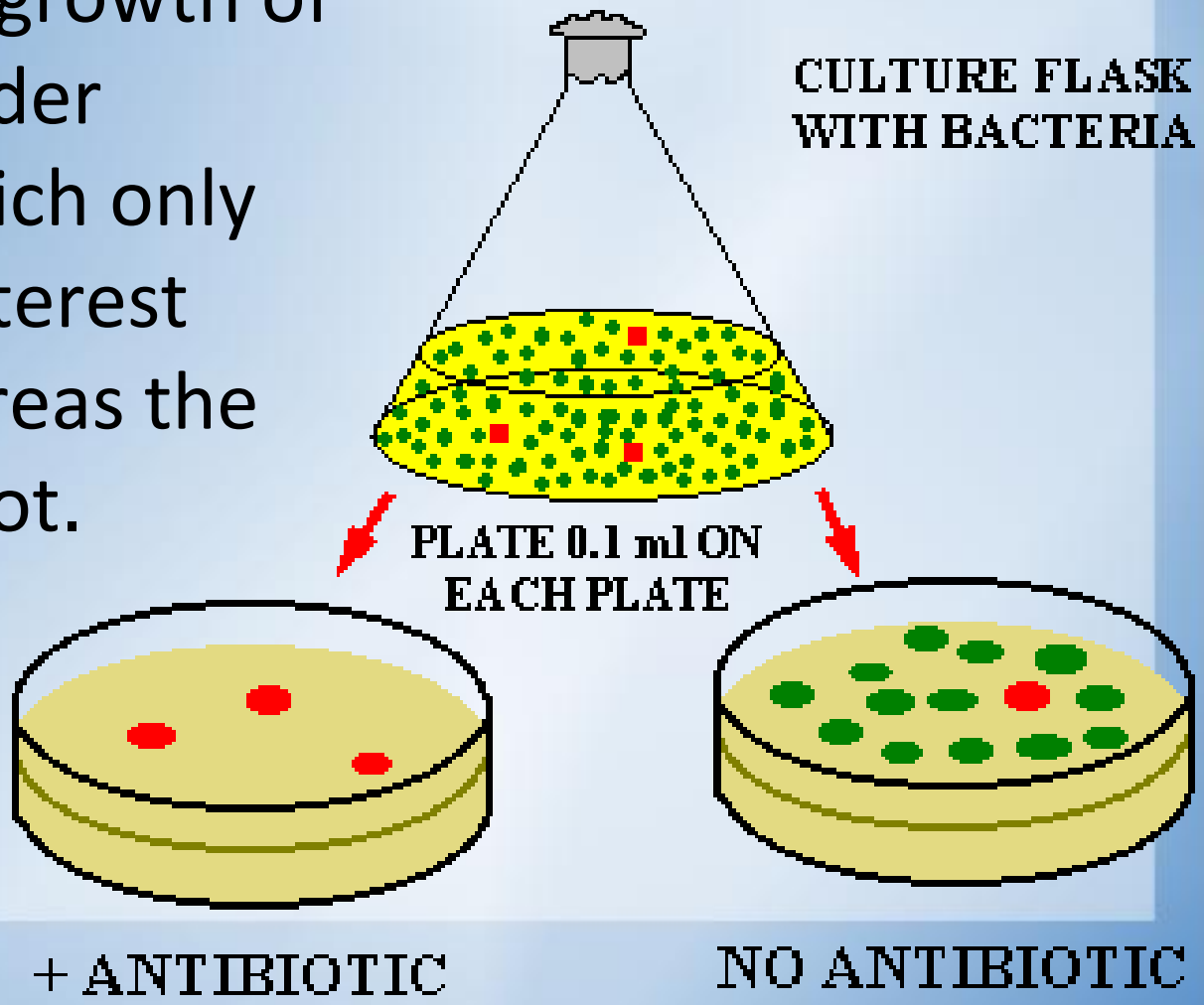
•The **adaptation hypothesis** proposes that the interaction of bacteriophage and bacterium is essential to the bacterium's acquisition of immunity to the phage. Exposure to the phage "induces" resistance in the bacteria.

•**Spontaneous mutation,** however, which occurs in the presence or absence of phage, is considered the primary source of genetic variation in bacteria.



Isolation of mutant cells

- Selection is the growth of the organism under conditions in which only the mutant of interest grows well, whereas the wild type does not.

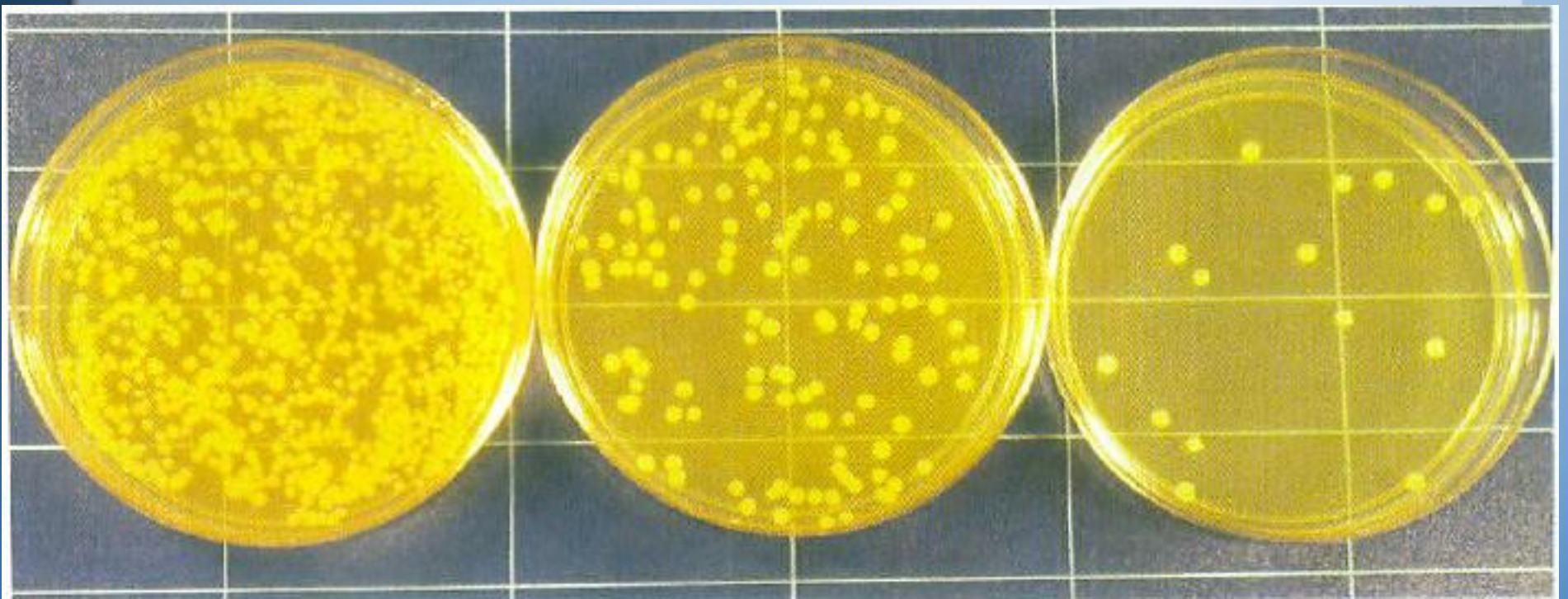


Prototrophy and auxotrophy

- A prototroph can synthesize all essential organic compounds and therefore can be grown on **minimal medium**. Through mutation, an auxotroph has lost the ability to synthesize one or more essential compounds and must be provided with them in the medium if it is to grow.

Bacterial growing

- Bacteria have three growth phases: lag phase, log phase (exponential growth), and stationary phase.



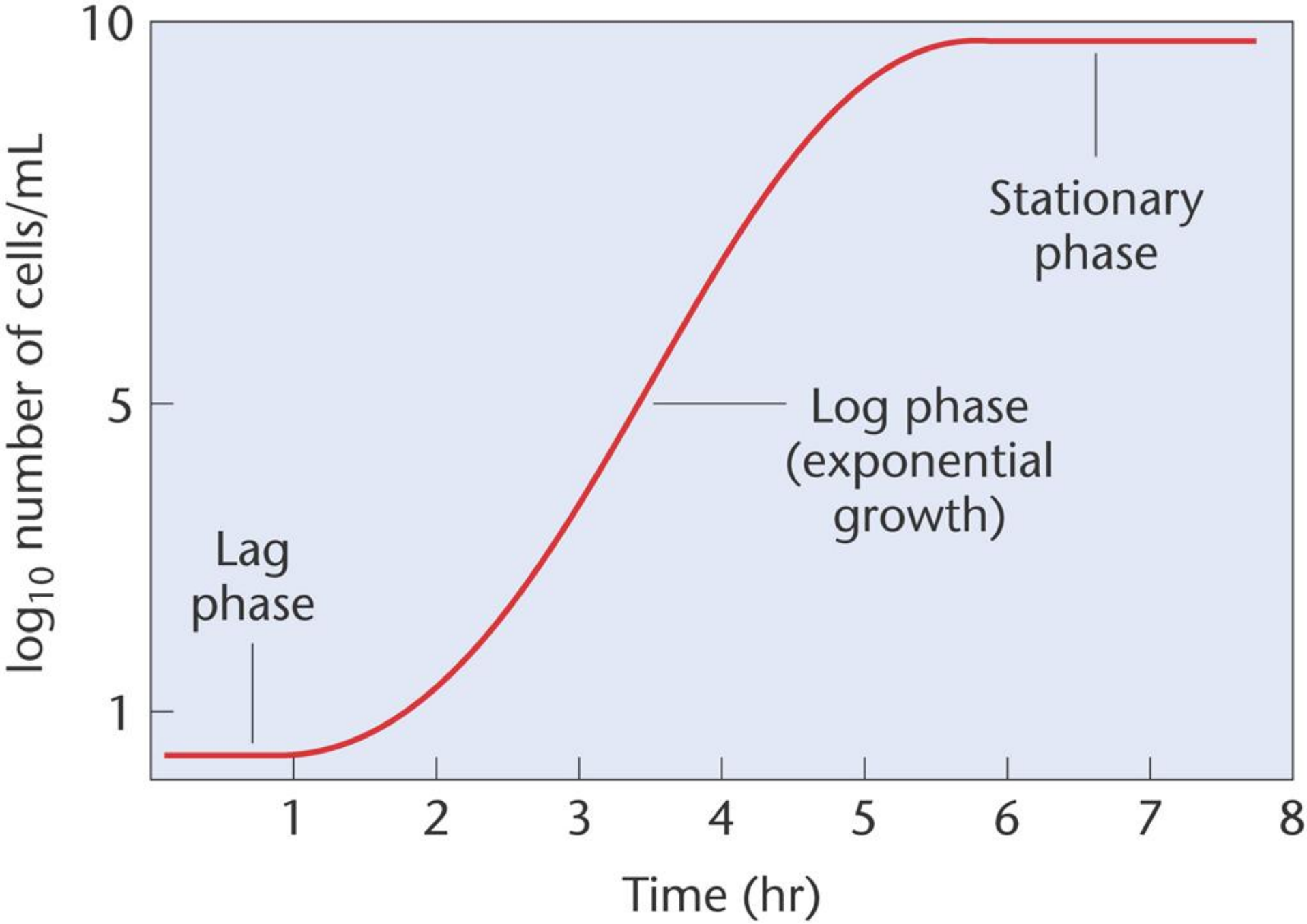
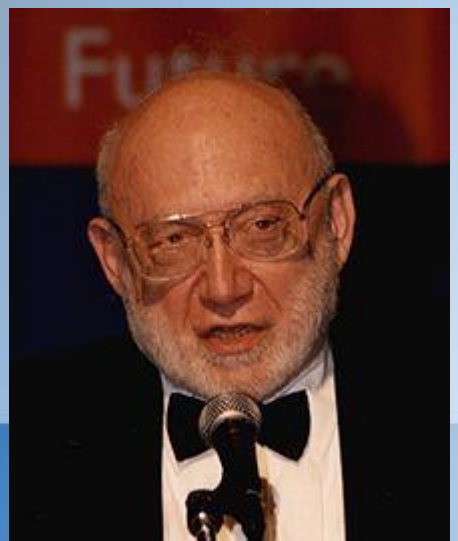
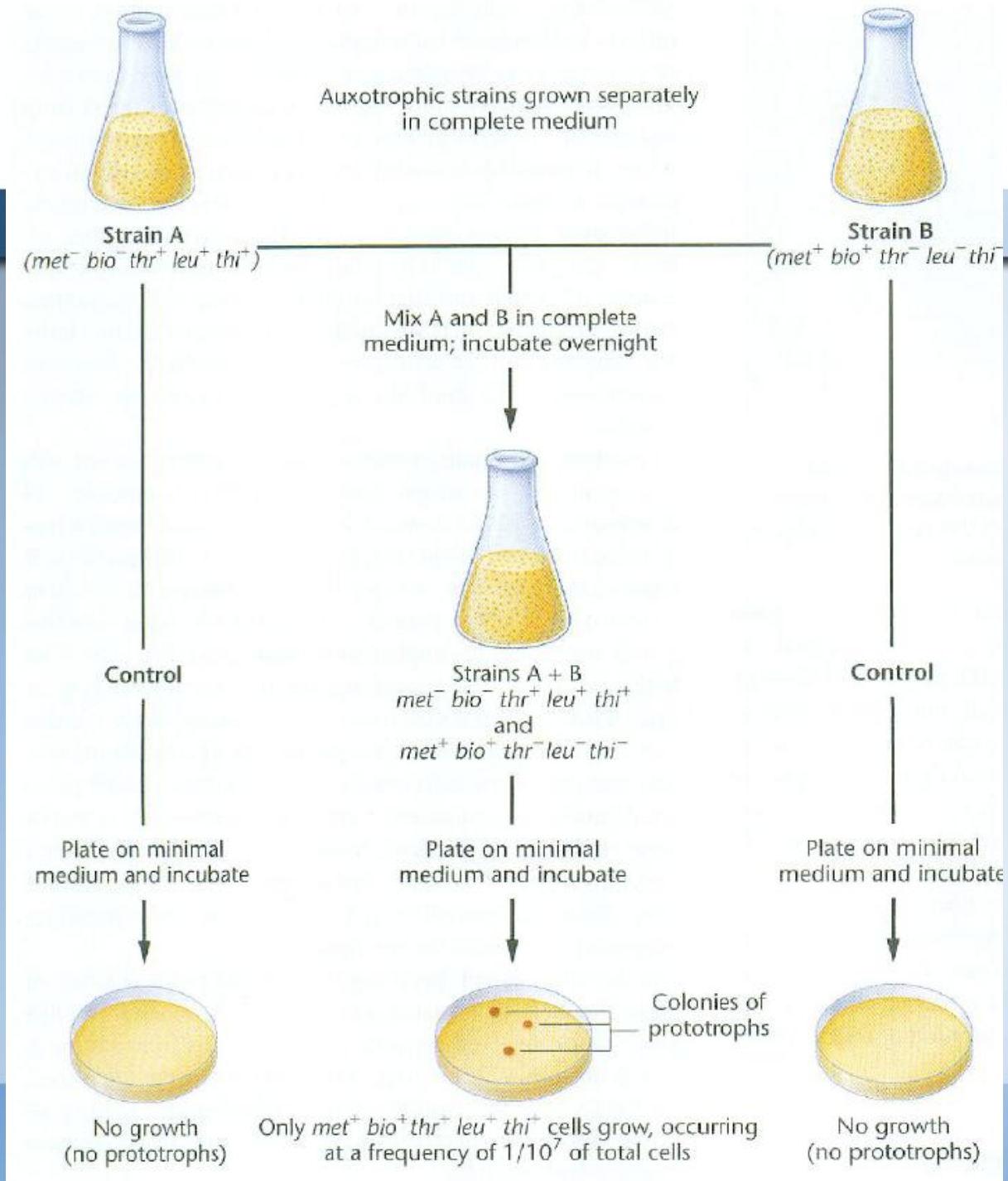


Figure 6-1

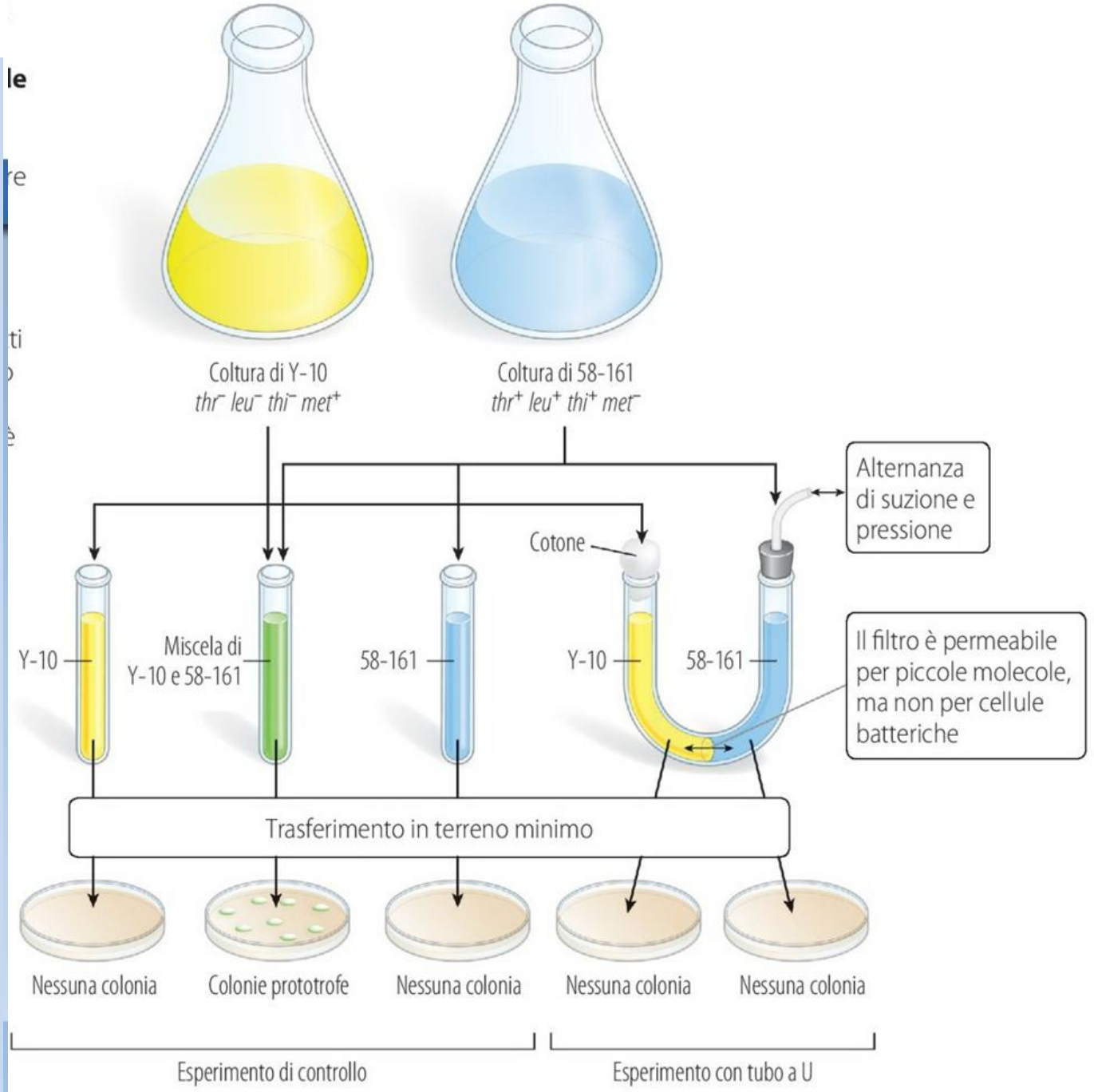
Conjugation Is One Means of Genetic Recombination in Bacteria

- Bacteria undergo conjugation, in which genetic information from one bacterium is transferred to another and recombines with the second bacterium's DNA.
- Lederberg and Tatum 1946

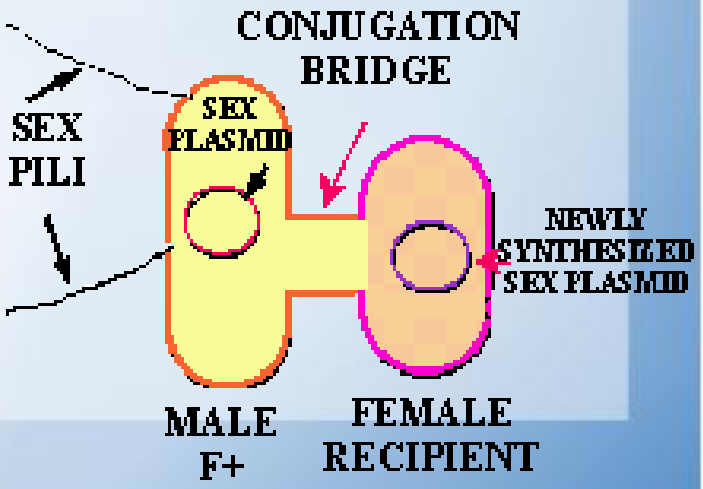


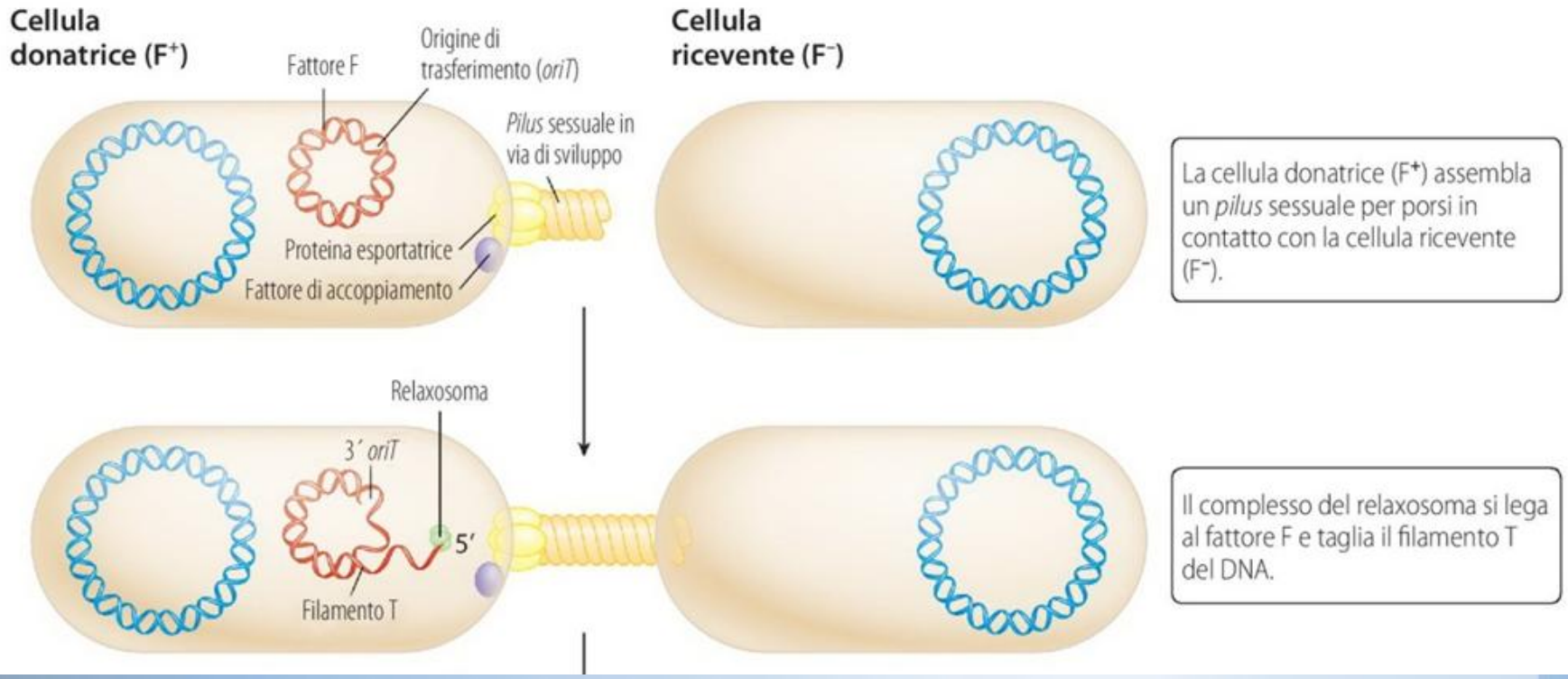

Auxotrophic strains grown separately in complete medium
Strain A
(met⁻ bio⁻ thr⁺ leu⁺ thi⁺)
Strain B
(met⁺ bio⁺ thr⁻ leu⁻ thi⁻)
Mix A and B in complete medium; incubate overnight
Control
Strains A + B
met⁻ bio⁻ thr⁺ leu⁺ thi⁺
and
met⁺ bio⁺ thr⁻ leu⁻ thi⁻
Control
Plate on minimal medium and incubate
Plate on minimal medium and incubate
Plate on minimal medium and incubate
Colonies of prototrophs
No growth (no prototrophs)
Only met⁺ bio⁺ thr⁻ leu⁻ thi⁻ cells grow, occurring at a frequency of 1/10⁷ of total cells
No growth (no prototrophs)

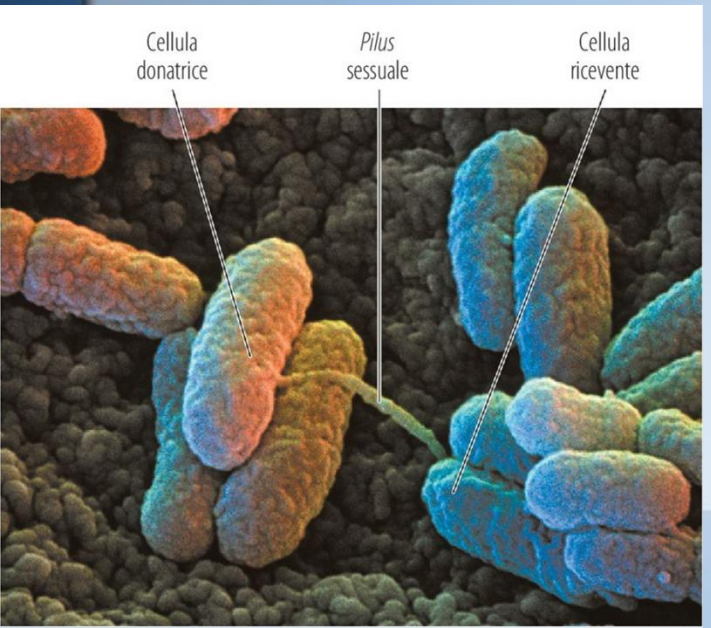
When strain A and strain B auxotrophs are grown in a common medium, but separated by a filter, no genetic recombination occurs and no prototrophs are produced. The apparatus shown is a Davis U-tube.

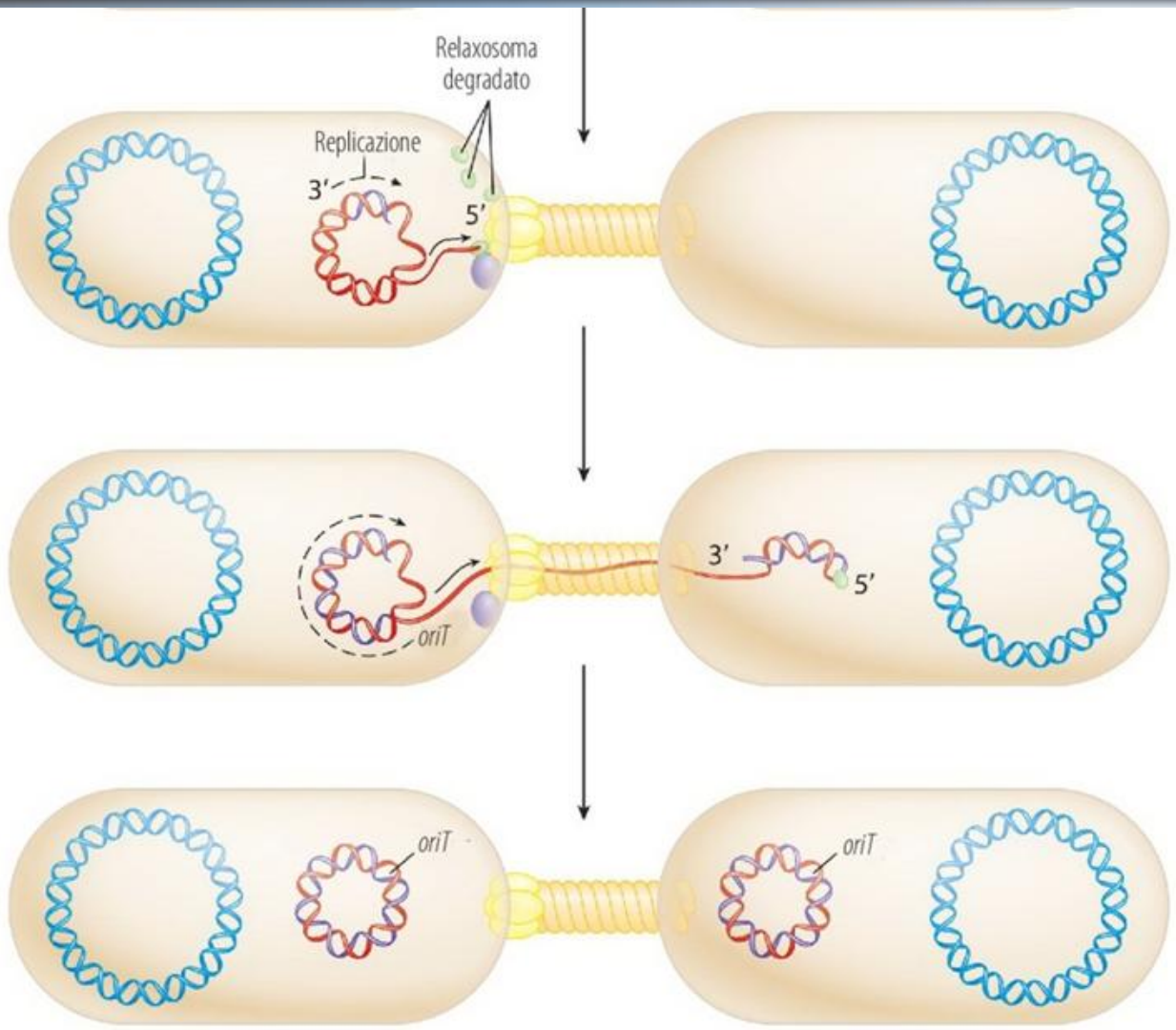


- In bacterial conjugation in *E. coli*, F^+ cells serve as DNA donors and F^- cells are the recipients. F^+ cells contain a **fertility factor** (F factor) that confers the ability to donate DNA during conjugation. Recipient cells are converted to F^+.
- Davis U-tube
- Pilus F




Cellula donatrice (F+)
Fattore F
Origine di trasferimento (oriT)
Cellula ricevente (F−)
Pilus sessuale in via di sviluppo
Proteina esportatrice
Fattore di accoppiamento
La cellula donatrice (F+) assembla un pilus sessuale per porsi in contatto con la cellula ricevente (F−).
Relaxosoma
3′ oriT
5′
Filamento T
Il complesso del relaxosoma si lega al fattore F e taglia il filamento T del DNA.


Cellula donatrice
Pilus sessuale
Cellula ricevente

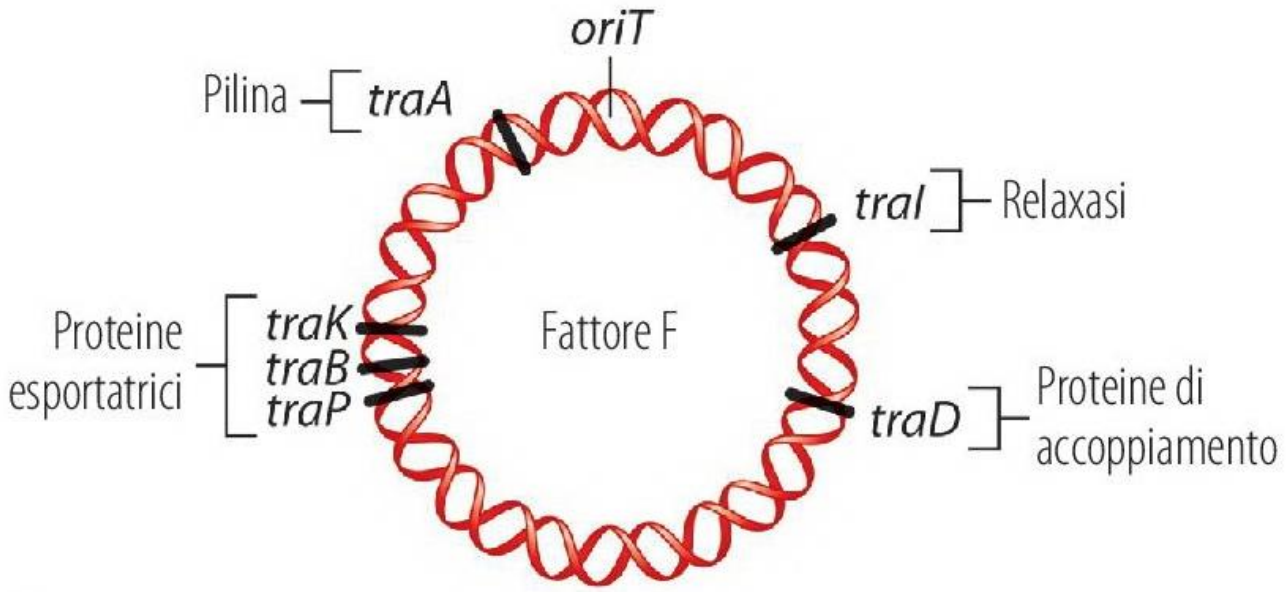


Il relaxosoma si degrada parzialmente, lasciando la relaxasi legata alle estremità 5′ del filamento T. Il complesso relaxasi-filamento T si lega a un fattore di accoppiamento per prepararsi all'esportazione. Comincia la replicazione a cerchio rotante del DNA del donatore.

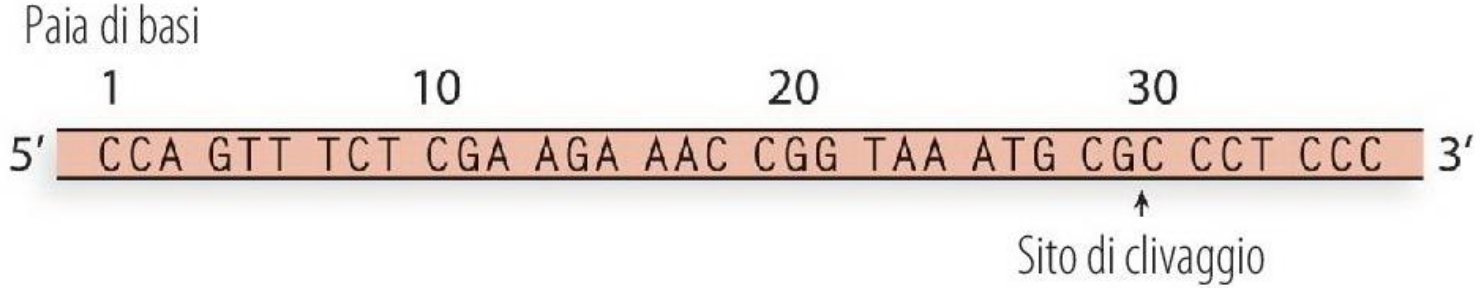
La proteina esportatrice muove il complesso relaxasi-filamento T nella cellula ricevente. La replicazione a cerchio rotante nel donatore svolge il filamento T nel ricevente, dove agisce da stampo per la replicazione del DNA

Il completamento della replicazione in entrambe le cellule lascia il donatore (F^+) immutato e converte la cellula ricevente in donatore F^+.

(**a**) Geni importanti nel trasferimento del fattore F



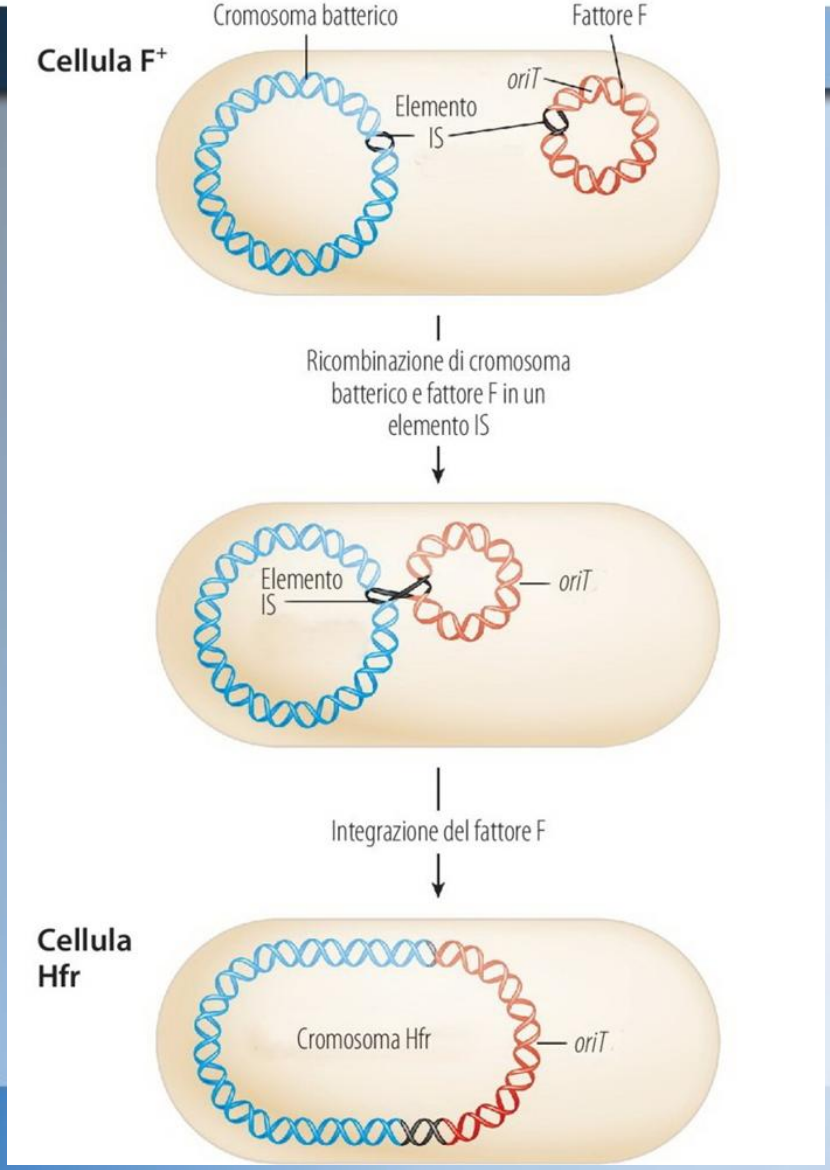
(**b**) Sequenza *oriT*

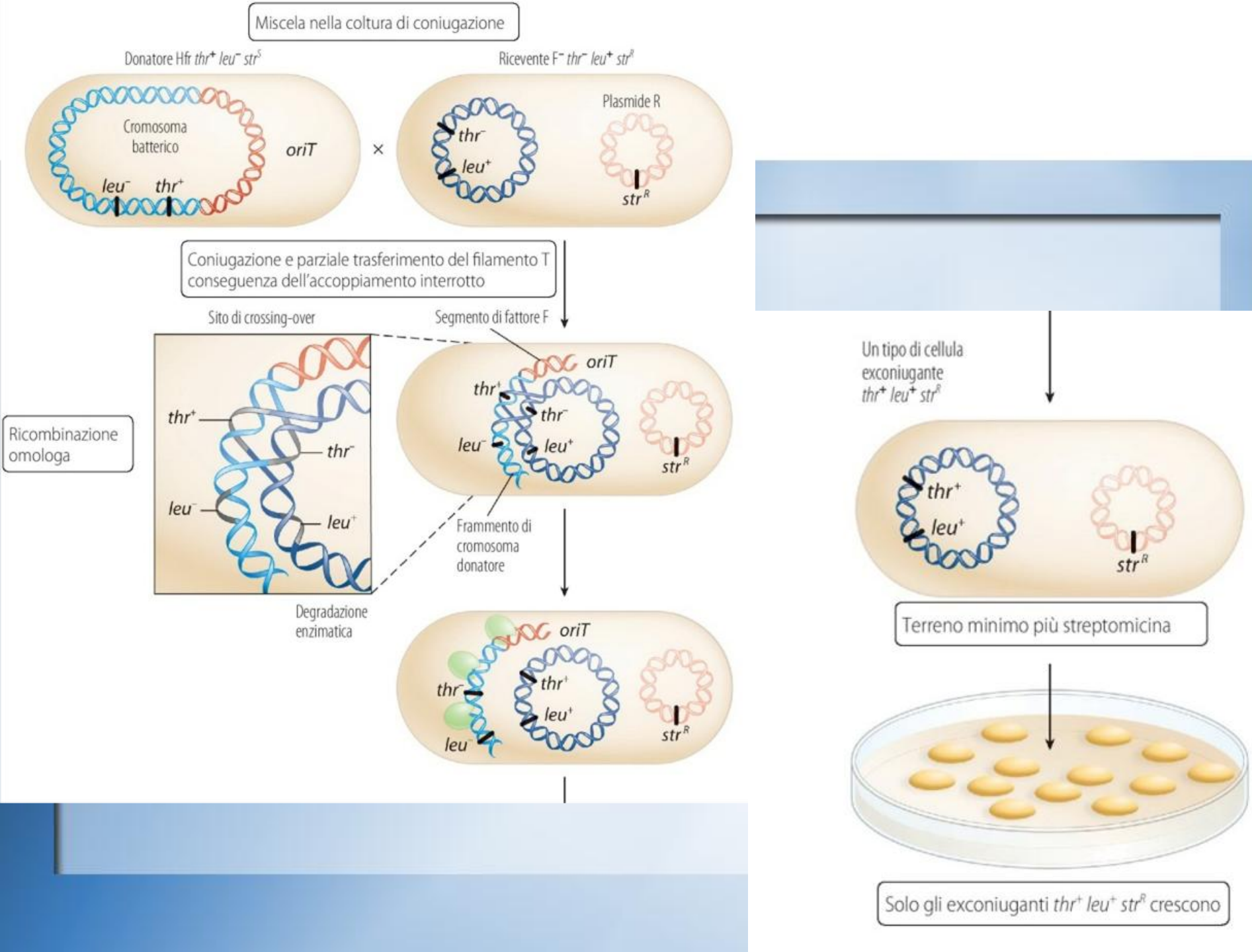


•Hfr bacteria and chromosome mapping

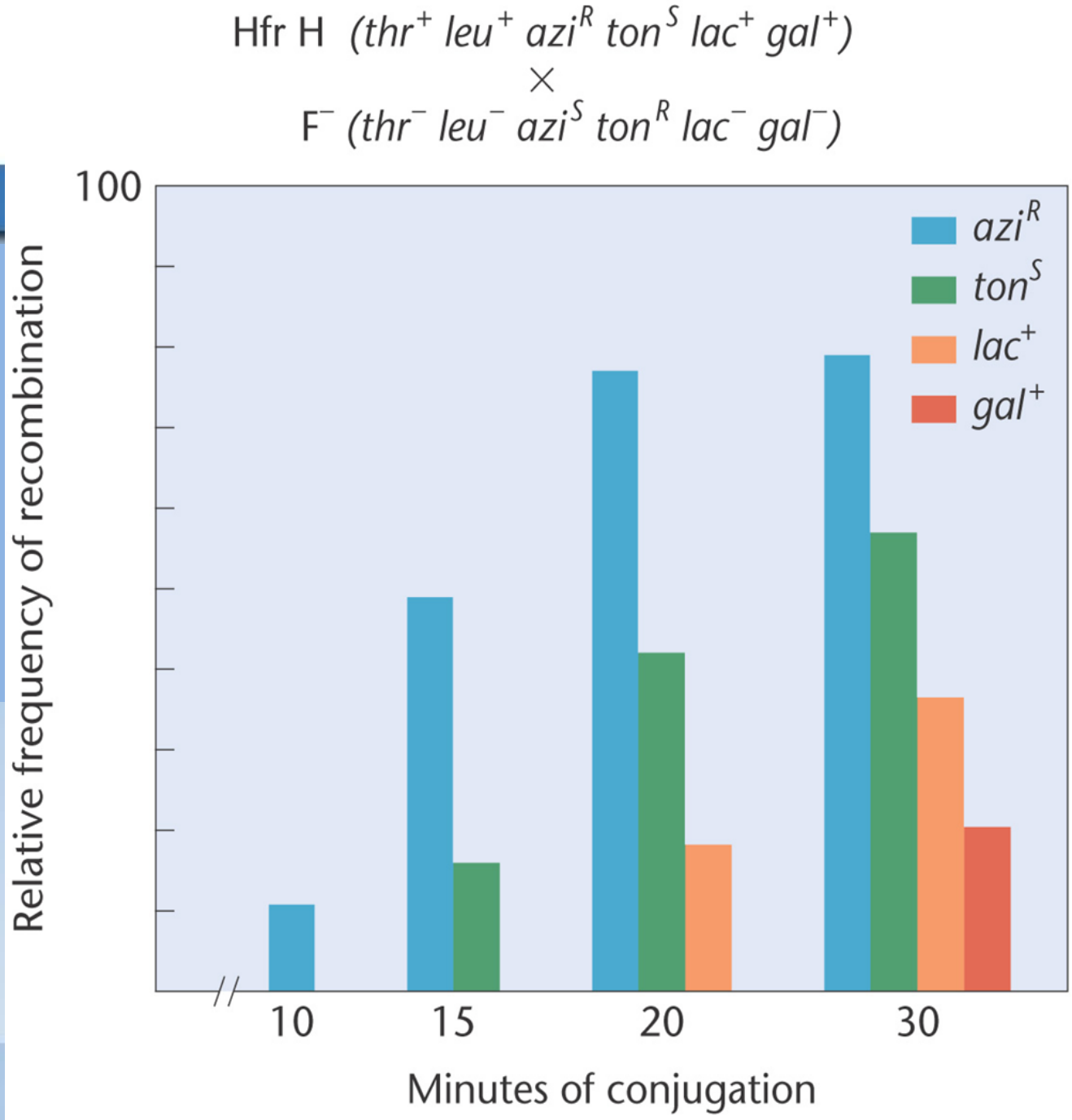
- An Hfr (high-frequency recombination) strain has the F factor integrated. An Hfr strain can donate genetic information to an F^- cell, but the recipient does not become F^+.
- Cavalli-Sforza 1950
- Wollmann and Jacob

.Ceppi hfr

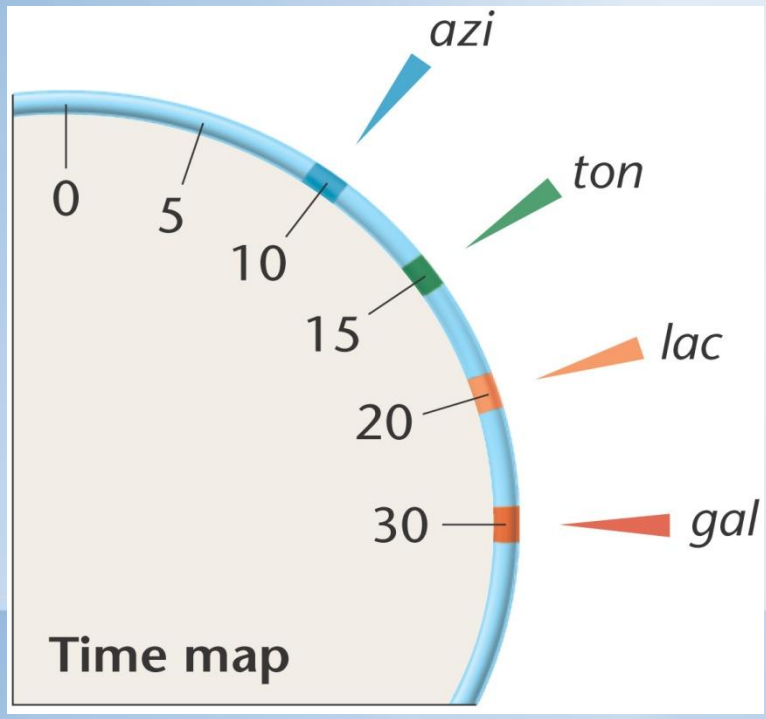

Cellula F+
Cromosoma batterico
Fattore F
oriT
Elemento
IS
Ricombinazione di cromosoma batterico e fattore F in un elemento IS
Elemento
IS
oriT
Integrazione del fattore F
Cellula
Hfr
Cromosoma Hfr
oriT


Miscela nella coltura di coniugazione
Donatore Hfr thr+ leu− strS
Ricevente F− thr− leu+ strR
Cromosoma batterico
oriT
leu−
thr+
×
thr−
leu+
Plasmide R
strR
Coniugazione e parziale trasferimento del filamento T conseguenza dell'accoppiamento interrotto
Sito di crossing-over
Segmento di fattore F
Ricombinazione omologa
thr+
thr−
leu−
leu+
oriT
strR
Frammento di cromosoma donatore
Degradazione enzimatica
Un tipo di cellula exconiugante thr+ leu+ strR
Terreno minimo più streptomicina
Solo gli exconiuganti thr+ leu+ strR crescono

- Interrupted matings demonstrated that specific genes in an Hfr strain are transferred and recombined sooner than others.



•The chromosome of an Hfr strain is transferred linearly, and the gene order and distance between genes can be predicted.

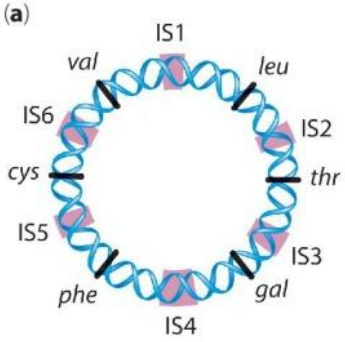


•Gene transfer by Hfr strains led to the understanding that **the *E. coli* chromosome is circular.** F^+ cells contain a fertility factor (F factor) that confers the ability to donate DNA during conjugation. Recipient cells are converted to F^+.

(a)

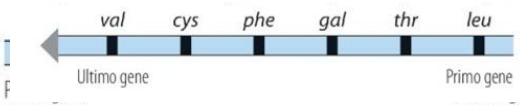
Hfr strain	Order of transfer (earliest → latest)													
H	*thr*	–	*leu*	–	*azi*	–	*ton*	–	*pro*	–	*lac*	–	*gal*	–	*thi*
1	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*	–	*pro*	–	*ton*	–	*azi*
2	*pro*	–	*ton*	–	*azi*	–	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*
7	*ton*	–	*azi*	–	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*	–	*pro*

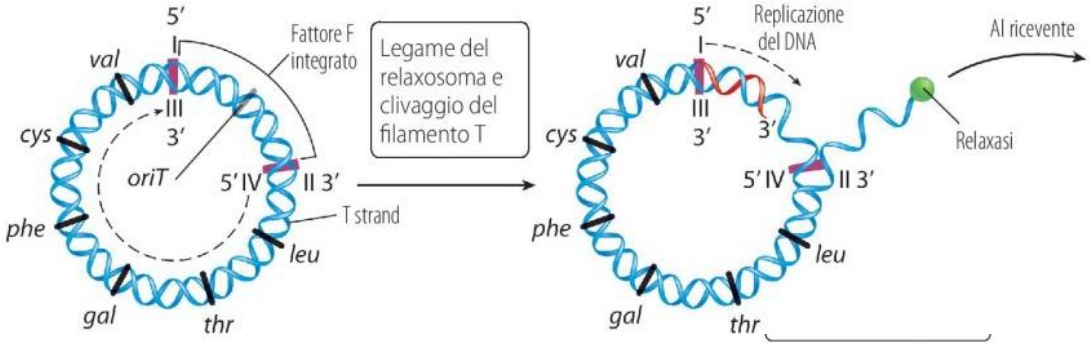
(**a**)



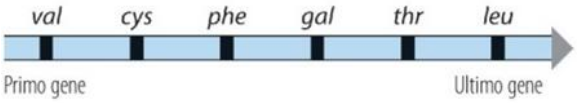
Integrazione dell'episoma presso l'elemento IS	Primo gene a trasferirsi
IS1	*leu* o *val*
IS2	*thr* o *leu*
IS3	*gal* o *thr*
IS4	*phe* o *gal*
IS5	*cys* o *phe*
IS6	*val* o *cys*

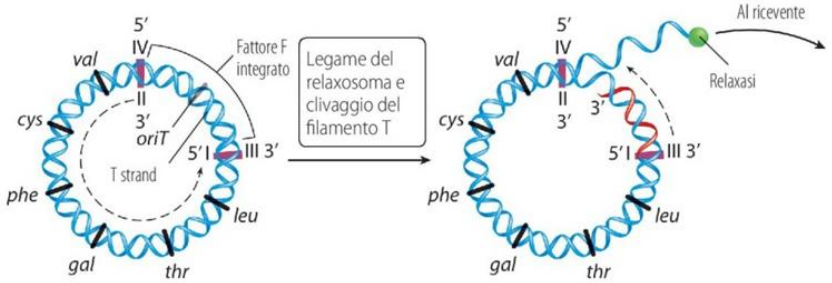
(**b**) Orientamento 1





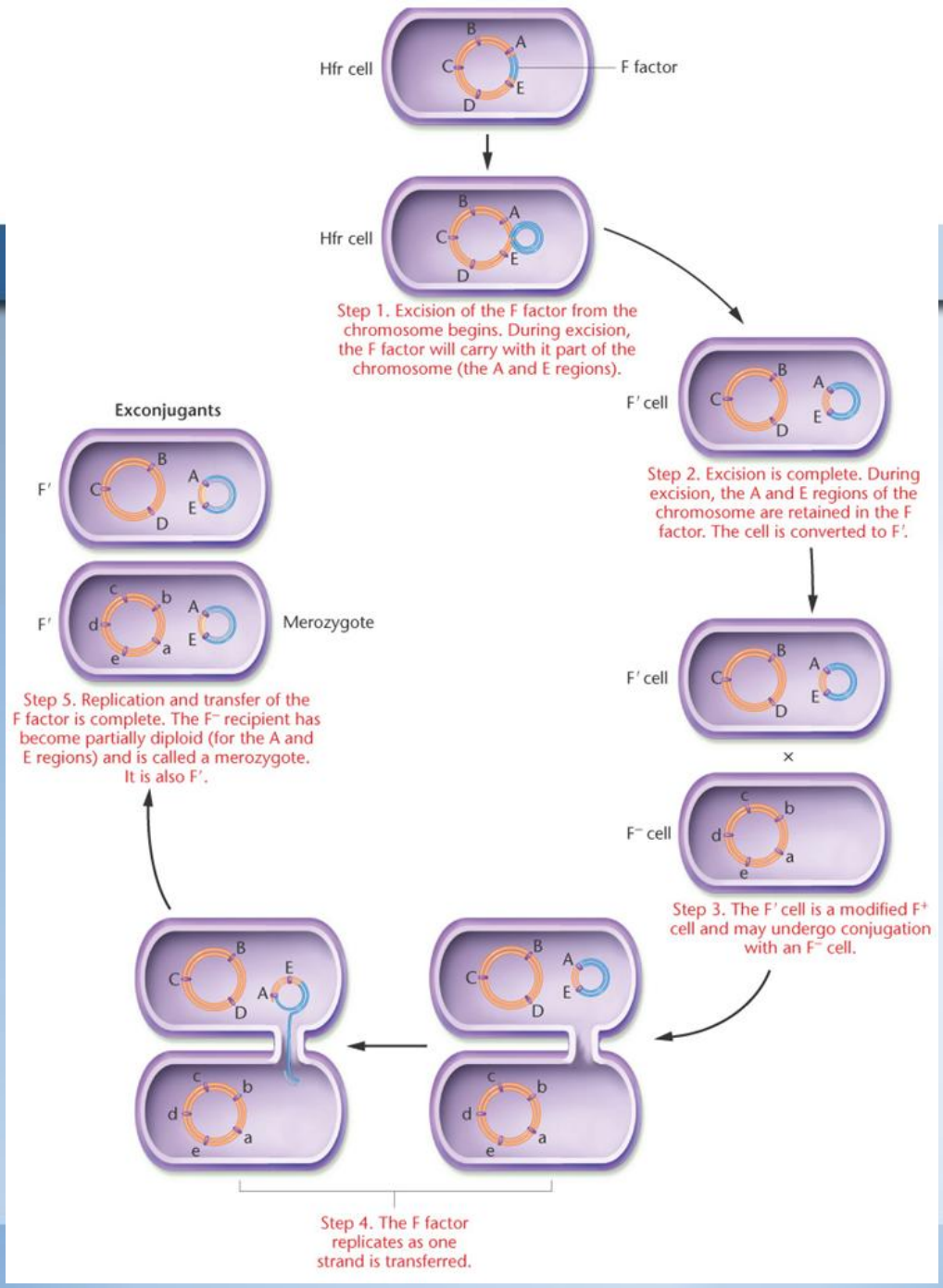
Nell'orientamento 1, *oriT* e il filamento T hanno estremità identificate con I e II





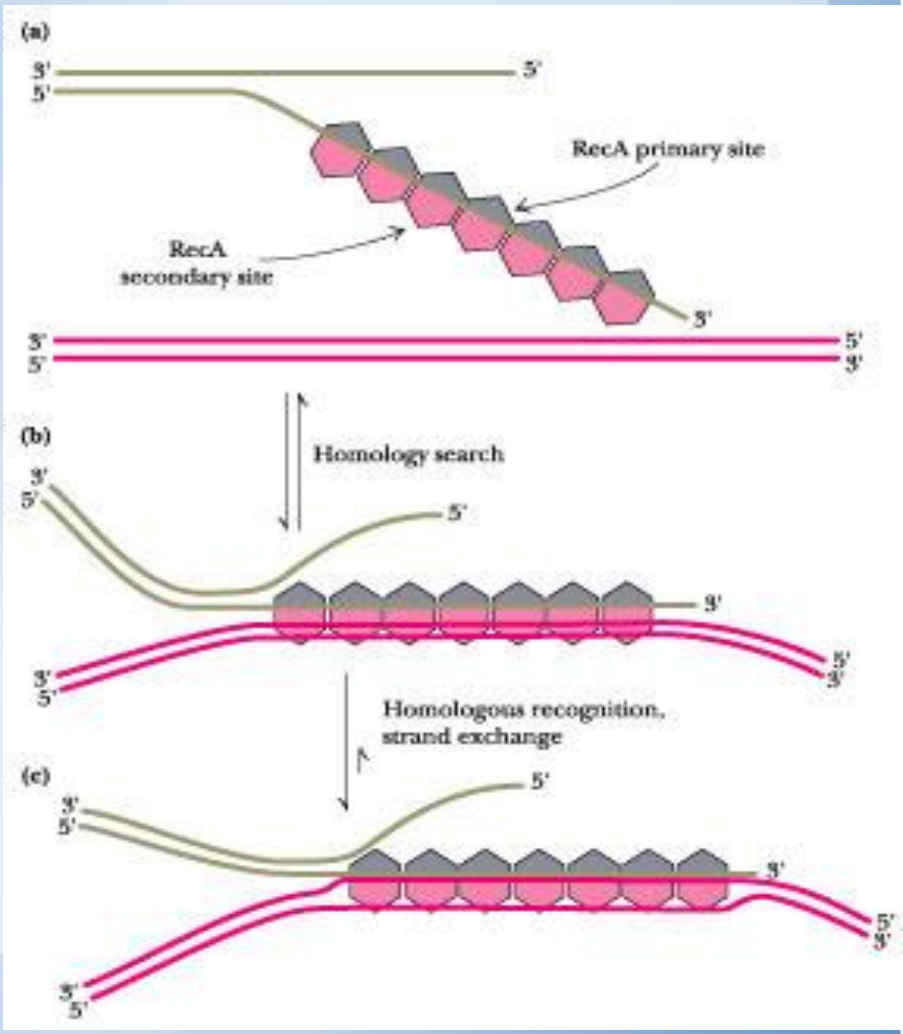
Nell'orientamento 2, *oriT* e il filamento T hanno estremità identificate con III e IV

•In some cases, an F factor is excised from the chromosome of an Hfr strain. In the process, the F factor (referred to as F’) often brings several adjoining genes with it. Transfer of an F’ to an F^- cell results in a partially diploid cell called a **merozygote**.


Hfr cell
F factor
Step 1. Excision of the F factor from the chromosome begins. During excision, the F factor will carry with it part of the chromosome (the A and E regions).
F′ cell
Step 2. Excision is complete. During excision, the A and E regions of the chromosome are retained in the F factor. The cell is converted to F′.
F′ cell
×
F⁻ cell
Step 3. The F′ cell is a modified F⁺ cell and may undergo conjugation with an F⁻ cell.
Step 4. The F factor replicates as one strand is transferred.
Exconjugants
F′
F′
Merozygote
Step 5. Replication and transfer of the F factor is complete. The F⁻ recipient has become partially diploid (for the A and E regions) and is called a merozygote. It is also F′.

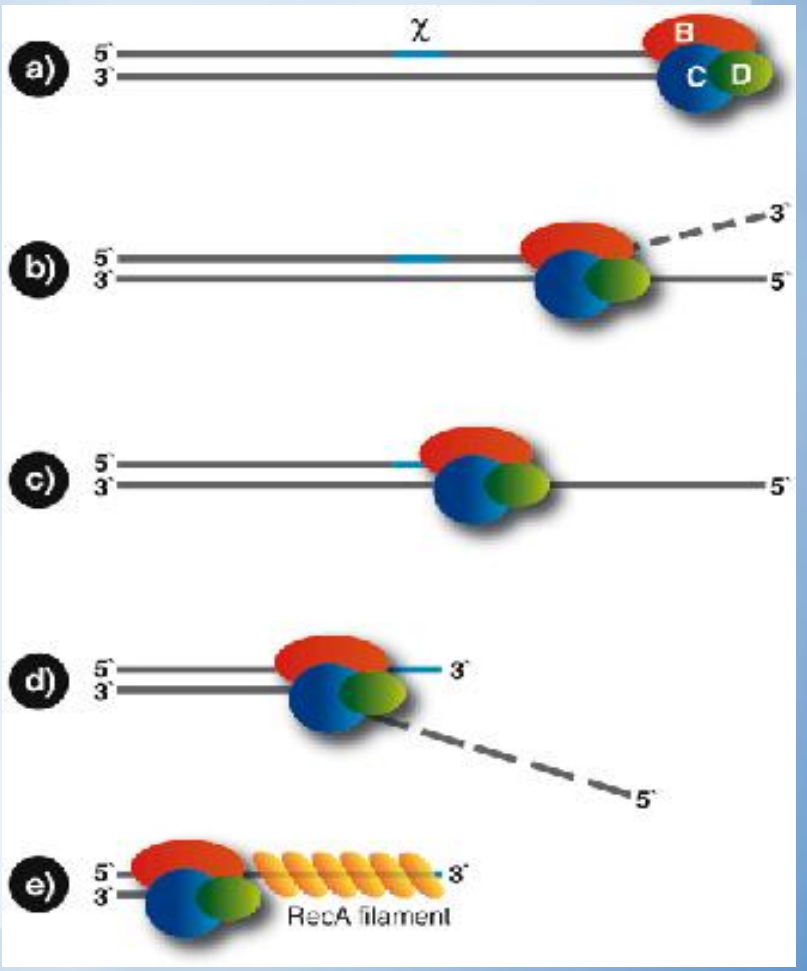
Mutational Analysis Led to the Discovery of the Rec Proteins Essential to Bacterial Recombination

•The RecA protein plays an important role in recombination involving single-strand displacement.



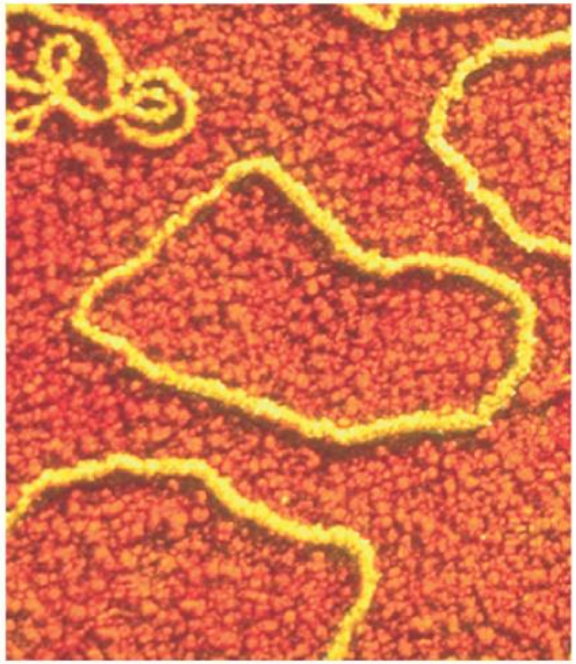
RecA is a 38 kilodalton Escherichia coli protein essential for the repair and maintenance of DNA. A RecA structural and functional homolog has been found in every species in which one has been seriously sought and serves as an archetype for this class of homologous DNA repair proteins. The homologous protein in Homo sapiens is called RAD51.

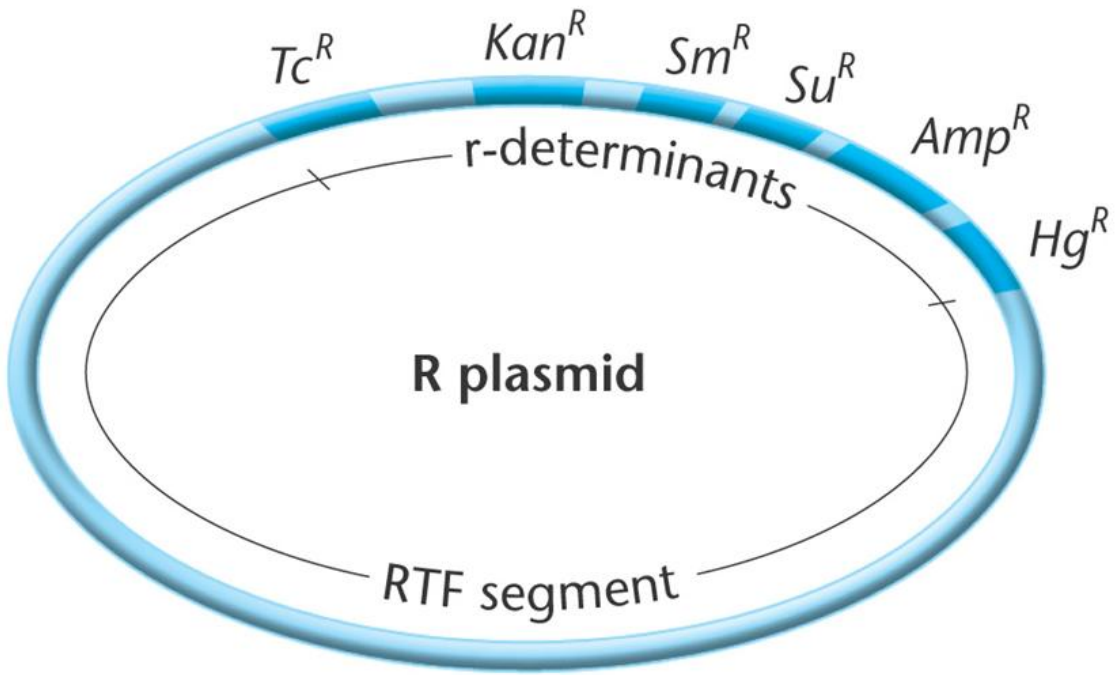
•The RecBCD protein is important for unwinding a double-stranded DNA molecule that serves as the source for genetic recombination. RecA then facilitates recombination.



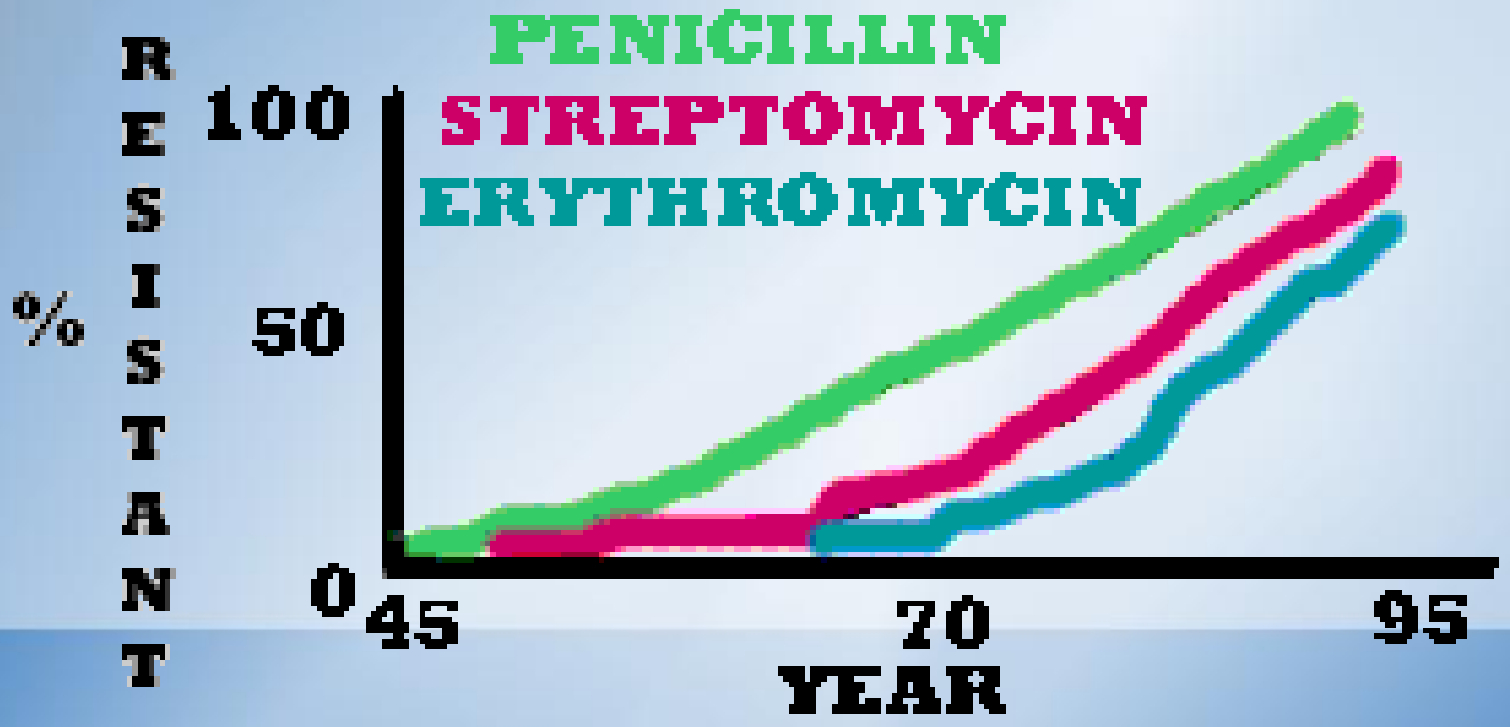
Plasmids

- Plasmids contain one or more genes and replicate independently of the bacterial chromosome.



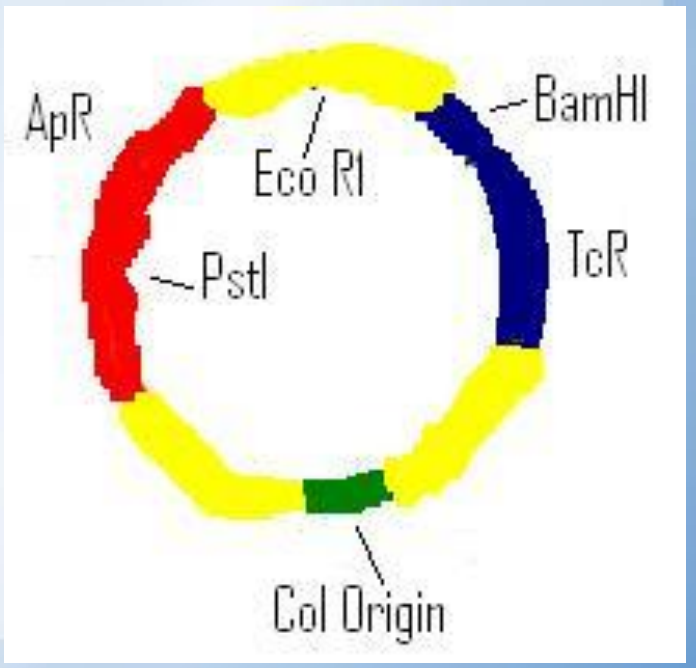


The conjugative transfer of antibiotic resistant plasmids between bacteria is a major problem facing the medical profession today



•Some examples

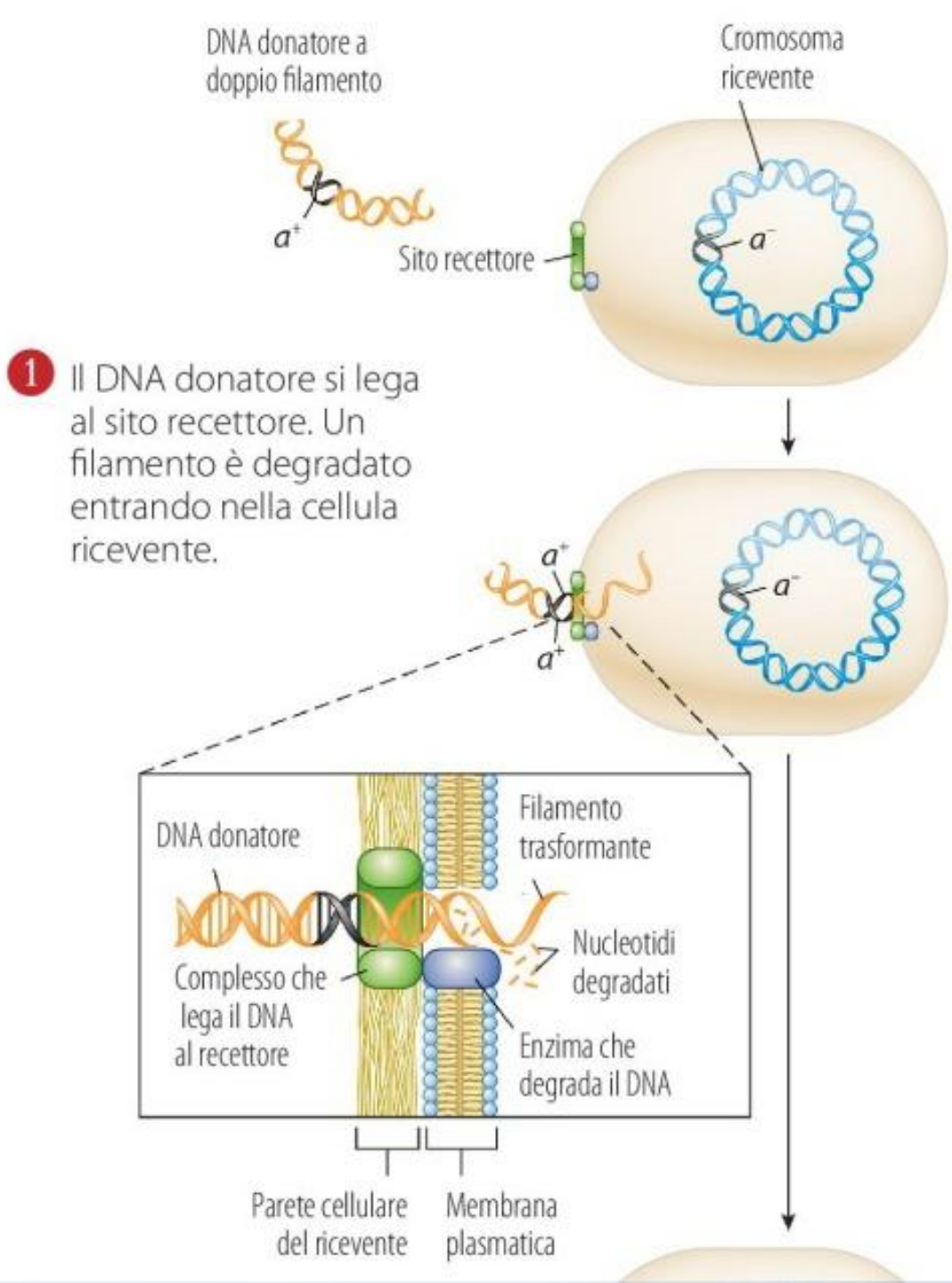
•F factors confer fertility, R plasmids confer antibiotic resistance, and Col plasmids encode colicins that can kill neighboring bacteria.

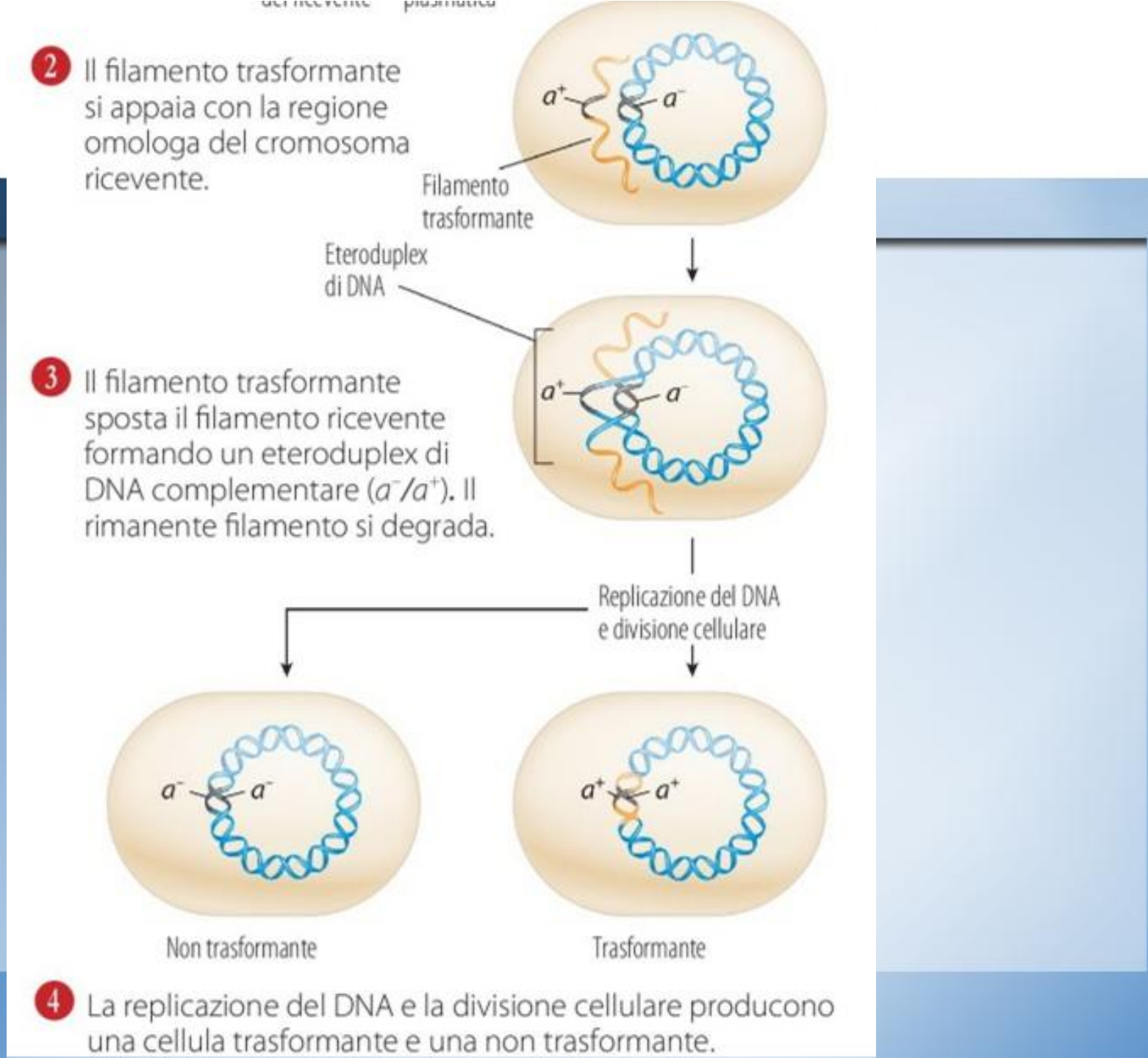


Transformation Is Another Process Leading to Genetic Recombination in Bacteria

- In transformation, small pieces of extracellular DNA are taken up by a living bacterial cell and integrated stably into the chromosome.

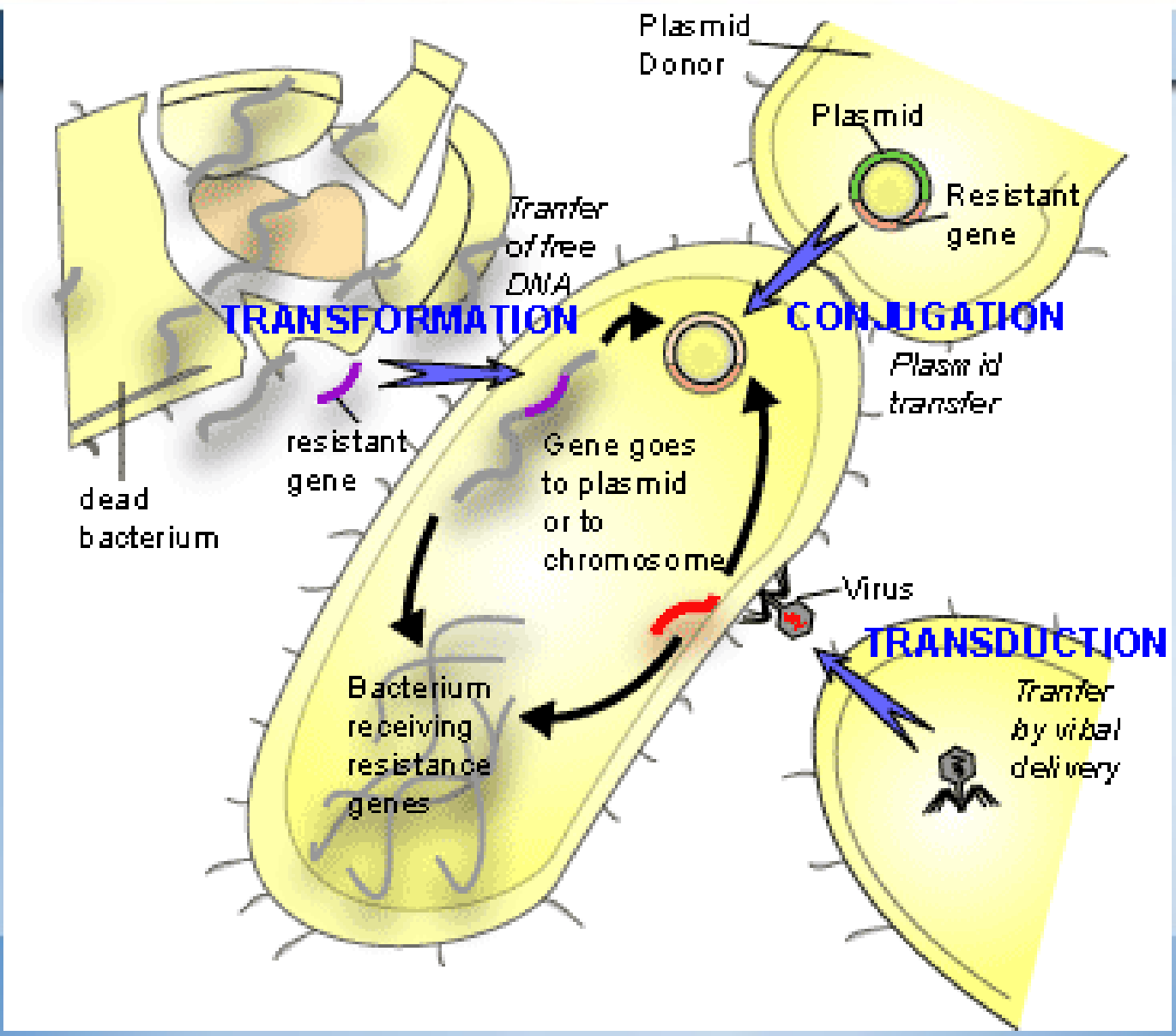
- Griffith
- Avery, McLeod, McCarthy

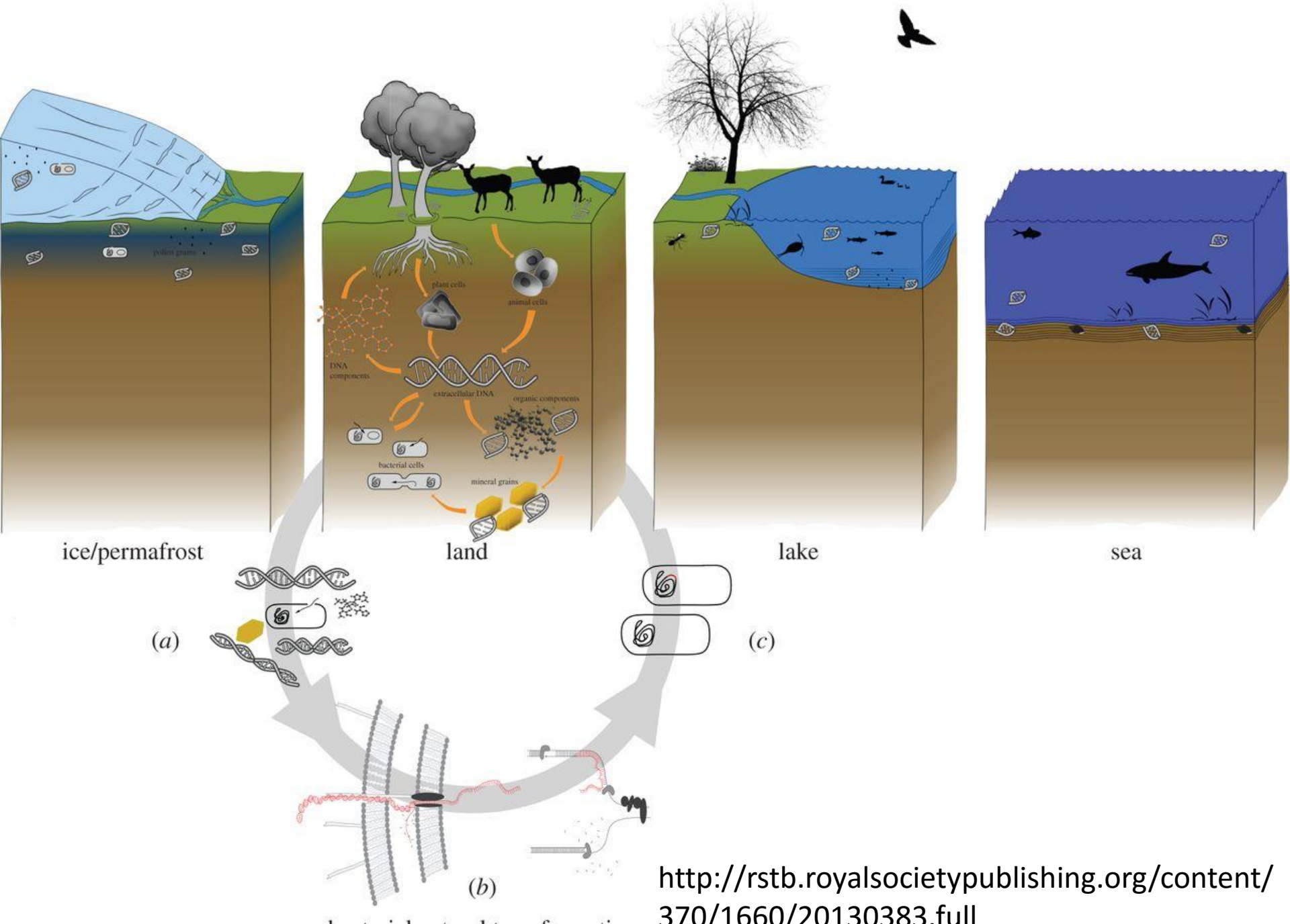



2 Il filamento trasformante si appaia con la regione omologa del cromosoma ricevente.
Filamento trasformante
Eteroduplex di DNA
3 Il filamento trasformante sposta il filamento ricevente formando un eteroduplex di DNA complementare (a^-/a^+). Il rimanente filamento si degrada.
Replicazione del DNA e divisione cellulare
Non trasformante
Trasformante
4 La replicazione del DNA e la divisione cellulare producono una cellula trasformante e una non trasformante.

•Once it is integrated into the chromosome, the recombinant region contains one host strand (present originally) and one mutant strand. Because these strands are from different sources, this region is referred to as a heteroduplex, and the two strands of DNA are not perfectly complementary in this region.

RIASSUNTO

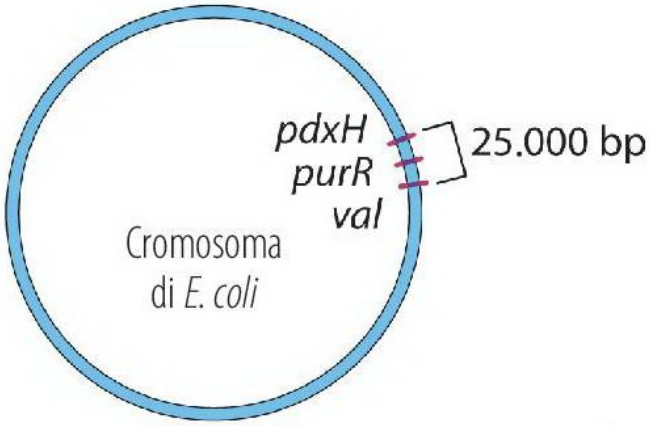

Plasmid
Donor
Plasmid
Resistant
gene
Tranfer
of free
DNA
TRANSFORMATION
CONJUGATION
Plasmid
transfer
resistant
gene
Gene goes
to plasmid
or to
chromosome
dead
bacterium
Virus
TRANSDUCTION
Tranfer
by viral
delivery
Bacterium
receiving
resistance
genes

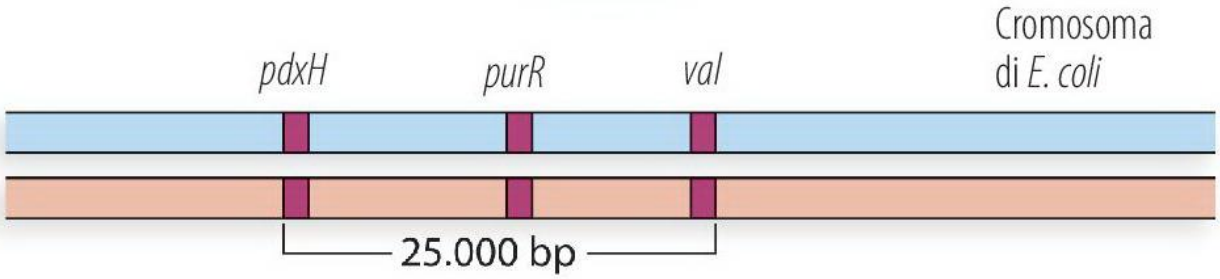


http://rstb.royalsocietypublishing.org/content/370/1660/20130383.full

MAPPATURA PER CO-COTRASFORMAZIONE

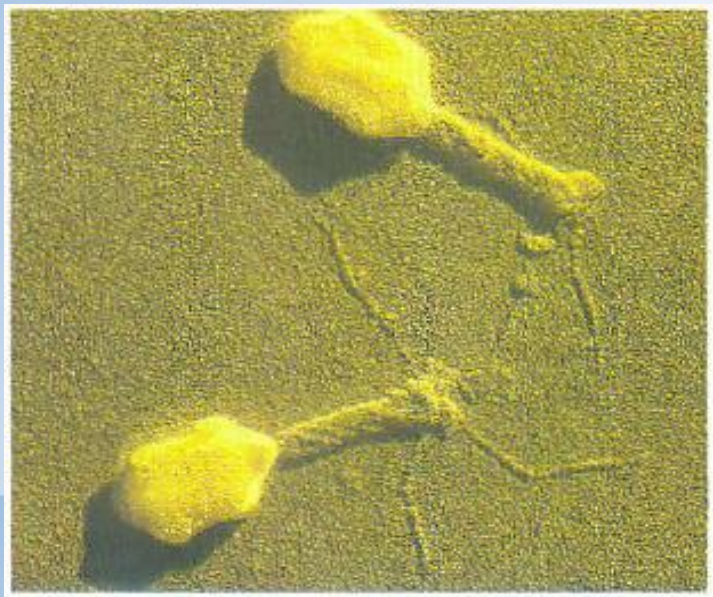
Esperimento	Geni co-trasformanti	Frequenza di co-trasformazione
1	*pdxH* e *val*	0,08
2	*purR* e *val*	0,81
3	*purR* e *pdxH*	0,68

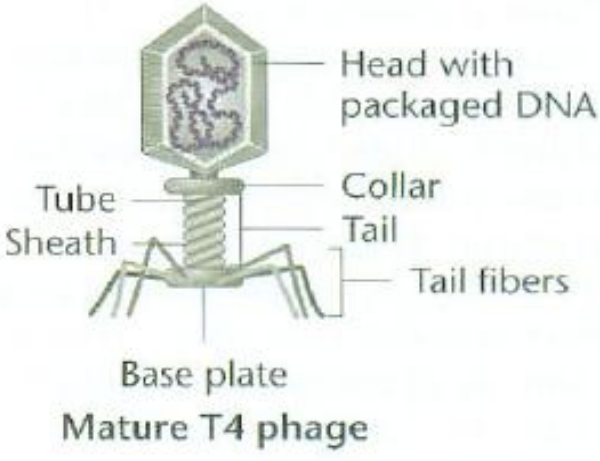




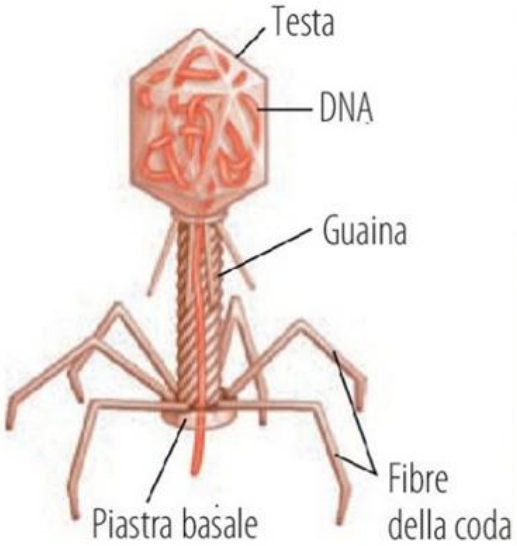
Bacteriophages Are Bacterial Viruses

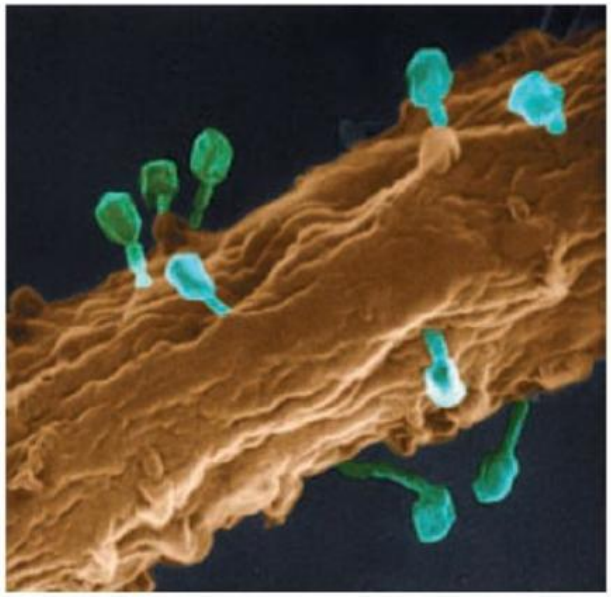
- Bacteriophages can infect a host bacterium and inject their DNA into its chromosome. The infected bacterium then produces more phage particles, which are released when the host cell is lysed.



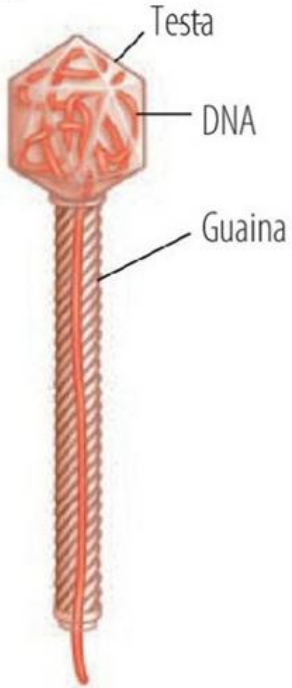


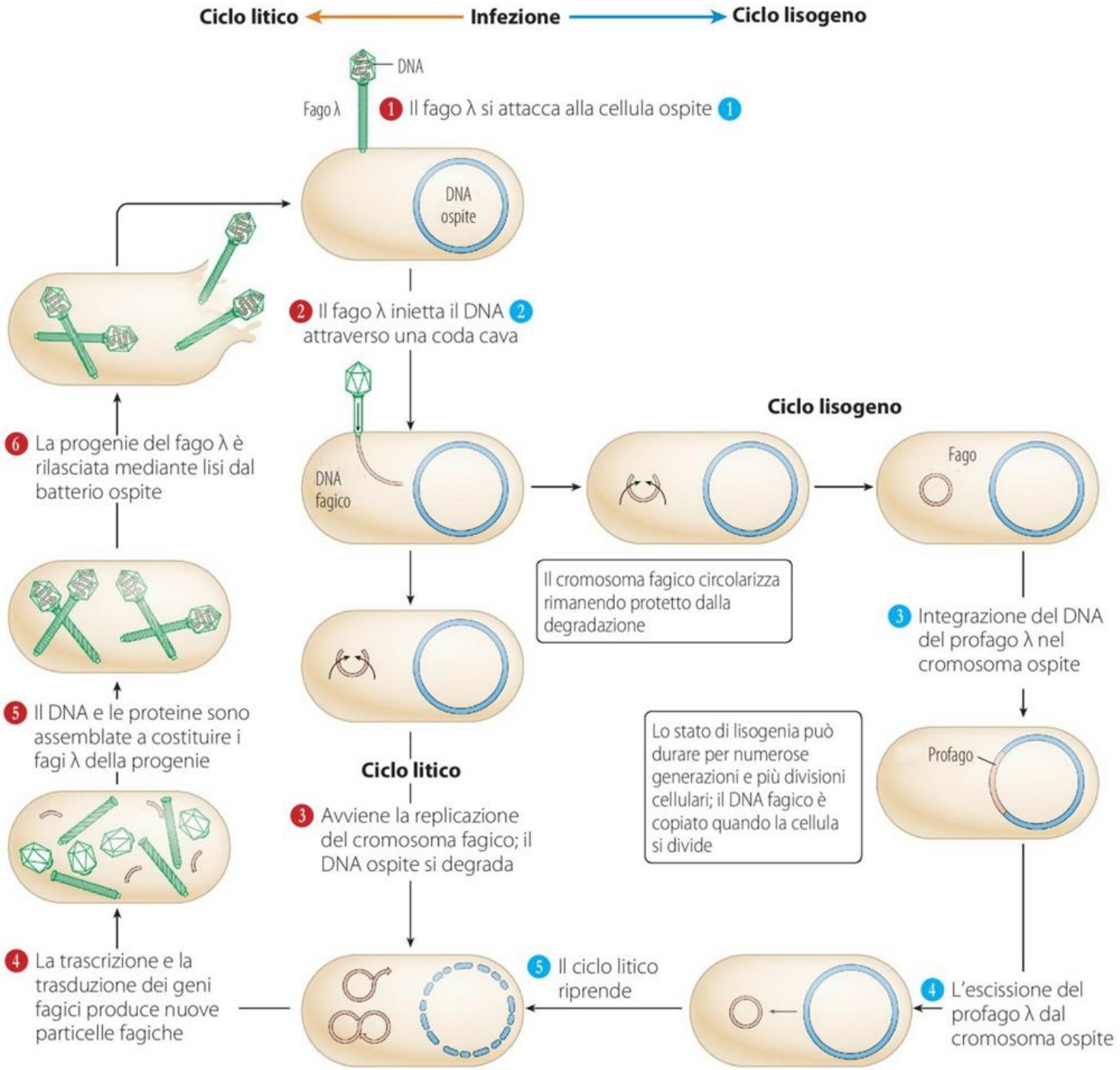
Fago T4





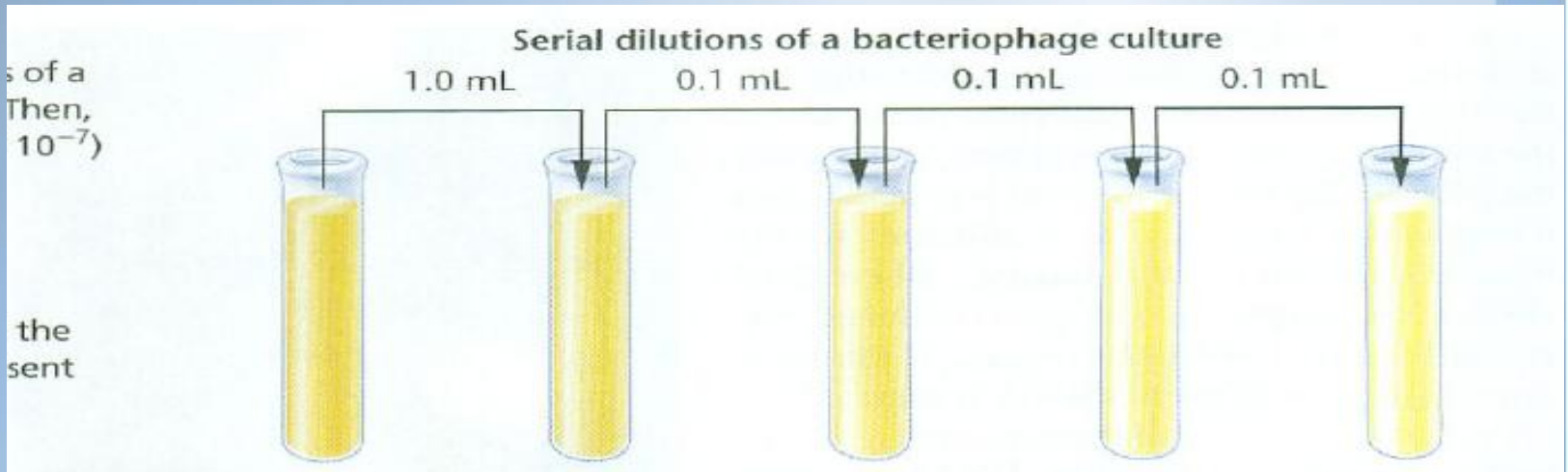
Fago λ



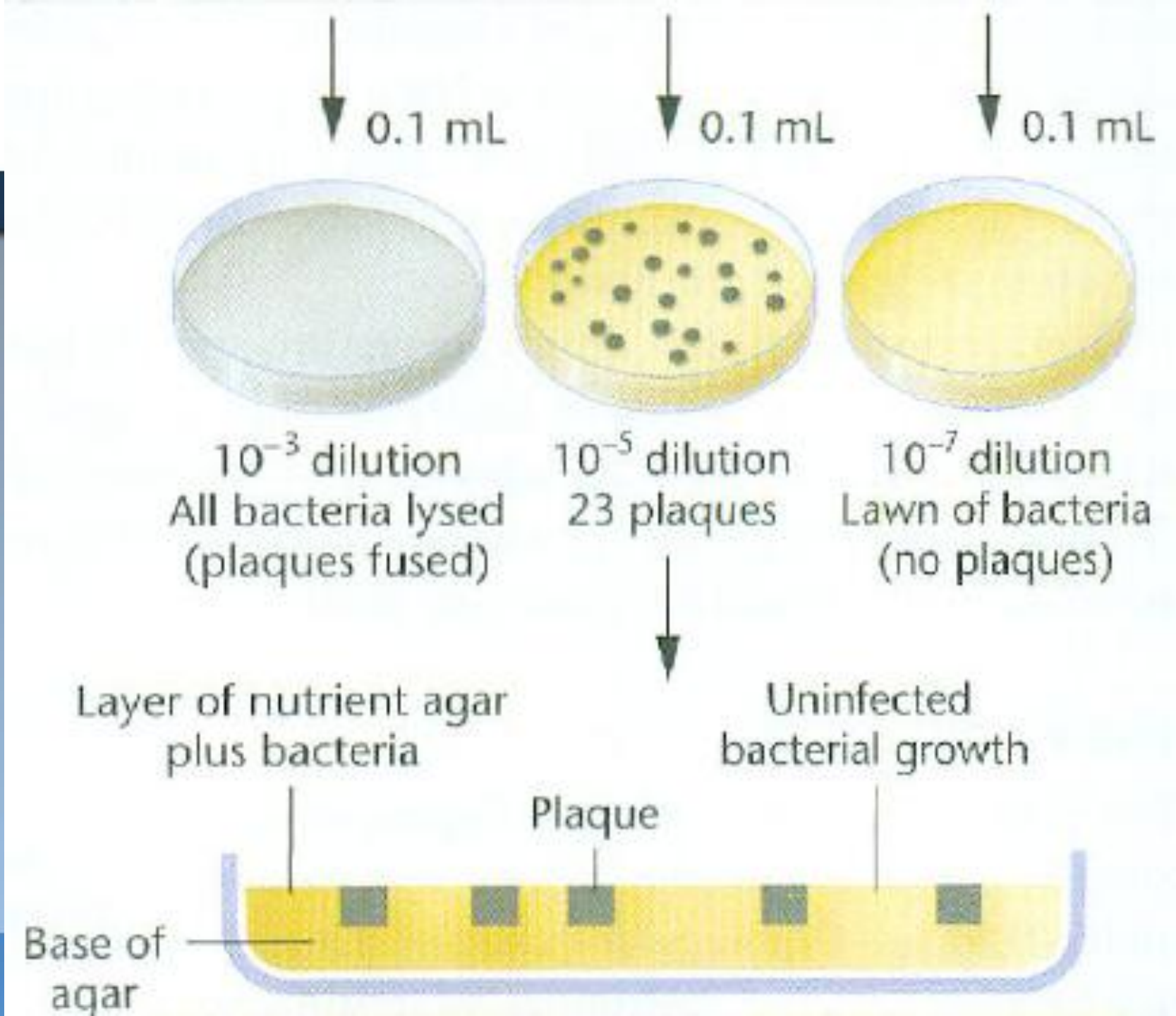

Ciclo litico
Infezione
Ciclo lisogeno
DNA
Fago λ
1 Il fago λ si attacca alla cellula ospite 1
DNA ospite
2 Il fago λ inietta il DNA 2 attraverso una coda cava
DNA fagico
Ciclo lisogeno
Fago
Il cromosoma fagico circolarizza rimanendo protetto dalla degradazione
3 Integrazione del DNA del profago λ nel cromosoma ospite
Profago
Lo stato di lisogenia può durare per numerose generazioni e più divisioni cellulari; il DNA fagico è copiato quando la cellula si divide
4 L'escissione del profago λ dal cromosoma ospite
5 Il ciclo litico riprende
Ciclo litico
3 Avviene la replicazione del cromosoma fagico; il DNA ospite si degrada
4 La trascrizione e la trasduzione dei geni fagici produce nuove particelle fagiche
5 Il DNA e le proteine sono assemblate a costituire i fagi λ della progenie
6 La progenie del fago λ è rilasciata mediante lisi dal batterio ospite

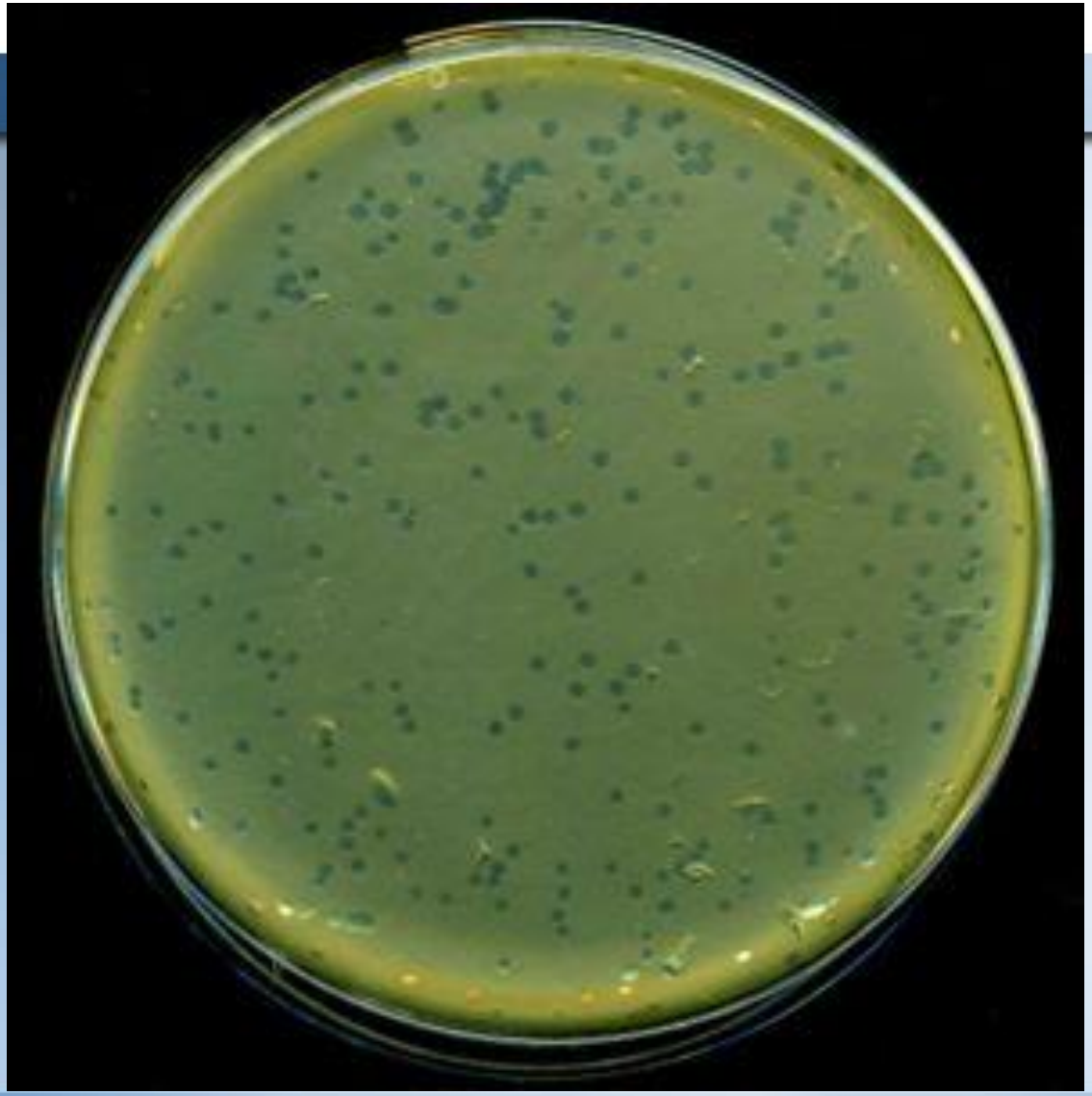
•The number of phages produced following the infection of bacteria can be determined by the **plaque assay**. This technique entails performing serial dilutions of virally infected bacteria, which are then poured onto agar plates.

- By counting the number of plaques (areas clear of bacteria) on the plates, the number of phages in the original culture can be determined.



Total volume	10 mL	10 mL	10 mL	10 mL	10mL
Dilution	0	10^{-1}	10^{-3}	10^{-5}	10^{-7}
Dilution factor	0	10	10^{3}	10^{5}	10^{7}

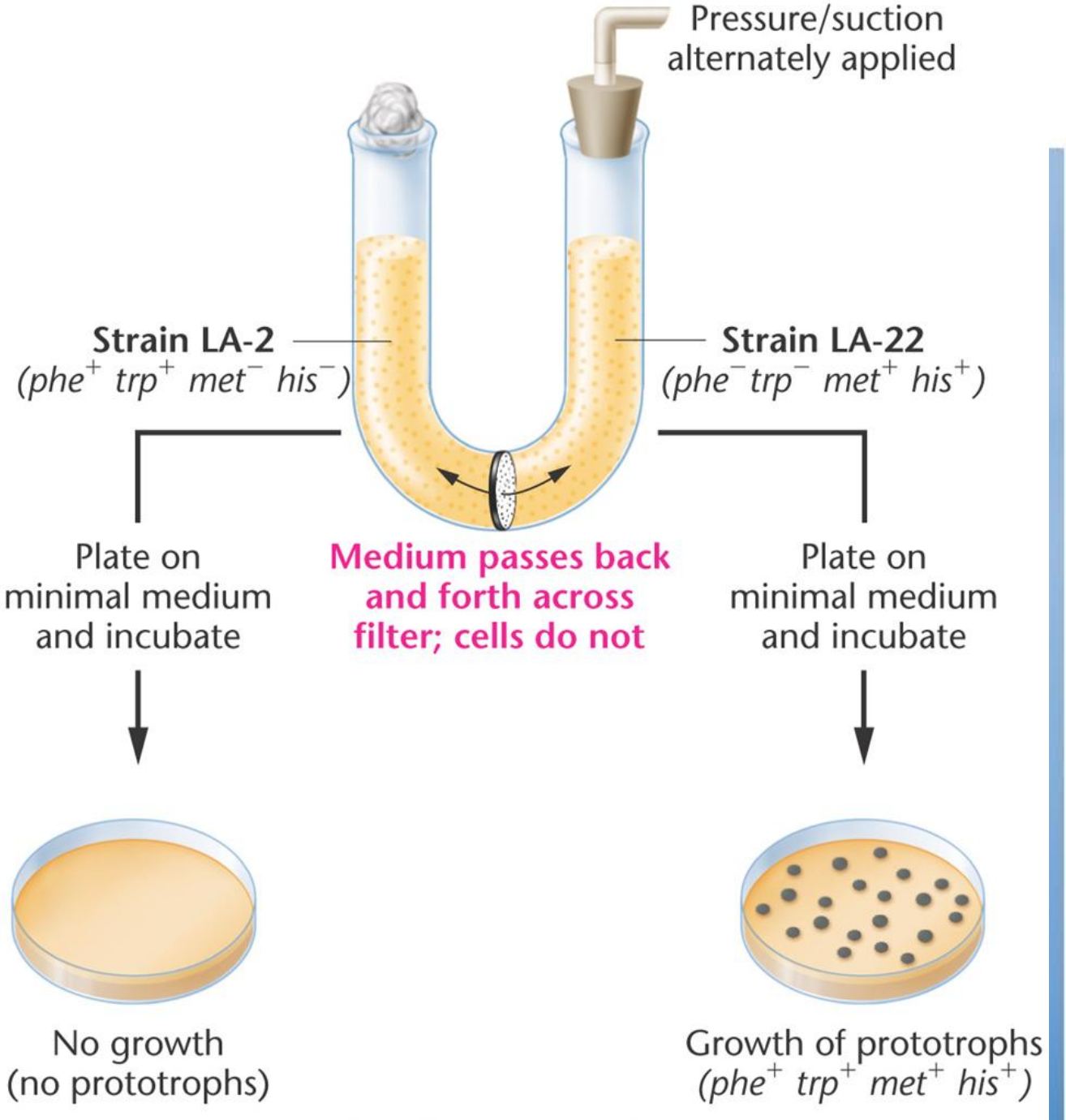

0.1 mL
0.1 mL
0.1 mL
10^{-3} dilution
All bacteria lysed
(plaques fused)
10^{-5} dilution
23 plaques
10^{-7} dilution
Lawn of bacteria
(no plaques)
Layer of nutrient agar
plus bacteria
Uninfected
bacterial growth
Plaque
Base of
agar



Transduction Is Virus-Mediated Bacterial DNA Transfer

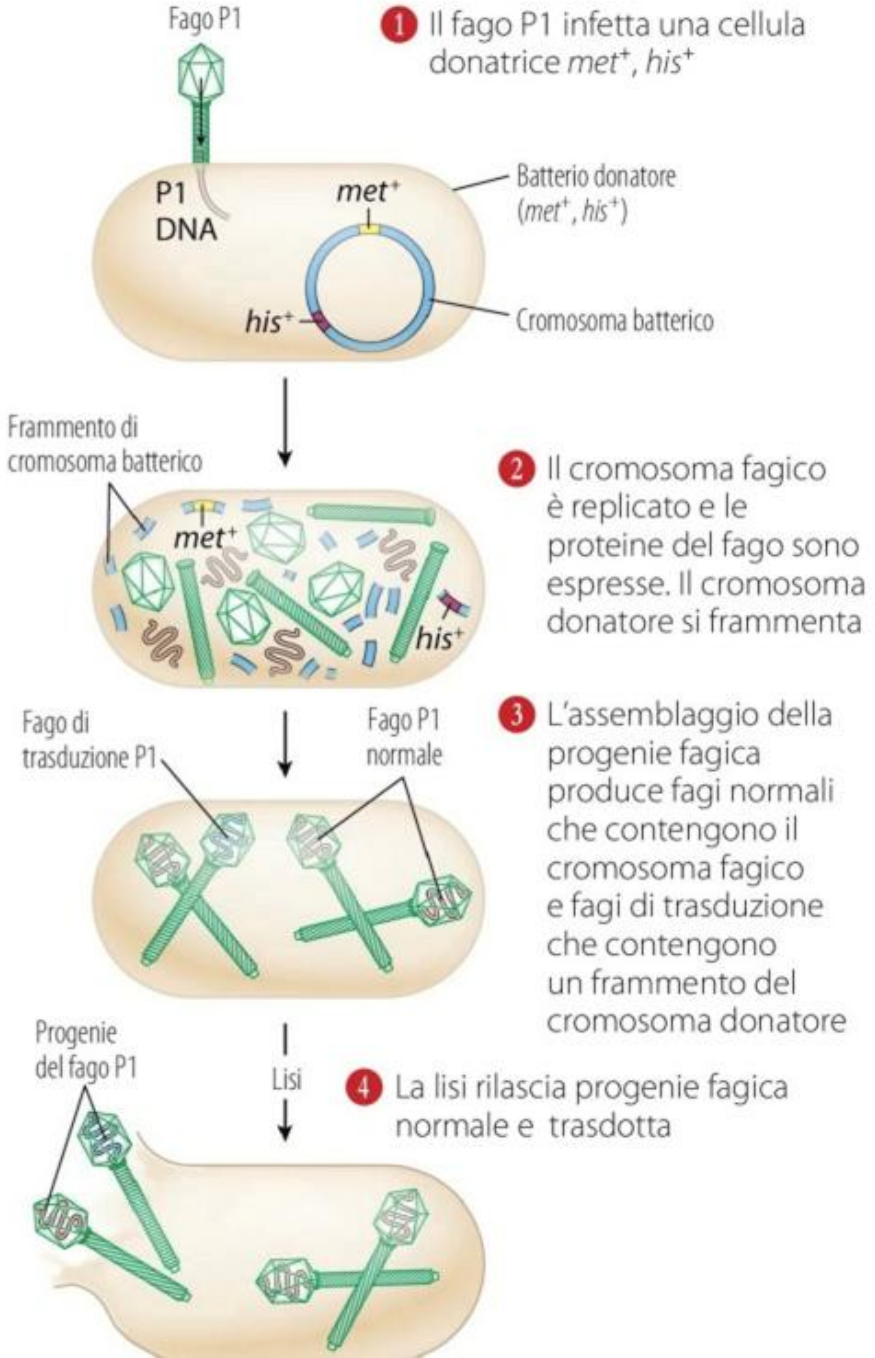
- Bacteriophages, which can themselves undergo genetic recombination, can be involved in a mode of bacterial genetic recombination called **transduction**.

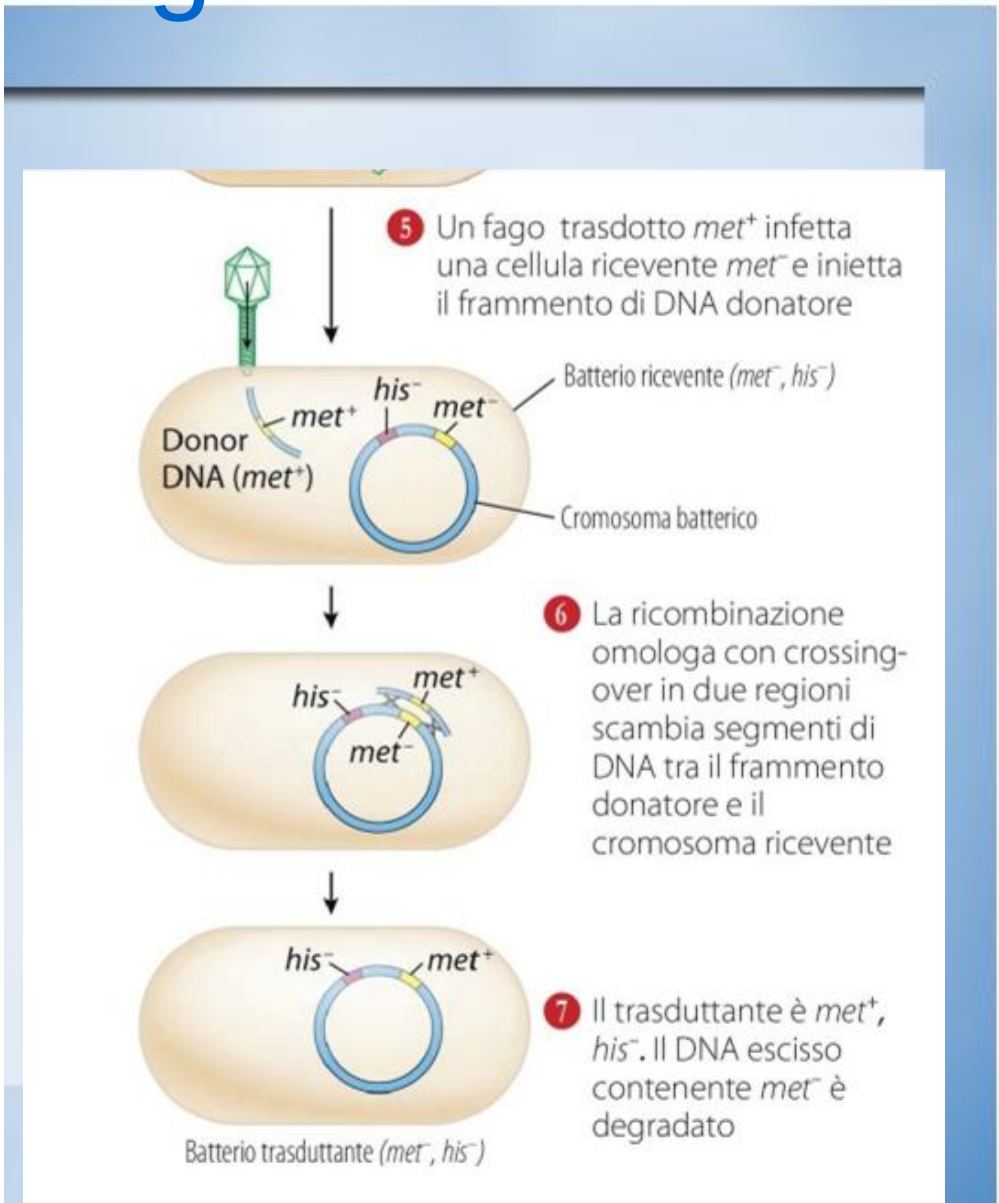
- The Lederberg-Zinder experiment led to the discovery of phage transduction in bacteria .



- In **generalized transduction**, bacterial DNA instead of phage DNA is packaged in a phage particle and is transferred to a recipient host. In **specialized transduction**, a small piece of bacterial DNA is packaged along with the phage DNA.

Trasduzione generalizzata





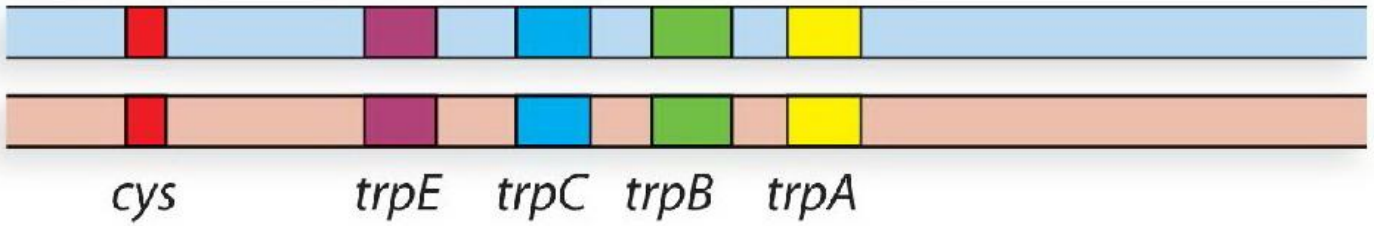
•Generalized transduction results in transfer of a large number of bacterial genes, whereas specialized transduction results in transfer of only a few bacterial genes.

Like transformation, generalized transduction can be used in linkage and chromosomal mapping (cotrasduzione).

(**a**) Frequenze di co-trasduzione

Genotipo del donatore	Genotipo del ricevente	Marcatore selezionato	Marcatore non selezionato	Percentuale di co-trasduzione del marcatore non selezionato con cys^+
$cys^+\ trpE^+$	$cys^-\ trpE^-$	cys^+	$trpE^+$	63
$cys^+\ trpC^+$	$cys^-\ trpC^-$	cys^+	$trpC^+$	53
$cys^+\ trpB^+$	$cys^-\ trpB^-$	cys^+	$trpB^+$	47
$cys^+\ trpA^+$	$cys^-\ trpA^-$	cys^+	$trpA^+$	46

(**b**) Mappa dell'operone *trp*



(**a**) La frequenza di co-trasduzione del gene cys^+ con ciascuno dei geni dell'operone *trp* è determinata in esperimenti separati con marcatore selezionato e non selezionato. (**b**) Mappa dell'operone *trp* proposta da Yanofsky.