number

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***Remember !**

we have 3 ways for gene transferring :

1. Transformation
2. transduction
3. conjugation

1. TRANSFORMATION

* Transform genes between bacteria.
* Bacteria can exchange genes which code for toxins or antibiotic resistance, or genes which code for virulence so they become more virulent.
* happened during transformation between two different cells : a piece of exogenous DNA enters to a recipient cell.

(competent بحيث تكون هذه الخلية)

*transformation: uptake of exogenous DNA into a recipient cell .

recombination with bacterial : (exogenous DNA) ثم يحدث لـ

chromosome

*cells which can undergo transformation have to be **competent** cell

***Remember !**

competent means that the cell can receive exogenous DNA by receptors at the end of the log phase .

*DNA transformation experiment was in 1928: " **Griffith experiment** :

Streptococcus pneumoniae (pneumococcus) استخدم بكتيريا ، استعمل الفئران والبكتيريا

he used mice because they are susceptible for streptococcus pneumonia

- we will use two different types of this bacteria : capsulated, uncapsulated.

NOTE : capsulated mentioned in the text book as encapsulated means have a capsule / en means inside (inside a capsule)

but uncapsulated mentioned as nonencapsulated which doesn't have a capsule

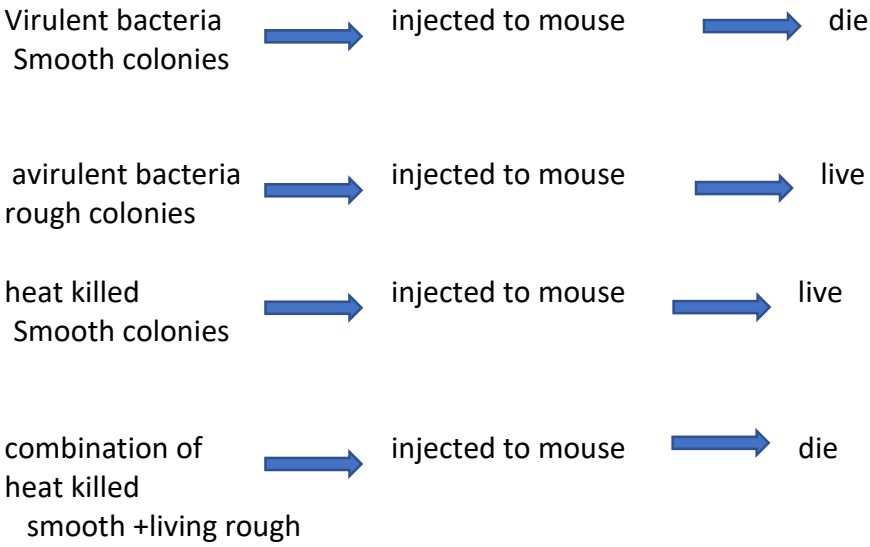
- capsule is a virulence factor

* bacteria with capsule: virulent bacteria/ can't be phagocytosed/smooth (S)

* bacteria without capsule : Avirulent/ phagocytosed / rough (R)

-When culturing bacteria that possess a capsule appear smooth colonies

- When culturing bacteria that not possess a capsule appear rough colonies.



<u>Why do the rats live in the last case rather than they die as expected ?!!</u>

the living bacteria was the uncapsulated (nonencapsulated) one , it takes some of the DNA of the dead bacteria (heat killed which primarily have the genes that are responsible of the production of capsules) so it produces it's own capsule using genes of the dead bacteria and become smooth (Anti Phagocytosis) , the DNA transferred be <u>Transformation</u>

Avery Macleod and Mccarty a 3 scientists repeat the same experiment but add a new step to ensure that the genetic material is the DNA not protein :
They have : rats , 2 types streptococcus pneumonia one heat killed smooth and the other is rough / the rat dead …
They tried to add some enzymes :
* amylase : it breaks the carbohydrates but the mouse die so transformation occur.
* protease : it breaks proteins but the mouse die so transformation occur
*RNAase : it breaks RNA but the mouse die so transformation occur
*** you may think that the transformation should stop because the RNA is broken , but remember the DNA is transferred (ready) no need for RNA .
* DNAaes :the mouse still alive so transformation does not occur

وبذلك تم تحديد DNAعلى أنها المادة الوراثية. **وهذا الاثبات الاول**

Hershey and Chase experiment 1952:
Used E.coli + bacteriophage (T2 phage)
*(virus can invade E.coli) الفاجّات
Bacteriophage : attach to the receptors on the bacterial cells then they inject the genetic material , protein coat and fibrous tail remain outside, DNA virus enters the cell then produced new viruses after the replication.
New virus produced called : **viral progeny**
هذه التجربة ساعدت في معرفة DNA على أنه المادة الوراثية لأن الغطاء الفيروسي مصنوع من بروتين فلم يدخل الى داخل البكتيريا .
They have 2 bacteriophages (they chose them with DNA genetic material / as viruses genetic material may be DNA or RNA) they labelled them : the first one was labelled by phosphorus radioactive

material because phosphor is a component of DNA / the other was labelled by sulfurous radioactive material because sulfur is a component of protein --- so we labelled the first by it's DNA and the second by it's protein

يعني الفايروس الي اختاروه مكون من بروتين و DNA بدهم يعرفوا مين من المكونات الي بينتقل للبكتيريا فعلموا كل واحد منهم بمادة مشعة وعملوا التجربة

Then they mixed with E-coli in test tubes and allowed to increase (reproduce)
after that they have centrifuged each tube :
first tube shows 2 layers :
a supernatant : which is the liquid contains viruses (DNA radioactive)
a precipitate : bacterial cells also DNA radioactive
that clearly means that the transferred material was DNA **وهذا كان الاثبات الثاني**
Second test tube also shows 2 test tubes :
a supernatant : which is the liquid contains viruses (NO DNA radioactive but protein radioactive)
a precipitate : bacterial cells but NO DNA radioactive and NO protein radioactive
so protein doesn't transferred !!
*transforming bacterial cell : transformation
* transforming bacterial DNA into eukaryotic cell : transfection
Transfection it is a transformation but the recipient is an eukaryotic cell (yeast , human …)
Transfection is important **in gene engineering**
NOTE : For better understanding see this video :
https://www.youtube.com/watch?v=iS06QuipbZM

2. TRANSDUCTION

transduction need **bacteriophage** !
A double strand DNA is transferred from donor cell into recipient cell by bacteriophage .
The first step in transduction is the attachment of bacteriophage to the cell then the virus injects it's genetic material into the bacterium , the genetic material of virus uses the ribosomes of the host cell to replicate itself , then packaging or assembling occur (heads and tails together forming new viruses) , the phage will lyse and release the progeny.
*The new virus called **transducing phage**

في bacteriophage infection وبالتحديد في عملية assembly وتجميع أجزاء progeny بعض الفيروسات بتحمل جزء من DNA على البكتيريا (النسبة 1/1000) وبالتالي ينتقل DNA من بكتيريا الى أخرى ،لكن هذا الفيروس لن يكون قادر على احداث العدوى لاحتوائه DNA البكتيريا وليس الفيروس transduction phage

*A good Example : B-lactamase gene that transferred to staphylococcus aureus by transduction, **NOTE : B-lactamase** are enzymes that have the ability to destroy B-lactam , B-lactam is the main component of many antibiotics like penicillin or cephalosporin.
*There are 2 cycles for bacteriophages :

1.lytic cycle: عملية حقن المادة الوراثية ثم تضاعفها ثم تجميع مكونات الفيروس ثم تحليله

2.lysogenic cycle : لكن بعض أنواع الفيروسات تتبع دورة أخرى وهي

***A temperate bacteriophage** : a virus which able to integrate bacterial DNA and undergo lysogenic cycle. Only temperate bacteriophage enter the lysogenic cycle not all bacteriophages !

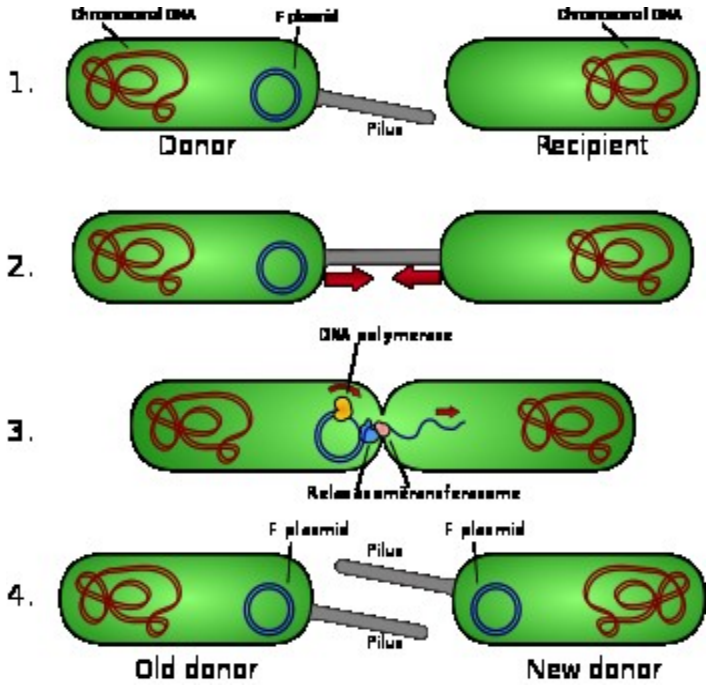
*In lysogenic cycle the attachment occur but the virus doesn't replicate it's components directly but instead of that the genetic material of viruses incorporated into the bacterial DNA so the viral genetics replicates with bacterial until the conditions change , then complete it's cycle with same steps as lytic cycle .
*When bacteriophage enter the cell and fuse with the DNA called prophage
فكل مرة الخلية تنقسم بتكون حاملة DNA للفيروس ،بذلك تكسب البكتيريا صفة جديدة وممكن تتحول لبكتيريا ممرضة .
البكتيريا الي مرت بالدورة الاندماجية بنسميها **lysogenized cell**
Examples of these genes' benefits or diseases :
1.bacterium diphtheria : Corynebacterium diphtheria produces the toxins that causes the disease because it has the gene of producing these toxins by transduction .
2.clostridium botulism : causes botulism (food poisoning)
3.Cholera toxins
4.Streptococcus pyogenes : produces toxins called erythrogenic toxin

*bacterial cells which produce the toxins called lysogenic (carrying viral genes)
هذه الجينات ممكن في أي لحظة يدخلوا الانقسام ،ويكونوا فيروسات جديدة

*bacterial strains that carry genes for viruses can make the disease so it is not lysogenic
أي أن المادة الوراثية في الفيروس تختلف عن البكتيريا

3. CONJUGATION

*we need **Fertility factor**
*What is fertility factor ?
It is the gene found in f plasmids that codes necessary proteins for conjugation (like pilin : the protein which makes the sex pilus.)
*we need two cells donor and recipient which are in direct contact via sex pilus
* F- , female, recipient , have fertility factor
*F+, male, donor , lack of fertility factor
*Steps :
1.Sex pilus attach to recipient cell
2.Retraction occurs to close cells together
3.Fertility factors code for a certain enzyme to make a nick or cleavage in the double strand plasmid
4.One of the strands migrated to the recipient cell
5.A complementary strand is synthesized in each cell



NOTE : after conjugation both cells are F+

الاقتران ليس بالضرورة انه يحدث بين نفس النوع من البكتيريا لأنه احنا عنا البكتيريا انواع مختلفة (genera) وتحت كل نوع عنا اصناف مختلفة (species)

F+ cell: plasmid in cytoplasm.

NOTE : in conjugation single strand DNA is transferred but in transduction double strand is transferred

HFR: cell: Highly Frequency Recombination

plasmid in chromosome(can highly recombination with other cells at recombination rate 1000

احتمالية انه يصير نقل للمادة الوراثية بين HFR و F- اكبر 1000 من انتقالها بين F-و F+ من غير ما يصير recombination

NOTE : The most frequently occurring naturally is conjugation than transduction than transformation .

We have 3 ways for gene transferring within the bacterial cell :

1.Transposons

يوجد جينات تسمى transposons ويطلق عليهم jumping genes

*they can move from plasmid to another plasmid /plasmid to chromosome/on the same chromosome /on the same plasmid /chromosome to plasmid

*transposons contribute in the spread of antibiotic resistance

*transposons found as integrated form in chromosome or in DNA

*transposons differ from plasmid that transposons can't replicate autonomously but plasmids can.

4 major domains of transposons :

1.Flanked inverted repeats (**palindromic sequences**)

Remember !

Palindromic means you can read from both sides .

2.transposase gene codes for the enzyme responsible for excision and integration for transposons
3. repressor gene regulate the expression for transposons and regulates genes responsible for translation of antibiotic resistance genes and genes of toxins production .
4.drug resistance gene or toxic production genes

interval repeats موجودة في نهاية transposons عبارة عن سلسلتين من القواعد النيتروجينية لكن باتجاهات مختلفة في نهاية كل جين.

2.Insertion sequence

smaller and shorter than transposons , they don't have the 4 major domains , causes mutation at their site of integration , they don't code for enzymes so they have less nucleotides

3. Programmed gene rearrangement :

Programmed gene rearrangement is the cause for antigenic variation by changing the antigens on the surface so they can evade the immune system .
Important example : Neisseria gonorrhea that causes **gonorrhea** (**STD** : sexually transmitted disease) السيلان

بالتالي ما بتعرف عليها جهاز المناعة ويصاب فيها الشخص أكثر من مرة لأنها بتغير antigen الموجود على سطحها. هذه الظاهرة تسمى antigenic variation

Another example : **borrelia recrutement** which cause relapsing fever due to programmed rearrangement that transfer some genes from latent expression to expression locus that activated
***Trypanosomes** are microorganisms which can do antigenic variation so it's hard to make antibiotics for them .
*Influenza viruses undergo antigenic variation

Normal flora called also microbial or microbiota

Normal flora some bacteria ,fungi protozoa which live normally in the body
* all normal flora are opportunistic
resident population :exist all the time like the one in intestine
transient population: take place in somewhere but not the original

carrier state :
The person has the disease but the symptoms are not clear and can transmit the disease
Asymptotic: بدون أعراض
Symptomatic : مع أعراض