



Microbiology

Doctor 2017 | Medicine | JU

● Sheet

○ Slides

DONE BY

Rawan Almujaibel

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Bayan Abusheikha

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

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DOCTOR

Anas Abu-Humaidan

In the previous lecture the Dr. talked about DNA structure and their 4 types of nitrogenous bases. Then he talked about bacterial DNA (chromosomes) and their replication process. Then, we ended our lecture by talking about Models of DNA replication, DNA mutations and their repair mechanisms.

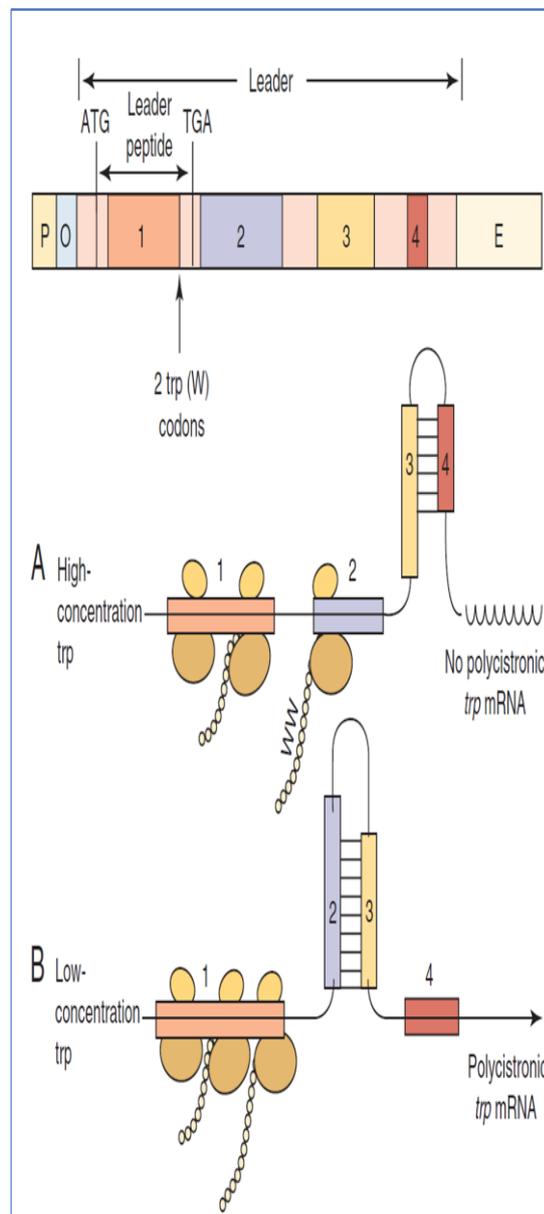
Regulation of transcription

We will talk about attenuation, as we talked about it briefly last lecture.

Attenuation is a regulatory mechanism of some biosynthetic pathways (eg. The tryptophan "trp" biosynthetic pathway) that controls the efficiency of transcription after transcription has been initiated but before mRNA synthesis of operon genes takes place, especially when the end product of the pathway is in short supply.

For example, under normal growth conditions, most trp mRNA transcripts terminate before they reach the structural gene of the trp operon. However, during conditions of severe tryptophan starvation, the premature termination of transcription is abolished, allowing expression of the operon at 10-fold higher levels than under normal conditions. The explanation for this phenomenon resides in the 162 bp regulatory sequences in front of the trp structural genes referred to as the leader sequence or trpL. The trp leader sequence can be transcribed into mRNA and subsequently translated into a 14 amino acid polypeptide with 2 adjacent tryptophan residues, a sequence that occurs rarely.

At the end of trpL and upstream of the regulatory signals, that controls translation for the trp structural genes, there is a structure called **Rho-independent terminator**. The DNA sequence of this region suggests that the encoded mRNA has a high probability of forming **stem loop secondary structure**. These have been named the **pause loop**, the **terminator loop** and the **anti-terminator loop**.



Attenuation of the trp operon uses the secondary structure of the mRNA to sense the amount of tryptophan in the cell (as trp-tRNA).

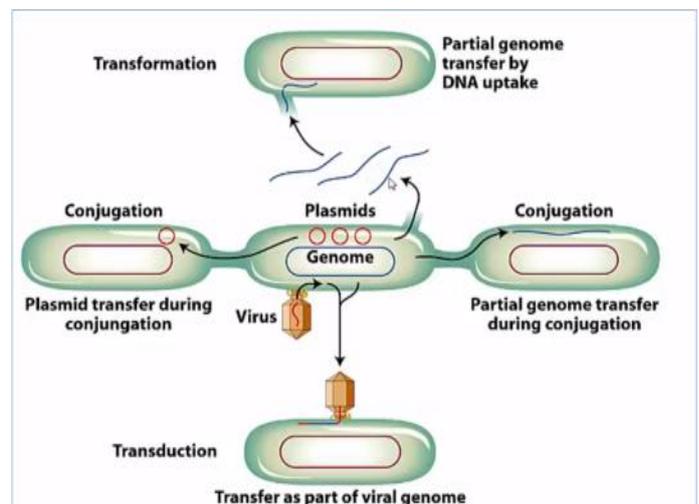
Prevention of transcription by a repressor protein is called **Negative control**. The opposite form of transcriptional regulation-initiation of transcription in response to binding of an activator protein is termed **Positive control**. Both forms of control are exerted over expression of the lac operon, genes associated with fermentation of lactose in E.coli.

So attenuation occurs when we have high number of tryptophan (the transcription is stopped), however attenuation stops when we have low number of tryptophan (transcription is happening and continue).

This video might help you understand more :
<https://www.youtube.com/watch?v=RQrdY07JkFU>

Horizontal Gene transfer (HGT)

Horizontal gene transfer is transferring DNA from one organism to another in a horizontal way and that DNA can be stably incorporated in the recipient permanently changing its genetic composition. There's no parental relationship between the two organisms which are sharing the gene)

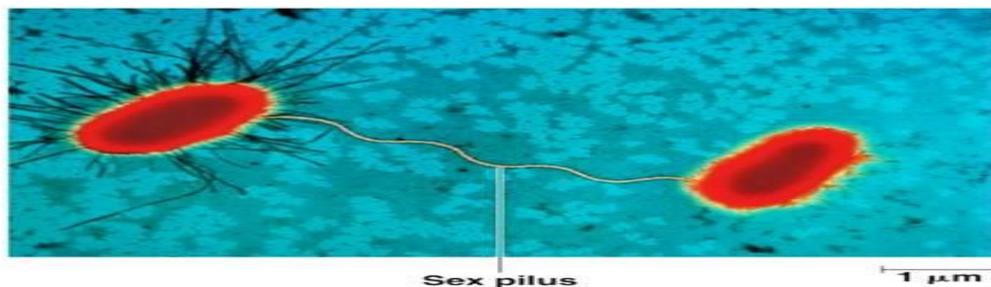
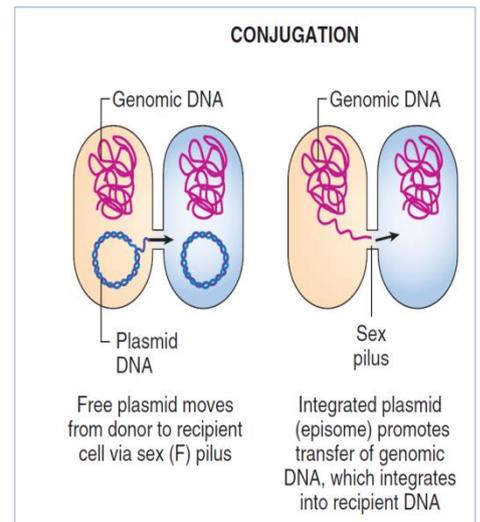


It is different from the vertical inheritance transfer as it is inheritance of parental gene, so it is passing genetic material from parent to daughter cells.

There are 3 broad mechanism mediate efficient movement of DNA between cells under (HGT):

1-Conjugation:

It requires donor -to-recipient cell physical contact to transfer only one strand of DNA. The recipient completes the structure of dsDNA by synthesizing the strand that completes that strand acquired from the donor.

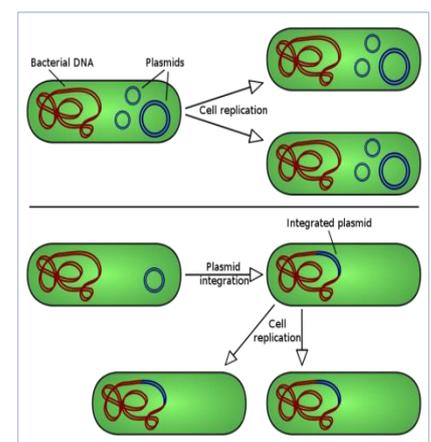


The Mechanism:

The donor cell produces sex pillus and extends their pillus to the recipient cell. Afterward, the donor cell retracts the pillus to bring the cells into close contact, and a pore forms in the adjoining cell membranes. Then, a single stranded of the plasmid (it is a circular DNA that is outside of the chromosomes DNA) is transferred to the recipient through the pore. In another form of conjugation, Parts of the genomic DNA is moving from donor to the recipient with the plasmid.

*the genetic material that can be replicate by itself called "replicon".

*If the genetic material that already parts of the genomic DNA it will be called "replisome"



Plasmids:

It is a small circular DNA molecule (1-200 kbp, it is coding for at least one gene) within a cell that is physically separated from a chromosomal DNA and can replicate independently. Plasmids can integrate themselves in chromosomal DNA using enzymes that cut the DNA and integrate itself within bacterial DNA.

Plasmids can be **Replicon** or **Epitome**.

They are used in Horizontal gene transfer and it is beneficial for the bacterial cell. Plasmids are not essential for bacterial life, however they are Important in the transfer of antibiotic resistance. Also, they can encode for virulence factor (ability of causing a disease).

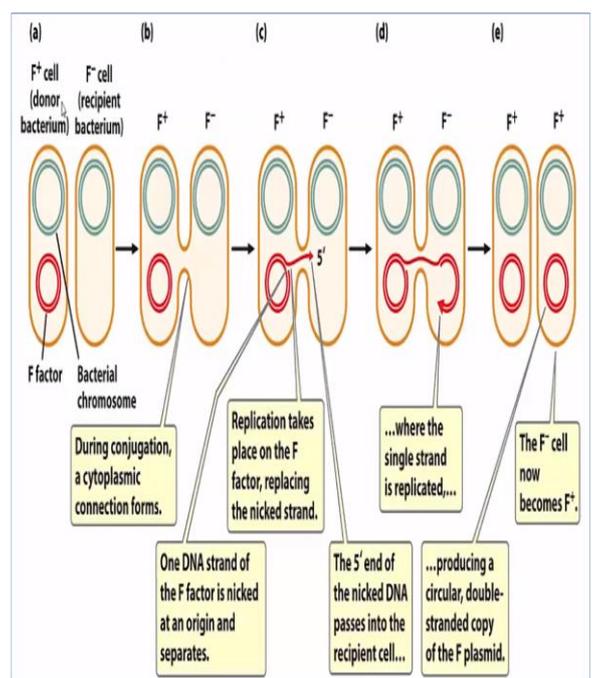
Plasmids classified by their "conjugativity" or their compatibility functions.

- Conjugativity means that plasmids can either be replicated in or transferred to the host microbes.
- Compatibility means the ability of plasmids to share similar replication and partition systems to be propagated stably in the same host cell line. So the failure of two co-resident plasmids to be stably inherited together in the absence of external selection is called incompatible plasmid. In the other word, if the introduction of a second plasmid negatively affects the inheritance of the first, the two are considered to be incompatible.

Examples

As you can see in the picture is the mechanism of plasmid mobilization, there is a donor bacteria that has the F factor (stands for fertility factor) we call it "F+ plasmid" and the recipient cell called "f- plasmid" as it does not have F factor inside it.

- 1- During conjugation a cytoplasmic membrane connection is formed.
- 2- Then F factor is cut or nicked at a specific region called the origin of transfer by a



protein assembly and separates.

- 3- Afterwards, replication is taking place on the F factor, so it replicating the nicked strand.
- 4- The 5' end of the nicked DNA passes into the F- cell.
- 5- In the F- cell the single strand is replicated producing a circle, double strand copy of the F plasmid.
- 6- Now the F- becomes F+.

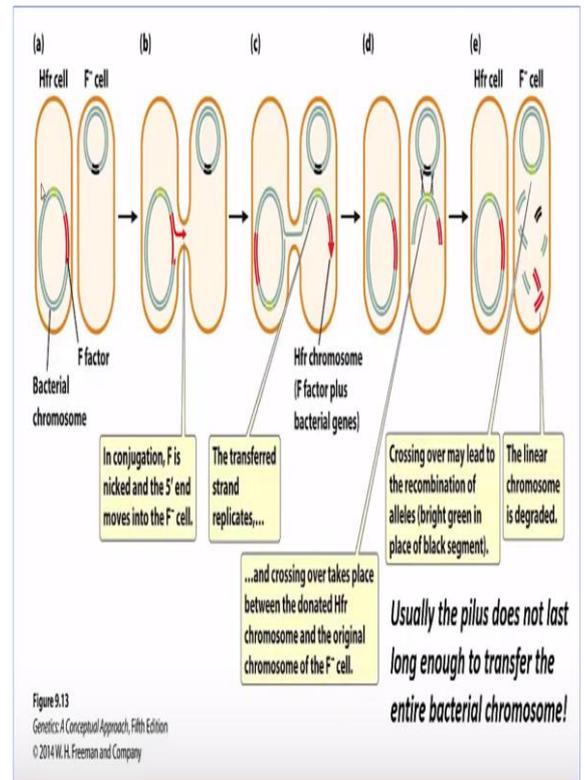
When the microbiologists did those experiments between donor and recipient bacteria, they found that the recipient bacteria were not F+ and at the same time they still have some recombination in their genes, if they didn't get the F factor how they did get their recombination?

The microbiologist found that the F factor can work in a different way from the above mechanism. So its integrated within the bacterial chromosomes so we will get another type called High frequency recombination. This way the F factor still is coding for the same things as for the pervious mechanism, however the mechanism is slightly different.

The mechanism is:

- 1- After being integrated in the bacterial chromosomes the cytoplasm membrane connection is formed with F- cell.
- 2- Then, one DNA strand is cut at the F origin , the free 5' end moves through the transfer pore, while the chromosome replicates, the donor strand is replicated as it enters the recipient (Hfr).
- 3- When the cells separate the donor cell chromosome is restored in the recipient integration events may lead to recombination of gene alleles (by crossing over) and Linear DNA is eventually degraded.
- 4- When many HFR and F- cell are mixed, conjugating pairs can form quickly.

NOTE: usually the pilus does not last long enough to transfer the entire bacterial chromosome.

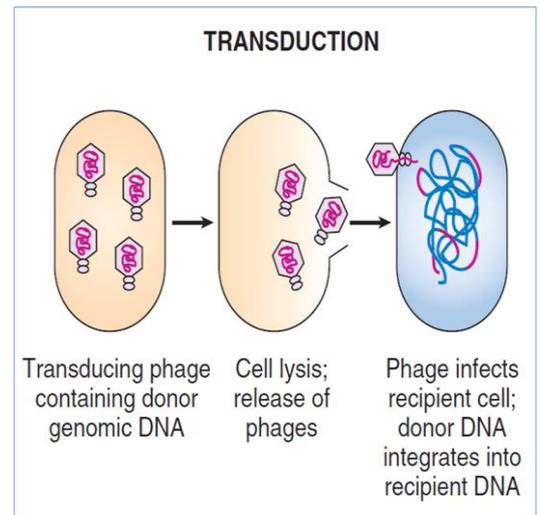


Coming back to the 2nd mechanism of DNA transfer is transduction ...

2-Transduction:

It is a phage –mediated genetic recombination in bacteria. In the simplest terms, a transducing phage is containing donor genomic DNA inside the cell. When the cell lyses, the phages is released, then the phages insert or integrates the genes into the recipient cell DNA.

An example is bacteriophages (are viruses that infect bacteria). They have 2 life cycles are called lytic cycle and lysogenic cycle:



First of all:

- 1- The phage attaches to the bacterial cell.
- 2- Then, the phage injects the cell with viral DNA.
- 3- The phage DNA inside the bacterial cell is circulated

"After injecting the DNA the cell can go 2 ways either to lytic or lysogenic cycle".

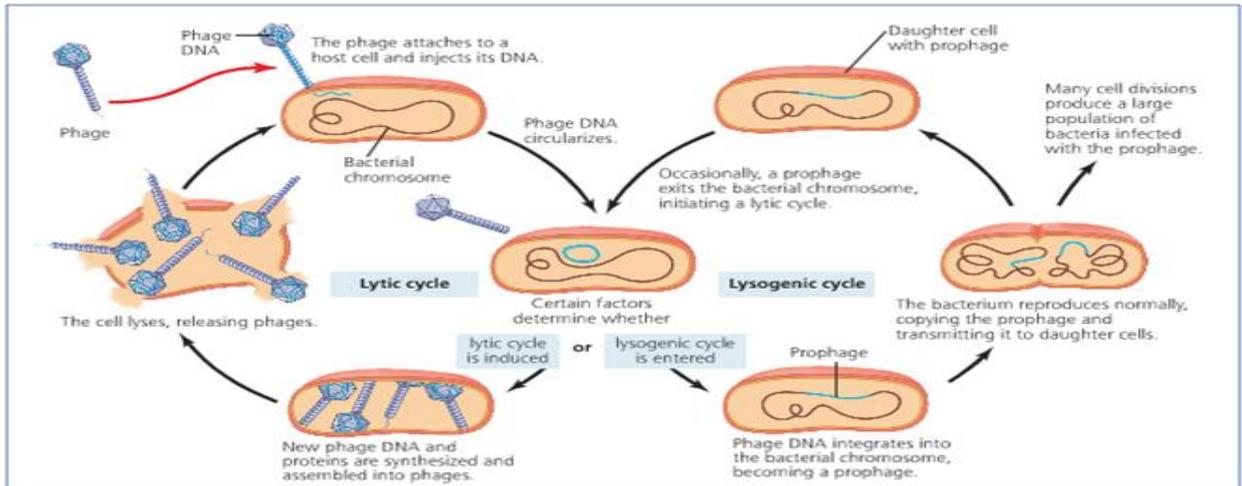
Lytic cycle:

- 4- Biosynthesis of viral nucleic acid and protein occurs.
- 5- Viral components are assembled into virions.
- 6- The plasma membrane breaks (lyses) and the virions is released from the host cell.

Lysogenic cycle:

- 4-When the phage DNA integrated into the bacterial chromosome, it becomes a prophage.
- 5-The bacterium reproduces normally copying the prophage and transmitting it to daughter cells.
- 6-Many cell divisions produce a large population of bacteria infected with the prophage.
- 7-Daughter cell with prophage is produced now, Ocassionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.

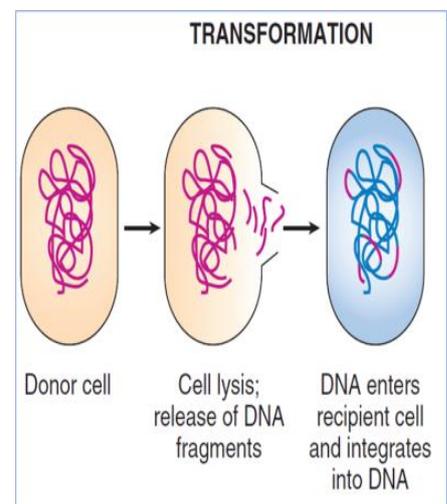
In real life and in researches transduction is a helpful tool to use to get a specific product or DNA.



3-Transformation:

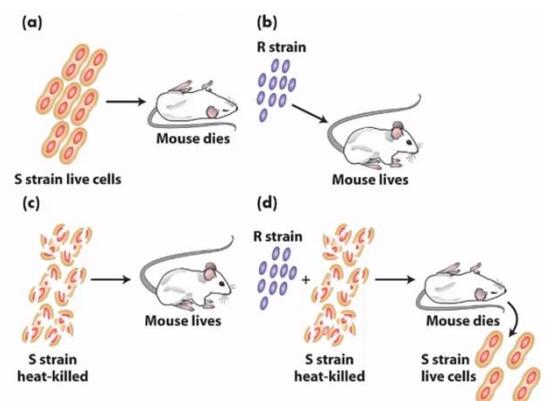
It is a genetic alteration of a naked cell resulting from the direct uptake or integration and incorporation of exogenous genetic material from it surrounding through the cell membrane into DNA.

Some bacteria are competent to uptake genetic material without any help. However, other bacteria need to be induced to do so, like using some certain chemicals or heat in an experimental environment (in genetic engineering).



Discovery of transformation transfer of DNA:

In 1928, an experiment has been done by a researcher called Griffith. He observed that virulent (causes a disease) Streptococcus bacteria in mice, when heat-inactivated and mixed with a non-virulent strain, could "transform" the non-virulent strain and make it virulent.



What he did is he brought 2 strains of streptococcus bacteria one of them virulence and the other is non-virulence strains. So, when he injected the mice with virulence strain they would die and when he injected them with the other

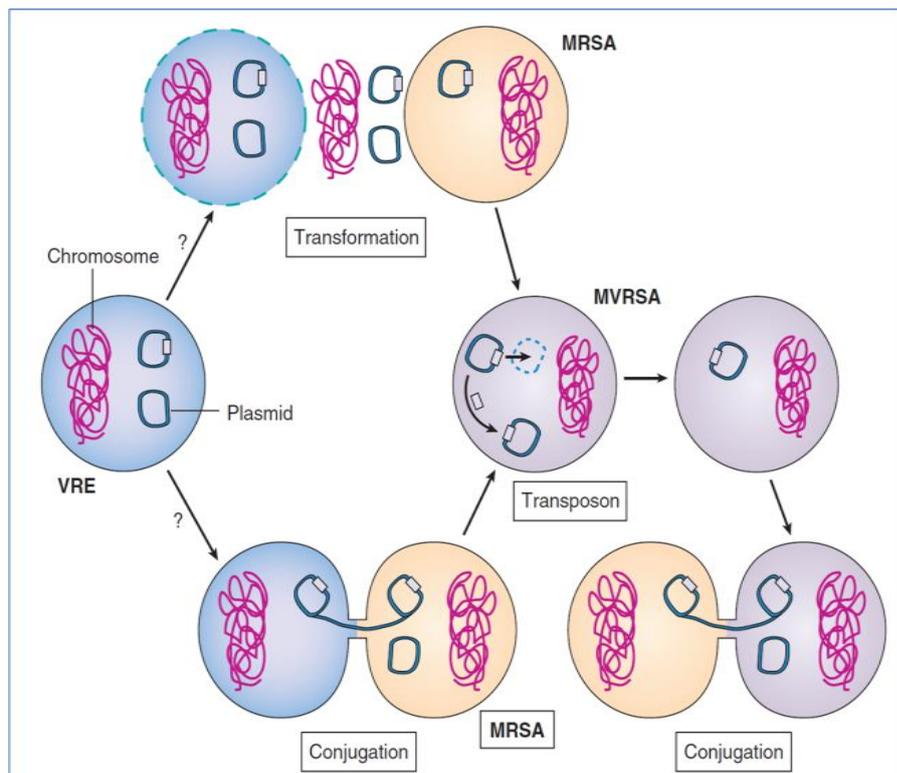
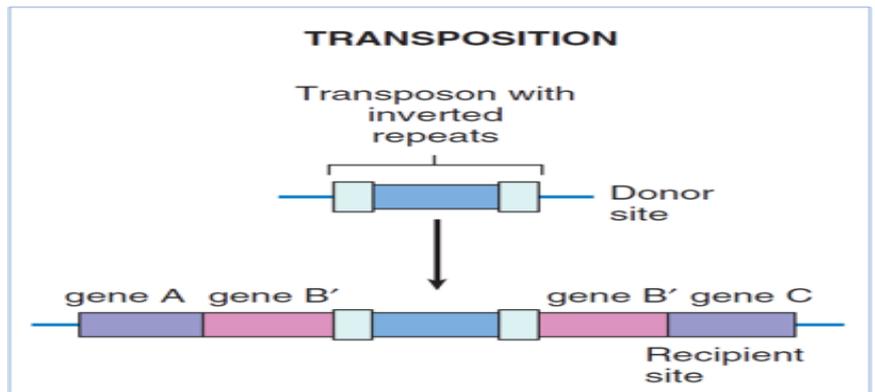
type they survive. Afterwards, he heated the virulence bacteria and injected again, surprisingly the mice survived. However, when he mixed it with normal strain the mice died. From this experiment he founded out the transformation transfer mechanism of DNA.

Explanation:

When the pathogenic bacteria died, it released its DNA into the environment. So the non-pathogenic bacteria took up the free DNA (pathogenic material) and became pathogenic (start forming capsules).

Transposition:

It is the transfer of a segment of DNA (that cuts itself) from one site to another specific site of a chromosome. In other words is the state of being removed and transposed from one place to another. It happens in eukaryotes as well in bacteria.



The implication of HGT:

Its importance appeared in antibiotics production for medical practitioners.

An example of bacterial resistance:

Methicillin-resistant Staphylococcus Aureus (MRSA) it is resistance to Methicillin. It has been a big scare in the medical field; especially it is transmitted very rapidly in the hospital environment. So that, they invented a Vancomycin is a certain antibiotic that is used against MRSA. Recently the researchers have noticed that MRSA has developed a resistance against Vancomycin as well.

The researchers found that the reasons behind MRSA's developing resistant to antibiotics are conjugation and transformation transfer for the genetic material.

Explanation:

In a case of co-infection Staphylococcus Aureus with MRSA and VRE, they co-infected some host and then through transformation which means that VRE died and it released its DNA, one of its plasmid that contains the antibiotics resistance (the multi-drug antibiotics resistance), went into MRSA and because it has transposons (the jumping gene), the genetic moves into new DNA plasmid. The new version of MRSA now in called MVERSA (Methicilin and Vancomycine resistance S.Aurous).

There is another mechanism can be used, which is happening through conjugation where simply the plasmid moves from VRE into MRSA, it inserts itself in the MRSA plasmid to make new type of bacterial resistance called VERSA. The cycle will continue, the infected bacteria is conjugating with other bacteria and so on. At the end the new genetic material will be transferred.

Another implication:

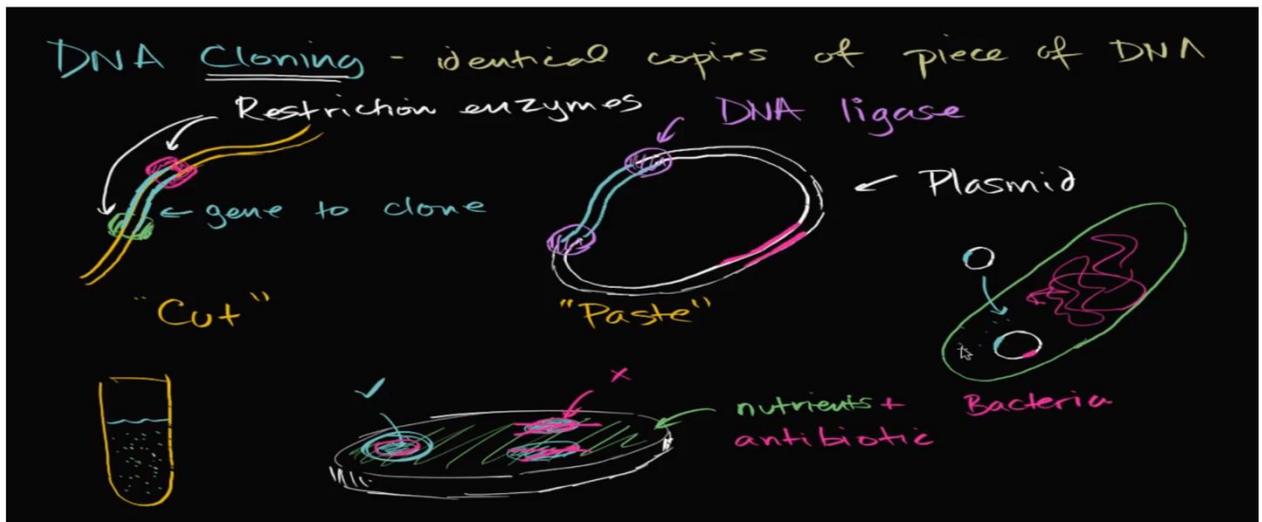
It is used as a tool in the laboratory so we manipulate it to use for our own purposes, for example:

In patients with diabetes type 1, they need to take insulin from quite young ages to regulate glucose rate.

In the past, the only source to get insulin to treat patients is from animal pancreases (excrete insulin form secretary cells) and give it to patient as a treatment medication. It is not an ideal way especially when we have quite a big population.

The researchers thought about another mechanism to produce more insulin by DNA cloning (getting 2 new identical copies of DNA from the old DNA). It is a mechanism of using HGT and by this we will be able to get big amounts of insulin for a cheap coast.

Since we have a gene and we know its sequence to clone. So, we use an enzyme (restriction endonuclease enzyme) that helps us cut the specific sequence that we need. Afterward, we paste the piece that we cut in another location (plasmid) by ligase enzyme. Then, we have the plasmid with the gene that we replicated and inserted in a bacteria (e.g. E. coli, since it has short doubling time). We grow these bacteria in a plate with nutrients. Then we put it in a broth. Then, we extracted the insulin and use it for our purposes.



How do we know colonies make insulin we want, because as we know not all bacteria can take up the plasmid?

While preparing the colonies we added another type of gene to the plasmid (antibiotic resistance gene), because when we plate the bacteria in antibiotics containing medium, only the one that is resist the antibiotics are the same one is contain the insulin gene.

BEST OF LUCK....

Some extra useful videos:

1- Lytic and lysogenic cycles of bacteriophages:

<https://www.youtube.com/watch?v=hFwA0aBX5bE>

2- Bacterial genetics part 1 (DR. recommended this video):

<https://www.youtube.com/watch?v=DWUxB7oxwTE>

3- Bacterial genetics part 2 (DR recommended this video):

<https://www.youtube.com/watch?v=vqe6wVcr0vg>