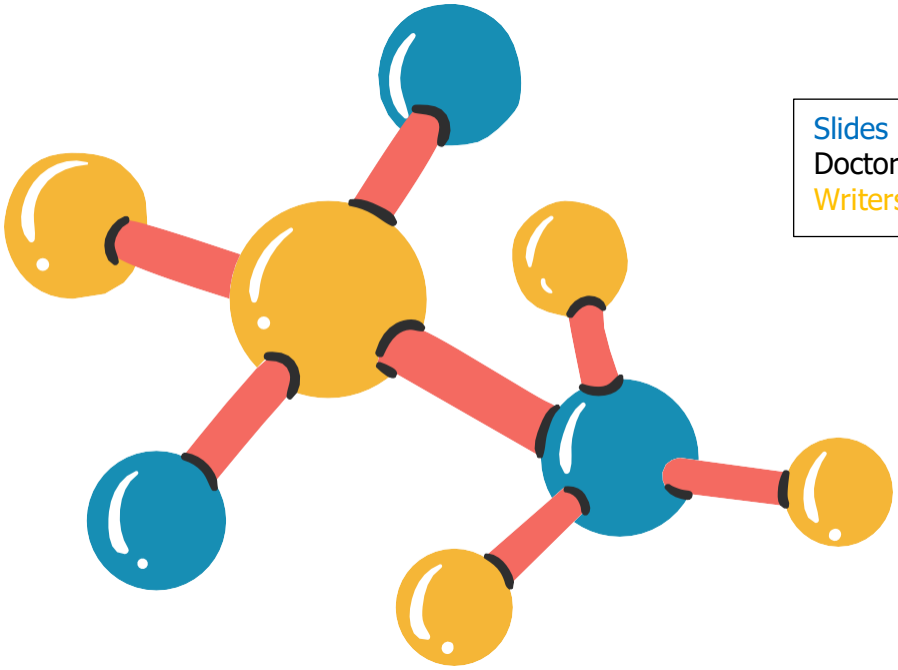
Sheet no.15

Biochemistry



Slides
Doctor`s notes
Writers` notes

Summer 2022

Writer: Aseel Rommaneh & Noor Abu Hantash

Corrector: Correct team

Doctor : Mamoun Ahram

Recombinant DNA (III)
CRISPR-CAS9

*It is such an excited topic, it is the future of medical treatment and molecular biology, gene editing, changing genes.

*You of course know about it and if not you are from Mars, read about it!!!

لأمانة النقل😔

What is CRISPR/Cas9?

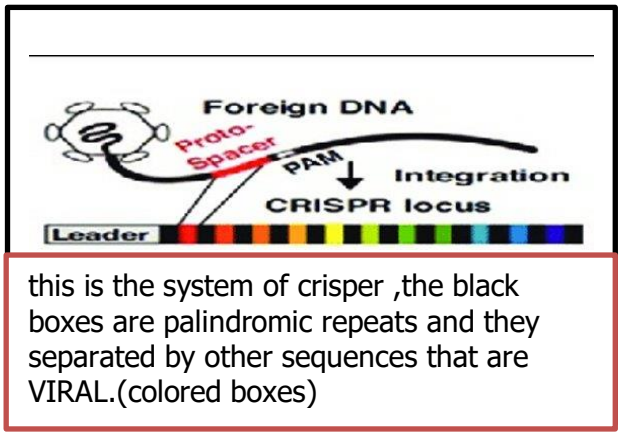
CRISPR is clustered regularly interspaced short palindromic repeats

*clustered:(groups of sequences, that exist bacteria),

* regulatory interspaced:(there are spaces between one repeat and another and they are regular)

* short

* palindromic متناظرة (the sequence from 5` → 3` on the first strand is the same to 5`→3` on the 2nd strand)like restriction sites.



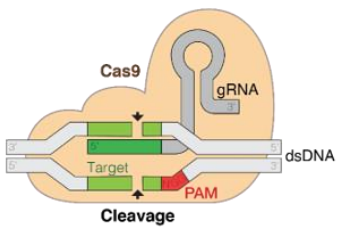
this is the system of crisper ,the black boxes are palindromic repeats and they separated by other sequences that are VIRAL.(colored boxes)

*repeats: repeated sequences

-It is a bacterial genetic system that constitutes the immune system of bacteria against phages.

-Cas9 is an RNA-guided nuclease that can either create single or double strand breaks.

*nuclease :an enzyme cleaves nucleic acids, it doesn't cleave any nucleic acid it has to be associated with RNA(to be RIBONUCLEASE).RNA leads nuclease to



This the nuclease(protein) associated with guide RNA(gRNA)

certain sequences to cleave.

*it can make single or double cuts(nicks).

-The nuclease is directed to its target sequence by a short RNA fragment known as a guide RNA (gRNA) or single guide RNA (sgRNA), which is complementary to the target segment of the genome.

The biological function(natural function)

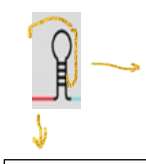
-When a phage infects a bacterial cell, the cell degrades the phage DNA into smaller pieces and integrates one of these fragments into the CRISPR cluster.

-When the phage infects the cell again, the cell transcribes the DNA into RNA (guide RNA or gRNA), which is integrated into the Cas9 nuclease and guides it to the phage DNA to degrade it.

*bacteria have immune system against bacteriophages(phages, bacterial viruses).

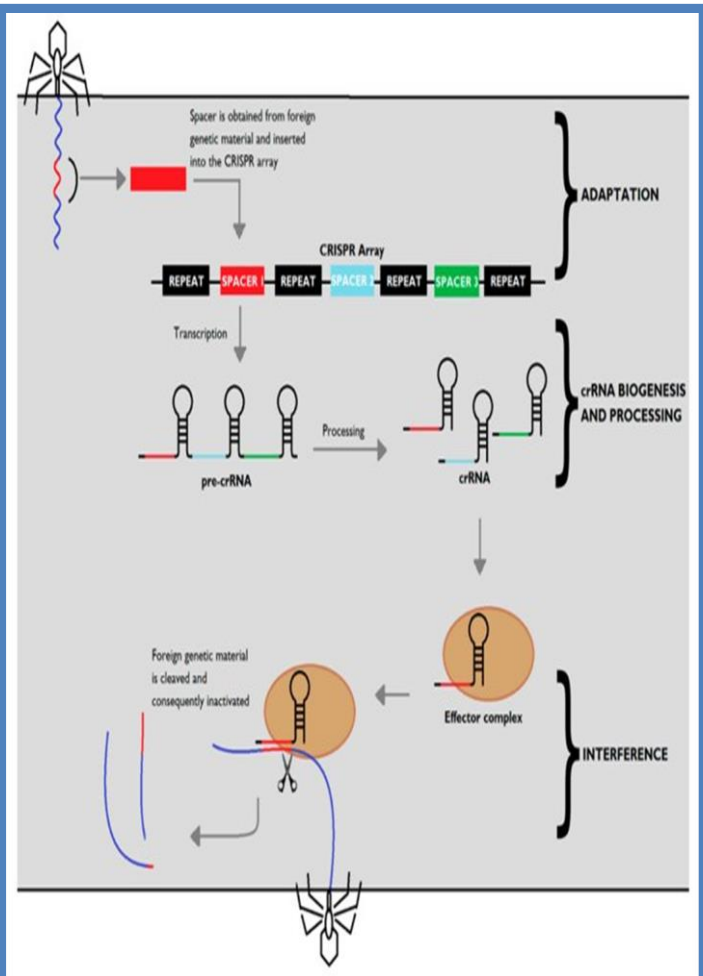
*bacteriophages infect bacteria by inserting their insects on cell membrane and inserting their DNA inside bacterial cells, this DNA is integrated into bacterial chromosome gets transcribed and translated and hijacks bacterial cell itself.

*bacterial cells have been evolved to invent a system to protect themselves from phages, like: restriction endonucleases and CRISPR-CAS9 system.



gRNA

crRNA



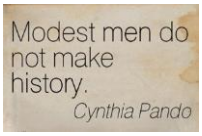
1)the bacterium activates enzymes like restriction endonuclease to cleave the DNA
2)the cleaved DNA is inserted to the bacterial chromosome, getting several sequences from different previous attacking phages(the coloured spacers) And they are separated by palindromic sequences(black) Forming CRISPR system(degraded DNA of phages separated by palindromic sequences)
3)the CRISPR(genetic)system is transcribed, producing several RNA molecule(CRISPR RNA)
4)CRISPR RNAs are cleaved to different RNA molecules called guide RNA (gRNA) containing the stem part(crispr RNA (crRNA)) and the looped with hydrogen bond part (the transcribed palindromic sequence)

*when another phage from the same type infects the bacterial cell again, the gRNA will bind to Cas9 and guides it to the DNA of phage to degrade the viral DNA, and NOT infect the bacteria!
*WHAT A BEAUTIFUL AND SOPHISTICATED SYSTEM!! 😶

*it learns us how our immune system learns of previous infections and exposures to antigens.

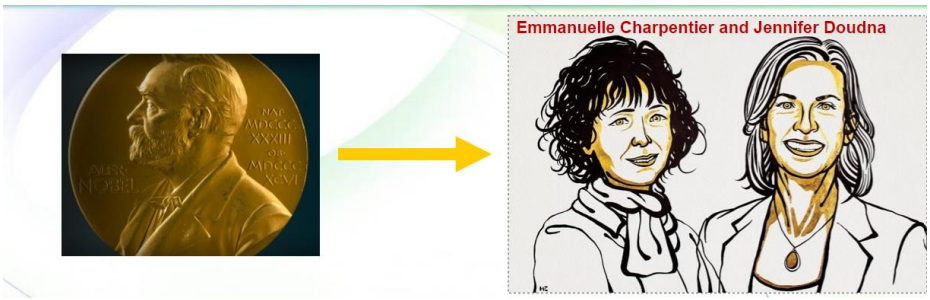
In 2020…

- A Portuguese scientist studied the biology of CRISPR-Cas9, but he is modest, it is not great achievement. Modest men don't make History.



*then French and American scientists took advantages of this system to edit genes and this is the pury of science to benefit from normal biological system, after 2 years they received noble prize.the doctor said:'its female power'.

The doctor is كغوو



**Remember from Molecular course: DNA repair mechanisms in human cells:

There are many repair mechanisms for DNA, here we will remind you with two of them.

*DNA breaks at different locations, for many reasons like X-ray hitting, the cell gets advantage of homology repair:

There are two mechanisms of homology repair:

1)Homology-directed repair: (HR)

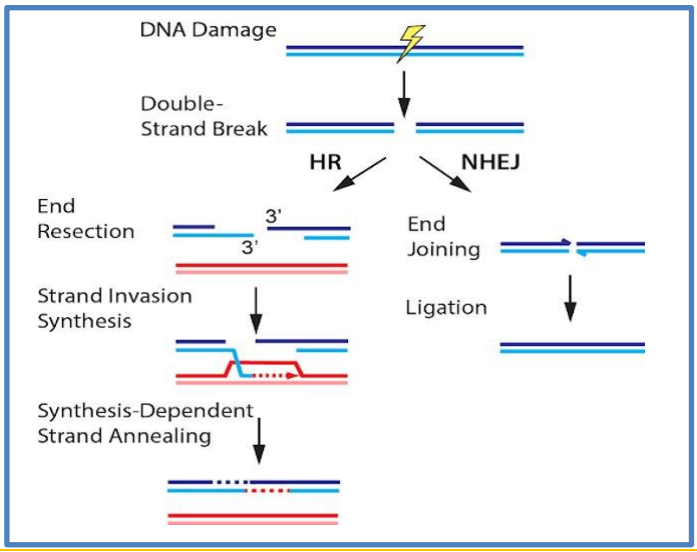
Our chromosomes are dipole(paternal & maternal),so if one chromosome is damage, there is a good chromosome

*it is an exchanging process, taking part of the good chromosome and puts in the damage chromosome, fulling the gap.

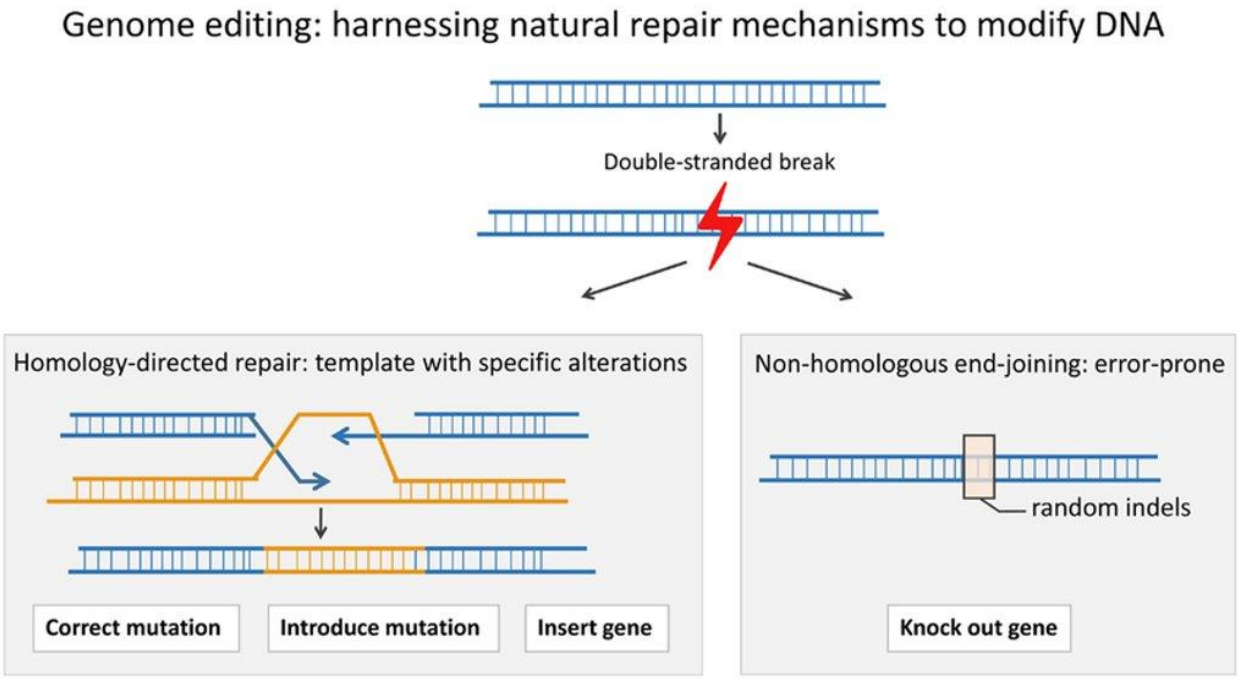
2) Non-homology end joining repair: (NHEJ)

There is a real damage, it doesn't depend on the presence of homologues chromosome,

*the 2 strands are glued together, the damage can still be noticed☹.



The consequences of DNA damage repair



HR: taking a piece of one strand from the proper chromosome and putting it in the damage chromosome
*it corrects mutations
*it is the replacement of a piece of chromosome and inserting a fragment of interest.

NHEJ:2 pieces are glued, creating indels.
*indels:insertion, deletion
You can notice that the DNA is repaired as its figure
*it causes insertion or deletion of nucleotides making a defective gene(mutated, not normal gene, doesn't function at all, or not properly)
*it is knocking out(removing) a gene from human genome

Last comparison!!

The system	**Homology directed repair**	**Non homology end joining repair**
The idea	take part of strand of good chromosome and put it in another to fill gap)	glue 2 strands together without needing to homologous chromosome
The results	Replacing piece of chromosomes or insert fragment into it	Having indels: insertion or deletion nucleotides that produce defective gene (non functional gene)
Need homologous chromosomes	Need	Don't need
The effect	100% repair	Not 100% because it make mutation

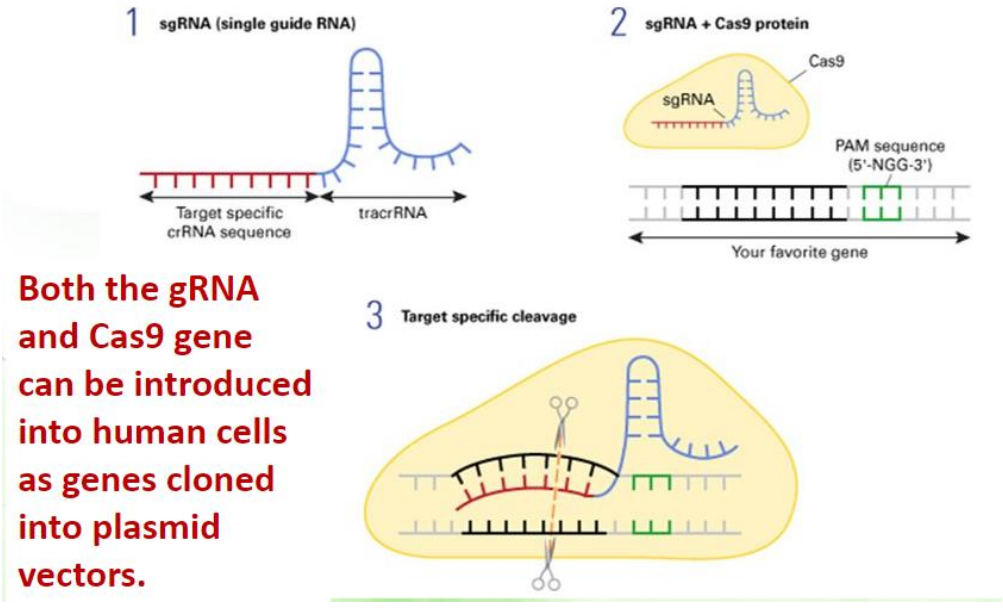
The steps of action

-How can we use HR combining with Cas9 in human cells?
To replace a gene, HR is activated by using Cas9 gene that can produce a protein (Cas9 nuclease) that does ONLY A SINGLE CUT(not 2), activating HR.
*the mechanism:
1)insertion of Cas9 gene along with RNA molecule that would be integrated into Cas9 protein*(forming Cas9 recombinant plasmid)

2)the plasmid is introduced to the cell, as the gRNA presented, it will guide the Cas9 to the region in the human chromosome that is complementary to the gene of interest (the gene we want to replace)

3)along with recombinant Cas9 gene, we have the introduction of the gene that we want to recombine into the chromosome::the part of DNA to replace the gene we are targeting
__we are telling the cell, we damage your chromosome(by Cas9), we are sorry, take this replacement, forget HR, there is one for you! ☐

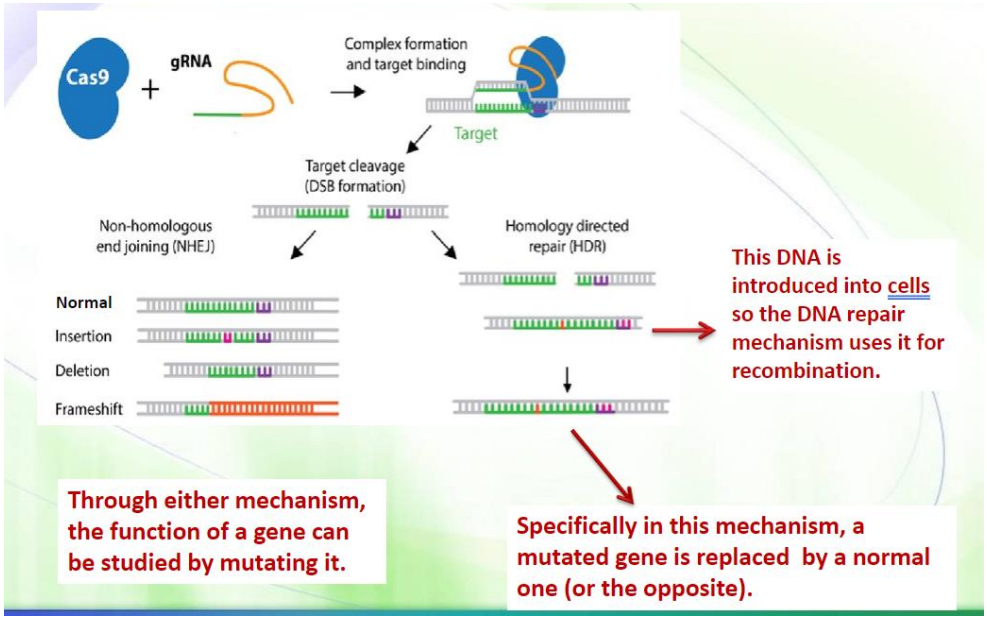
**the conclusion:
When the HR is activated, the cell does Cas9 to chromosome(could be human, mouse,…chromosome) we want to damage, after damaging the chromosome at certain location and activating HR, then HR use the fragment we introduce as DNA to replace the damaged DNA.We are tunning cells.



Gene editing

*what are the usages for Cas9?
1)fixation:we use the introduced DNA rather than the Homologues chromosome, so we replace the defective gene and put the good gene instead.
2)studying the effects of the gene if it is defected.
3)we can do the opposite of point 1, we can remove the good gene away and insert the defective gene instead, to recognize the function and important of the removed good gene.

**Cas9 can also activates NHEJ, so the system will glue the 2 strands of DNA causing indels(insertion, deletion)mutation, making a frameshift mutation,so the gene is disturbing.
We can use Cas9 activating NHEJ to:
1)damaging a cancerous gene
2)studying a certain gene by disturbing and mutating it.



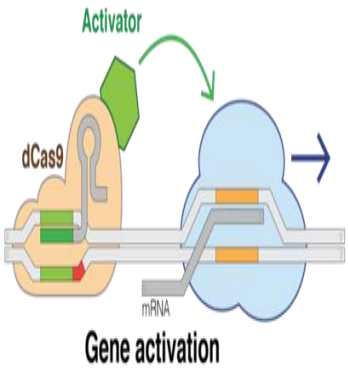
Other creative uses

*Transcriptional regulatory factors can be added to a "dead" Cas9 (dCas9), enabling them to turn genes on or off or adjust its level of activity.

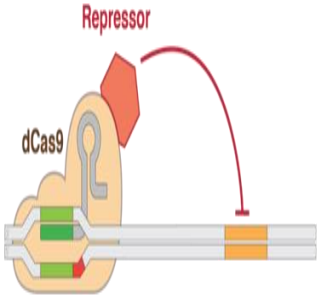
*GFP can be added to visualize genes

We can use Cas9 also to guid certain proteins, like transcriptional factors to a gene to activate or repress it.

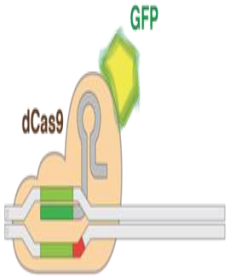
*there is a Cas9 that is associated with gRNA, but the Cas9 is defective, so we do genetic engineering, we take Cas9 protein goes to DNA of interest but without cutting it.
→it could be bound to:
1)activator: to activate transcription, we take it to gene we want to activate
2)repressor: to depress transcription, take it to PPE,
promoter region,
silencer on certain location and depress it.
3)Green florescence protein (GFP):so we can detect cells that takes up the Cas9 protein plasmid that contains Cas9 and study what will happen to the cell if this or that happened.



Gene activation

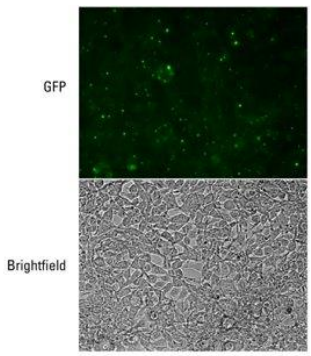


Gene repression



Visualisation

**we can see cells bound to Cas9 under microscope :

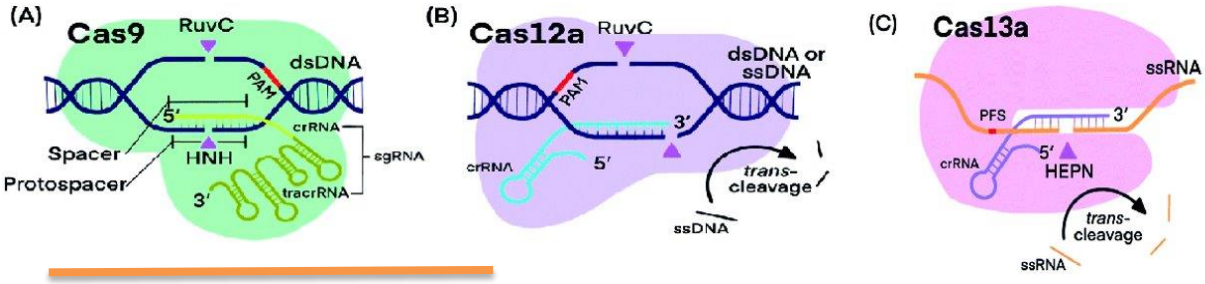


Under florescence microscope, the cells that fluoresces are the one with Cas9 protein

Cells under LM

Other Cas enzymes

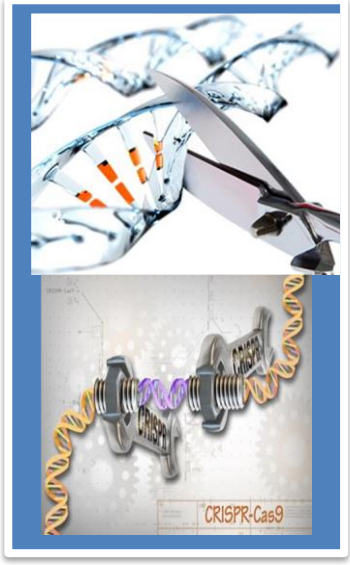
Since Cas9 has been discovered, other cas proteins were discovered.
1)our lovely Cas9: it introduces blunt cuts.
2)Cas12a: a smaller enzyme, it introduces staggered cuts rather than blunt ones
3)Cas13a:it is specific for RNA endonucleases
**different enzymes have been discovered but the scientists trying to be creative.



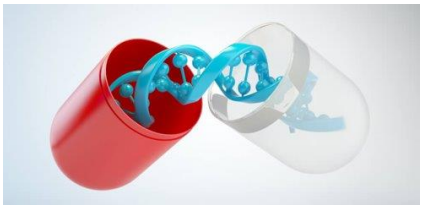
-let`s see some application of CRISPR-Cas9:

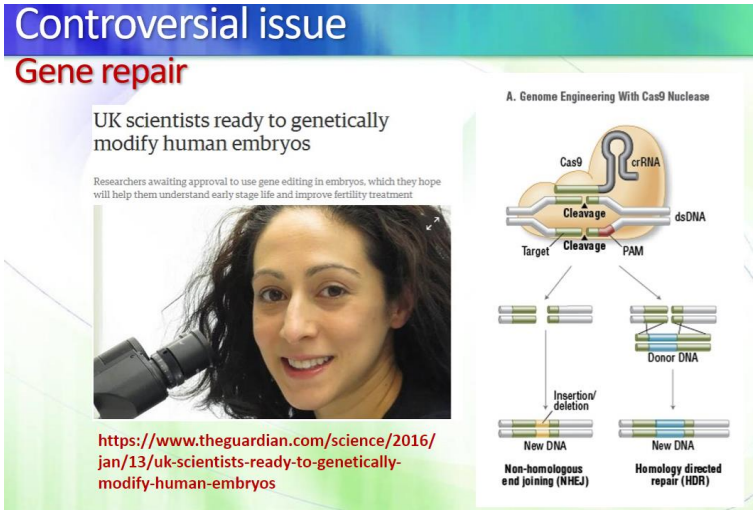
**scientists are trying to use our fingerprints,
editing our genes

*changes DNA, cuts certain regions to disturb it or fix it, using CRISPR as tools, wrenches, bolts



**for future uses, they are looking for using pills of DNA rather than chemicals one.





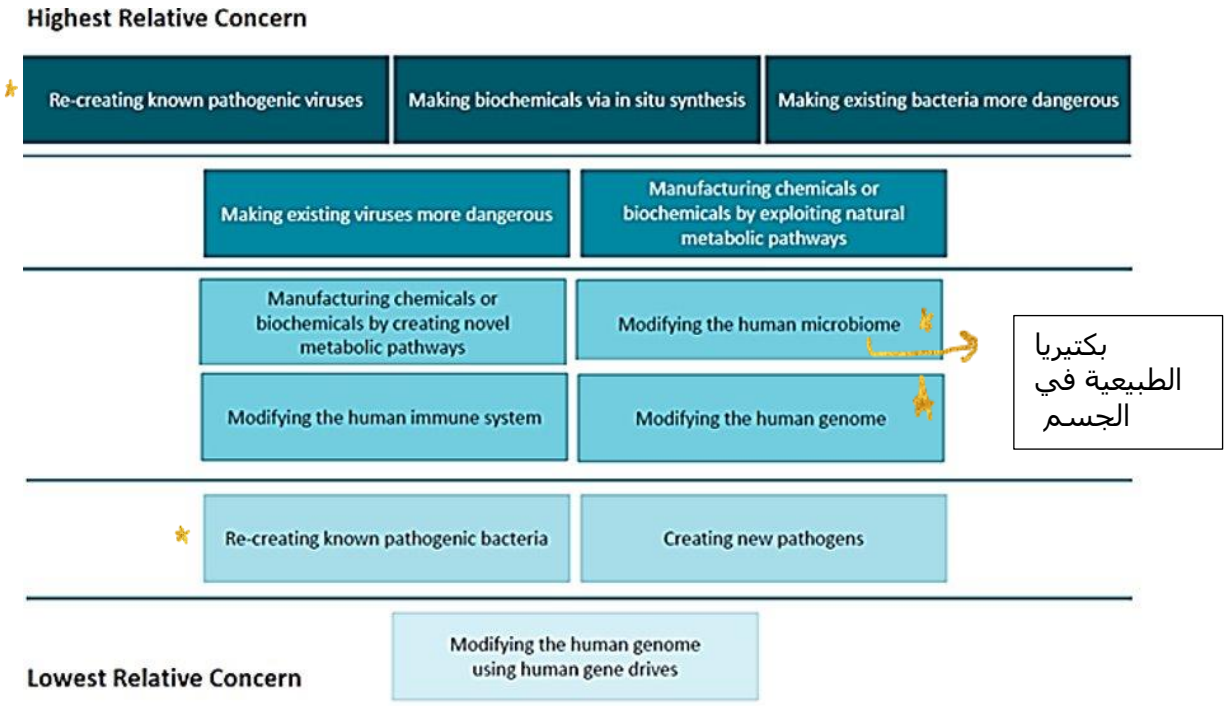
Scientists try to be creative with molecular techniques, this scientist-in the upside slide-genetically modified embryos using CRISPR-CAS9.So, they are trying to do many things some of them is really cool, earth shattering, great things to fix DNA and treat diseases.

Also, bad things happen the Chinese scientist-downside slide- modify the DNA of twins, he disturbed CCR5 gene(important for the entry of HIV) by damaging this gene, these girls will be protected against HIV, but it is also important for intelligence, we wont know what will happen.Actually, he did unethical experiment and he imprisoned 3 years.

*Bioterrorism

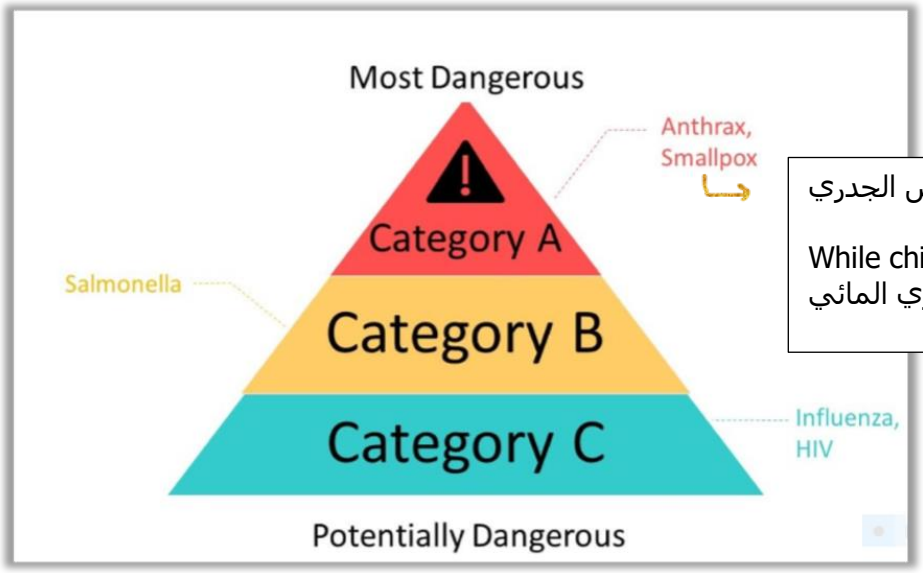
الإرهاب الحيويّ

Here, there are some examples:



بكتيريا الطبيعية في الجسم

***the Chart bellow classifies the danger of some viruses:**



فيروس الجدري

While chickenpox: الجدري المائي

Category A is the most dangerous
Category B isn't too dangerous, it is bad but not widely separated, like HIV.

Doctor Mamoun says to you: الله معاكم 🤝

PAST PAPERS:

Scientists have discovered a new protein hormone that is released from some cells and is transported to other parts of the plant, enter cells, and regulate gene expression, you aim to create mutations in the hormone gene, you can do this by:
a. Allow cells to express specific restriction endonuclease
b. Create a recombinant DNA with a glutathione-S-transferase gene conjugated to the hormone gene
c. Activation of homologous recombination following introduction of CRISPR-Cas9 system
d. Activation of non- homologous end joining following introduction of CRISPR-Cas9 system
e. Target the gene with specific primes

One of the following is NOT true regarding the CRISPR part of the CRISPR/Cas9 system?
A) It contains repeated palindromic sequences
B) It's a part of the bacterial genome
C) It contains DNA fragments of bacteriophage DNA
D) It's transcribed and translated
E) It encodes Cas9 protein

During gene editing by the CRISPR/Cas9 system, the insertion/deletion mutations (indels) are created by
a. The guide RNA (ORNA)
b. The CRISPR part of the system
c. Homology-directed DNA repair system
d. The non-homologous end joining DNA repair system
e. The Cas9 part of the system

10)One of the following is not true in regards to the CRISPR-Cas9 system :

A. It contains DNA fragments of bacteriophage DNA
B. It is part of bacterial genome
C. It encodes Cas9 protein
D. It contains repeated palindromic sequence

You want to purify this hormone, but you do not have an antibody that can help you purify it. You can do this:
A. Protein tagging
B. CRISPR-Cas9 system
C. Proteins expression in bacteria
D. Immunoprecipitation
E. Reporter gene assay

D
E
D
C
A

"يرفع الله الذين
امنوا منكم والذين
أوتوا العلم درجات"