Doctor 022

Biochemistry

Sheet no. 3

DNA REPAIR MECHANISMS IN HUMAN CELLS

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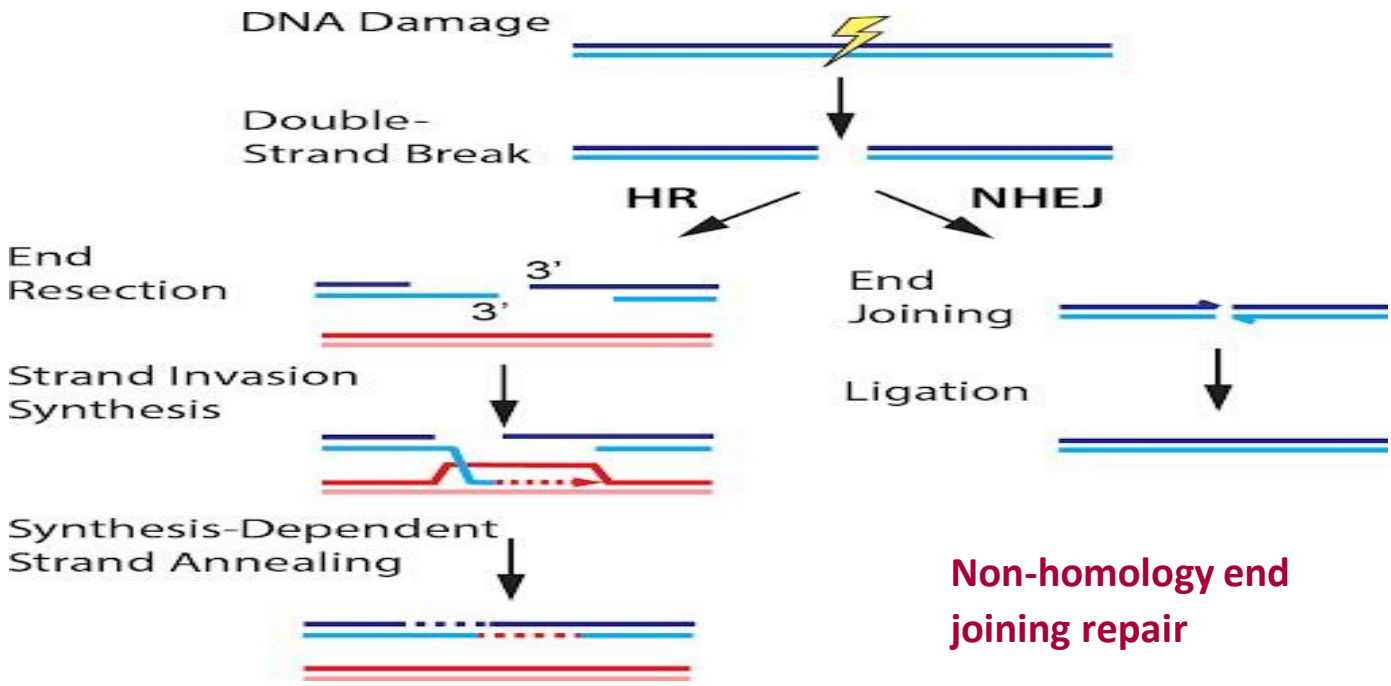
DNA REPAIR MECHANISMS IN HUMAN CELLS



Homology-directed repair

Non-homology end joining repair

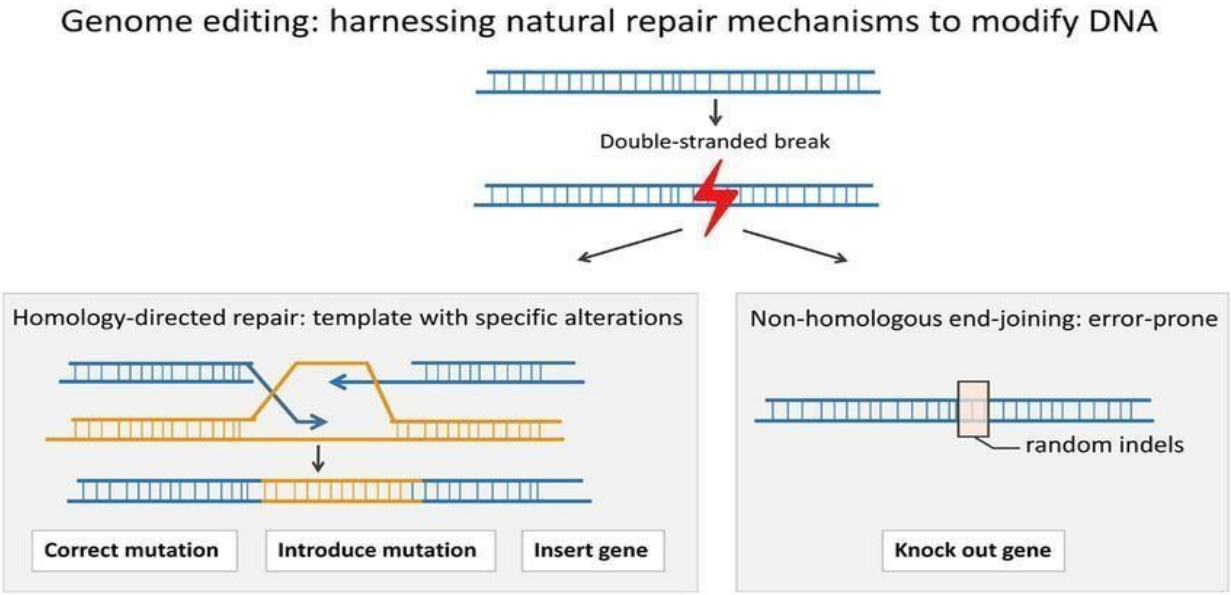
Our body can get mutated,for example cells DNA can get mutated thousand of times,but we do not get infected because we have repair mechanisms,especially skin cells because these cells exposed to the sun also bladder cells (transitional epithelium) these cells exposed to toxic material

In the molecular biology course, which you perfectly enjoyed, we talked about those 2 repair systems, homology-directed repair (on the left) and non-homology end joining repair (on the right).

Homology-directed repair: our cells are diploid, so we can take advantage of the existence of a homologous chromosome, so we take a part from the homologous chromosome and put it in the damaged site then we just fill the gap.So when apart of DNA is lost the cell use the complementary strand so it is more accurate.

Non-homology end joining repair: here, we don't depend on the homologous chromosome, it is close to the concept of a "glue".So in this mechanism it will induce error (indels mutation) like adding or removing nucelotide so different codon so different amino acid

THE CONSEQUENCES OF DNA DAMAGE REPAIR

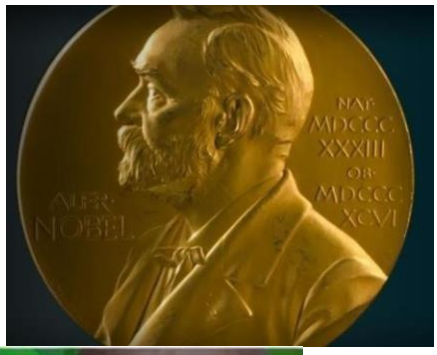


So, when we use non-homology end joining repair, there will be indels (insertion/deletion mutations), it's like when you glue a broken vase, you can still see that it was broken and glued, which will lead to a non- or miss-functional gene, so it's like knocking out a gene from the human body.

Using homology-directed repair, it's like you are fixing a mutation by inserting a DNA fragment, and this sentence seems iconic (inserting a DNA fragment), because this is exactly what they did. **IN 2020…**

One of the weirdest concepts to be written in a sheet.

-writer



Years ago, Portuguese scientist Francis Mojica discovered CRISPR system in bacteria, 2 years later, Emmanuelle Charpentier and Jennifer Duodna took advantage of this system by entering the field of gene editing, and they won Nobel prize, “women power”, the doctor said, and REMEMBER, MODEST MEN DO NOT MAKE HISTORY!

WHAT IS CRISPR/CAS9?

(Future of medical treatment, enjoy!) vary dangerous technology but promising in treatment.

CRISPR is (an abbreviation for): clustered regularly interspaced short palindromic repeats.

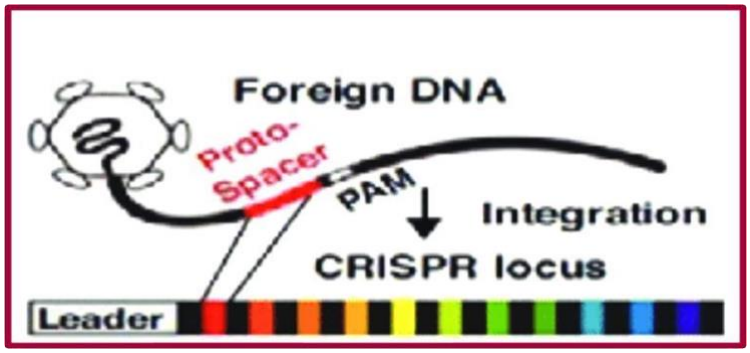
Quick elaboration: 1-repeats: repeated sequences/2-palindromic: you can read them from 5➐3 in the same exact order in both strands/ 3-short: are you looking for an elaboration? /4-regularly interspaced: there are spaces between them, and those spaces are specific between each repeat and the another(regularly) /5-clusters: all located as clusters (groups of sequences).

It is a bacterial genetic system that constitutes the immune system of bacteria against phages like virusrs

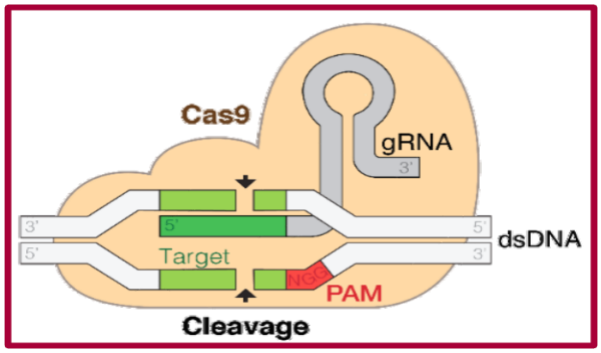
Cas9 is an RNA-guided nuclease (it cannot cleave, unless there is an RNA molecule associated with it, that guides it to certain sequences) that can either create single or double strand breaks.

Side note: we can also call it a Ribonuclease, which means a nuclease attached to an RNA molecule.

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.

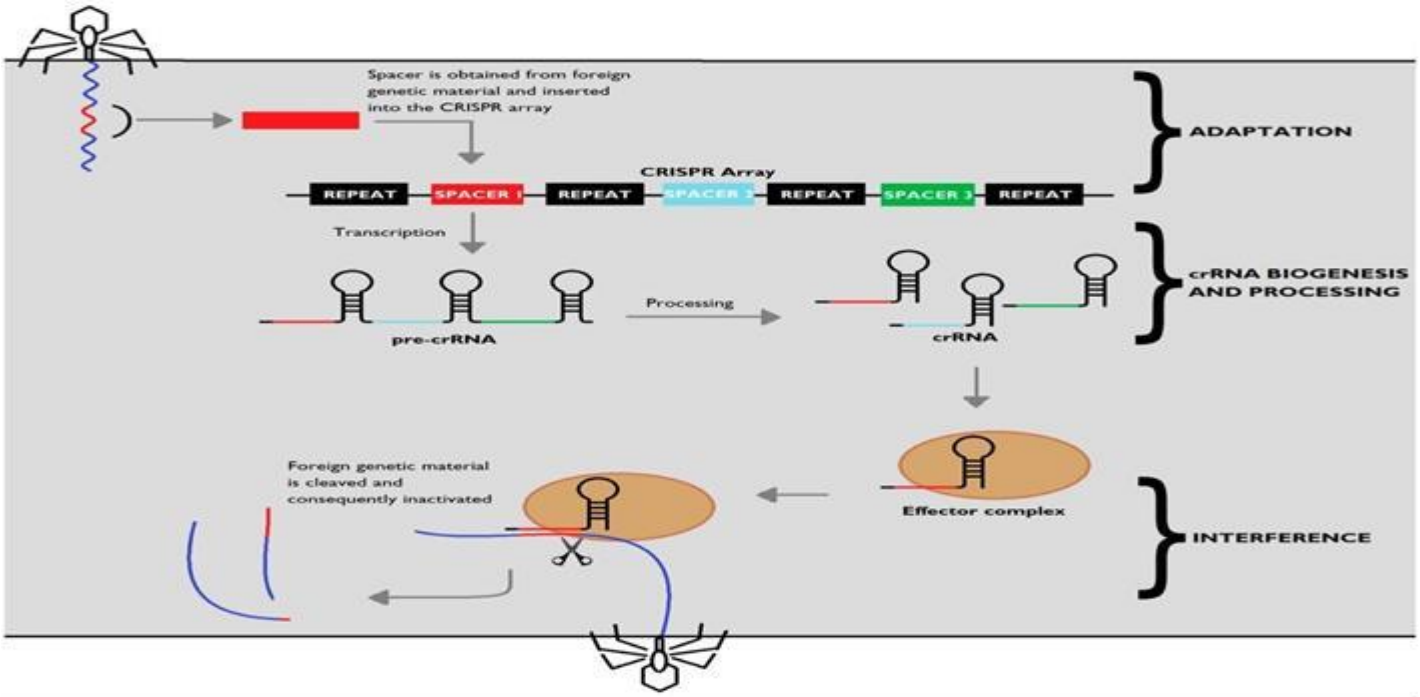


CRISPR



Cas9

THE BIOLIGICAL FUNCTION (the concept)



What happens normally without immune response?

The bacteriophage inserts its DNA into bacteria, and this DNA integrates with bacterial DNA and gets transcribed and translated, so the phage almost “hijacks” the bacterial cell.

One of the ways that are used by bacteria to defend themselves- that had already been mentioned- is the restriction endonucleases, CRISPR is a similar idea.note that the bacteria do not use CAS9 when it get infected for the first time (;

- **When a phage infects a bacterial cell, the cell chops off the phage DNA into smaller pieces and integrates one of these fragments into the CRISPR cluster. (each space between repeats will contains a piece of a different bacteriophage that have infected the bacterium before).**

- **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.**

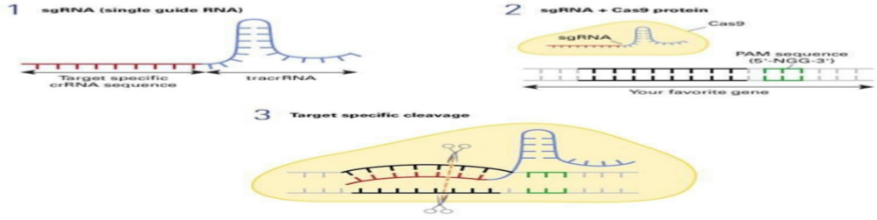
Note: please try to understand every step in the figure above….

THE STEPS OF ACTION

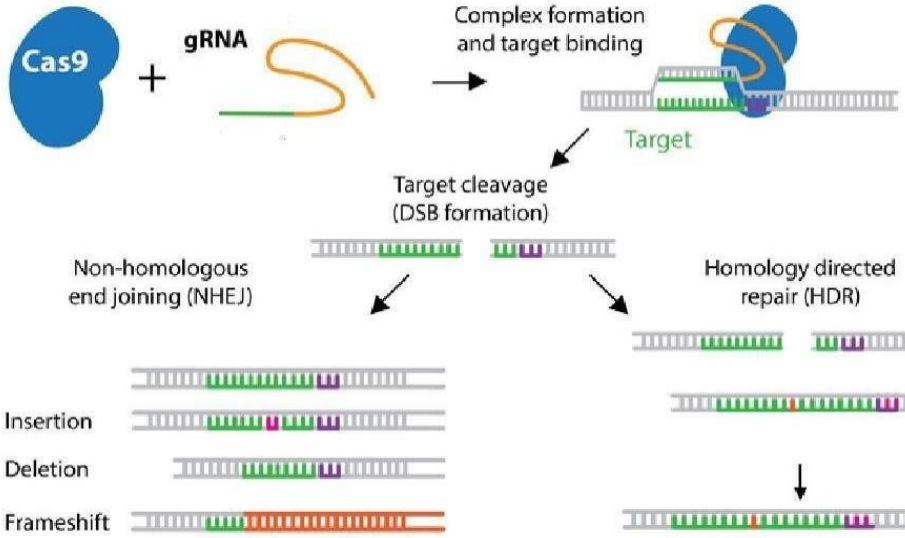
So to replace a gene in the human genome the homology- directed repairing system is activated, and it is activated by using a Cas9 gene that can produce a Cas9 nuclease that can only create one cut ,not 2 cuts because that what activates the homology-directed repairing system .but how does Cas9 do that?

Using recombinant DNA technology, we insert the Cas9 gene into a plasmid, along with the RNA molecule that would be integrated with Cas9 protein, so this recombinant gene will be introduced into human cells, and upon translation, the Cas9 protein will be able to function as it will be bound to RNA, and this RNA will guide it to a specific sequence (gene of interest that we want to replace Or cut) in the human genome which is complementary to the DNA

Along with Cas9 recombinant gene, you have the introduction of the gene that we want to recombine with the chromosome (the gene we wan toadd),sowejustFOOL^{the} cell,oops,we damaged ^{you} Take this replacement and forget about your homologous chromosome (what a glorious dialog).



GENE EDITING



This DNA is introduced into cells so the DNA repair mechanism uses it for recombination

(Fooling the cell)

So, when the cell normally does homology-directed repair, there is a chance of having a defective gene, so what we do when we fool the cell is that we just introduce the normal gene,(fixe mutation)

Through either mechanism, the function of a gene can be studied by mutating it. (So, we take the good gene out and replace it with a mutated one, ok not going to deny it, we are bad.)

Sometimes, after Cas9 cleave the DNA, the cell might go the other way and use non-homology end joining repair, so we will have indels (insertion/deletion which lead to frameshift mutations).

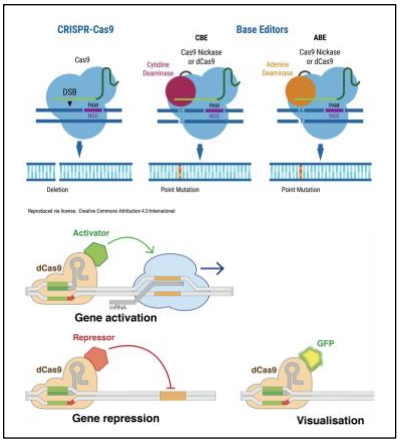
OTHER CREATIVE USES

(we can gide Cas9 to specific site to the DNA but not make a cut by disrupt his nuclases activity and carry with him another enzyme and it will bind to DNA)

Bace editing

if deaminated,C is converted to U ,which is read by the DNA plymerase as T changing CG to TA.(DNA repair mechnisim covert U to T so we change DNA)

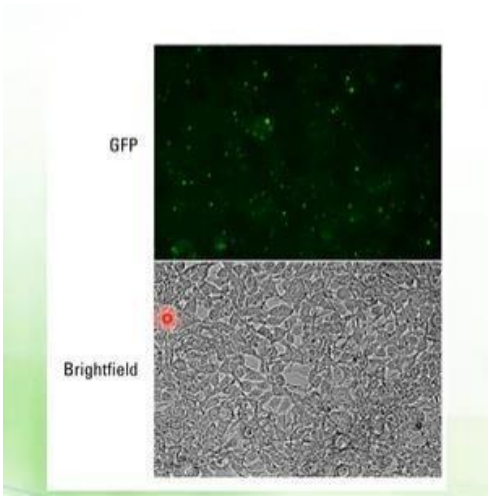
***if deaminated,A is converted into inosine, which read by the DNA polymerase as G changing AT to GC.**



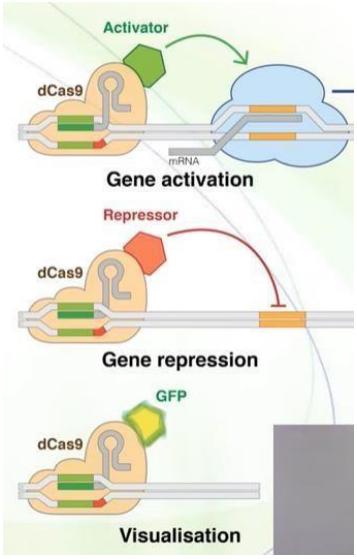
Transcriptional regulatory factors can be added to a "dead" Cas9 (dCas9), enabling them to turn genes on and off or adjust its level of activity.

Simply, we have a mutated Cas9, so it will not cut DNA, but it still can be bound to a gRNA to travel around our genome and attach to specific sequences in it, and we attach it to an activator or a repressor so we can turn genes on and off.

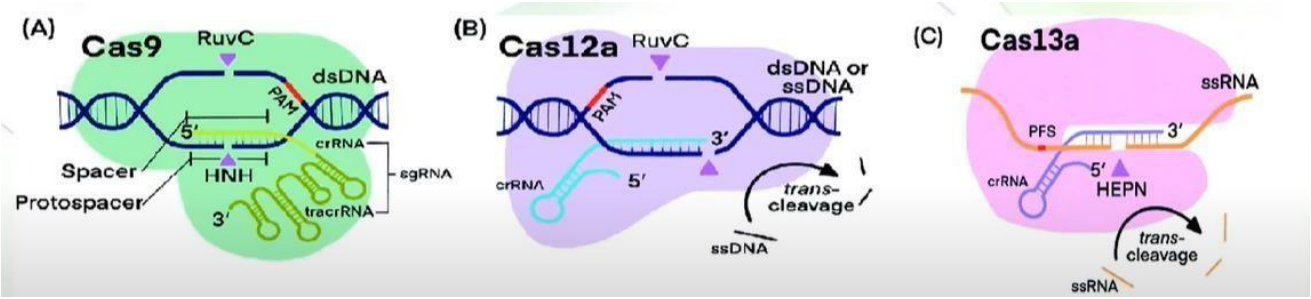
GFP (green fluorescent protein) can be added to visualize genes. (it is attached the same way as the previous point). - in the figure below the cells that fluoresce are the one that have the C9 protein-.



these photos are related to the previous topic.



OTHER CAS ENZYMES



Since Cas9 system has been discovered, we discovered some similar enzymes, such as:

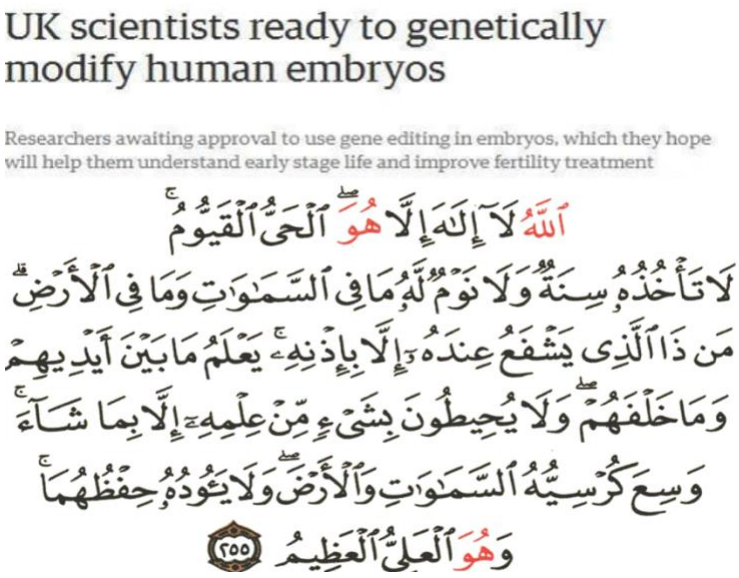
Cas12a: a smaller enzyme that introduces staggered cut. (not on the same line)- unlike the Cas9 that creates blunt cuts- (notice the figure).

Cas13a: an RNA endonuclease. Target RNA

With these random beautiful photos, this is what scientists are trying to do, they are trying to edit DNA using CRISPR/Cas9 system to get rid of some genes or adding some useful genes, and as genetic science is escalating rapidly, who knows, we might reach the level of TAKING this system as a pill, deleting the laboratory boring pictures of our minds.

CONTROVERSIAL ISSUE/ THE DARK SIDE OF SCIENCE

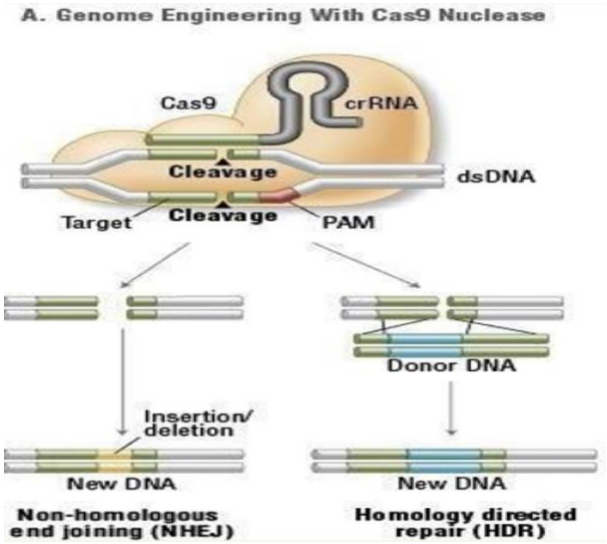
Btw, scientists are going outrageous with this system, go and read, its beautiful!



UK scientists ready to genetically modify human embryos

Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment

ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلْحَىُّ ٱلْقَيُّومُ ۚ
لَا تَأْخُذُهُۥ سِنَةٌ وَلَا نَوْمٌ ۚ لَّهُۥ مَا فِى ٱلسَّمَٰوَٰتِ وَمَا فِى ٱلْأَرْضِ ۗ
مَن ذَا ٱلَّذِى يَشْفَعُ عِندَهُۥٓ إِلَّا بِإِذْنِهِۦ ۚ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ
وَمَا خَلْفَهُمْ ۖ وَلَا يُحِيطُونَ بِشَىْءٍ مِّنْ عِلْمِهِۦٓ إِلَّا بِمَا شَآءَ ۚ
وَسِعَ كُرْسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلْأَرْضَ ۖ وَلَا يَـُٔودُهُۥ حِفْظُهُمَا ۚ
وَهُوَ ٱلْعَلِىُّ ٱلْعَظِيمُ ﴿٢٥٥﴾

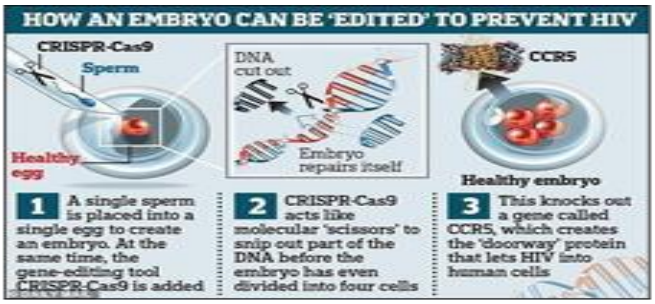


https://www.theguardian.com/science/2016/jan/13/uk-scientists- ready-to-genetically-modifyhuman-embryos

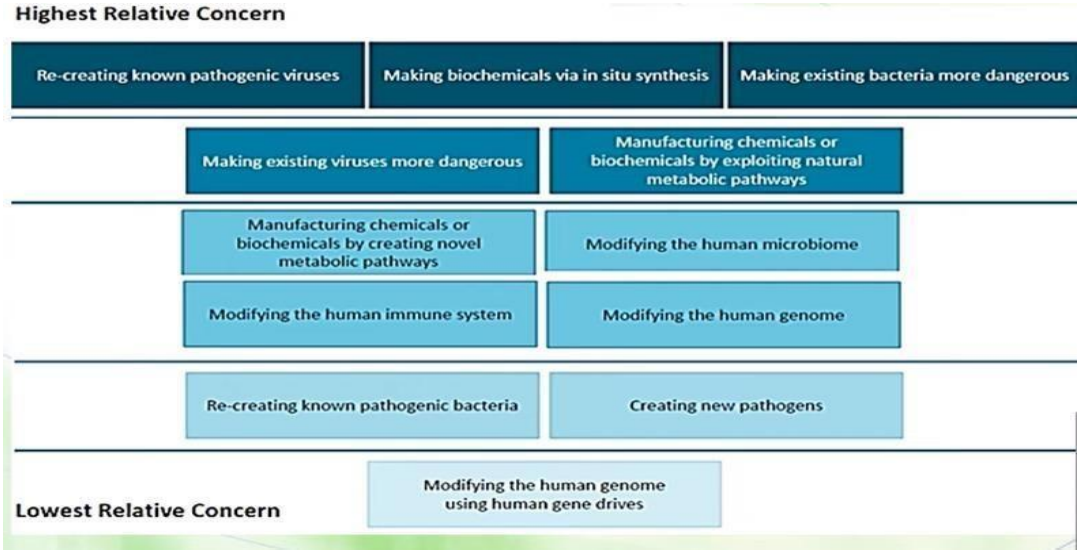
https://www.theguardian.com/wo rld/2019/dec/30/gene-editing- chinese-scientist-he-jiankuijailed-three-years

this Chinese doctor used CRISPR/Cas9 to knockout

the gene that causes HIV to enter our cells from 2 twin girls, but the gene seems to be important in other things like intelligence, so this was reported to be unethical and he was punished, read more! So, you thought the lecture was going to end when the doctor showed some links and newspaper screenshots, well I thought the same lol, stay focused.



BIOTERRORISIM (not important currently)



What scientist are trying to do seems cool, but it still can be used for bioterrorisim, there are many things that can be done including modifying the human genome, modifying the human microbiome, recreating known pathogenic bacteria or making existing bacteria more dangerous, etc., this all can be used in biological weapons and other ways to hurt humanity.