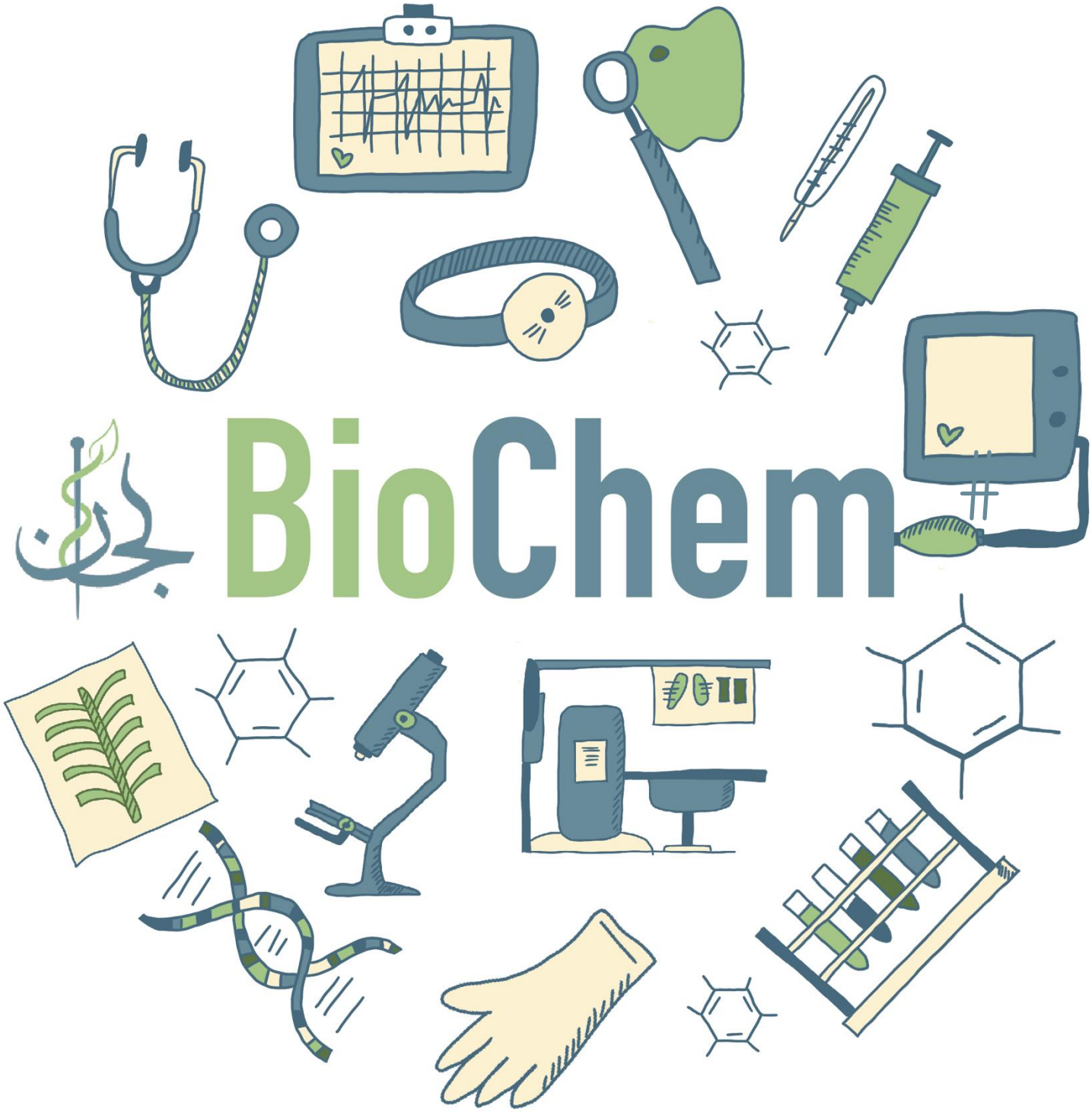

BioChem

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Recombinant DNA-based molecular techniques :(The third part)

An exciting and new technology that known is as CRISPR-CAS9 system.

This system has changed the fields of not only molecular biology but also medicine at a hole!

It is a bacterial system that composed of two components: a genetic system known as CRISPR and an enzyme known as CAS9.

Genome editing by CRISPR/Cas9:

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats.

For more clarification: -

It is short sequence that is repeated over and over again and these sequences are Palindromic.

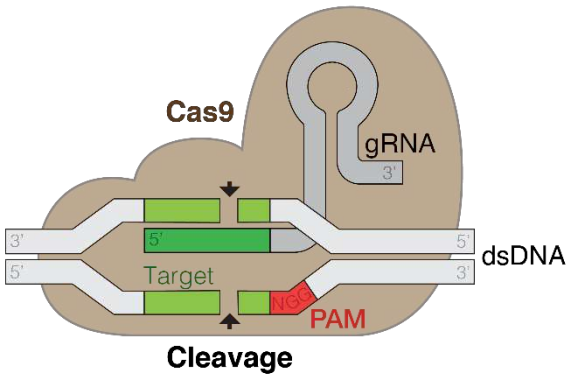
They are interspaced meaning that they are separated by other sequences and this separation is regular and all of these repeats are clustered within one part of the genome.

CRISPR system is a genetic system that is part of the bacterial genome (the bacterial chromosome) and it contains different fragments from different bacteriophages.

THIS WILL BE CLARIFIED LATER ON, IN THIS SHEET!

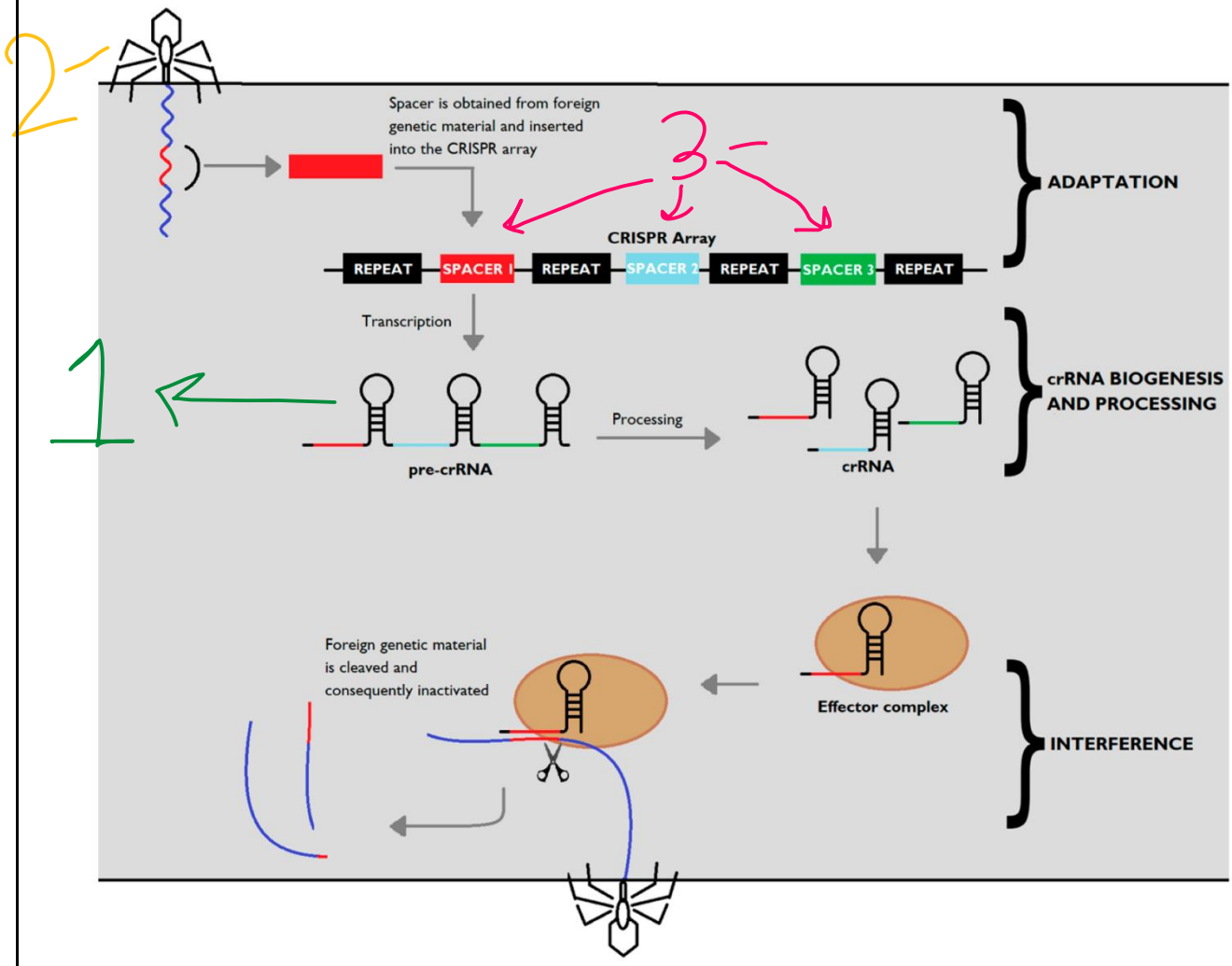
It constitutes the immune system of bacteria against phages. We can say that the bacteria created this system in order to protect themselves against the bacteriophages or the viruses that infect these bacterial cells.

Cas9 is a RNA-guided nuclease that can either create single or double strand breaks. It is a ribonucleoprotein which means that we have two parts: protein part (CAS9) and a ribonucleic acid part (RNA MOLECULES PRODUCED BY CRISPR SYSTEM).



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

Now please look at the figure below in order to understand the process:-



You can see that we have Palindromic sequences so the RNA will have this shape and hydrogen bonds would form between the different Palindromic sequences.

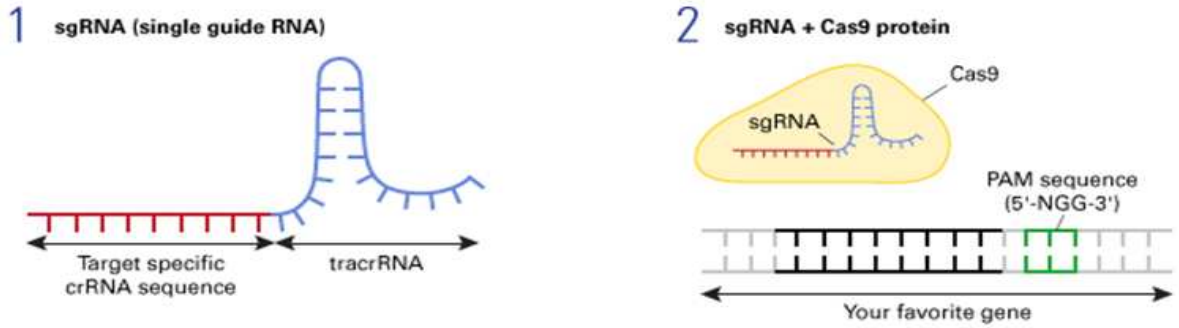
[remember, the important thing about the palindromic sequences is that they are read the same on both strands from 5' to 3' so they are complementary to each other]

When a phage infects a bacterial cell by inserting its genetic material into this bacterial cell, the bacteriophage's DNA is cut into different parts and fragments where one of these fragments is taken and is inserted into the cluster region. If another bacteriophage infects the same cell the same thing will take place, in other words the cell will chop off the phage DNA into smaller pieces and integrate one of these fragments into the CRISPR cluster. the two pieces of DNA in the CRISPR cluster that taken from different phages are separated by repeated sequences (they are regularly interspaced) as you can see in the figure, spacer1, spacer2 and spacer3 -.

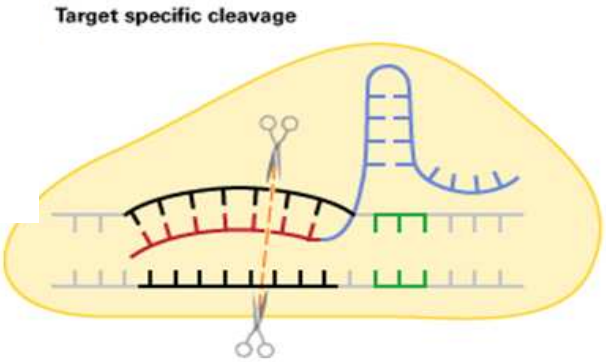
When the phage infects the cell again (REINFECTION), the cell transcribes the region of CRISPR in the DNA into RNA (gRNA).

So we have these RNA molecules and they get processed to give us different CRISPR RNA molecules, each one of them is integrated into the Cas9 nuclease (it becomes a ribonucleoprotein) and guides it to the phage because we have complementary base pairing between the bacteriophage DNA and the RNA which is now part of the Cas9 to degrade it, so now the bacteriophage cannot overcome the bacterial cell and this is how the bacteria can protect itself against the bacteriophage.

The steps of action:



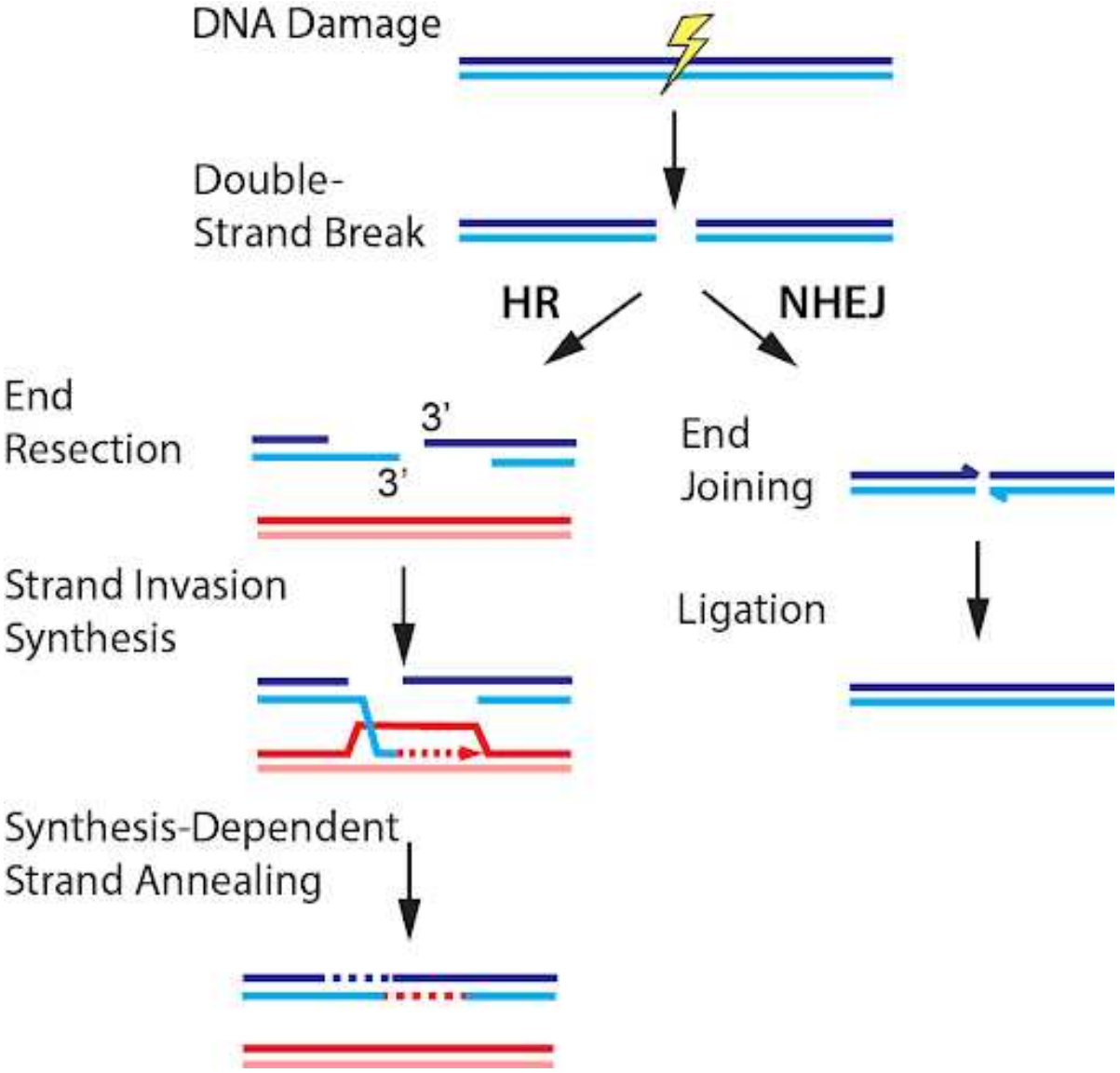
Both the gRNA and Cas9 gene can be introduced into human cells as genes cloned into plasmid vectors.



We can take advantage of the CRISPR-CAS9 system by inserting RNA molecule of our interest into the Cas9 system, so we guide the cas9 nuclease into a region of interest which must be complementary to the RNA molecule that is now part of the cas9 protein as a result we can cleave this region.

So we create a Recombinant DNA molecules (plasmids) that contain the gene which would be transcribed to this guide RNA and the plasmid would also produce Cas9 as well by introducing the bacterial gene into the human cell, so Cas9 will do the same thing as it does in bacteria and we can now cleave (cut) the human DNA at this particular site

A DNA repair mechanism in human cells:



Remember when we have talked about DNA repair mechanisms, so how we can repair the double-strands break (Cas9 creates double-strands break)?

They can be repaired by recombinational repair mechanism known as homologous recombination (HR) which is taking advantage of the cell being diploid (having two copies of every chromosome-2n) so if one chromosome is damaged the cell can exchange between the genetic material from the homologous chromosome filling in the gap created by the Cas9, repairing the DNA.

The other type of repair mechanism is known as non-homologous end joining (NHEJ): in this case the cell glues the two strands with each other, and the chromosome sequence is continued but the mutation might be created.

So what are the benefits of CRISPR-Cas9?

We use it to fix the mutations in the DNA and to study the benefits of some genes, so how does that happen?

1-First thing the cas9 and gRNA are introduced (the guided RNA) which guides it to the complementary DNA sequence, then this complex acts as an endonuclease and DNA is cut.

2-Then this broken DNA can undergo either non homologous end joining (NHEJ) (which creates mutations) or homologous end joining (HEJ):

A-If it undergoes NHEJ procedure, we can use it to know the benefits of specific gene by creating a mutation in the place where the gene is cut and rejoined (gluing the ends with each other) so it becomes dysfunctional.

B-If it undergoes HEJ procedure, we can fix mutation or disturbed a gene:

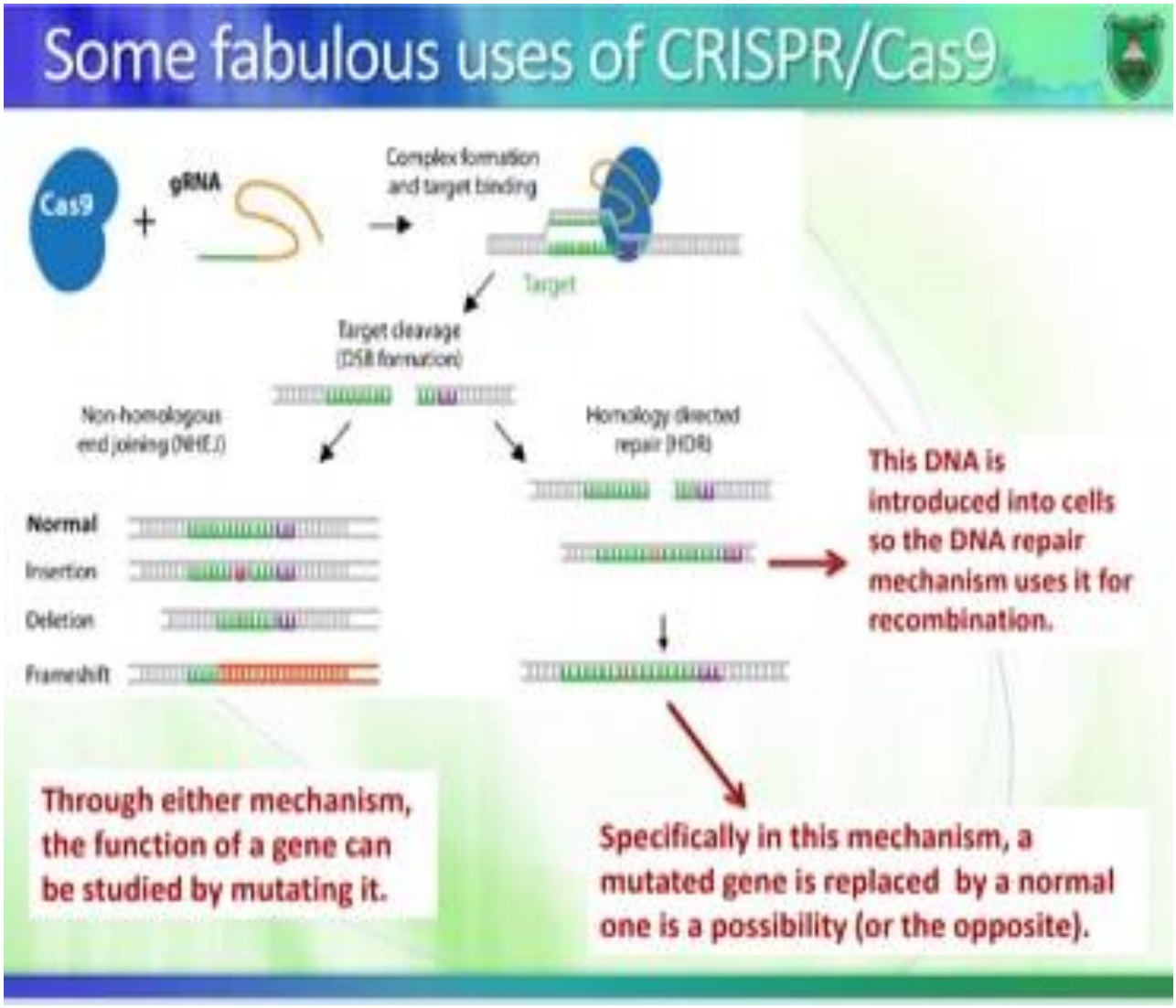
*-If we want to fix: after the cas9 and gRNA system works as endonuclease and cuts the DNA we insert a gene with the correct sequence instead of the mutated one, and to disturbed as gene (to know its benefit for an example) we replace the gene with correct sequence with mutated one.

So to summarize:

After the DNA has been cut it can go either HEJ system or NHEJ:

****Through either mechanism, the function of a gene can be studied by mutating it (this sentence includes both HEJ and NHEJ)**

HEJ: **Specifically in this mechanism, a mutated gene is replaced by a normal one is a possibility (or the opposite**)



This video is very helpful and helps you to understand what is written above (its only 4min watch it):

https://www.youtube.com/watch?v=4YKFw2KZA5o

Just know this, it is not required (just to benefit yourself):-

A Chinese scientist makes modifications in 2-baby girl embryos, where he used the cas9 and gRNA to cut DNA in the position of ccr5 gene making it dysfunctional, as you know if a cell has the ccr5 receptor on its surface then HIV virus (AIDS) can infect the cell, and since ccr5 isn't presented they are immune against HIV infection, in addition a new research shows that by removing ccr5 the human intelligence increases! But that's not always good since the complex can move and target another parts of the DNA and creating new mutations!

Summary:

When a virus attack a bacterial cell:

1-Its DNA is cut into small pieces and only one piece goes to CRISPR system.

2-When virus attacks again the DNA piece is transcribed into guided RNA.

3-Then gRNA attracts the cas9 enzyme.

4-Finally, this complex attacks the virus DNA and act as endonuclease and the virus is killed.

*Cas9+gRNA form a complex that is used to fix mutations and stops some genes from working allowing us to know their benefits.

**Note: the Blue color refers to the doctor words, the Black refers to slides words, Red refers to summaries in our own words.

SHORT QUIZ

1. The Underlined “R” in the word CRISPR refers to:

a) repeats b)regularly c) RNA d)none of these are correct

2.Through both HEJ and NHEJ, the function of a gene can be studied by..... it

a) exonuclease b)endonuclease c)mutating d) none of these

3 . In NHEJ we take the advantage of the cell being diploid

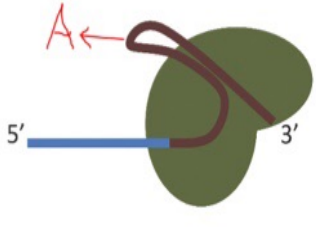
A) true. B) false

4. The CRISPR function in bacteria is acting as immune system

A) true. B) false

5. The RNA which is referred by the letter “A” is :

a) mRNA b) gRNA c) sgRNA d) b and c are correct



ANSWERS

Q1	Q2	Q3	Q4	Q5
B	C	B	A	D