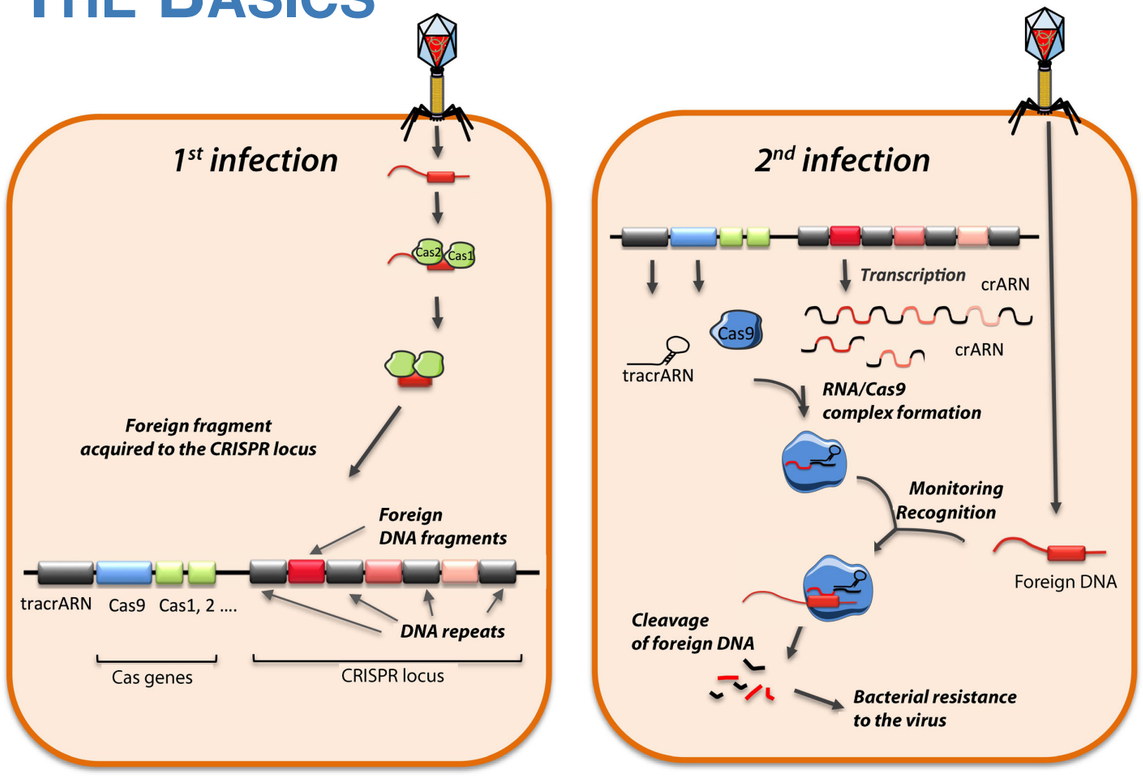
CRISPR-Cas9 TARGETED GENOME EDITING

BRAM LOGGHE

THE BASICS



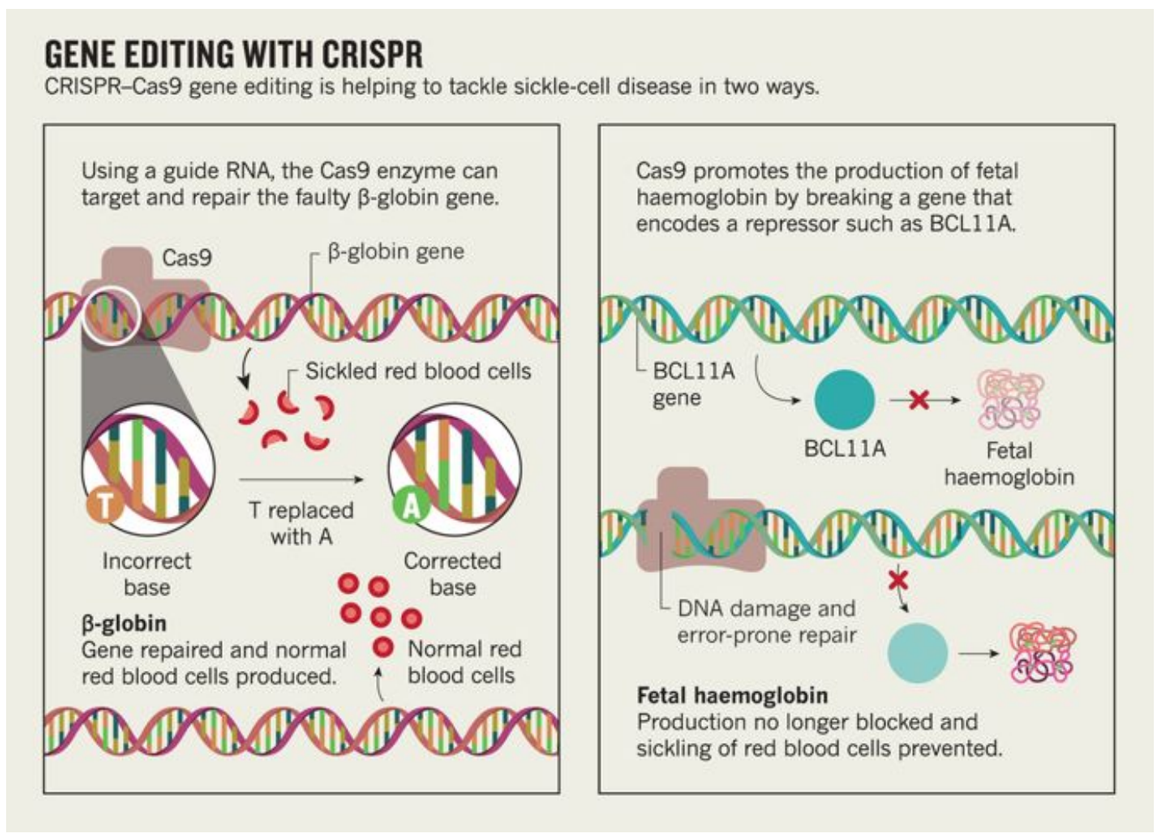
The CRISPR/Cas system exists of several components:

- CRISPR locus:
  Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- Cas9: nuclease
- Guide RNA

When a bacterium is infected by a virus and survives, a fragment of this viral DNA is inserted in the CRISPR locus without its PAM (**P**rotospacer **A**djacent **M**otif) sequence. Cas9 uses this sequence 1) as a flag to confirm it is detecting foreign DNA and 2) as cleavage site. Those fragments in the CRISPR locus are transcribed into RNA, processed and serve as a guide for Cas9 to target the foreign viral complementary DNA. Only if the PAM is present in the target DNA it breaks the double helix. This is to prevent breaking down its own CRISPR locus.

GENOME EDITING

These principles can be used to target and modify eukaryotic DNA by using a custom guide RNA sequence that is complementary to the target DNA of interest. This system will induce a double-stranded break which triggers the DNA repair mechanisms which will try to repair this by 1) either non-homologous end joining (NHEJ) that introduces indels leading to a premature stop-codon or 2) homology-directed recombination (HR) that is knocking-in donor DNA.



CRISPR Therapeutics is using this technology as a new therapy for sickle cell disease. Their approach is to unleash fetal haemoglobin production (HbF) because this haemoglobin does not include β-globin which is mutated in this disease. After birth a gene called BCL11A encodes for a suppressor which inhibits the transcription of HbF. CRISPR Therapeutics is focusing on this gene by inserting a modified Cas9 protein with its corresponding guide RNA specific for this BCL11A gene. When inserted the Cas9 protein does as described above and induces a double strand break. This will trigger the DNA repair mechanisms by using NHEJ and disrupting the function of the repressor, allowing cells to produce HbF. Another option is by using the HR repair mechanism. Here, Cas9 is used to break the helix amounting to the β-globin and knocking-in a corrected gene instead. This method requires more work and is prone to mistakes.

FUTURE

The use of this technology could be a major breakthrough in various domains. This system can be used for diagnosis as well. The cleavage sites of Cas9 could be inactivated and a fluorescent protein could be attached. This will make Cas9 stick on the target DNA and visualize its position. The same method could be used to turn the transcription of certain sequences off or on.

A disadvantage of this technique might be the off-target mutations when the guide RNA is not 100% accurate. A solution to prevent this problem could be by editing Cas9 so it can only break one of the two strands. This means that two Cas9 proteins are required at the same place before genome editing can be performed.

REFERENCES

1. Duroux-Richard I, Giovannangeli C, Apparailly F. CRISPR-Cas9: A revolution in genome editing in rheumatic diseases. Joint Bone Spine. 2017;84(1):1-4.
2. Crispr Genome Editing, Cas9 Crispr | TransOMIC [Internet]. Transomic.com. 2018 [cited 8 November 2018]. Available from: http://www.transomic.com/Products/CRISPR-Genome-Editing.aspx#1b536e10-d943-4168-8e55-af2ce6f3be06,7230592
3. What is CRISPR-Cas9? [Internet]. Yourgenome.org. 2018 [cited 8 November 2018]. Available from: https://www.yourgenome.org/facts/what-is-crispr-cas9
4. Hemoglobinopathies [Internet]. Crisprtx.com. 2018 [cited 8 November 2018]. Available from: http://www.crisprtx.com/programs/hemoglobinopathies
5. Bourzac K, Pauling L, Itano H, Singer S, Wells I, Ingram V et al. Gene therapy: Erasing sickle-cell disease [Internet]. Nature. 2018 [cited 9 November 2018]. Available from: https://www.nature.com/articles/549S28a
6. http://www.freepik.com