Advances in Microbiology and Related Sciences for Sustainable Development: The CRSPR Cas9 Revolution

By

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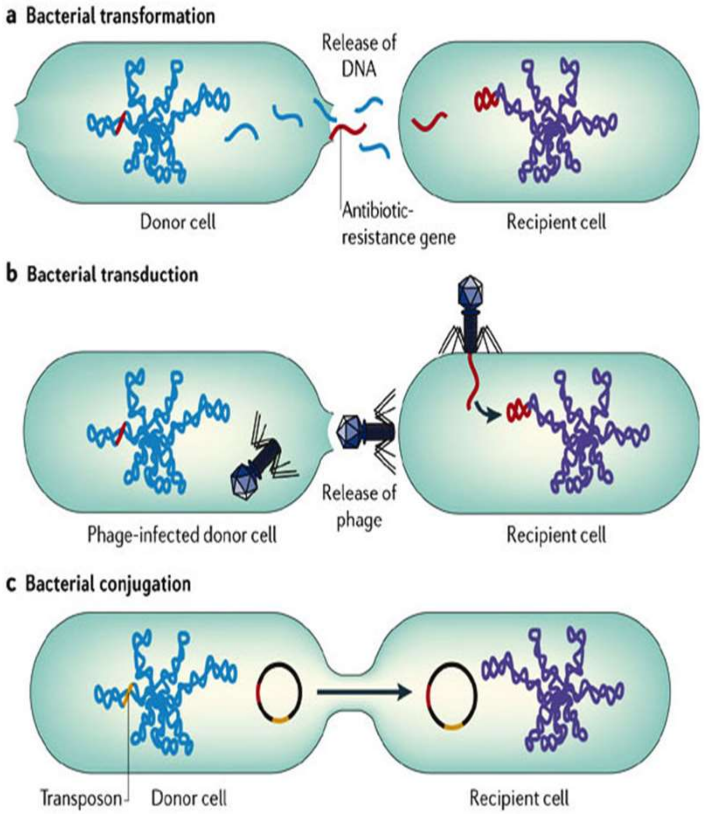
Outline

- **Introduction/Background**
- **Historical: CASPR Cas - Dimensional Narratives, Perspectives and Developments**
- **CASPR Cas applications, ethics and sustainable development**
- **Conclusion(s)**

MICROBIOLOGY AND TRANSFER OF GENETIC INFORMATION

Gene gain via Horizontal Gene Transfer (mostly prokaryotes)



Microbial Patterns

Janeway & Medzhitov 2002 Ann Rev Immunol 20 197-216

Pathogen-associated molecular patterns (PAMPS)

- Conserved microbial molecules shared by many pathogens
- Include:
  - Bacterial lipopolysaccharides
  - Peptidoglycan
  - Zymosan
  - Flagellin
  - Unmethylated CpG DNA

Pattern Recognition Receptors (PRR)

- Include:
  - Toll like receptors
  - Receptors for apoptotic cells
  - Receptors for opsonins
  - Receptors for coagulation and complement proteins

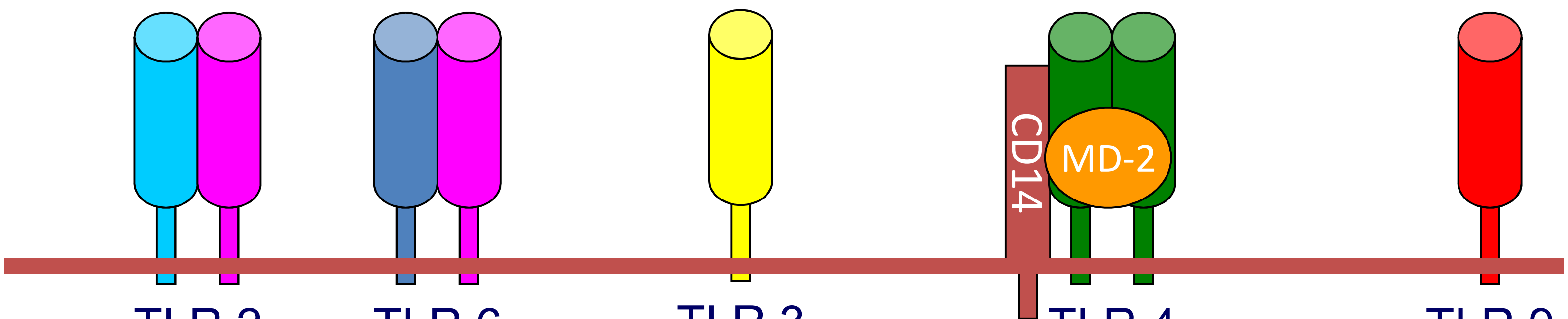
Type 1 PAMPS and their PRR

Peptidoglycan (Gram + bacteria)
Lipoproteins
Lipoarabinomannan (Mycobacteria)
LPS (Leptospira)
LPS (Porphyromonas)
Glycophosphatylinositol - (*T. Cruzi*)
Zymosan (Yeast)

dsDNA

LPS
Lipotechoic acid -
(Gram + bacteria)
RSV F protein

Unmethylated
CpG DNA



TLR 2
TLR 1

TLR 6
TLR 2

TLR 3

TLR 4

TLR 9

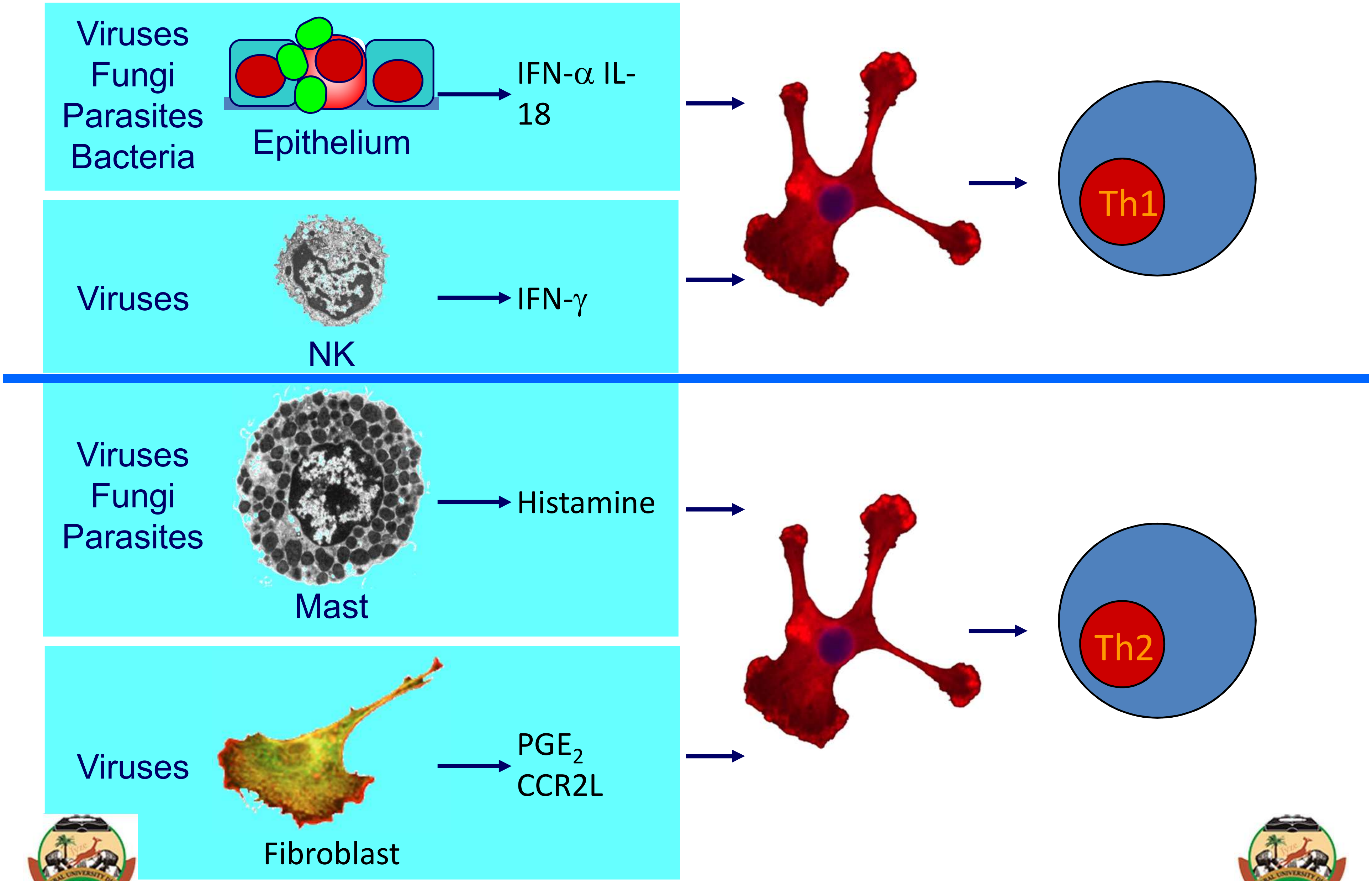
Low level IL-12p70
Some ligands
induce IL-10
or IL-12p35

High
IL-12p70
IFN-α

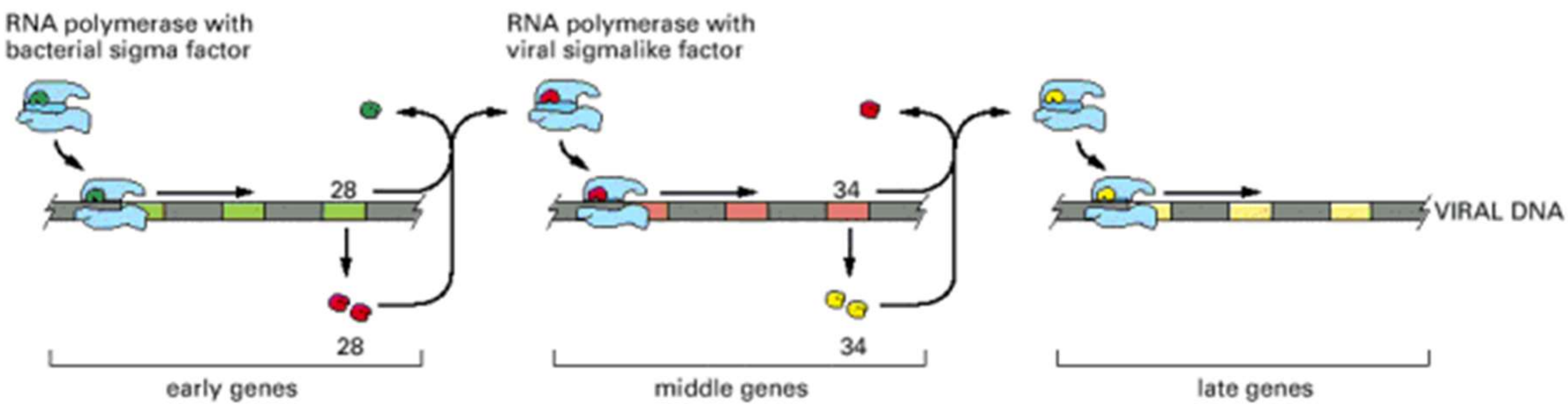
High
IL-12p70

High
IL-12p70
IFN-α

Sources of modulatory tissue factors



Interchangeable RNAP Subunits and Strategy to Control Gene Expression by Bacterial viruses/Bacteriophages



The bacterial virus SPO1, which infects the bacterium *B. subtilis,* uses the bacterial polymerase to transcribe its early genes immediately after the viral DNA enters the cell. One of the early genes, called 28, encodes a **sigma-like factor** that binds to RNA polymerase and displaces the bacterial sigma factor. This new RNAP specifically initiates transcription of the SPO1 “middle” genes. One of the middle genes encodes a second sigma-like factor, 34, that displaces the 28 product and directs RNAP to transcribe the “late” genes. This last set of genes produces the proteins that package the virus chromosome into a virus coat and lyse the cell.

Molecular Biology of the cell © 2002 by Bruce A *et al.*

Transduction

- Transfer of bacterial genes via viruses
  - Donor to recipient
  - Virus: Bacteriophages
- Types
  - Generalized - Random pieces of host cell DNA (any genes); Packaged with phage during lytic cycle
  - Specialized - Only certain specific bacterial genes are transferred e.g. toxins producing
- Replication Cycle
  - Lytic
  - Lysogenic
  - Abortive/Defective phage

Bacteriophage Structure

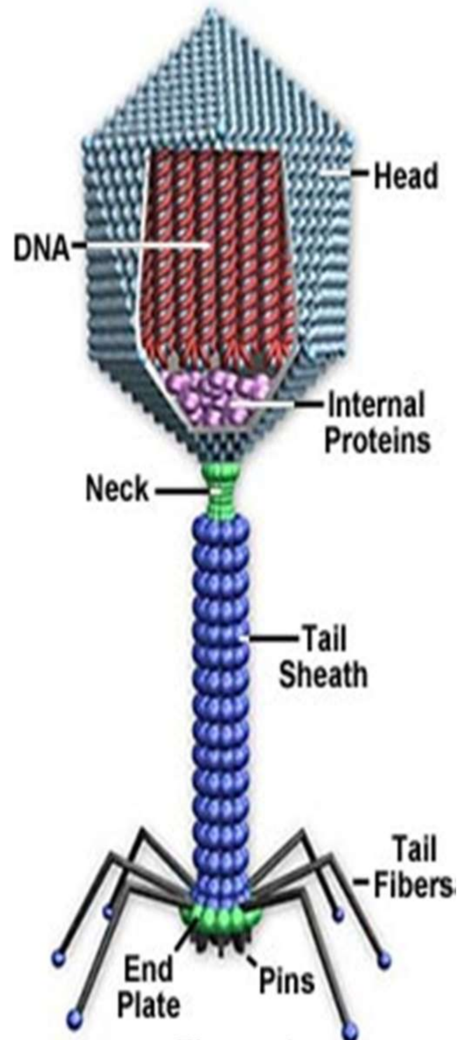


Figure 1

Recombinases and Further Developments

Cre-LoxP System – Used to creat transgenic animals (Henderson CJ 1998; Kobayashi E, Suzuki T & Yamamoto M, et al 2013

CRISPR-Cas System : Clustered Regulated Interspaced Short Palandronic Repeats – CRISPR-associated – used to create transgenic mosquito to control malaria , Smilder AL et al. 2023

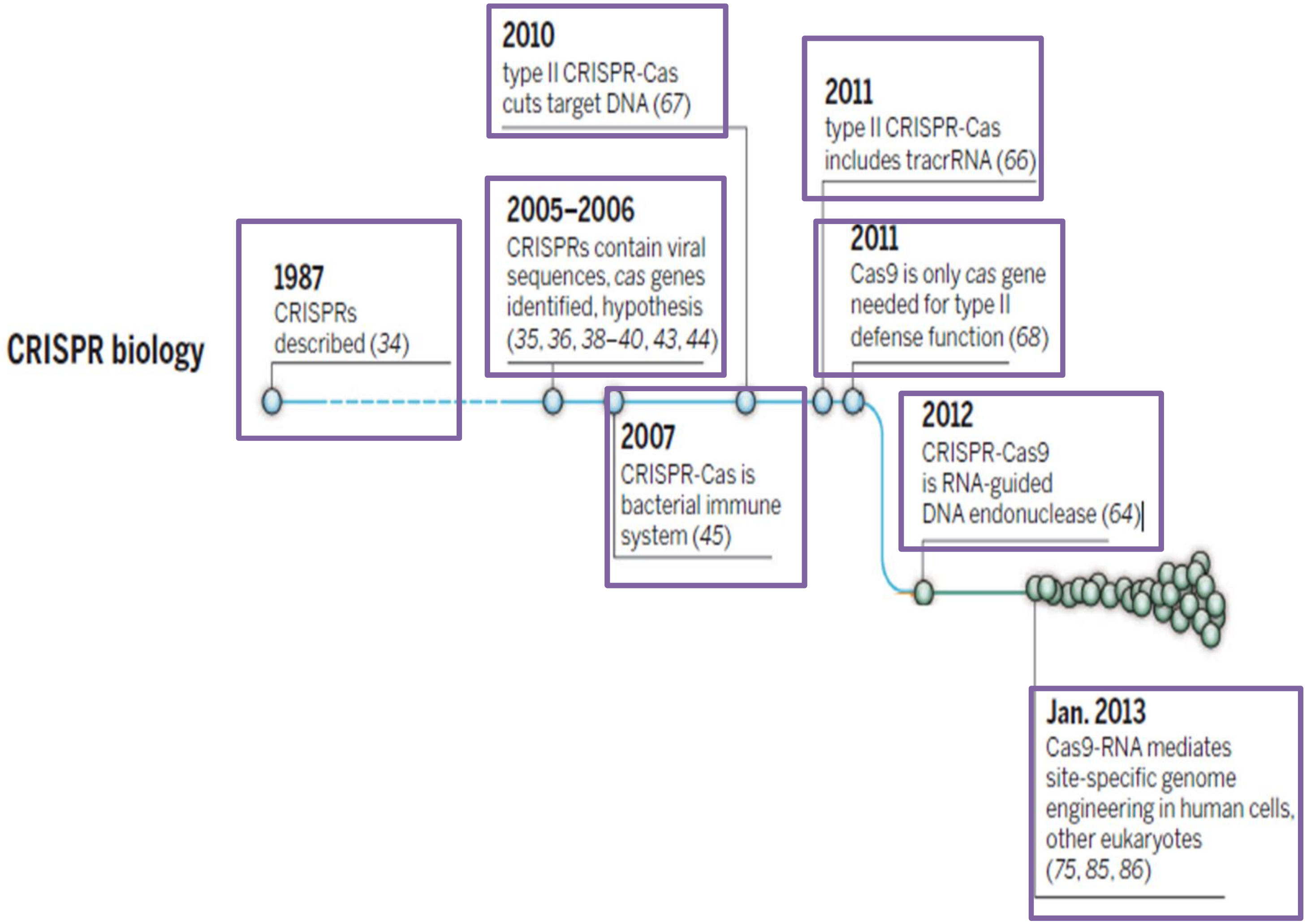
CRISPR-Cas used with Artificial Intelligence to predict the onand off-target activity of RNA-targeting CRISPR tools – enhancing precission in gene editing, Sanjana et al 2023.

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

A family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria and archaea.

- A prokaryote is a single-celled organism (Bacteria and Archaea) that lacks a nucleus and other membrane-bound organelles.
- CRISPR sequences are derived from DNA fragments of bacteriophages that had previously infected the prokaryote. (They are used to detect and destroy DNA from similar bacteriophages during subsequent infections.)
- A bacteriophage is a duplodnaviria virus that infects and replicates within bacteria and archaea.

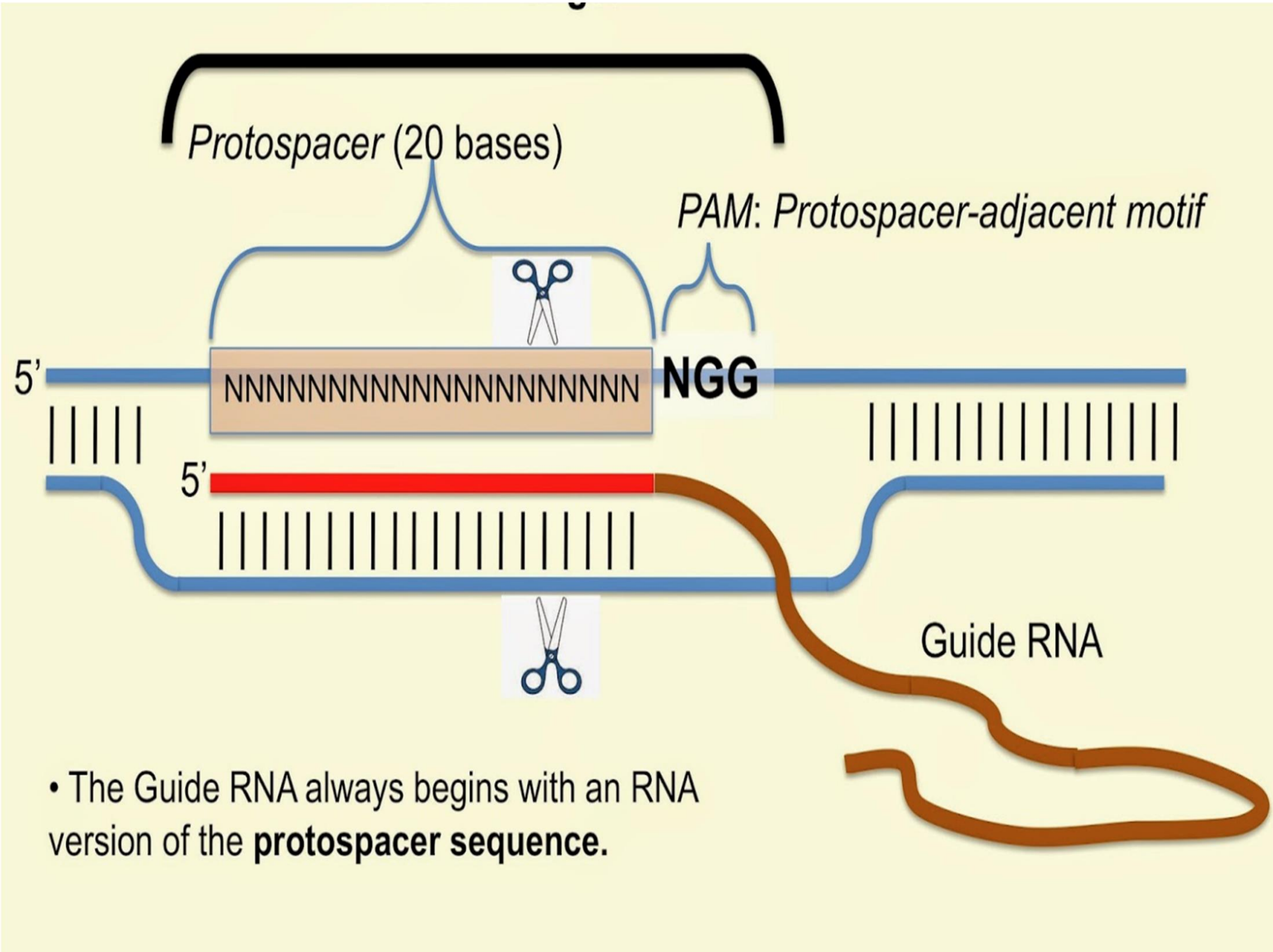
CRISPR Cas and Historical Perspective

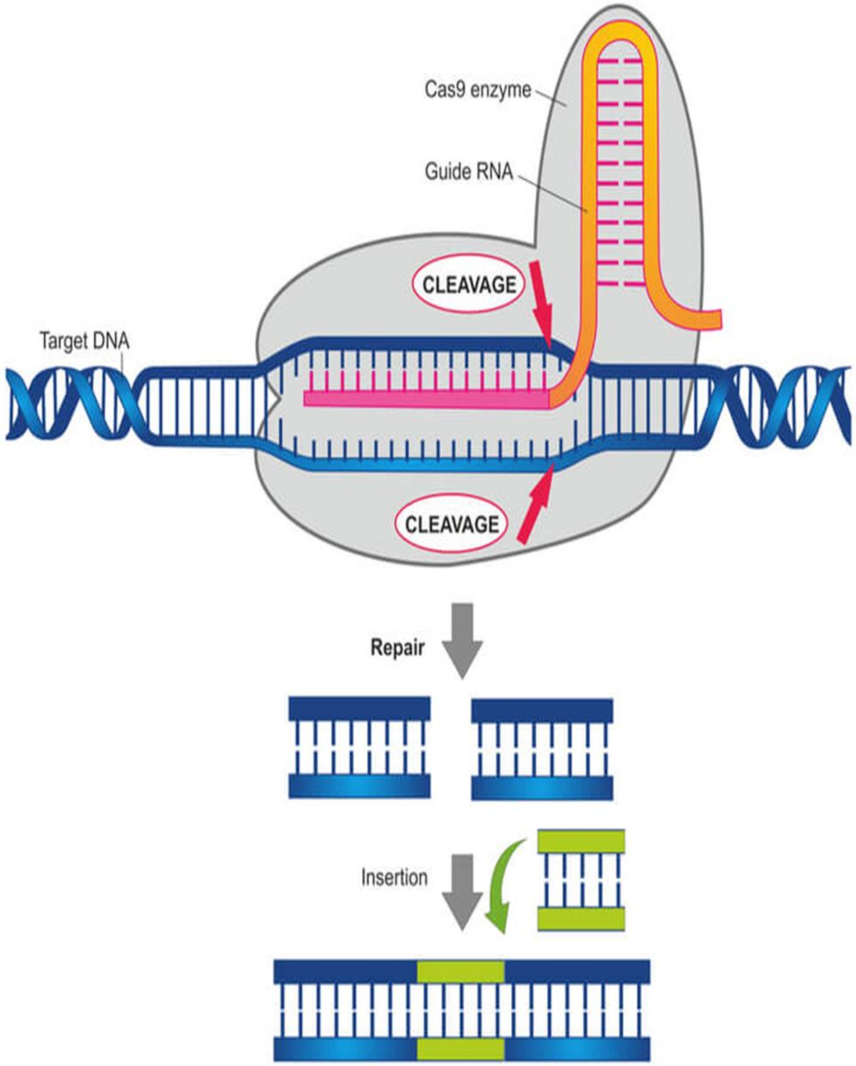


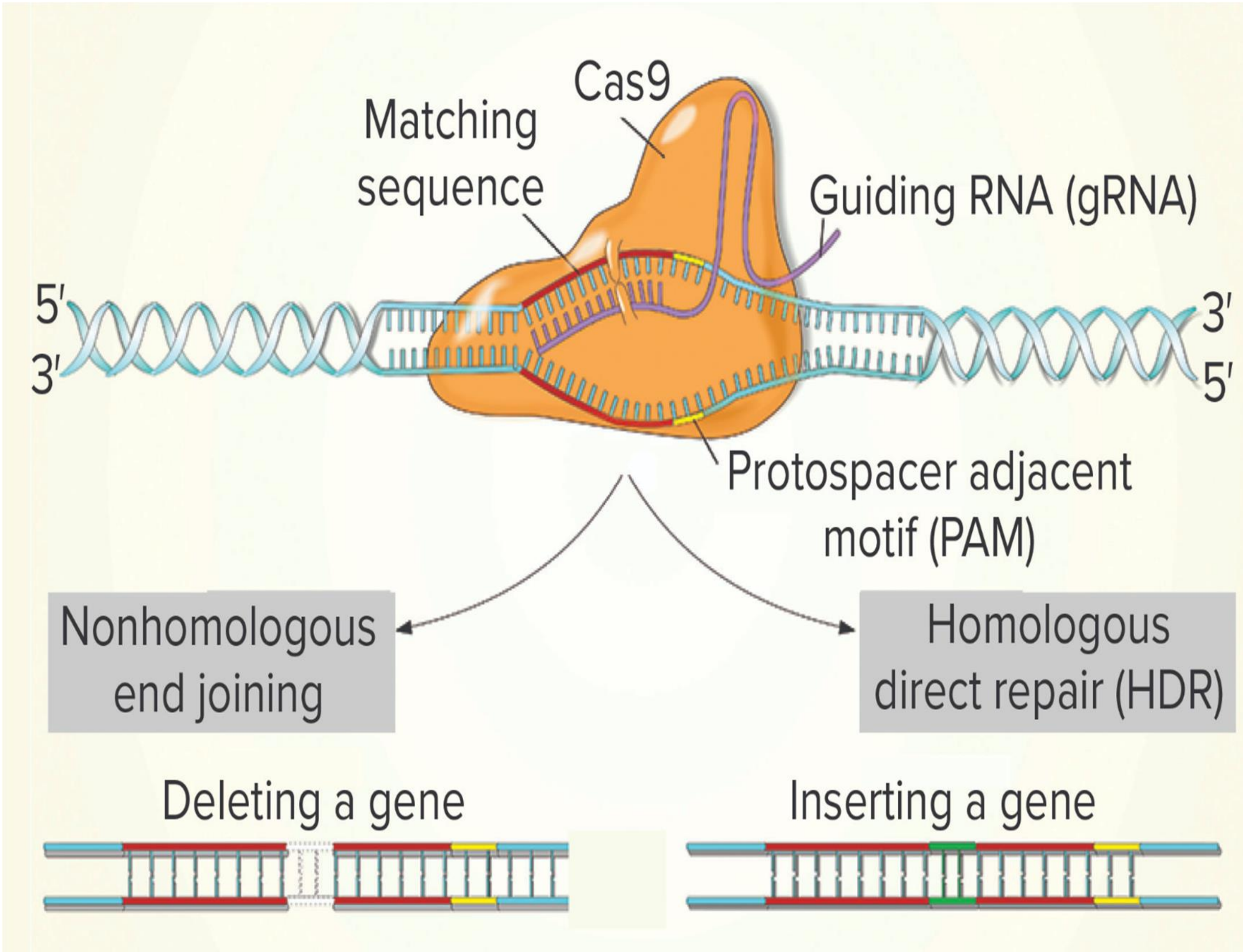
Doudna, J.A., and Charpentier, E. (2014) Science. 346: 1258096–1258096

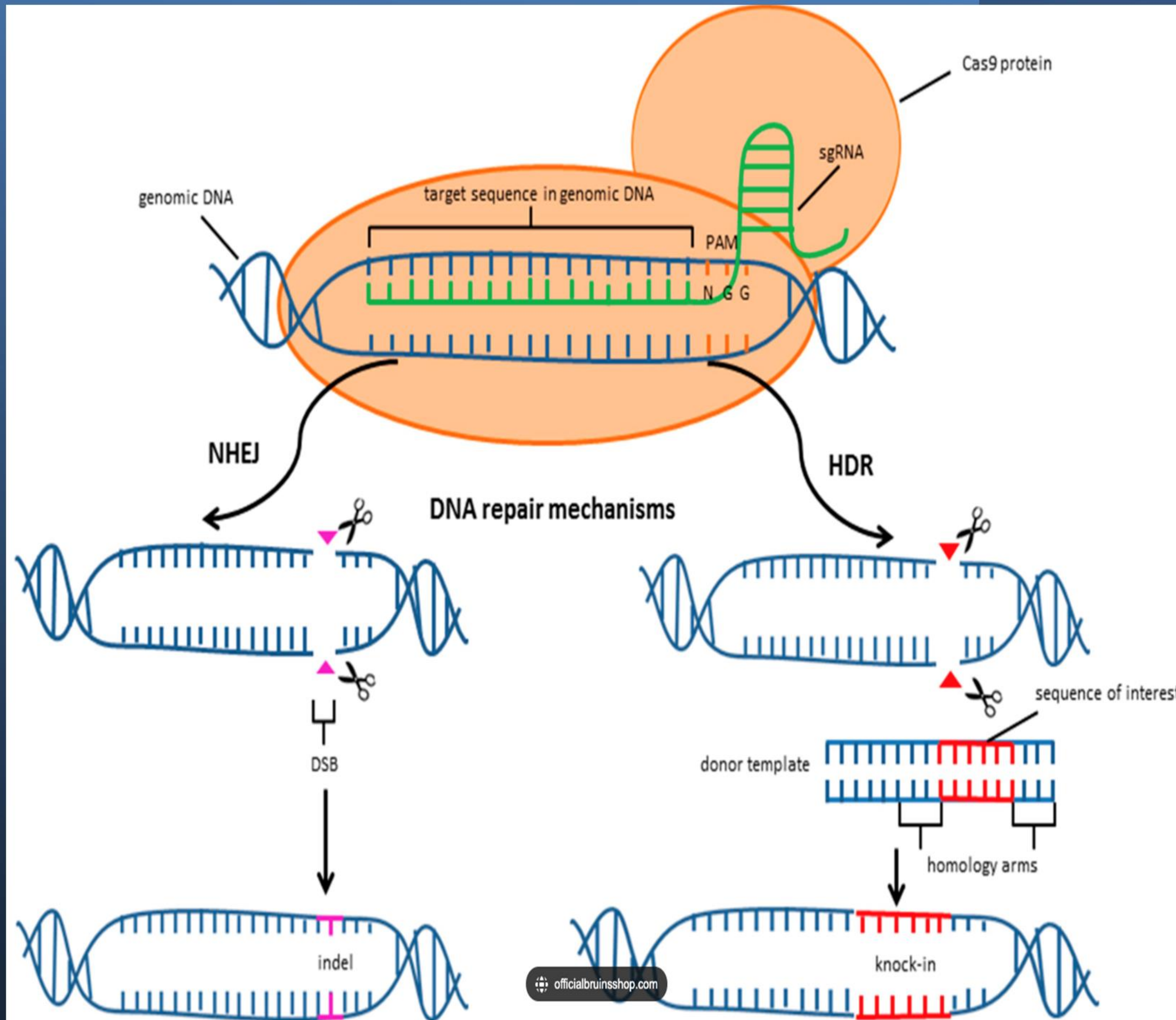
Cas9 ("CRISPR-associated protein 9")

- An enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence.

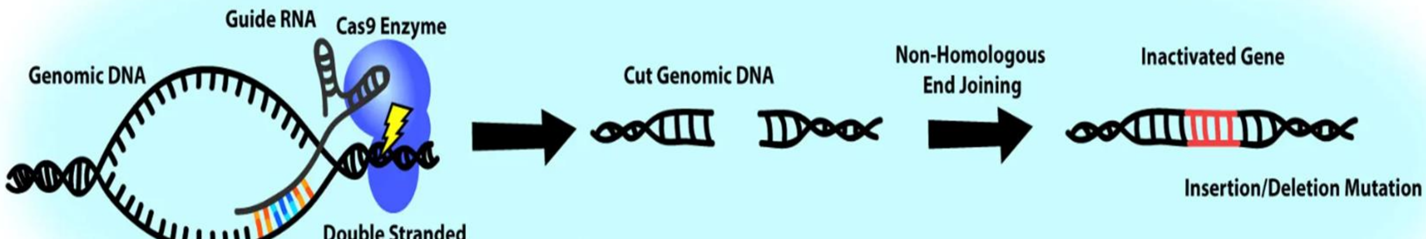

Protospacer (20 bases)
PAM: Protospacer-adjacent motif
5'
NNNNNNNNNNNNNNNNNNNN
NGG
5'
Guide RNA
• The Guide RNA always begins with an RNA version of the protospacer sequence.


Cas9 enzyme
Guide RNA
CLEAVAGE
Target DNA
CLEAVAGE
Repair
Insertion


Cas9
Matching sequence
Guiding RNA (gRNA)
5′
3′
3′
5′
Protospacer adjacent motif (PAM)
Nonhomologous end joining
Homologous direct repair (HDR)
Deleting a gene
Inserting a gene

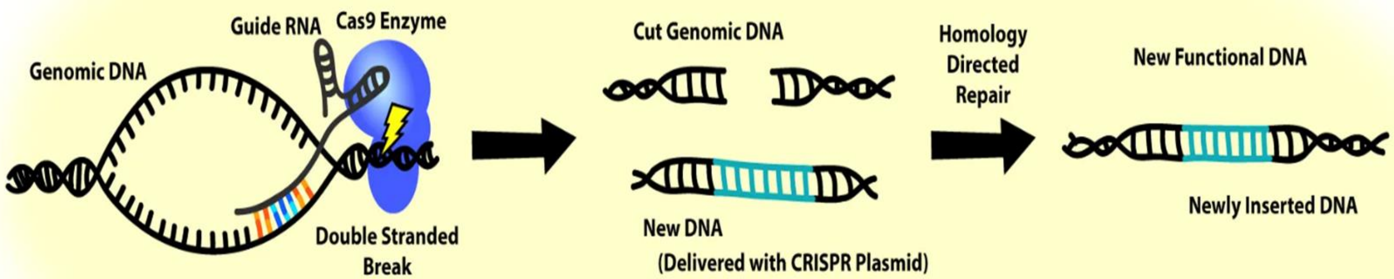

Cas9 protein
sgRNA
genomic DNA
target sequence in genomic DNA
PAM
N G G
NHEJ
HDR
DNA repair mechanisms
DSB
sequence of interest
donor template
homology arms
indel
knock-in
officialbruinsshop.com

Gene Silencing with CRISPR



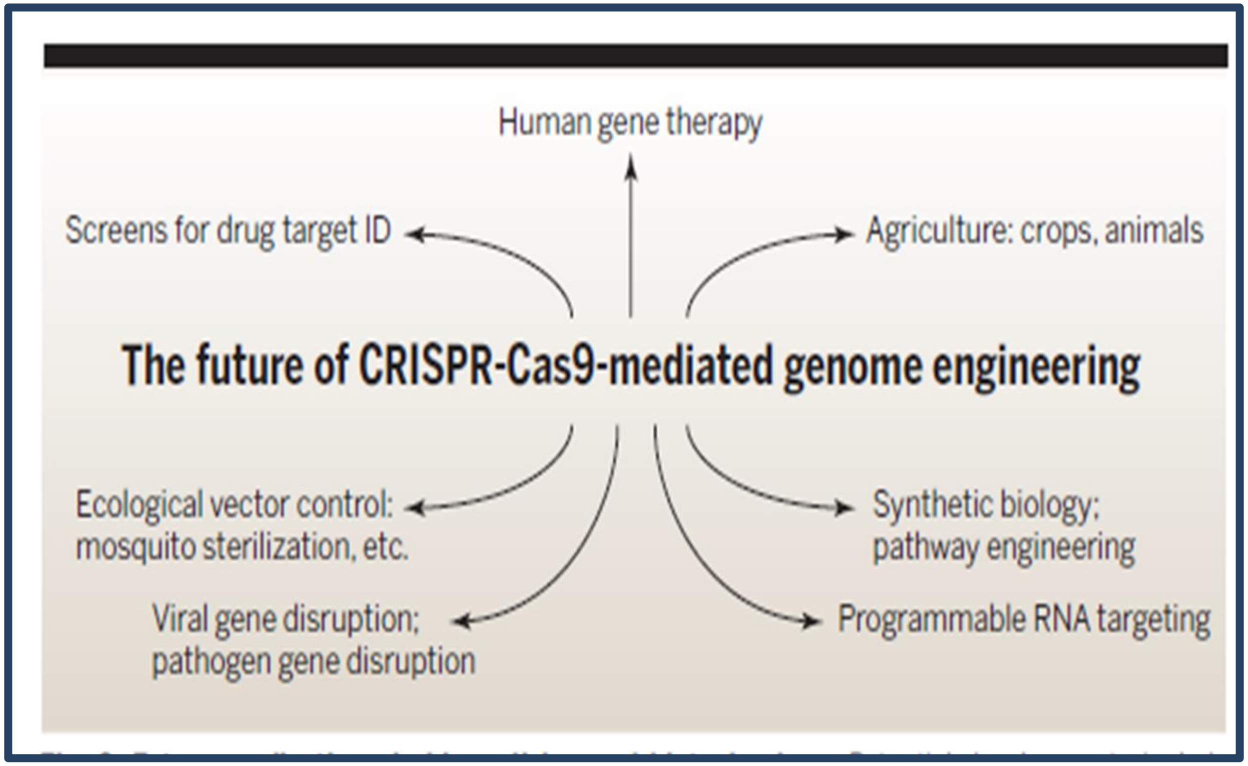
After the Cas9 enzyme is guided to the right location in the genome and cuts the DNA, the cell's natural repair mechanisms take over. The cut is fixed by non-homologous end joining. This process is error-prone and does not perfectly replace the cut DNA, often resulting in an insertion or deletion mutation which silences the gene.

Gene Insertion with CRISPR



To insert a gene, the new gene is added into the original CRISPR plasmid. It is designed to line up perfectly with the cut DNA strands, so the cell uses a different technique, homology directed repair, to incorporate a new stretch of DNA into the genome.

Future applications



''Yes, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) CRISPR technology allows for precise and efficient gene editing by cutting and modifying specific sequences of DNA. This has the potential to revolutionize fields such as medicine, agriculture, and biotechnology by enabling scientists to create genetically modified organisms (GMOs) with desirable traits, or to cure genetic diseases by editing out faulty genes'' J Knoblet

Doudna, J.A., and Charpentier, E. (2014) Science. 346: 1258096–1258096

CRISPR Cas and Application

- Agriculture – plant breeding and crop resistant, plant protection (viral!!!)
- Medical and Pharmaceutical – gene therapy e.g sickle cell anaemia, disease treatment (cancer & Alzheimer's)
- Environment – Biofuels, environmental protection (clean up), pesticides/
- Industry – food in particular, Drugs (discovery & development)

CRISPR-edited crops breaking new ground in Africa?

- Evaluation of Striga resistance in Low Germination Stimulant 1 (LGS1) mutant sorghum - Prof. Steven Runo; Professor of Molecular Biology, Kenyatta University
- A field of sorghum in Botswana. Gene editing has created sorghum plants that are resistant to a destructive parasite called witchweed
- Application of reproductive biotechnologies to develop a transgenic goat as a model for genetic control of animal diseases Specifics of the target gene(s) and phenotype(s) - Wilkister Nakami Nabulindo, PhD Graduate Fellow, International Livestock Research Institution
- Gene editing to control maize lethal necrosis in Africa for improved maize productivity and grain harvests - James Kamau Karanja Senior Research Scientist, Kenya Agriculture and Livestock Research Organization (KALRO) National Agricultural Research Laboratories (NARL), Kabete
- CGIAR research program on roots, tubers and banana (CRP-RTB) - Dr. Leena Tripathi, Principal Scientist, International Institute of Tropical Agriculture (IITA)
- Modulation of energy homeostasis in maize to develop lines tolerant to drought, genotoxic and oxidative stresses - Dr. Elizabeth Njuguna, Former doctoral fellow, VIB-UGENT Center for Plant Systems Biology, Ghent University, Belgium &Plant Transformation Laboratory, Kenyatta University, Kenya
- Accelerating African Swine Fever Virus (ASFV) vaccine development via CRISPR-Cas9 and synthetic biology technologies - Dr. Hussein Abkallo, Post-Doctoral Fellow Affiliation: Vaccine Biosciences/ Animal and Human Health (AHH)/ International Livestock Research Institute (ILRI)

https://africenter.isaaa.org/wp-content/uploads/2021/04/GENOME-EDITING-IN-AFRICA-FINAL.pdf

- Addional information:
- Tripathi L et al. Genome Editing for Sustainable Agriculture in Africa. Front Genome Ed. **2022** May 12;4:876697.
- Heidi Ledford (**2024**) CRISPR-edited crops break new ground in Africa - Scientists in the global south use the popular technique to protect local crops against local threats. https://www.nature.com/articles/d41586-024-00176-8

CFISPR Cas and Ethics

The potential ethical and social issues of CRISPR: the use of CRISPR also raises ethical concerns, such as –

- the potential for unintended consequences or the creation of genetically modified organisms which may have unforeseen ecological effects. These concerns have led to calls for careful regulation and oversight of CRISPR research and development.

Overall, while CRISPR has the potential to be a game-changer in many fields, it is important to proceed with caution and consider the potential implications and ethical considerations associated with this technology.

CFISPR Cas and Ethics

The potential ethical and social issues of CRISPR include:

1. Safety concerns: CRISPR gene editing can result in unintended consequences, such as off-target mutations or unexpected gene interactions.
2. Unequal access to gene editing: a concern that CRISPR could create disparities in access to healthcare.
3. Slippery and dangerous slope towards eugenics selective breeding of humans (designer babies).
4. Long-term consequences on biodiversity: CRISPR in agriculture and ecology could have long-term effects on biodiversity, as genetically modified organisms are introduced into ecosystems. There is a potential risk of altering ecosystems in ways that may have unforeseen devastating consequences.
5. Informed consent: If CRISPR is applied to humans, there is a concern that informed consent could be compromised. The complexity of the technology may make it difficult for patients to fully understand the risks and benefits of gene editing, potentially leading to uninformed consent, especially by overzealous applier.
6. Implications on cultural and religious beliefs: CRISPR gene editing has the potential to challenge cultural and religious beliefs about the sanctity of life and naturalness of the human genome.

Overall, these ethical and social issues highlight the importance of careful regulation and oversight of CRISPR research and development, as well as the need for ongoing dialogue and engagement with stakeholders in science, medicine, and society as a whole.

Sustainable Development Goals (SDGs)

- The sustainable Development Goals (SDGs) are aim to change/transform the world and involve actions to:

1. terminate/eliminate/end poverty and inequality
2. Protect the planet earth
3. ensure all people have access and enjoy health, justice and prosperity

The SDGs are 17 in total, ie SDG1-17

Sustainable Development Goals (SDGs)

- The 17 SDGs:

https://unosd.un.org/content/sustainable-development-goals-sdgs

Sustainable Development Goals (SDGs)

- Sustainable is qualified by Sustainability
- Sustainability is the capacity to support and uphold a process that is safer and ethically sound in a time scale
- There are 3 recognized key concepts of sustainability:

1. Economic Sustainability
2. Environmental Sustainability
3. Social Sustainability

These key concepts could crystallize into Food, health and security.

Resources/References

- Owen, J A, Punt, J and Strandford, S A. 2013 *Kuby Immunology 7th Ed* WH Freeman: New York

- Sompayrac L (2010) *How the Immune System Works 4th Ed* Blackwell

- Campbell and Reece (2005) *Biology 7th Ed* Pearson Education Inc.; Chapter 43

- Fox, S (2010) *Human Physiology 11th* Ed McGraw Hill; Chapter 15
- Bhaya, D., Davison, M., Barrangou, R. 2011. CRISPR-Cas System in Bacteria and Archaea: Versatile Small RNAs for Adaptative Defense and Regulation. Annual Review of Genetics. 45:273-297.
- Bortesi, L., and Fischer, R. 2014. *The CRISPR/Cas9 system for plant genome editing and beyond.* Biotechnol. Adv. *33*: 41–52.
- Doudna, J.A., and Charpentier, E. 2014. The new frontier of genome engineering with CRISPR-Cas9. Science. 346: 1258096–1258096.
- Horvath,P., Barrangou,R. 2010. CRISPR/Cas, the immune System of Bacteria and Archaea. Science: 327: 167-170.
- Pennisi, E. 2013. The CRISPR Craze. Science. 341: 833-836.

CRISPR Cas With Take Home Goodies

CRISPR technology has the potential to render sustainable developments by revolutionizing several fields of study, especially those related to genetics and biotechnology. Some of the fields that see the most potential in CRISPR include:

1. Medicine: CRISPR has the potential to transform the way we treat genetic diseases, by allowing scientists to target and modify specific genes responsible for these diseases. This could lead to new treatments and potentially even cures for a wide range of genetic disorders.
2. Agriculture: CRISPR could be used to create crops that are more resistant to pests, diseases, and environmental stressors, as well as to develop new plant varieties with improved nutritional content or other desirable traits.

3. Biotechnology: CRISPR has already been used to create new bioproducts, such as biofuels and bioplastics. It also has the potential to be used in a wide range of other applications, such as in the production of industrial enzymes or other chemicals.

4. Ecology: CRISPR could be used to help protect threatened or endangered species by modifying their genomes to make them more resistant to environmental stressors or disease.

5. Basic research: CRISPR has also opened up new avenues for basic research in genetics, by allowing scientists to more easily study the functions of specific genes and the effects of gene mutations. Overall, CRISPR has the potential to transform several fields of study, by enabling precise and efficient gene editing and creating new opportunities for scientific discovery and innovation.

Resources/References

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816-21.
doi: 10.1126/science.1225829
http://science.sciencemag.org/content/337/6096/816.long

Shengdar Q Tsai, et al. (2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nature Biotechnology*, 33: 187–197. doi: 10.1038/nbt.3117 http://www.nature.com/nbt/journal/v33/n2/full/nbt.3117.html

'**John Knoblett**: Is CRISPR a **disruptive technology**? Chatbot

Rewriting the Code of Life' by Michael Specter in *New Yorker* January 2, 2017 issue
http://www.newyorker.com/magazine/2017/01/02/rewriting-the-code-of-life

'Do CRISPR Enthusiasts have their head in the sand about the safety of gene editing?' by Sharon Begley in *STAT News* July 18, 2016
https://www.statnews.com/2016/07/18/crispr-off-target-effects/

'Genetically Engineer Almost Anything' by Tim De Chant and Eleanor Nelsen in *NOVA Next* July 17, 2014
http://www.pbs.org/wgbh/nova/next/evolution/crispr-gene-drives/

THANK YOU