CRISPR-Cas and the brave new world of Genetic Engineering

Bob Dorsett, MD
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June 2026

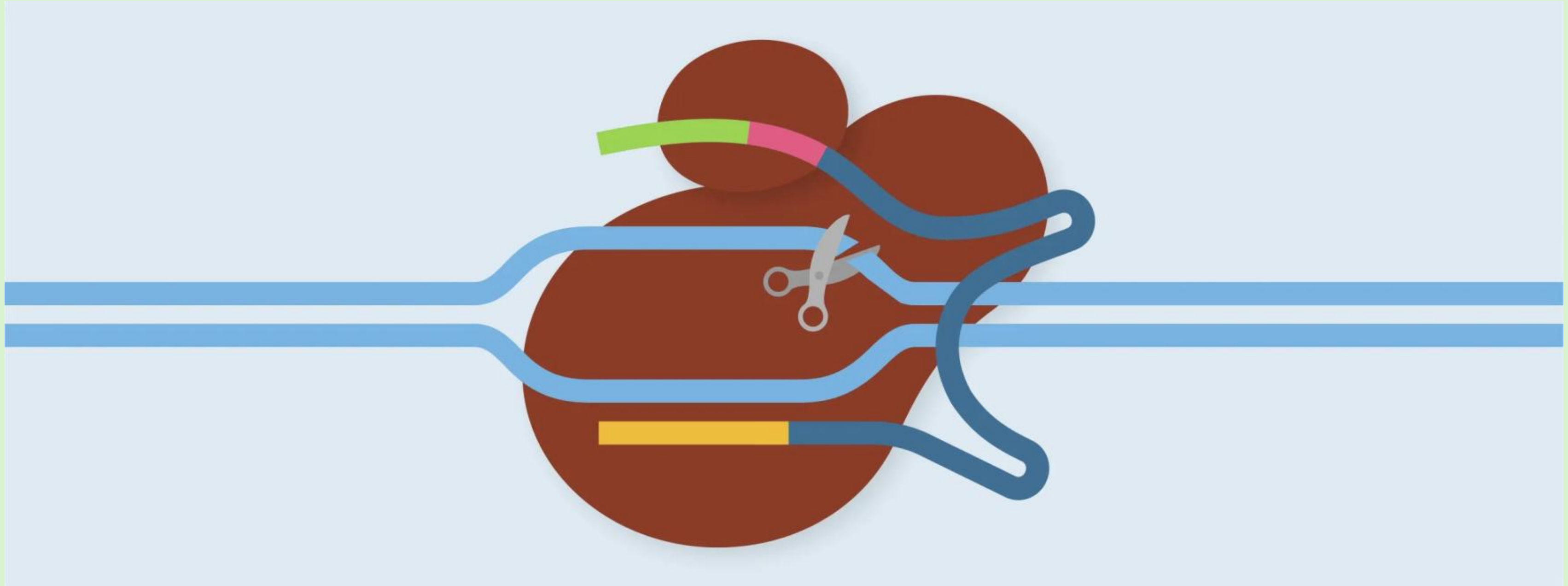

image: Laura Olivares Boldú
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Outline

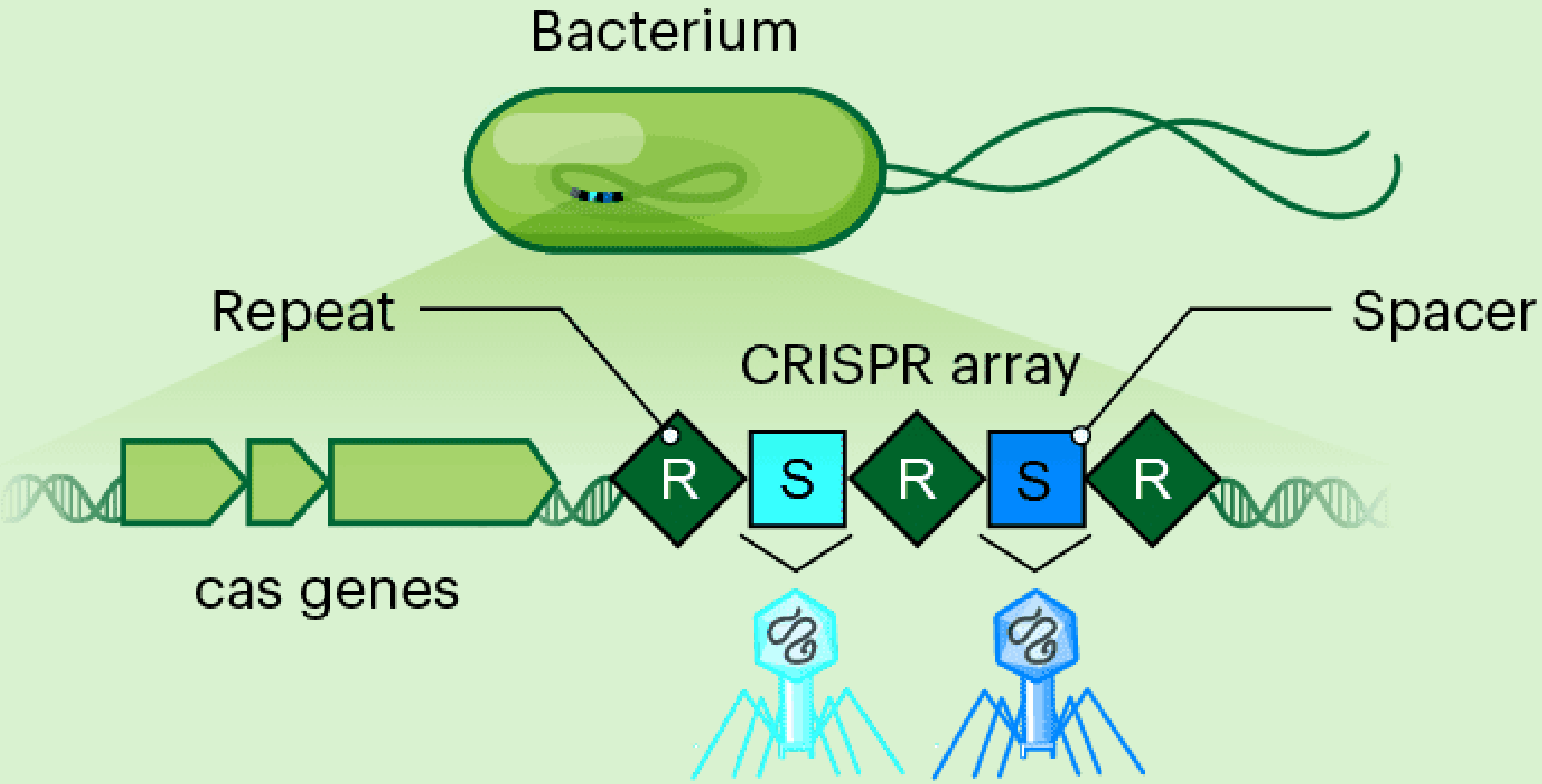
- Introduction
    - What is genetic engineering?
- Background molecular genetics
- Case study: sickle cell disease
- Bacterial origins of genetic engineering
- The gene repair toolkit
    - CRISPR-Cas9 and homologous repair
    - Base editing
    - Prime editing
- The future (and the ethical concerns ahead)

Over the past twenty years researchers have developed chemical tools to edit DNA. (Nature had it all figured out two or three billion years ago.) Those tools open a whole new realm of possibilities. We have the potential to cure genetic diseases, improve crops and livestock, enhance human capabilities, restore extinct species, create new life forms, and much more. All of which raise a whole lot of practical and ethical questions . . .

<u>Glossary of terms</u>

- **DNA**: life's library, stores genetic information
- **RNA**: the cell's multipurpose tool for reading and editing information
- **nucleotides (aka bases)**: the chemical components of DNA and RNA
- **complementary base pair**: Nature's solution to information transfer
- **codon**: the triplet of bases in DNA that designates a particular amino acid, e.g. TAC => Methionine
- **gene**: the sequence of codons on DNA that determines the amino acid sequence in a protein
- **genetic code**: how Nature translates base sequences to build proteins
- **mutation**: a change in the nucleotide sequence on DNA
- **Central Dogma**: information flows from DNA -> RNA -> protein
- **transcription**: the process copying information on DNA into RNA
- **reverse transcription**: copying information from RNA into DNA
- **bacteriophage (aka phage)**: a virus that infects bacteria

CRISPR-Cas: Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats with **C**RISPR-**As**sociated genes. An adaptive immune system invented by archaea two or three billion years ago. Discovered by humans 40 years ago. Understood 20 years ago. Applied to medicine in 2012. Cured sickle cell disease in 2019. Awarded the Nobel Prize in 2020.



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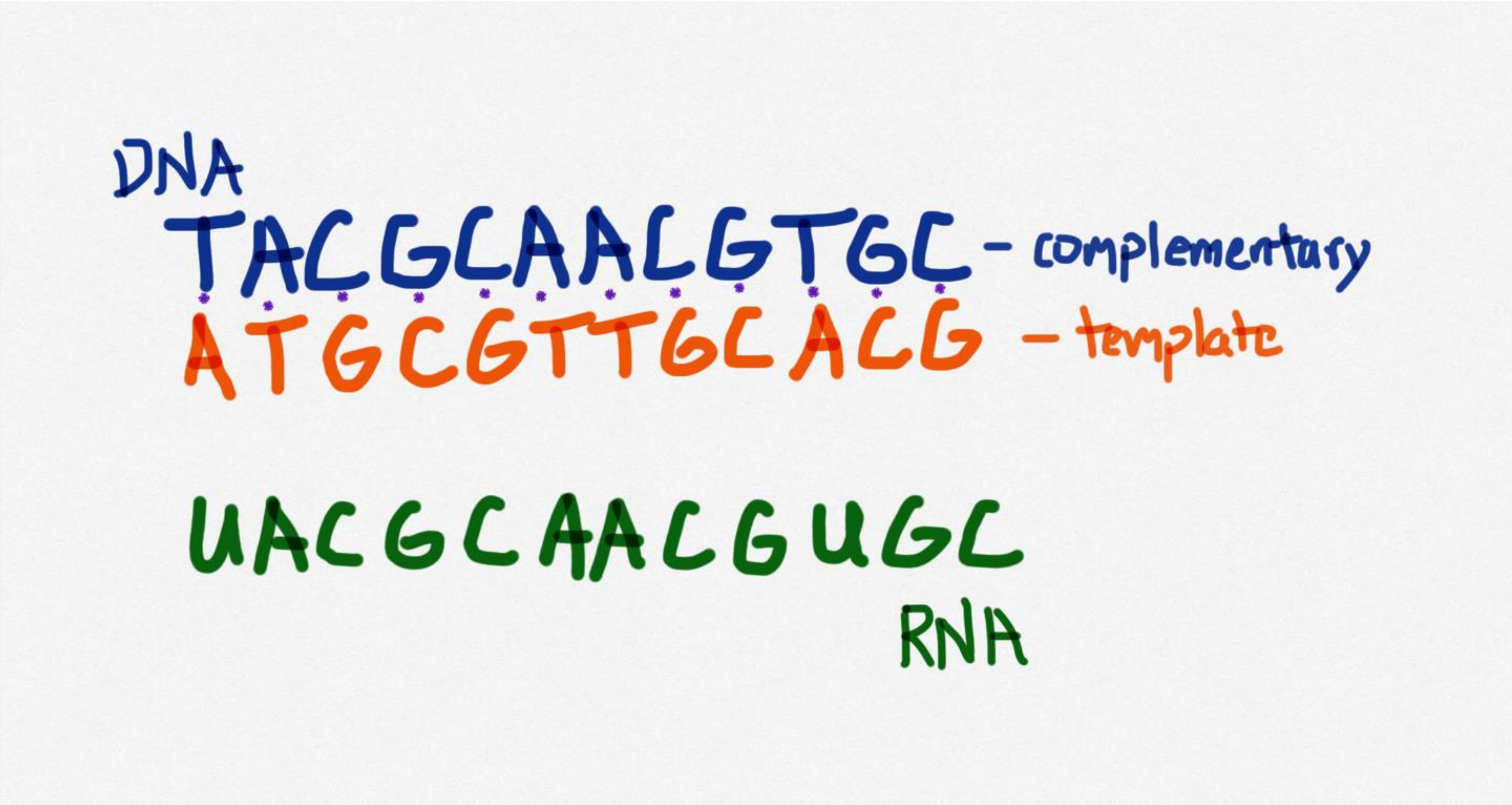
Jennifer Doudna and Emmanuelle Charpentier
2020 Nobel Prize in Chemistry

image: Nobel
Committee

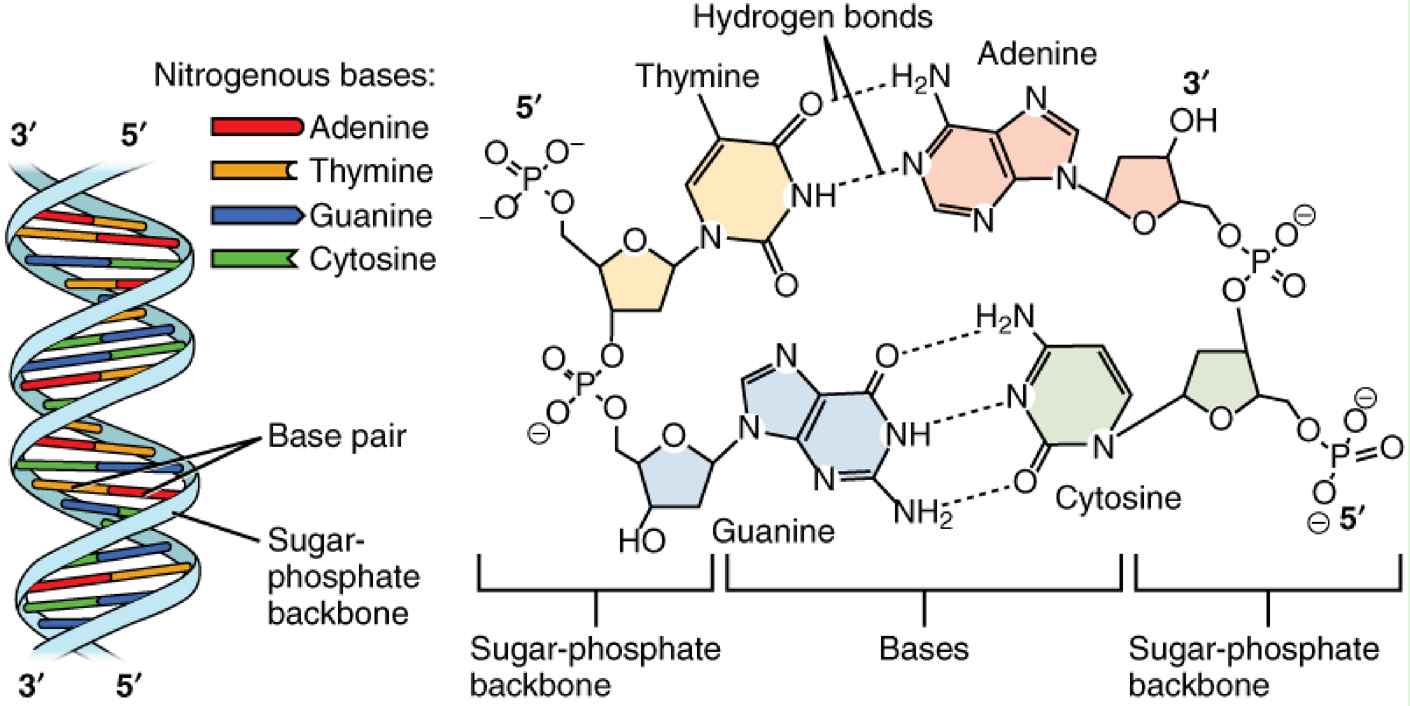
Molecular Genetics in Brief

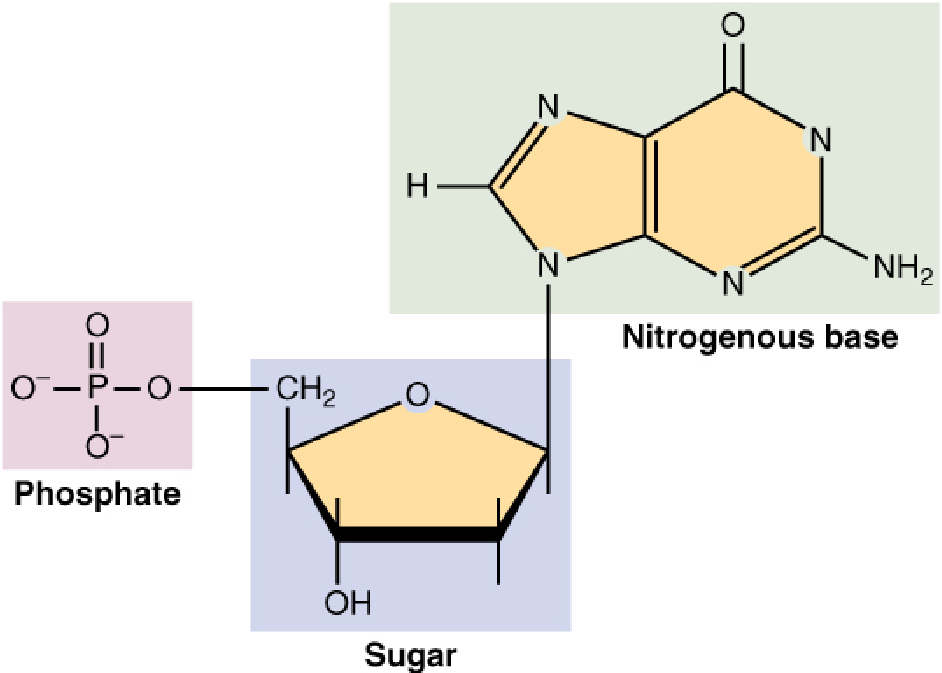
Nucleotide sequences in DNA and RNA showing complementary base pairing. The human genome has about three billion base pairs coding about 40,000 genes.



The nucleotides and complementary base pair bonding

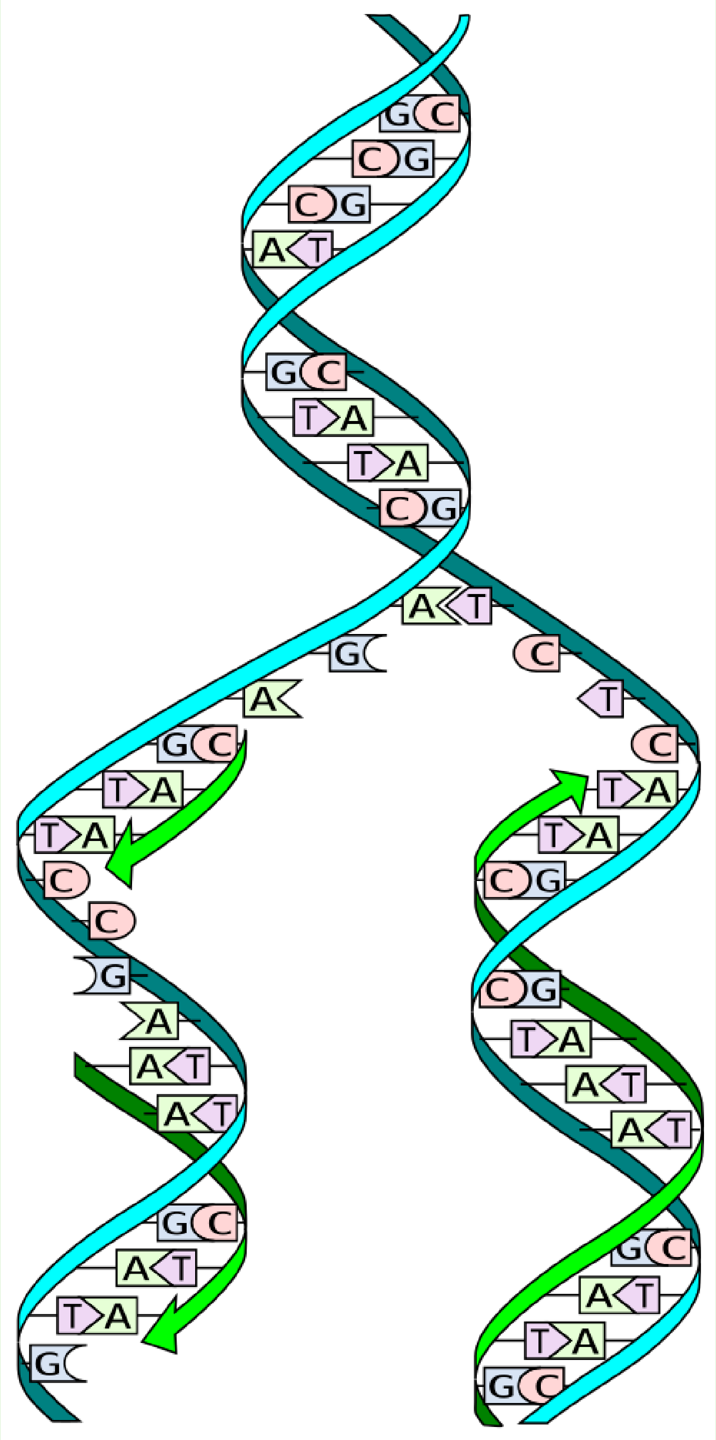
image: Wikipedia



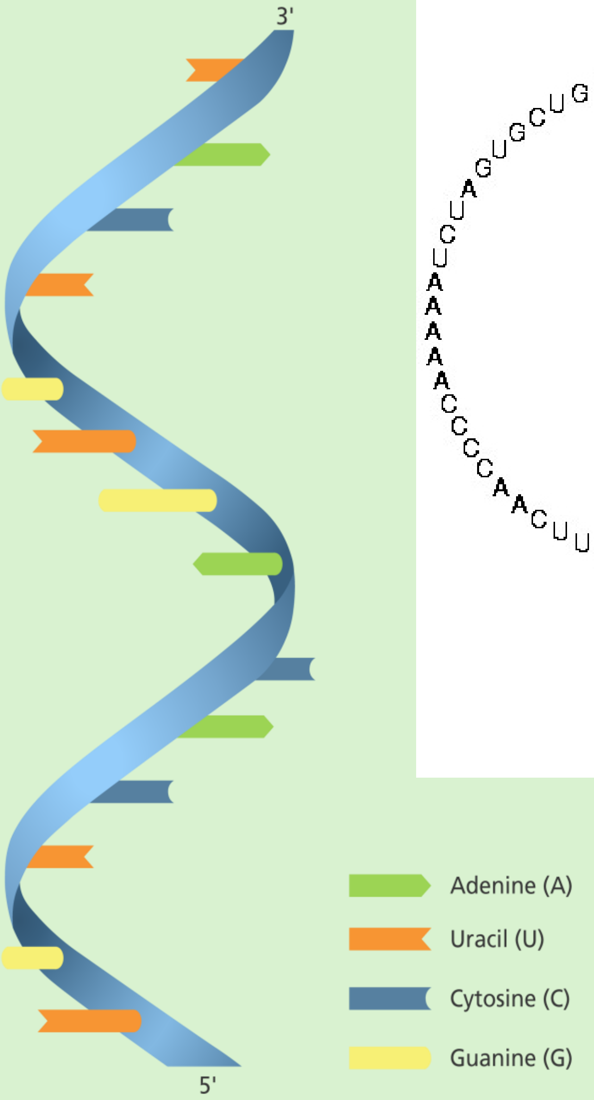


<u>DNA molecule during replication</u>.
DNA carries all the information necessary for cell function. Replication is the mechanism that copies genetic information into the next generation.

Image: Wikipedia molecular biology



RNA: the all-purpose tool. If the cell was a computer, RNA is the CPU running the programs encoded in DNA. RNA transforms information from DNA into molecular activity and can itself catalyze those activities. In the CRISPR system, RNA finds the exact location on DNA that needs repair and then facilitates that repair.



Nucleotide sequence on RNA
image: Laura Olivares Boldú
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RNA, like proteins, can form tertiary structures

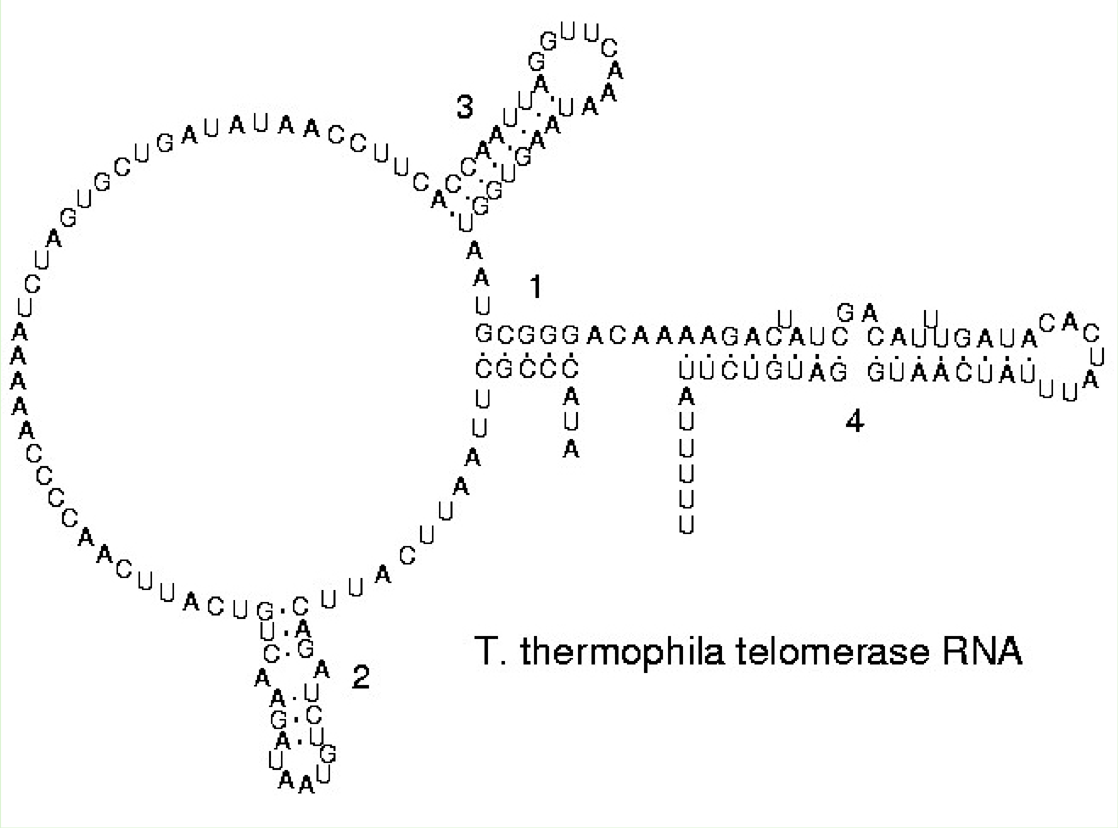


image: Wikipedia

<u>The Central Dogma</u>: information is stored in DNA and then transcribed to RNA. That information guides protein production (translation). Various proteins control essential cell functions: structure, movement, immune response, the flow of materials through cell membranes and the rates of chemical reactions.

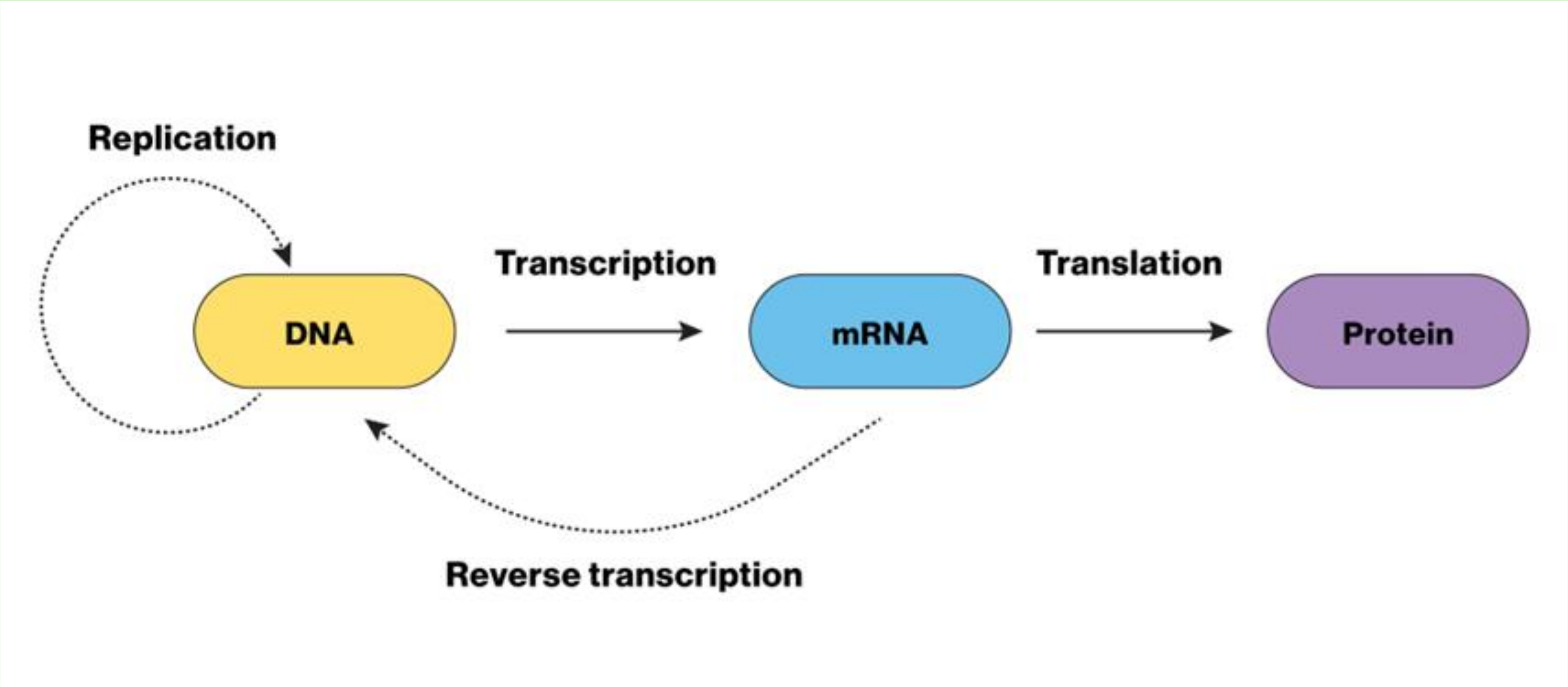
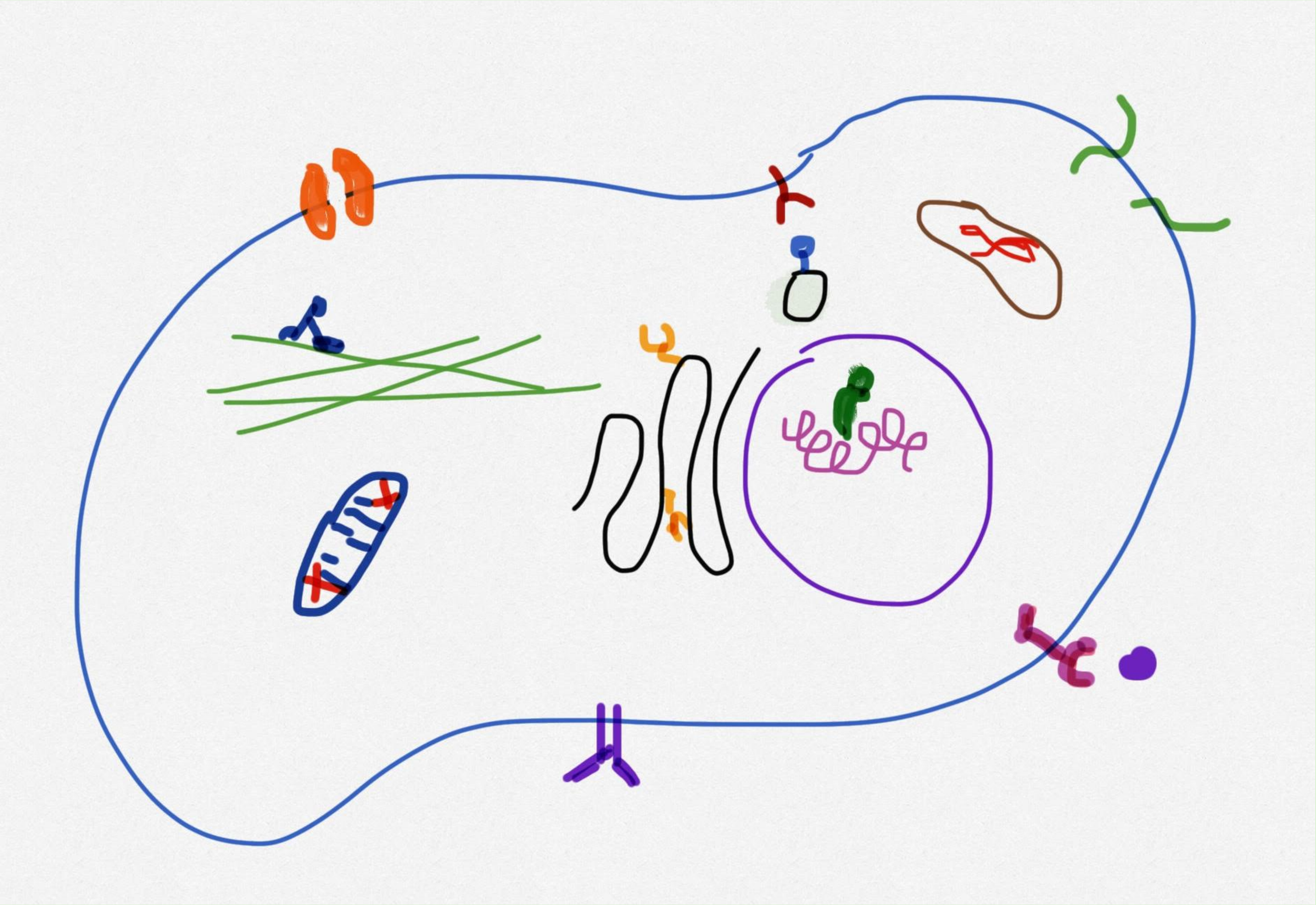


image by Google

Proteins provide the structure and function of a living cell



From DNA blueprint to functional protein

1. The sequence of nucleotides in a gene determines the sequence of amino acids in a protein.
2. The sequence of amino acids in a protein determines the 3D shape of the protein.
3. The 3D shape of the protein determines the protein's function.

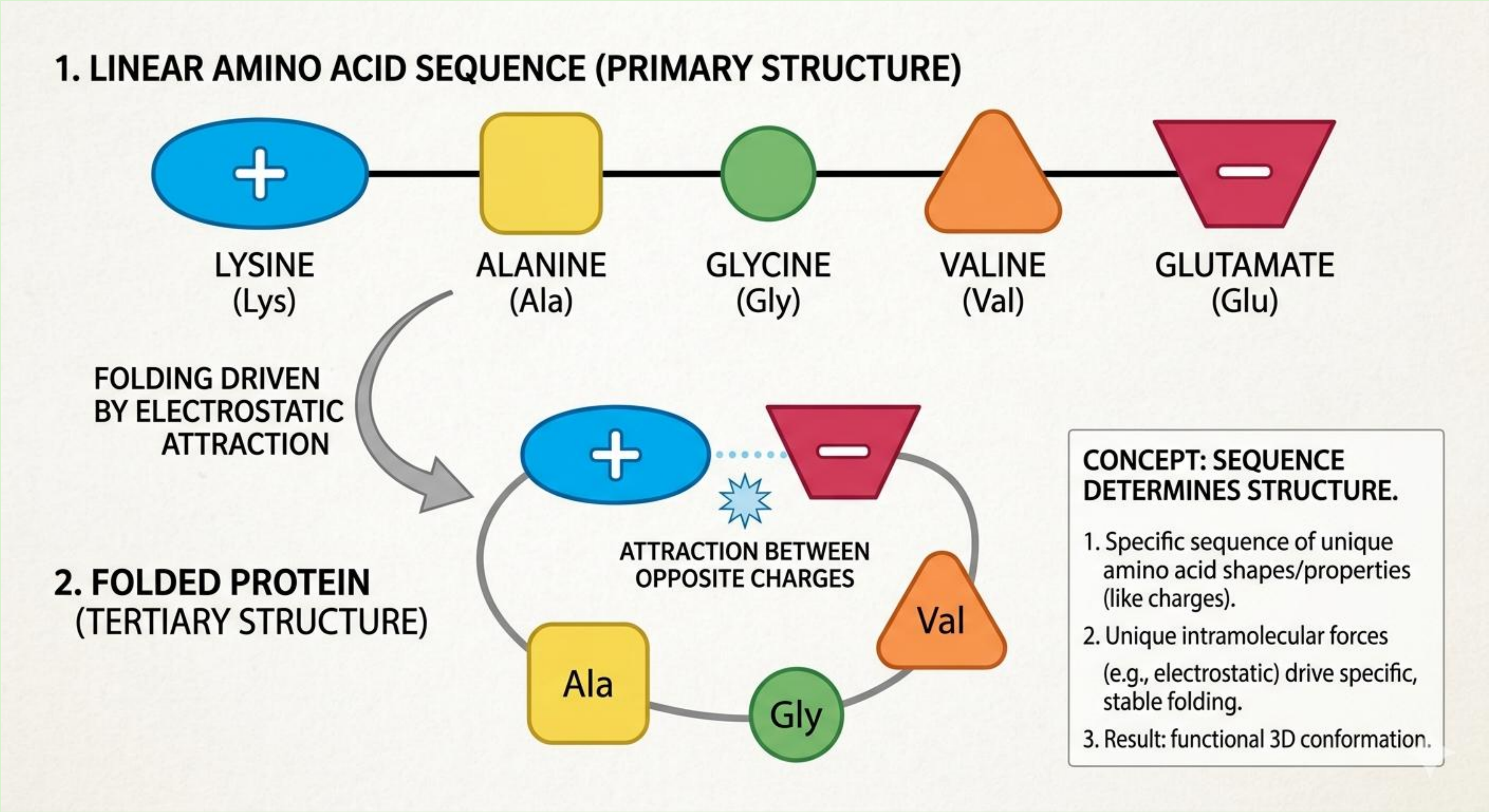
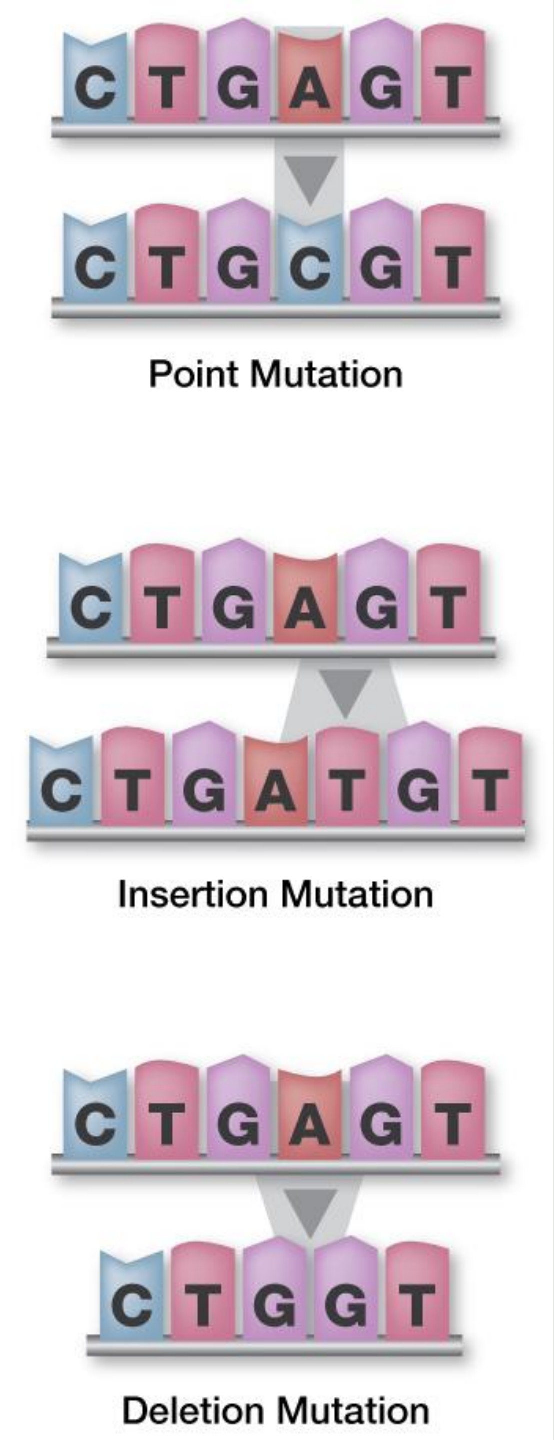


image: Google Nano Banana 2

Mutations are changes in the sequence of nucleotides on DNA. They typically result from errors in replication but may also occur because of environmental insults like viral infections, chemical agents, and radiation. If the mutation occurs in an active gene it may change protein structure and function.

image: U. Utah Genetics



How a change in amino acid sequence may change the structure of a protein

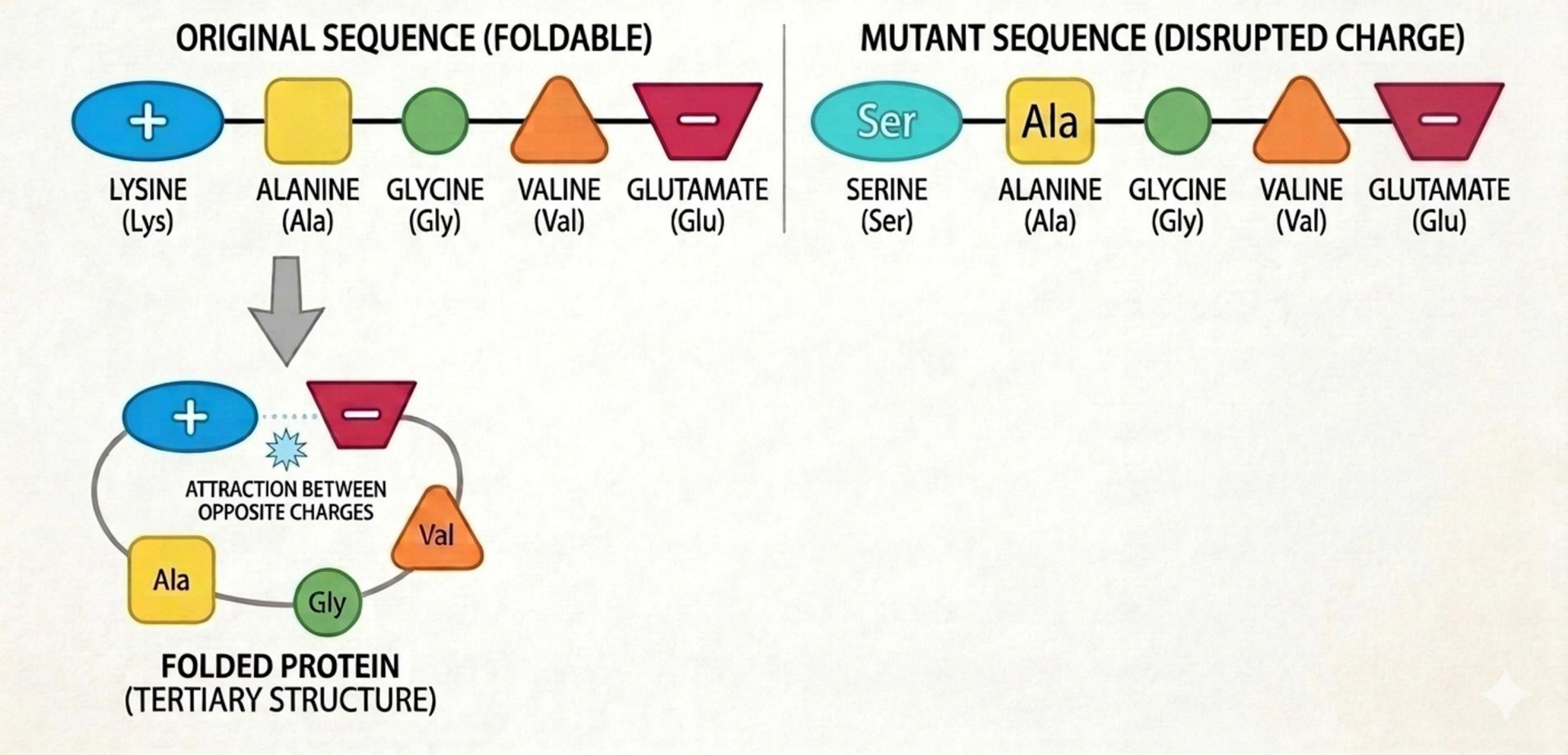


image: Google Nano Banana 2

Sickle Cell Disease

The mutation that causes sickle cell disease. Image below shows codons 124 through 128 bracketing the sickle cell mutation in hemoglobin A. HbA has 141 amino acids total.

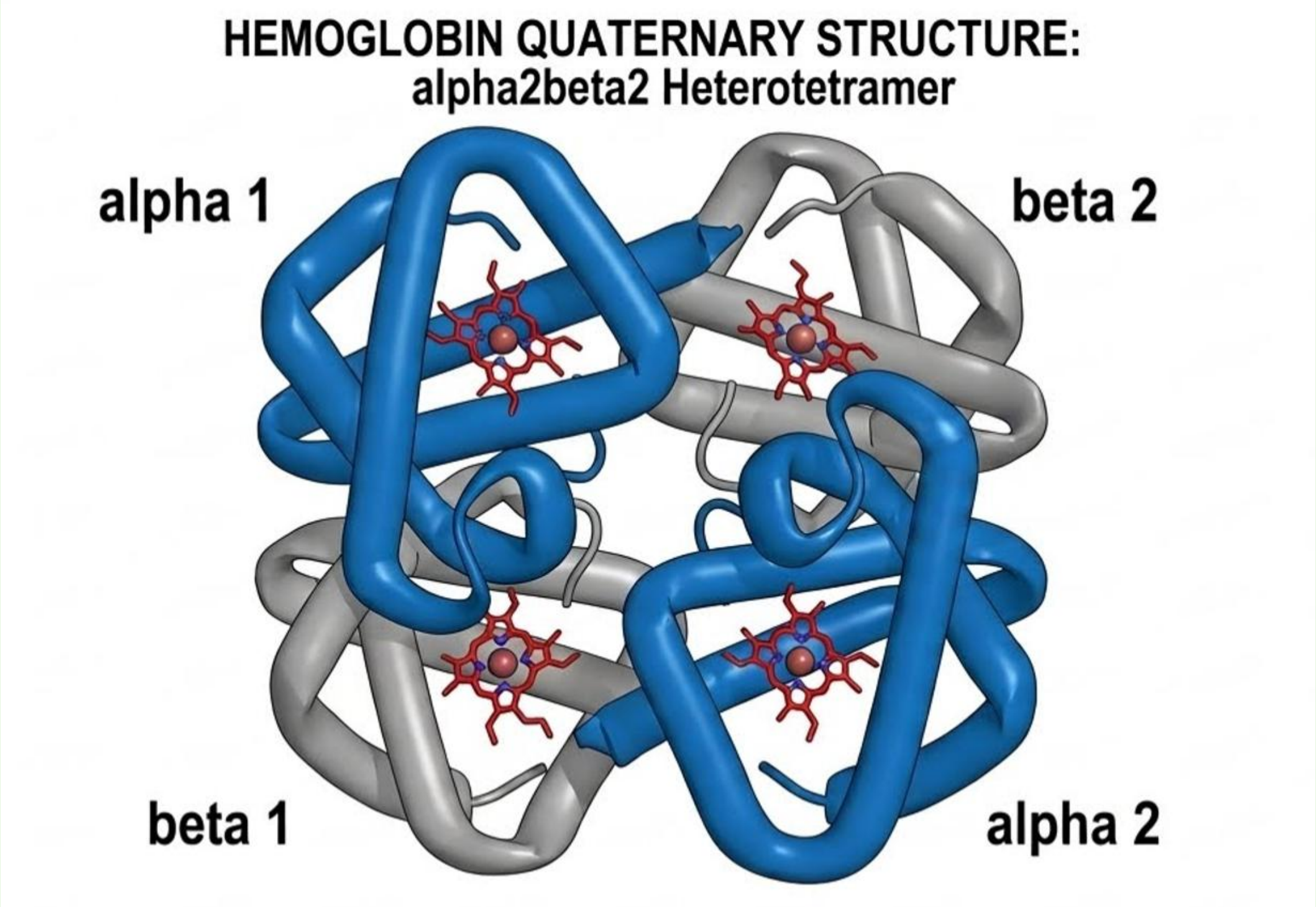
Normal DNA (HbA):

A C C T G A G A G A G C T G T

Sickle DNA (HbS):

A C C T G A G T G A G C T G T

The hemoglobin molecule comprises two alpha and two beta proteins. The sickle mutation bends the tail of HbA outward. (Left tail sits above the red heme group in upper left of this image.) The tail is sticky, and it can cross-link to a neighboring hemoglobin molecule.



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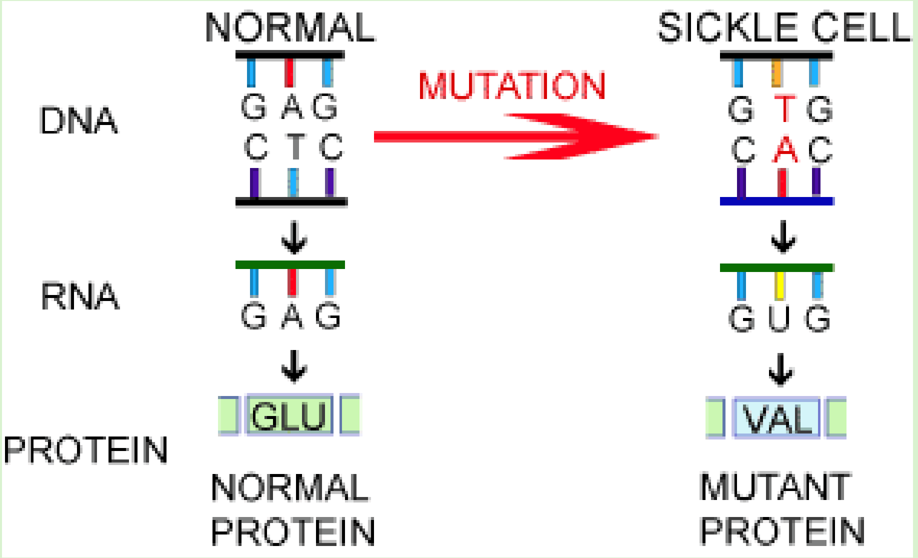
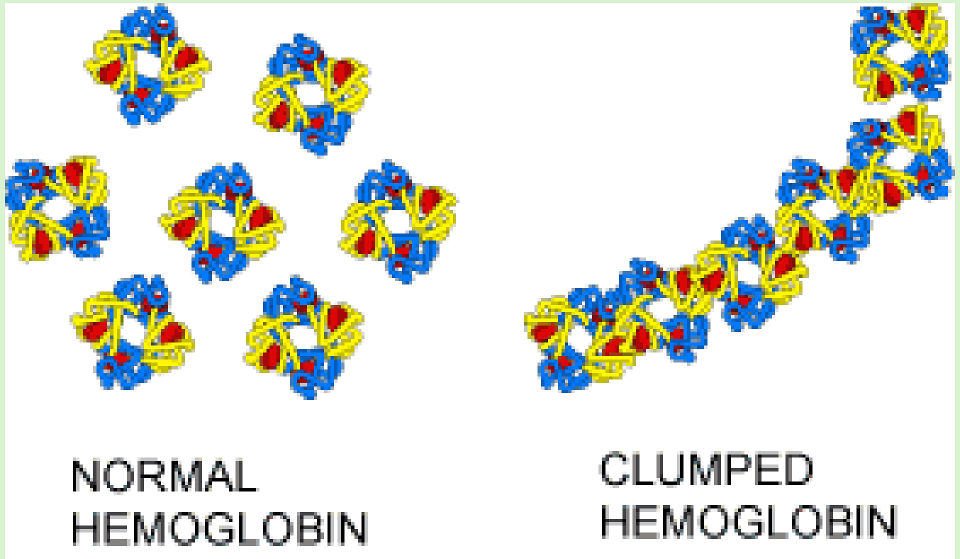
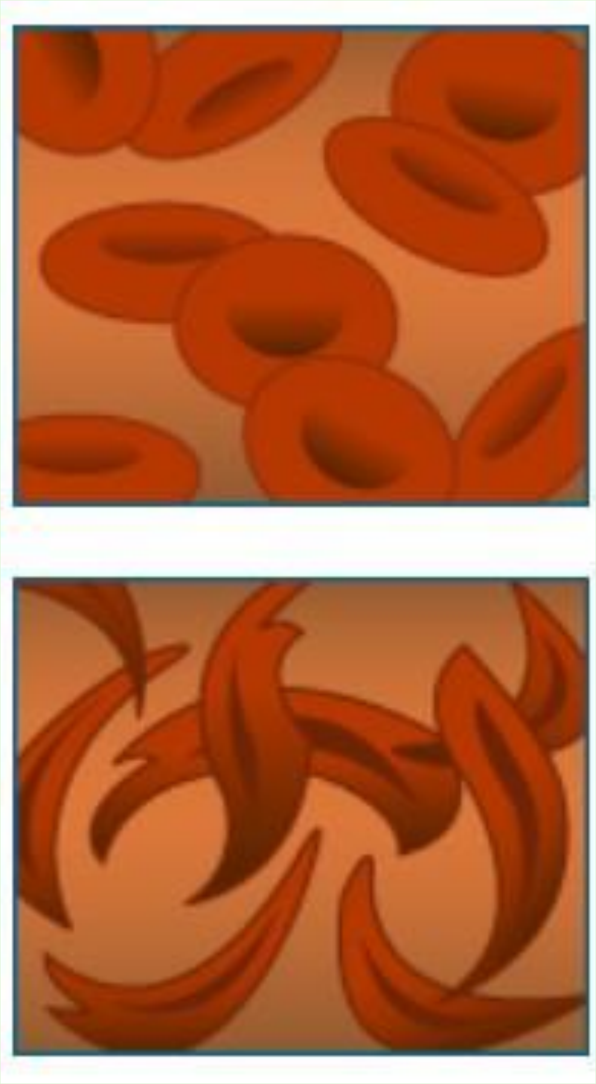
The sickle cell mutation in the hemoglobin alpha gene and its consequences.







images: UC Berkeley

How to correct the mutation?

We need a system that can:

1. Find its way from the environment into the cell.
2. Locate the mutation from among ~ 3 billion base pairs on DNA.
3. Restore the normal sequence of nucleotides in the gene.
4. And do all of the above without damaging the cell or causing other mutations.

The currently available repair systems, in order of increasing precision:

- CRISPR-Cas and homologous repair
- Base editing (a sophisticated CRISPR-Cas) or
- Prime editing (an even more precise CRISPR-Cas)

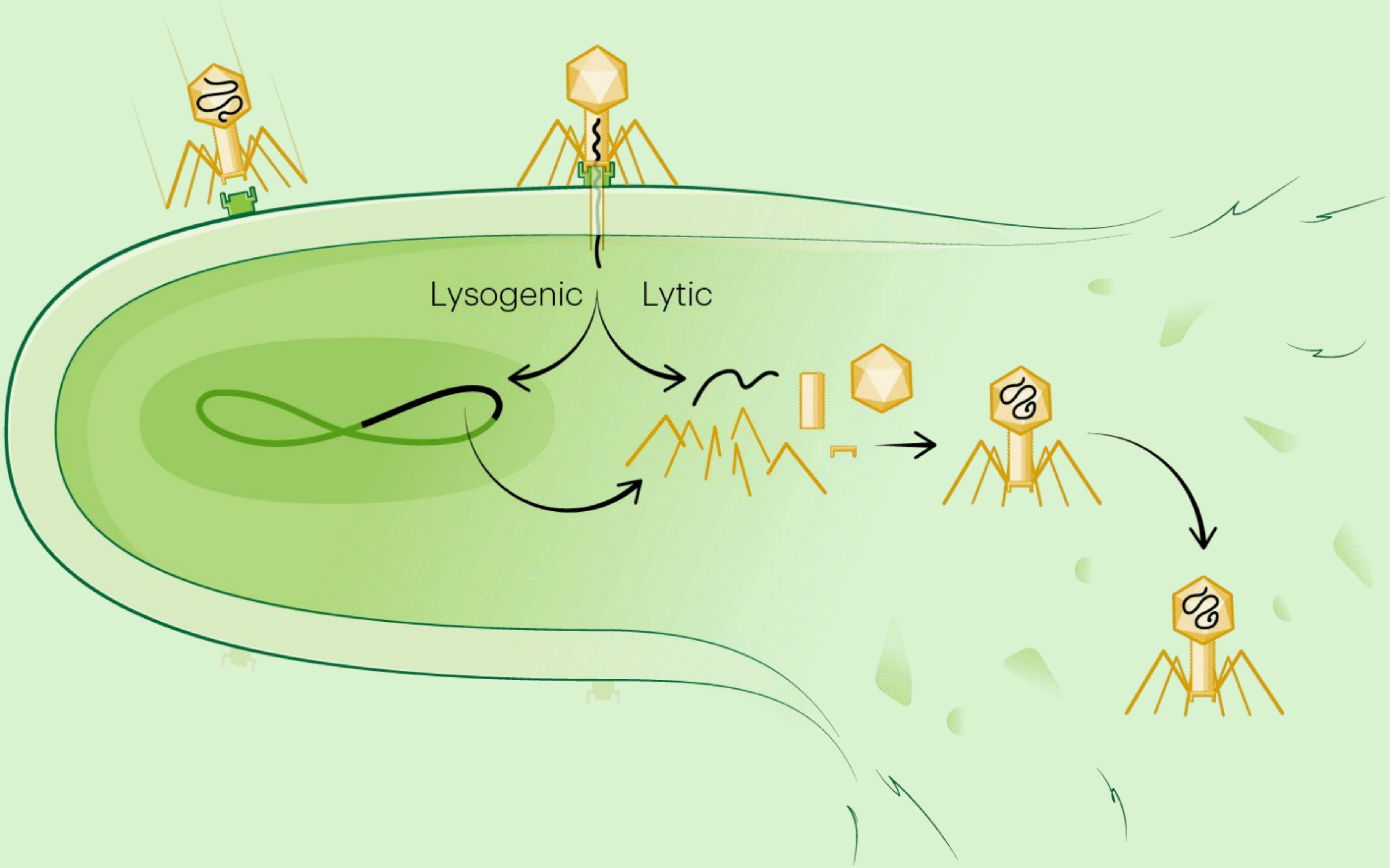
All of these tools rely on the detailed genetic maps provided by the Human Genome Project and its successors. We know the nucleotide sequences of all the genes in a human cell. CRISPR relies on that information to locate genes and to provide the correct nucleotide sequence needed to repair mutations.

The evolutionary origins of CRISPR-Cas

Archaea invented CRISPR-Cas to defend themselves against bacteriophages, viruses that infect bacteria. CRISPR provides an immune memory for bacteria.

1. In the original phage infection, bacterial restriction enzymes chop up the genome of the invading phage.
2. If the bacterium survives it creates a DNA copy of a segment of phage DNA.
3. That phage DNA segment is incorporated into CRISPR. It is passed along to future generations of bacteria.
4. When progeny bacteria later on encounter that same phage they transcribe the appropriate viral segment on CRISPR to produce multiple RNA copies complementary to the phage DNA.
5. The CRISPR RNA segment guides the Cas protein to the invading phage genome and Cas destroys the phage DNA.

Typical life history of a phage



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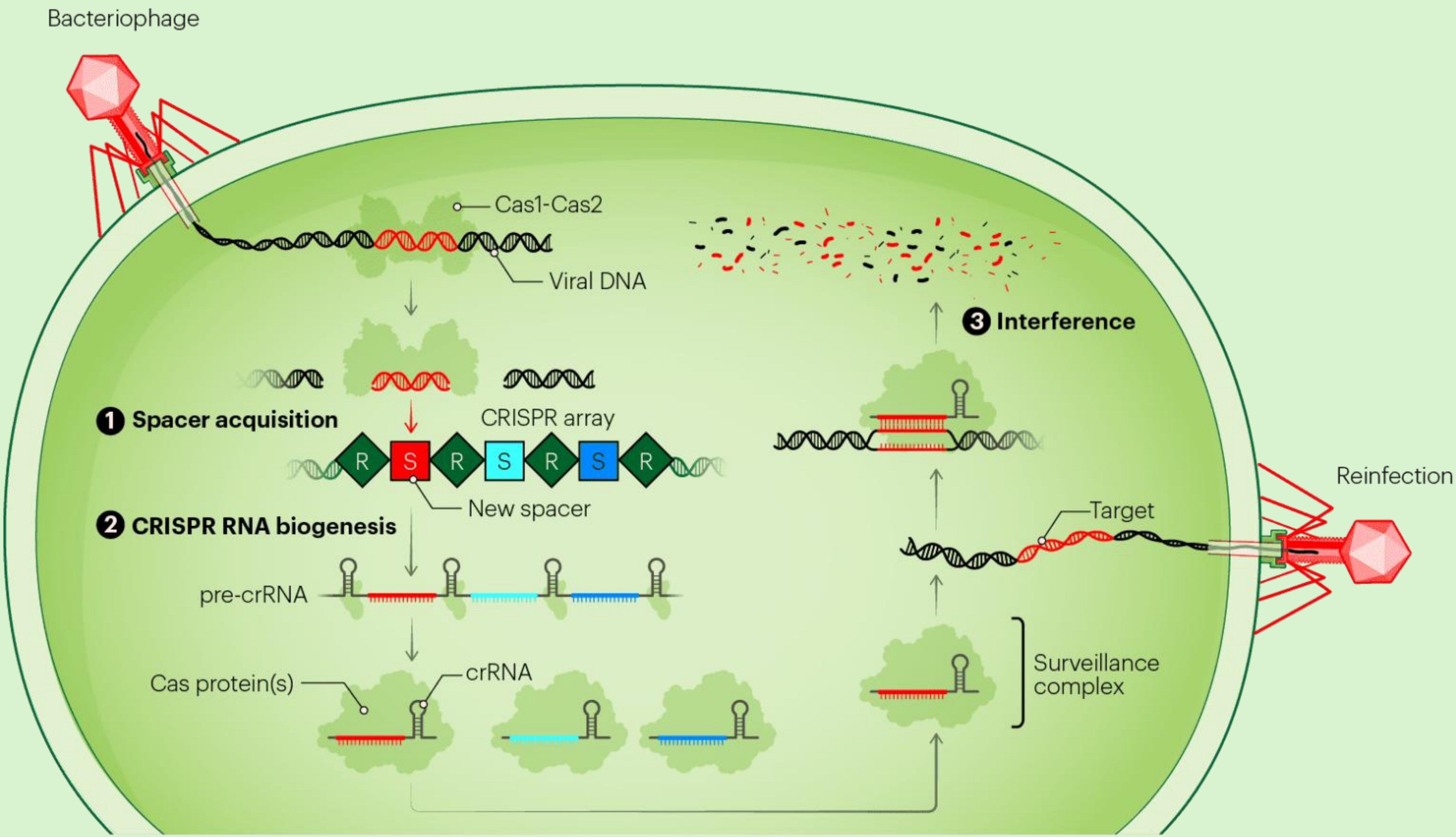
CRISPR-Cas immune system in bacteria



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Gene Engineering with CRISPR-Cas

Bacterial tools to the rescue in human cells:

Jennifer Doudna, Emmanuele Charpentier and their respective laboratories figured out how to modify the bacterial CRISPR-Cas system to

1. Locate a particular gene in the human genome.
2. Remove the mutated nucleotide sequence, and
3. Create the conditions by which the cell's own DNA repair mechanisms would replace the mutation with a normal nucleotide sequence.

Anatomy of a CRISPR-Cas system

- crRNA = CRISPR RNA
- tracrRNA = linker RNA, binds CRISPR RNA to Cas (the big blue blob)
- spacer = CRISPR probe segment, 20 nucleotides
- protospacer = DNA target, the base sequence that the spacer is targeting
- PAM = base triplet that labels self vs. foreign DNA

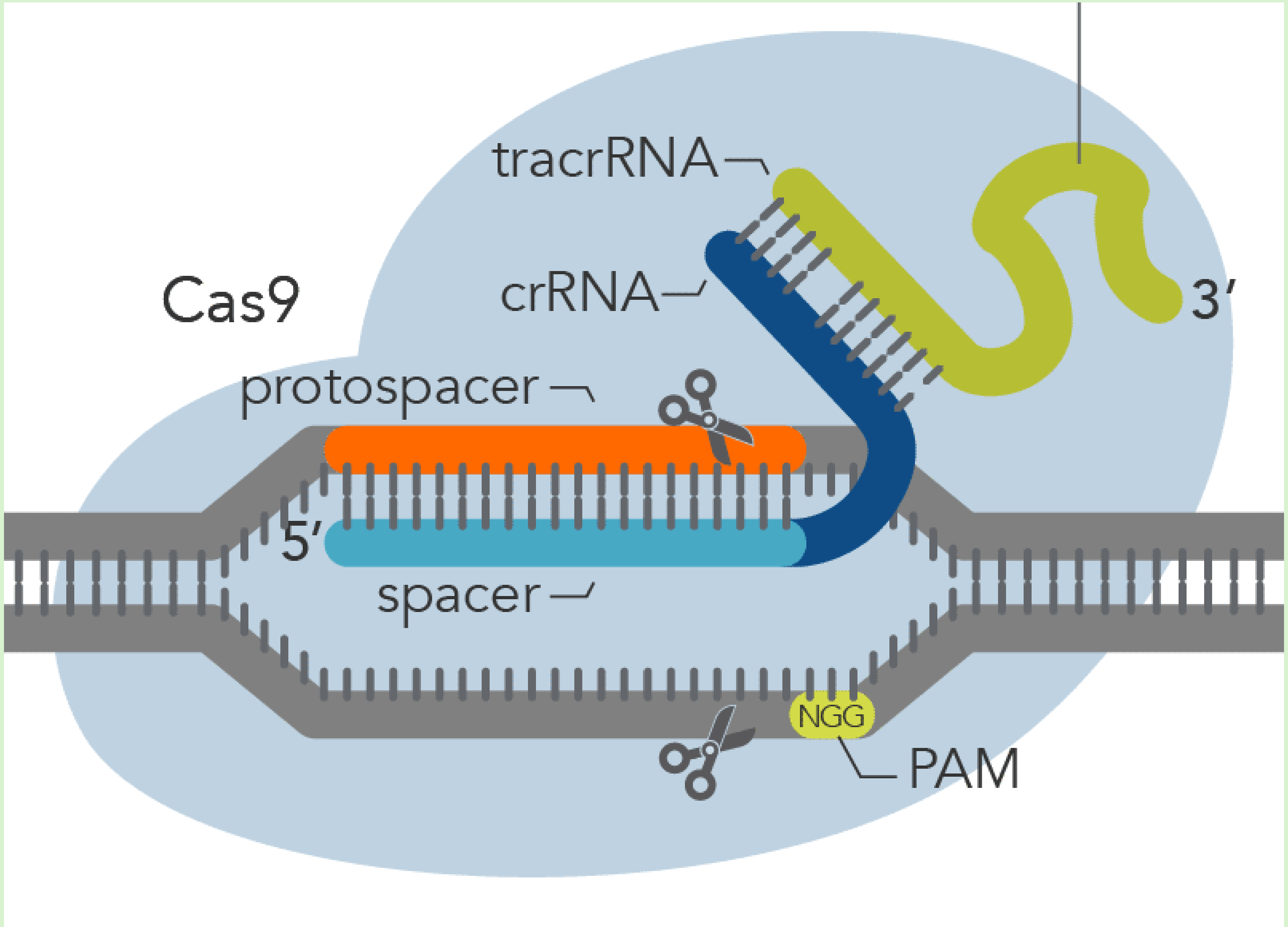
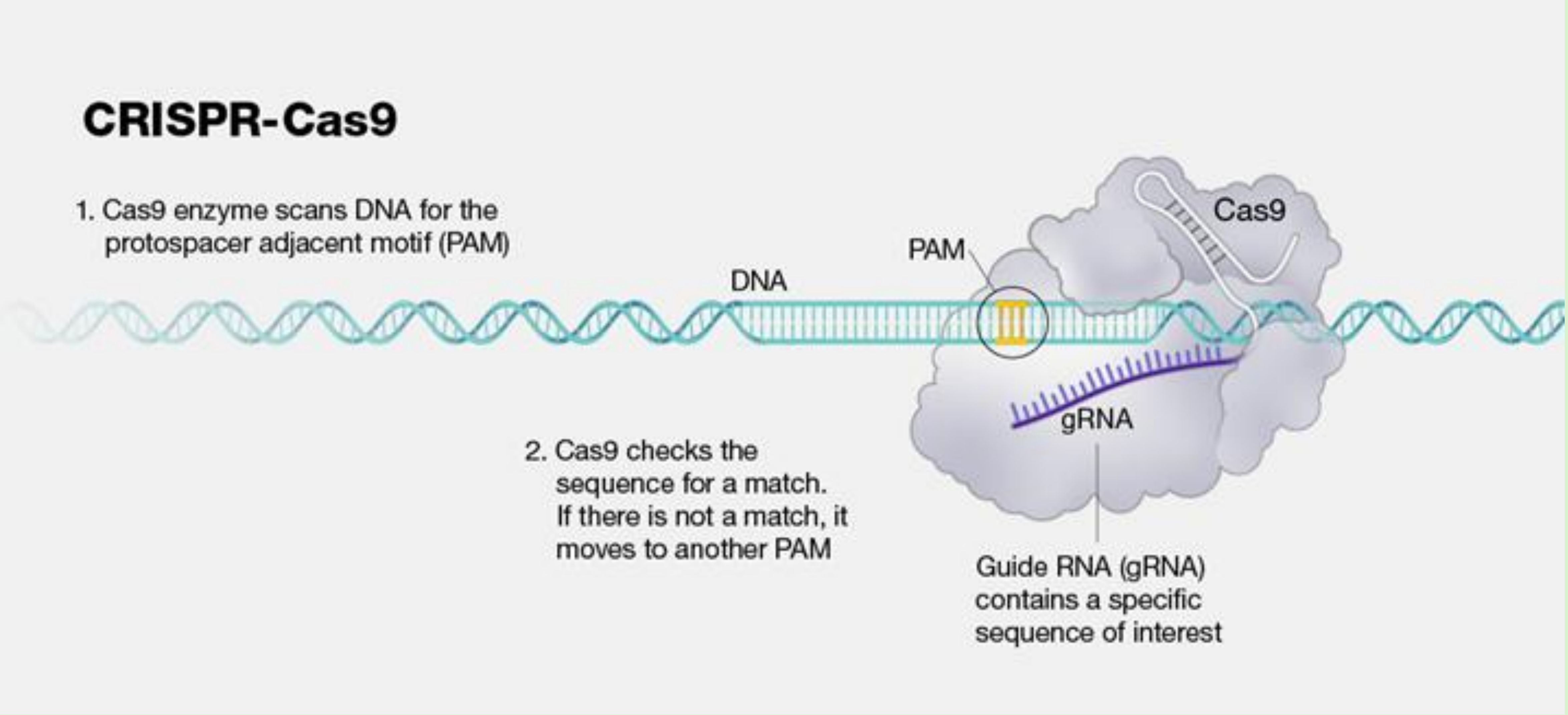
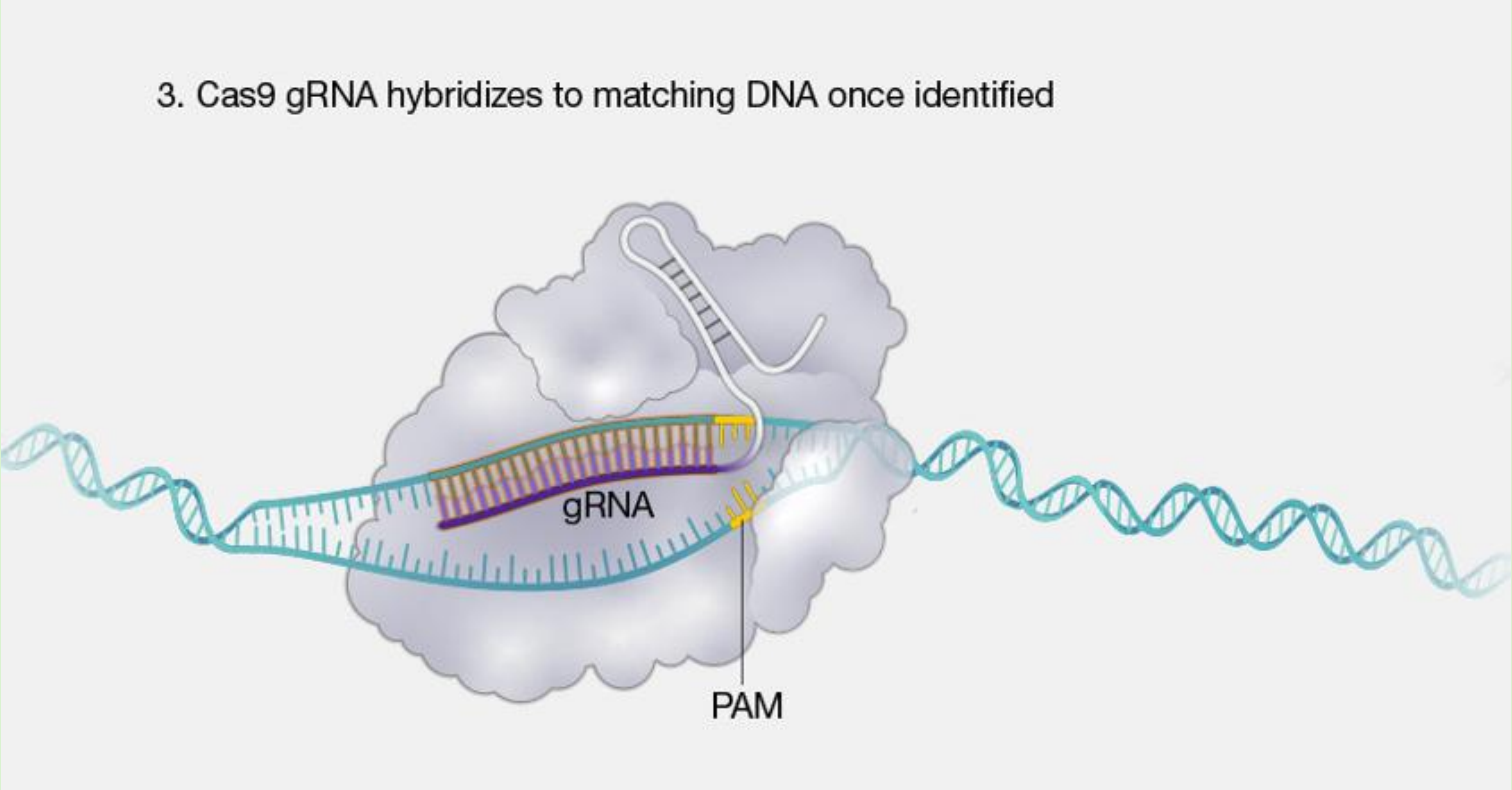


image: Integrated DNA Technologies

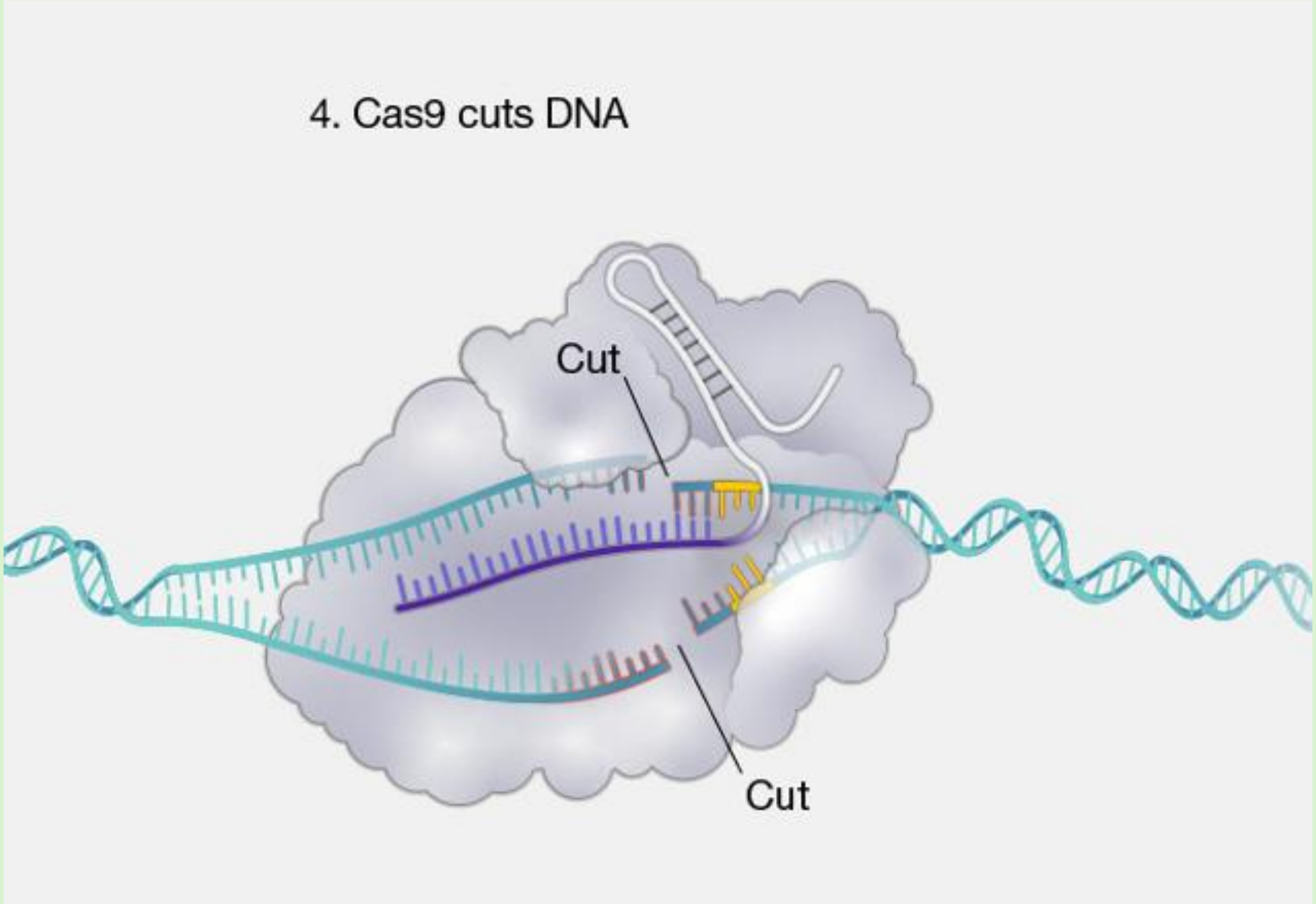
First steps in CRISPR-Cas editing. Cas carries CRISPR to a PAM sequence. PAM tells Cas that this is foreign DNA.



<u>Next steps</u>: CAS separates the DNA strands. If the guide (spacer) CRISPR RNA binds to the DNA target sequence (!!phage alert!!) it activates Cas scissors.



CRISPR-Cas finishes its mission. Cas cuts both DNA strands. After this, the cell's own DNA repair machinery takes over.



Native CRISPR-Cas leaves damaged DNA. That's its job in bacteria, after all – to destroy phage DNA. And that may be enough for some purposes. Destroying cancer-causing genes, for instance, can help treat the cancer. But if you want to edit genes, e.g. repair mutations, CRISPR-Cas needs help.

First attempts at CRISPR-Cas gene therapy used homologous repair. Cells have innate DNA-repair mechanisms, dedicated protein systems that identify and repair breaks in the DNA. Those systems use DNA on the homologous chromosome as a template for the repair. Each human cell, after all, has two copies of DNA – one from mom and one from dad. If the homologous DNA has a normal nucleotide sequence, you can end up with a normal gene in the repair. Repairs, though, are prone to mutation.

One trick to facilitate a desired result: flood the cell with normal segments of DNA bracketing the CRISPR target. Then the innate repair system is more likely to use a normal template and make the correct repair.

Victoria Gray. First person cured of sickle cell disease, after 34 years suffering episodes of excruciating pain and life-threatening arteriolar blockage from sickle cell disease.

New Tools in the Toolbox

CRISPR-Cas v.2: Base Editing. Use CRISPR-Cas as a guide, and attach an enzyme for targeted nucleotide transformation. This process requires a subsequent round of replication before the cell's repair system reverses the mutation.

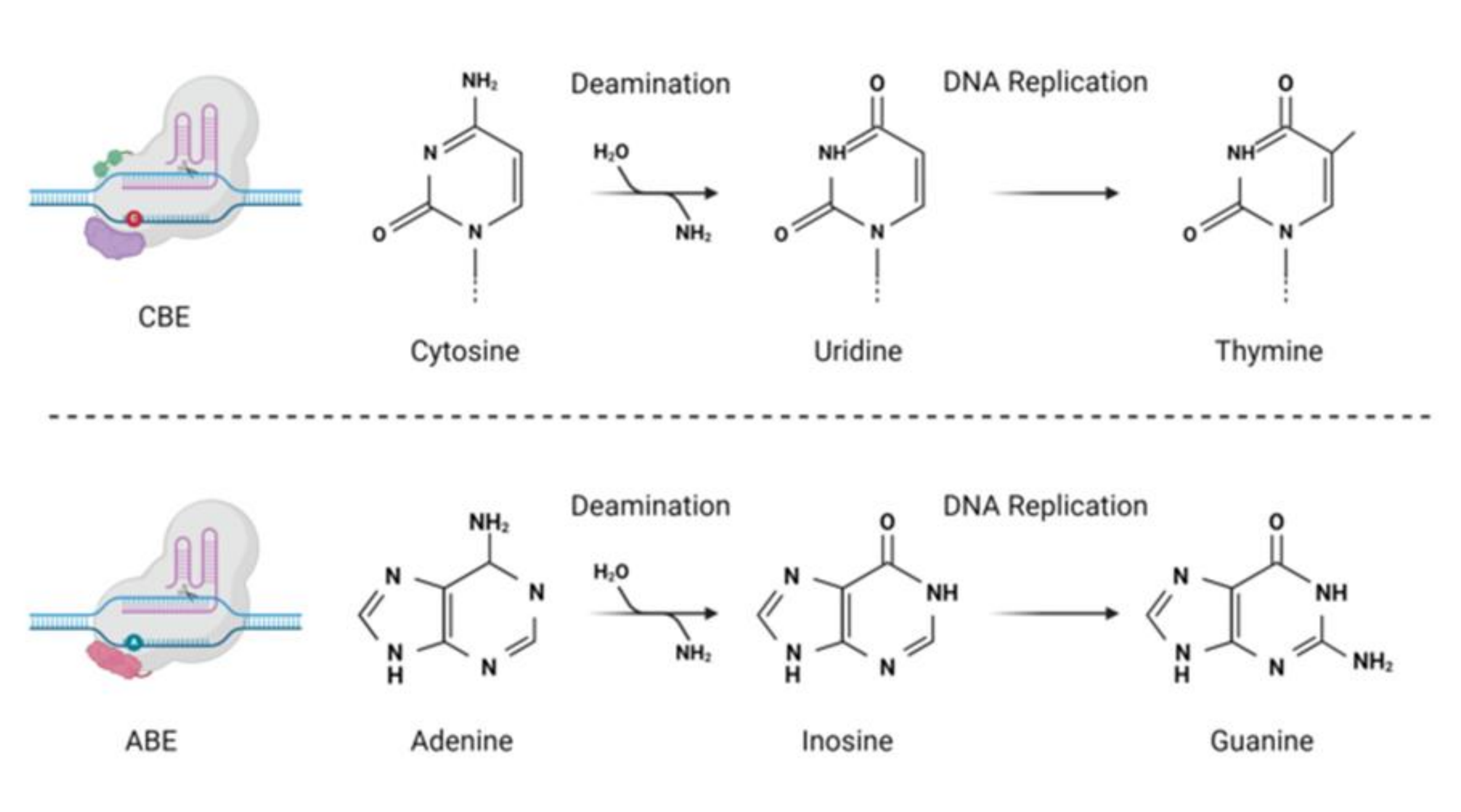


image: CRISPR Medicine

<u>The process of base editing.</u> Needs UGI to fend off the cell's innate repair system.

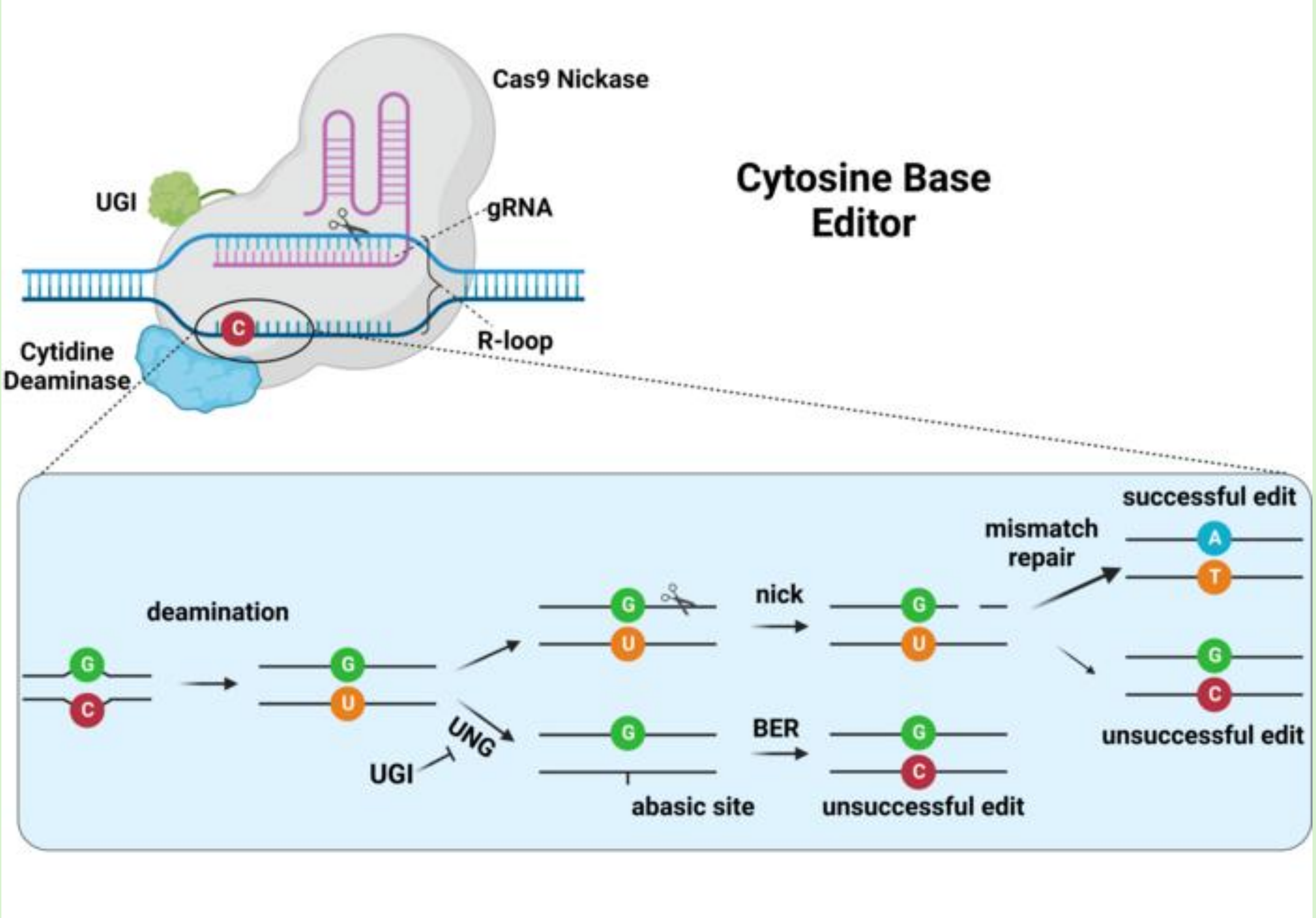
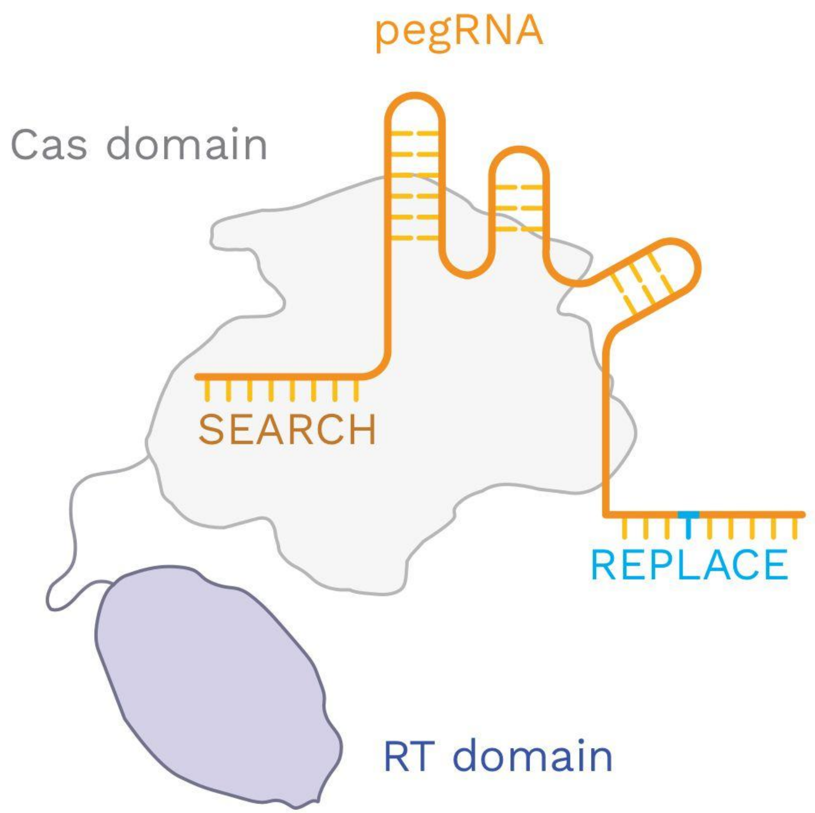


image: CRISPR Medicine

CRISPR-Cas v.3: Prime Editing.

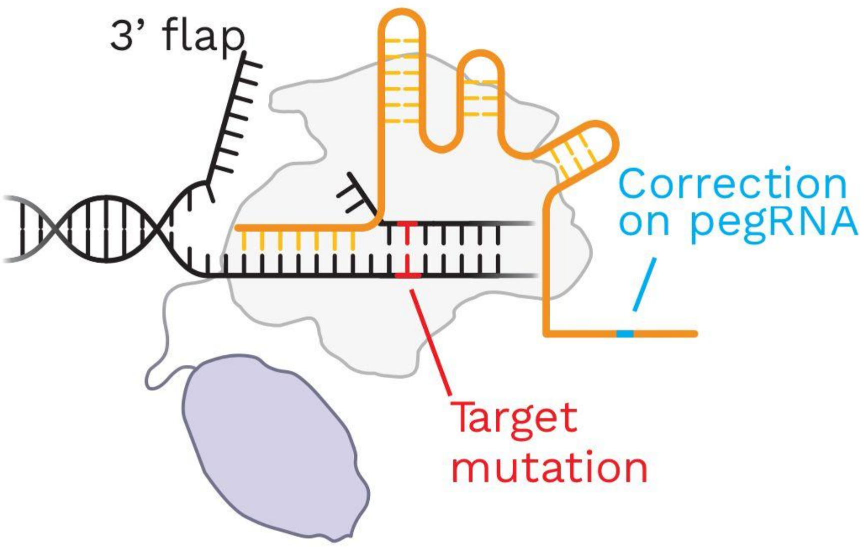
CRISPR RNA carries normal code and RT. No need for additional DNA repair tools.

images: Prime Medicine



Search

Prime Editor complex initiates search for target DNA

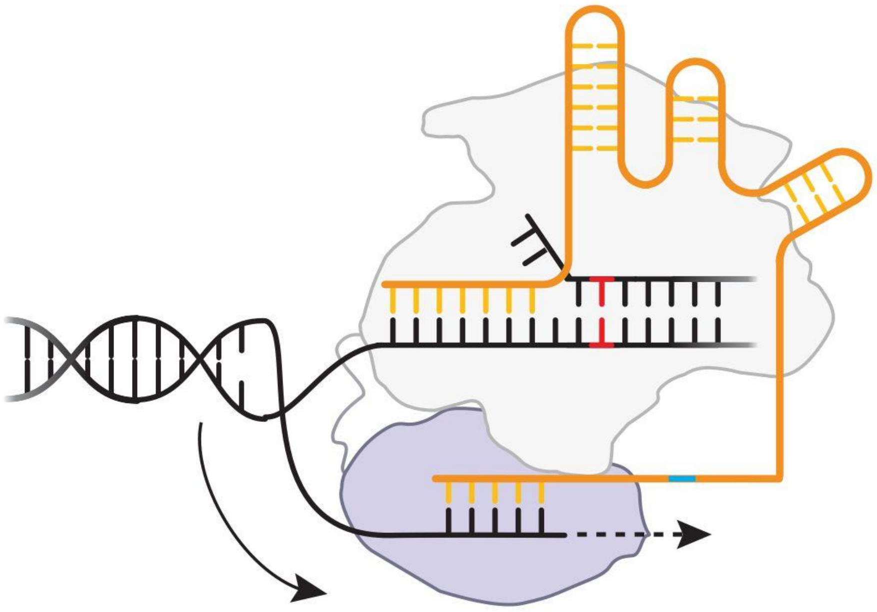


Find & nick

Prime Editor complex finds DNA with target mutation, nicks one strand

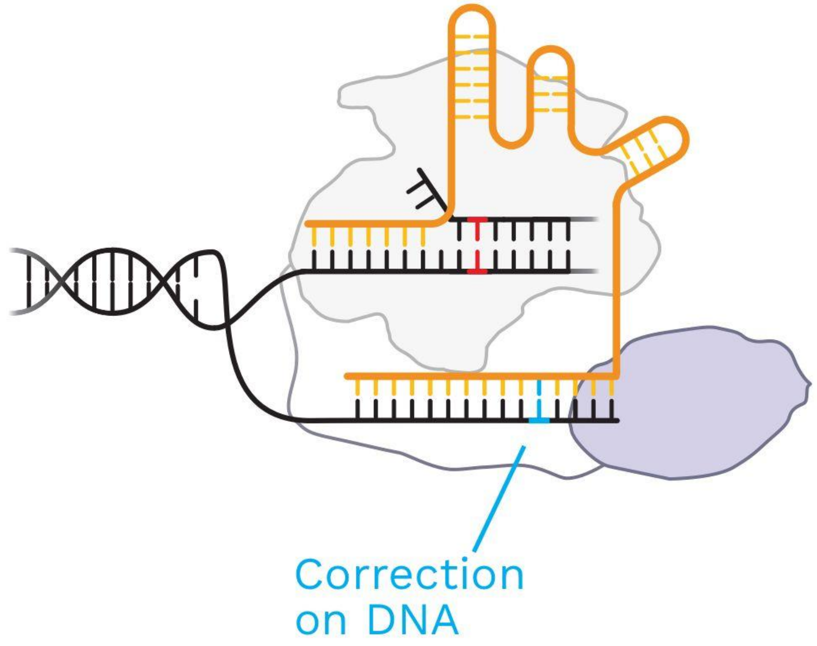
Finishing the job. Reverse transcription replaces mutated DNA with normal code.

images: Prime Medicine



Prime

Nicked DNA strand primes the RT domain for DNA synthesis



Replace

Prime Editor complex copies in corrective DNA sequence

Dangers inherent in CRISPR-Cas technology:

- unintended consequences
- biological weapons

Ethical questions:

- How accurate does a gene editing system have to be before using it in humans?
- What applications are acceptable?
  - repair non-lethal mutations?
  - preventative repairs, before the onset of any disease?
  - create new gene combinations / new life forms?
  - recover lost species?
  - create more productive livestock and crops?
  - create designer babies, i.e. allow parents to choose their children's traits?
  - and many, many more . . .

References

Doudna, Jennifer. 2020. Nobel Lecture. https://www.youtube.com/watch?v=KSrSIErIxMQ

Doudna, Jennifer. CRISPR-Cas in Nature. 2026. Innovative Genomics Institute. https://innovativegenomics.org/crisprpedia/crispr-in-nature/. The story of the bacterial origins of the CRISPR-Cas immune system.

IGI. 2026. CRISPR Made Simple. https://innovativegenomics.org/crispr-made-simple/. An educational library with lots of resources explaining CRISPR and its applications.

Thank you!

. . . and there’s a whole lot more to this story. Check out the IGI references, especially.

. . . and an appreciation. We recognize the Nobel Laureates, and rightly so. Under their leadership (and graciously acknowledged by the Laureates) are many hundreds of post-docs, graduate students, lab techs, and other support staff who spend countless hours at work in the research labs. Sustaining all that effort, at least until recently, have been the National Institutes of Health, the National Science Foundation, and the great research universities, all funded by the American people. I hope you share the wonder of these accomplishments and consider that it might be worthwhile to continue . . .

The genetic code as carried on mRNA. Read from the inside out. Translation reads the code in triplets. For example, the triplet UGG codes for Tryptophan (TRP at 3 o'clock on the diagram). Other amino acids have redundant codes, e.g. GGU, GGC, GGA, GGG all for Glycine (12 o'clock). Note also the STOP codes at 2 o'clock.

All life on earth uses this code, from viruses to humans, mushrooms to oak trees.

image: U. Utah Genetics / Miller and Levine, Biology