Clustered Regularly Interspaced Short Palindromic Repeats

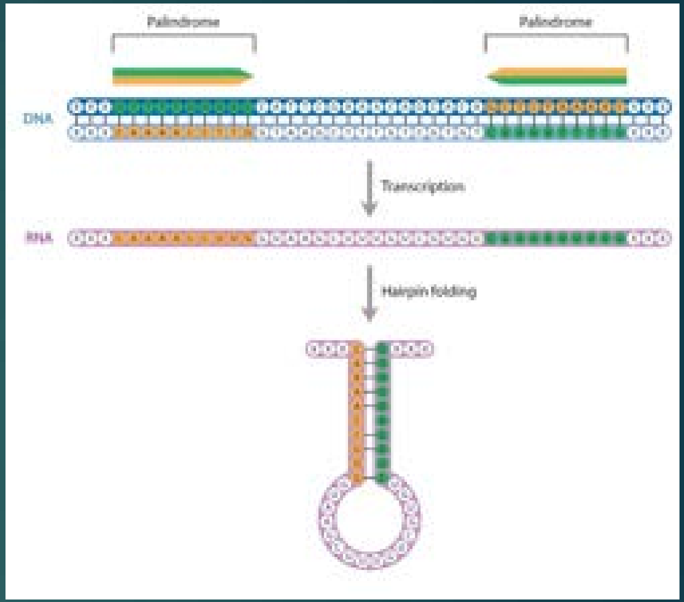
CRISPR Technology: From Procaryotic Origins to Eukaryotic Applications

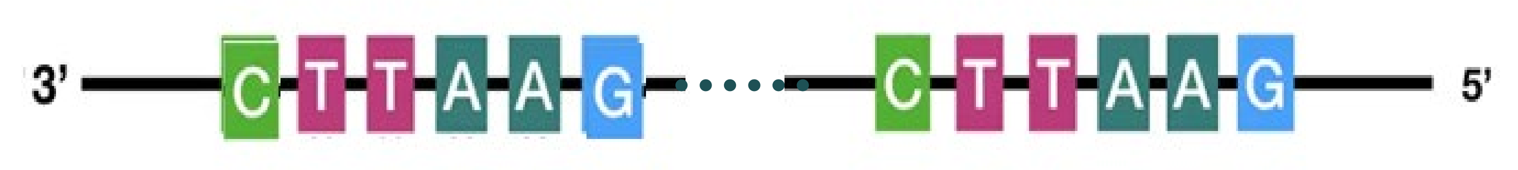
AN OVERVIEW OF CRISPR'S JOURNEY IN GENETIC ENGINEERING

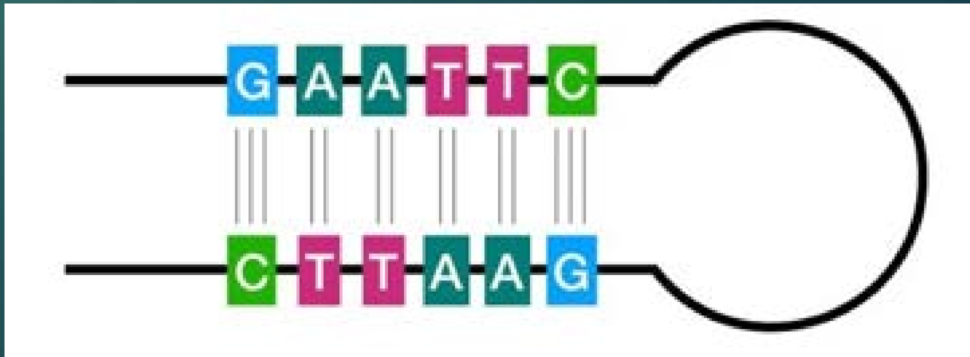
Leathem Mehaffey VCLLI, Spring 2026

What's a Palindrome??

- Palindromes are texts that can be read forwards and backwards.
  - *A man, a plan, a canal, Panama*
  - *Step on no pets*
  - *Able was I ere I saw Elba*
- Palindromic sequences in RNA refer to self-complementary sequences that can fold back on themselves to form hairpin or stem-loop structures. In biology, these structures are critical for the functionality, stability, and processing of RNA molecules.







CRISPR in the News:

The New York Times

Scientists Find Form of Crispr Gene Editing With New Capabilities
A common bacterium contains molecules that target RNA, not DNA. If it can be harnessed for use in humans, the process may lead to new forms of bioengineering.

Gene Editing Spurs Hope for Transplanting Pig Organs Into Humans
Geneticists have created piglets free of retroviruses, an important step toward creating a new supply of organs for transplant patients.

Scientists Can Design ‘Better’ Babies. Should They?
Advances in reproductive technology have put genetic choices within reach of prospective parents. But critics warn of ethical peril.

Science,Feb 2020
CRISPR takes on cancer

Baby Is Healed With World’s First Personalized Gene-Editing
The technique used on a 9½-month-old boy with a rare condition The technique used on a 9½-month-old boy with a rare conditionhas the potential to help people with thousands of other uncommon genetic diseases.

Scientists Find Form of Crispr Gene Editing With New Capabilities
A common bacterium contains molecules that target RNA, not DNA. If it can be harnessed for use in humans, the process may lead to new forms of bioengineering.

As D.I.Y. Gene Editing Gains Popularity, ‘Someone Is Going to Get Hurt’
After a virus was created from mail-order DNA, scientists are sounding the alarm about the genetic tinkering carried out in garages and living rooms.

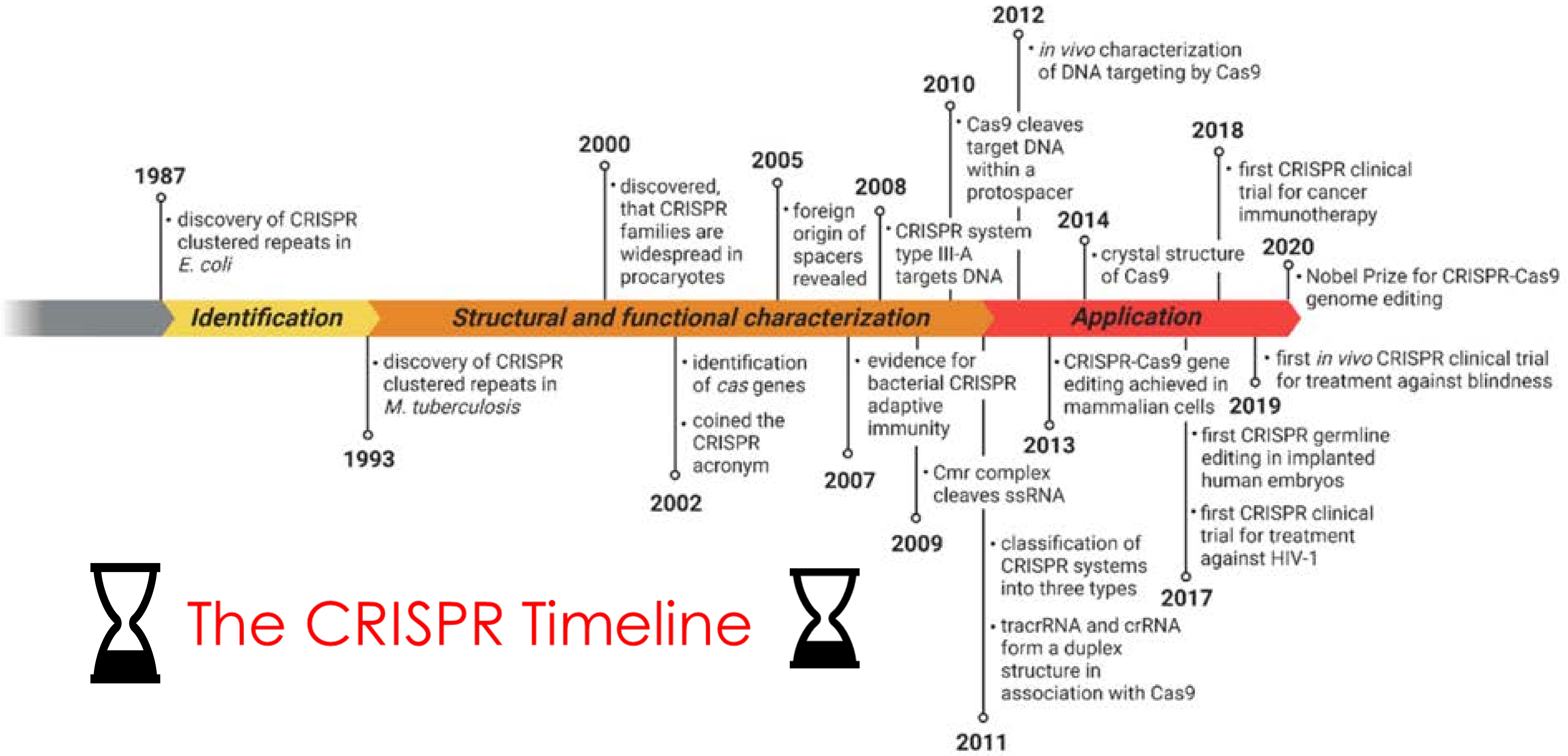
Science, June 10
Scientists Can Design ‘Better’ Babies. Should They?
Advances in reproductive technology have put genetic choices within reach of prospective parents. But critics warn of ethical peril.

Science, January 2025
A new ‘mini-CRISPR’ flexes its editing power in monkey muscles
The downsized DNA-slicing machinery may reach more tissues to take aim at more diseases

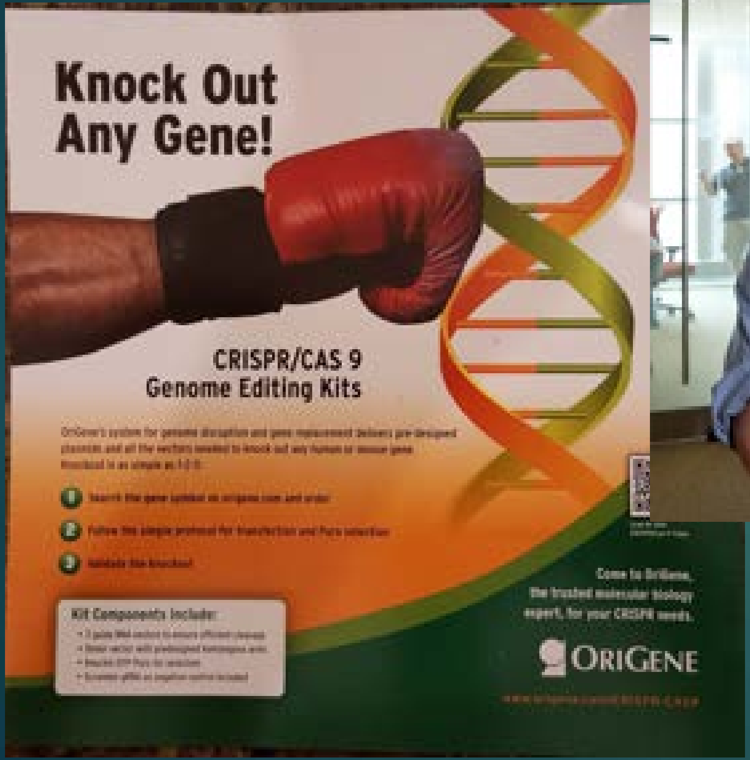
Today's Agenda

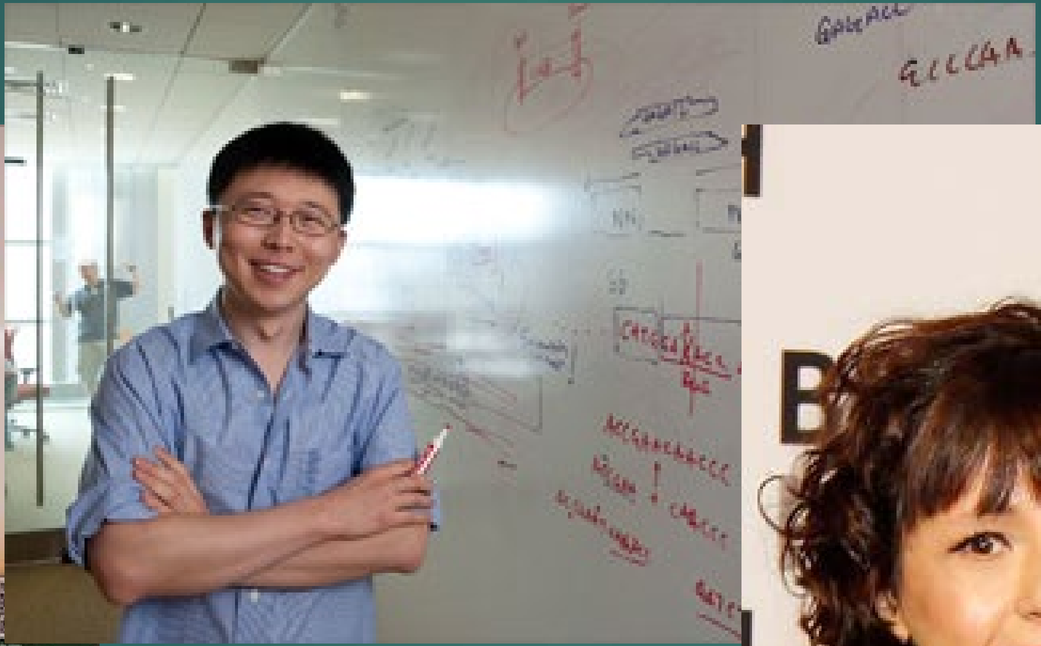
- History of CRISPR
- How CRISPR Works on DNA
- Advances in CRISPR Technology
- Clinical Uses of CRISPR
- Ethical Considerations and Regulatory Challenges
- Future Directions in CRISPR-Based Therapies

The CRISPR Timeline



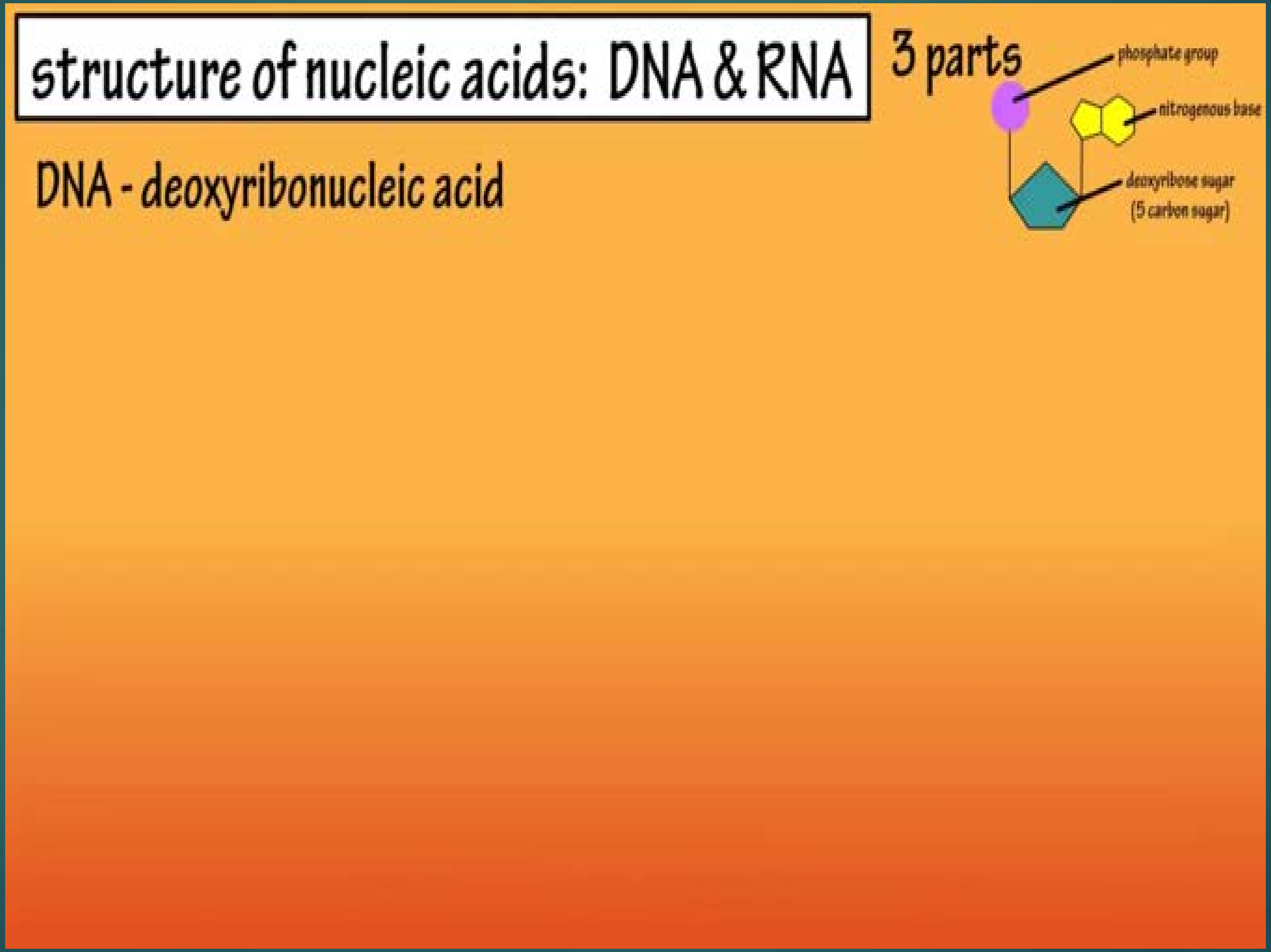
The CRISPR-CAS 9 System: Early Applications in Eukaryotes and a Patent Dispute





So: How Does CRISPR Work??

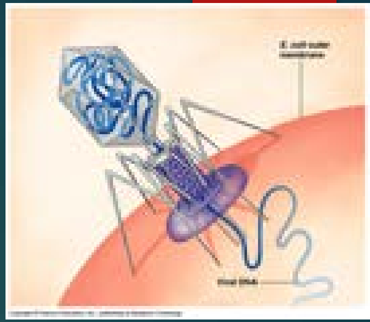
First, we need a short primer on DNA



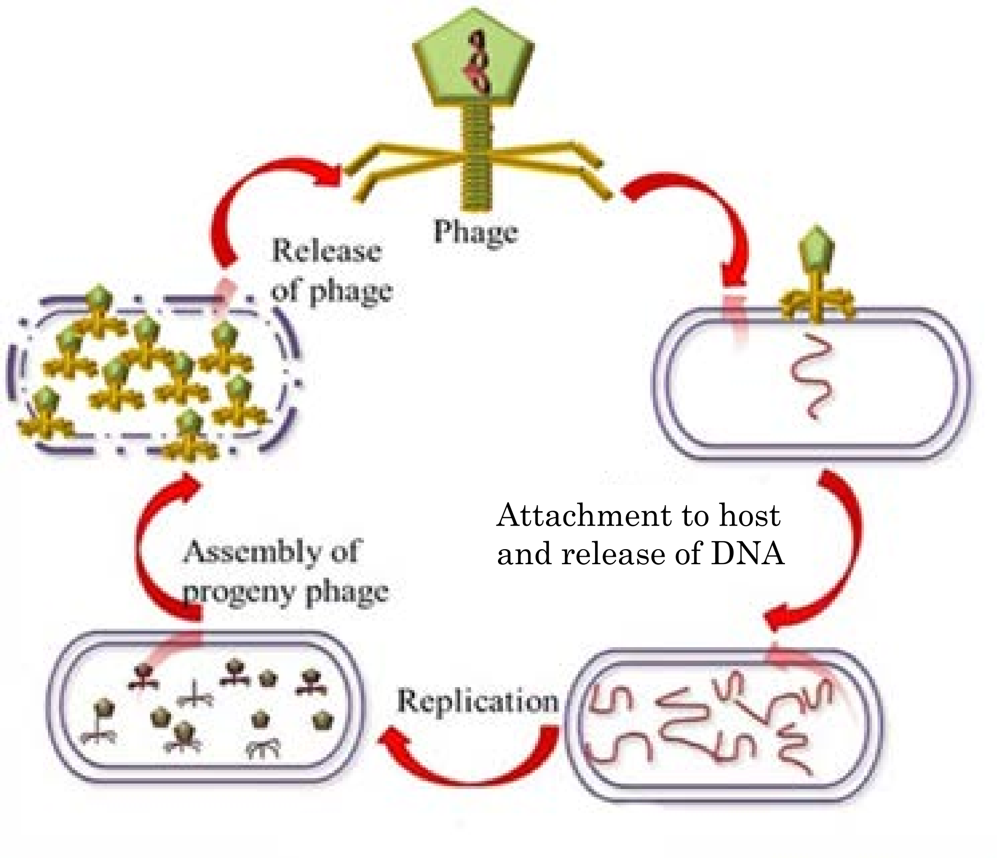
What's the takeaway from this??

- Base pairing in DNA is unique:
  - Adenine *always* bonds to Thymine
  - Guanine *always* bonds to Cytosine
- Genes are sequences of DNA. The code in a gene lies in the sequence of base pairs.
- It follows that if you know the sequence in a gene or even a part of the sequence, you can design a "probe" with complementary bases which will seek out that gene in the DNA in the nucleus of a cell.
  - This is the basis of PCR and other molecular techniques such as ancestry tracing.
  - ***It is also the basis of CRISPR***.

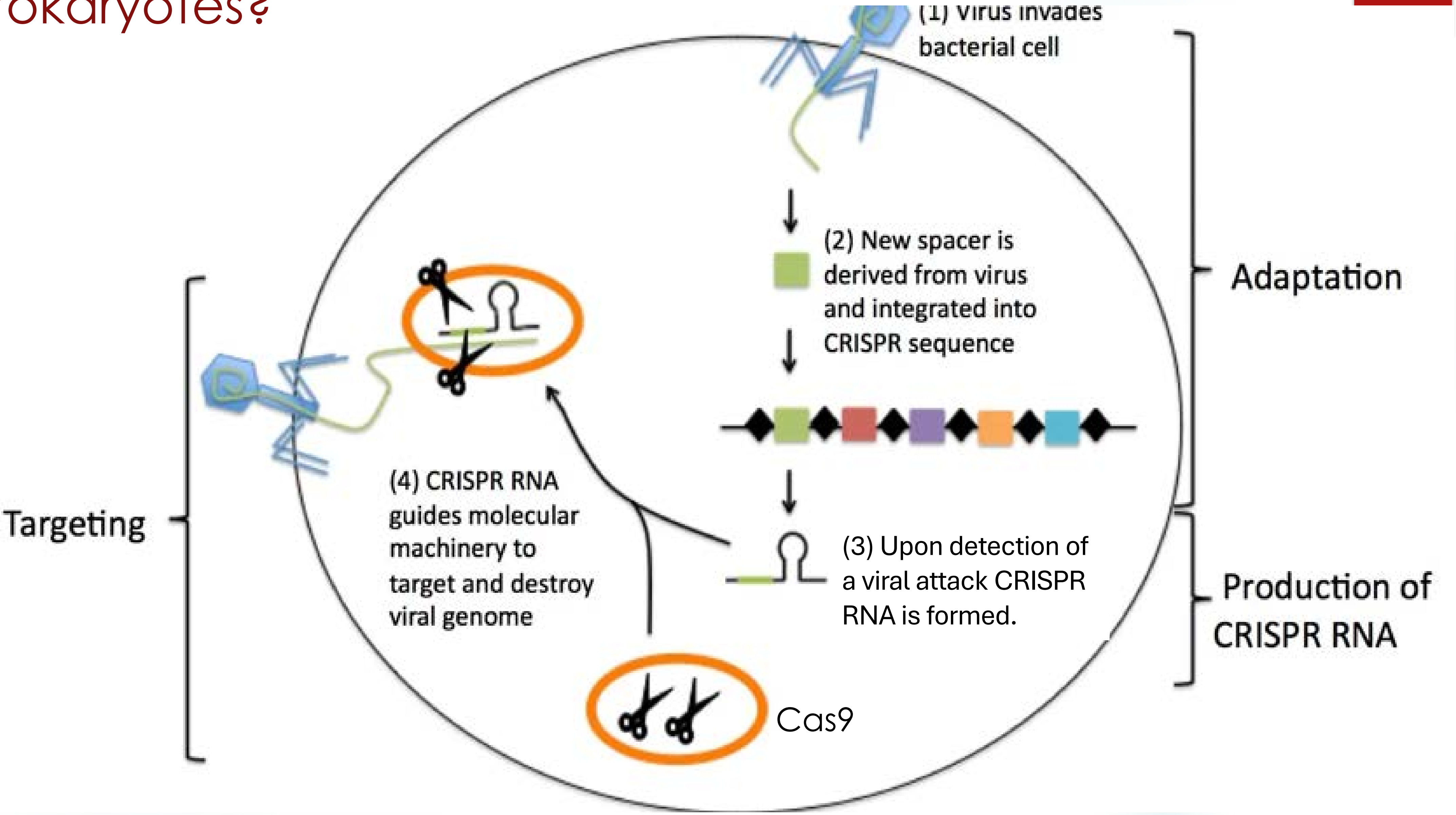
CRISPR In Prokaryote Cells



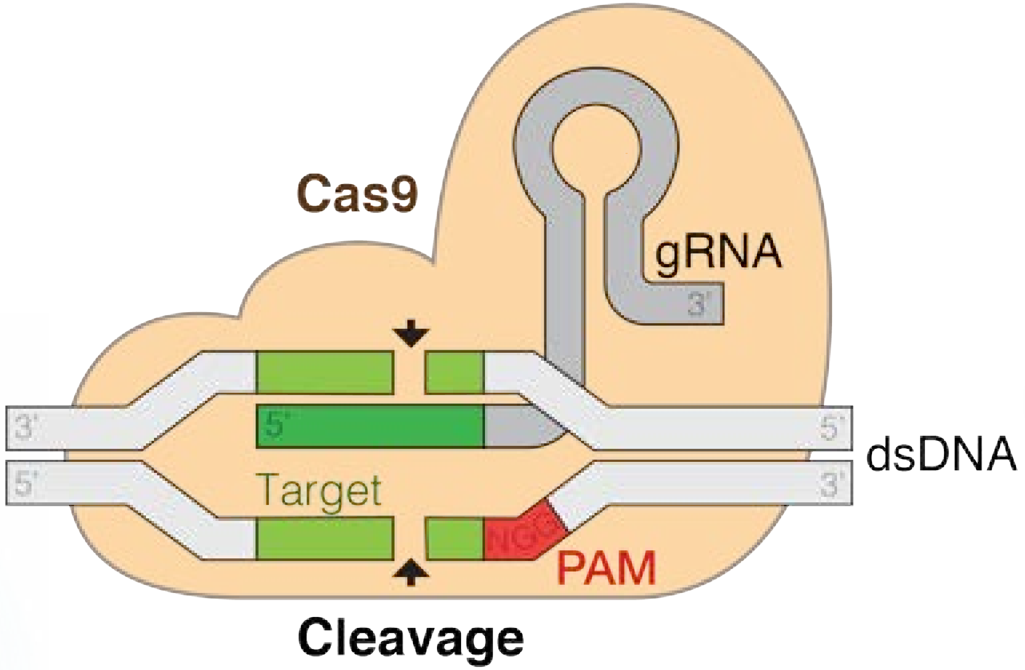
- Viruses act by inserting their nuclear (genetic) material (DNA or RNA) into the cell.
- The viral genetic material takes over the protein and nucleic acid synthetic machinery of the cell to make more viruses.
- Eventually the new viruses erupt from the cell, destroying it, and go on to infect other cells.
- Viruses that attack bacterial cells are called *bacteriophages*.
- CRISPR is based on the bacterial cell's "immune" or defensive system to protect against viruses (or allospecific plasmids).



How Does CRISPR work in prokaryotes?



Role of the PAM*

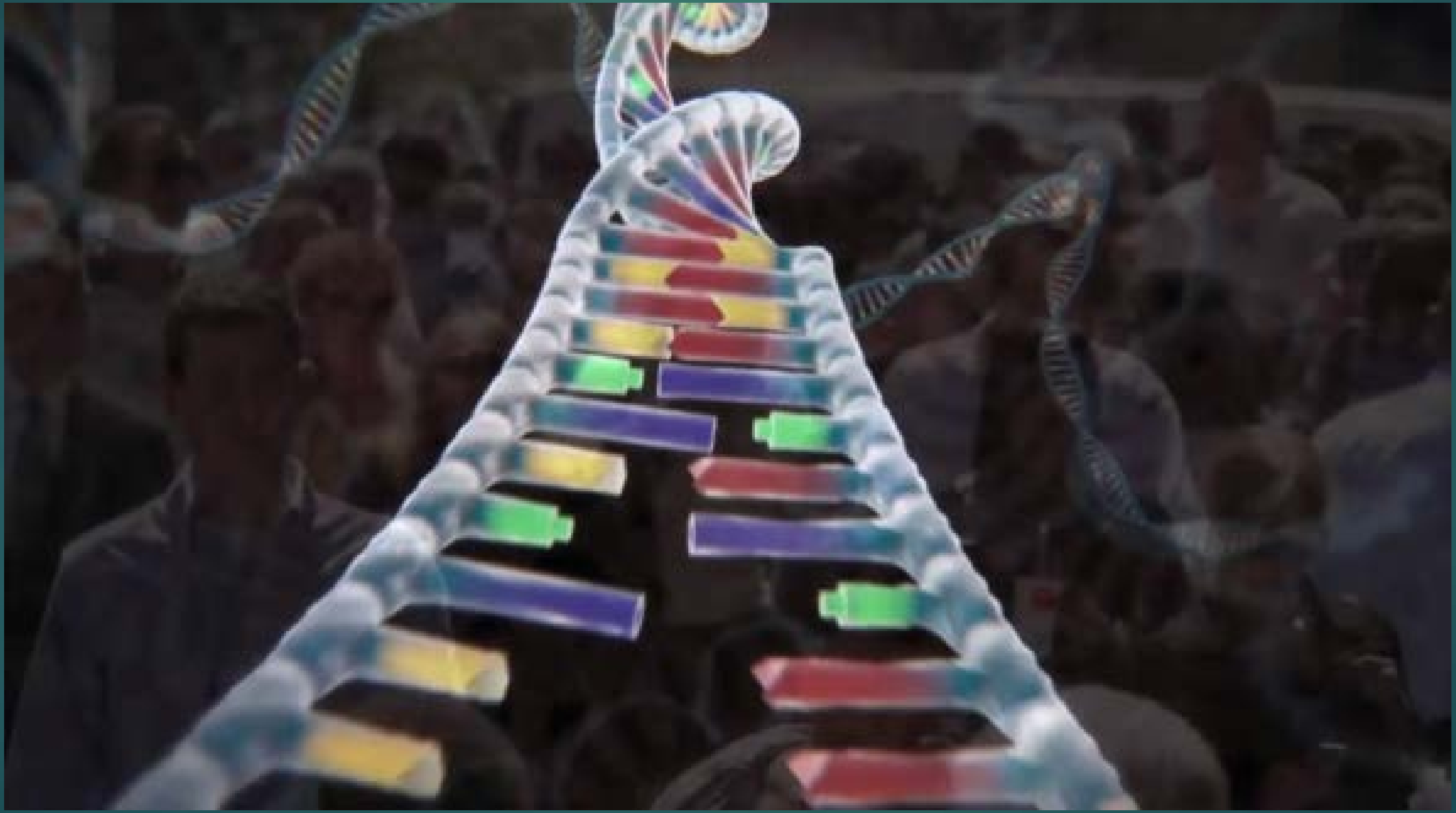


- Cas9 will not bind to the target DNA unless there is a PAM sequence adjacent to the site on the bacterial or viral DNA. The spacer in the bacteria's own CRISPR loci does not contain a PAM sequence and will thus not be cut by the nuclease. But the protospacer in the invading virus or plasmid does contain the PAM sequence and will thus be cleaved by the Cas9 nuclease.

*Protospacer Adjacent Motif

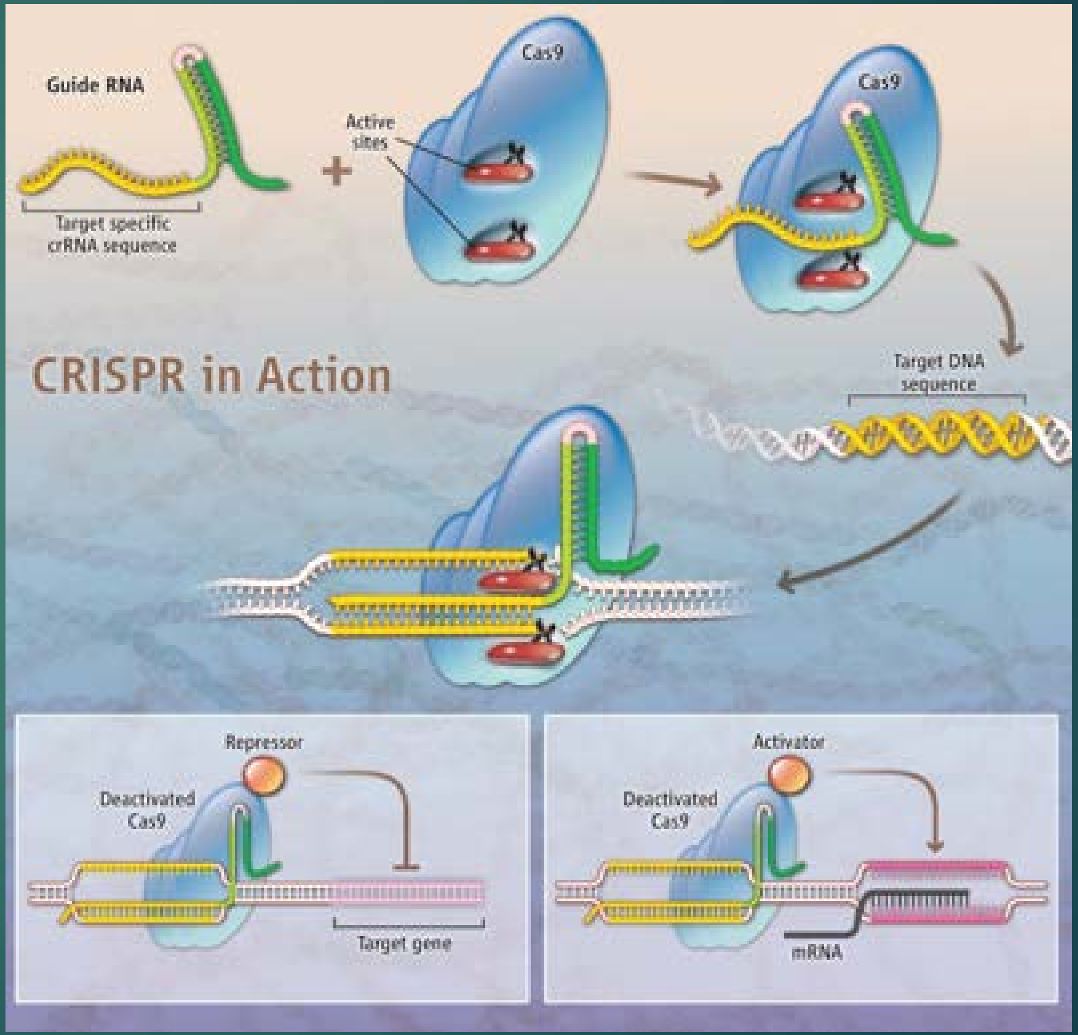
CRISPR in action

Taking advantage of CRISPR system:



DNA surgeon.

With just a guide RNA and a protein called Cas9, researchers first showed that the CRISPR system can home in on and cut specific DNA, knocking out a gene or enabling part of it to be replaced by substitute DNA. More recently, Cas9 modifications have made possible the repression (lower left) or activation (lower right) of specific genes.



Published by AAAS [Jennifer Doudna explains CRISPR (16 min)]

Elizabeth Pennisi Science 2013;341:833-836

Science AAAS

SUMMARY: how CRISPR-Cas9 works

01

Target Recognition

gRNA binds the complementary DNA sequence, directing Cas9 to the precise site.

02

Cas9 Binding

Cas9 associates with the gRNA–DNA complex, positioning for cleavage.

03

DNA Cleavage

Cas9 creates a double-strand break at the target site.

04

DNA Repair Initiation

Cellular repair pathways activate in response to the break.

05

NHEJ Pathway

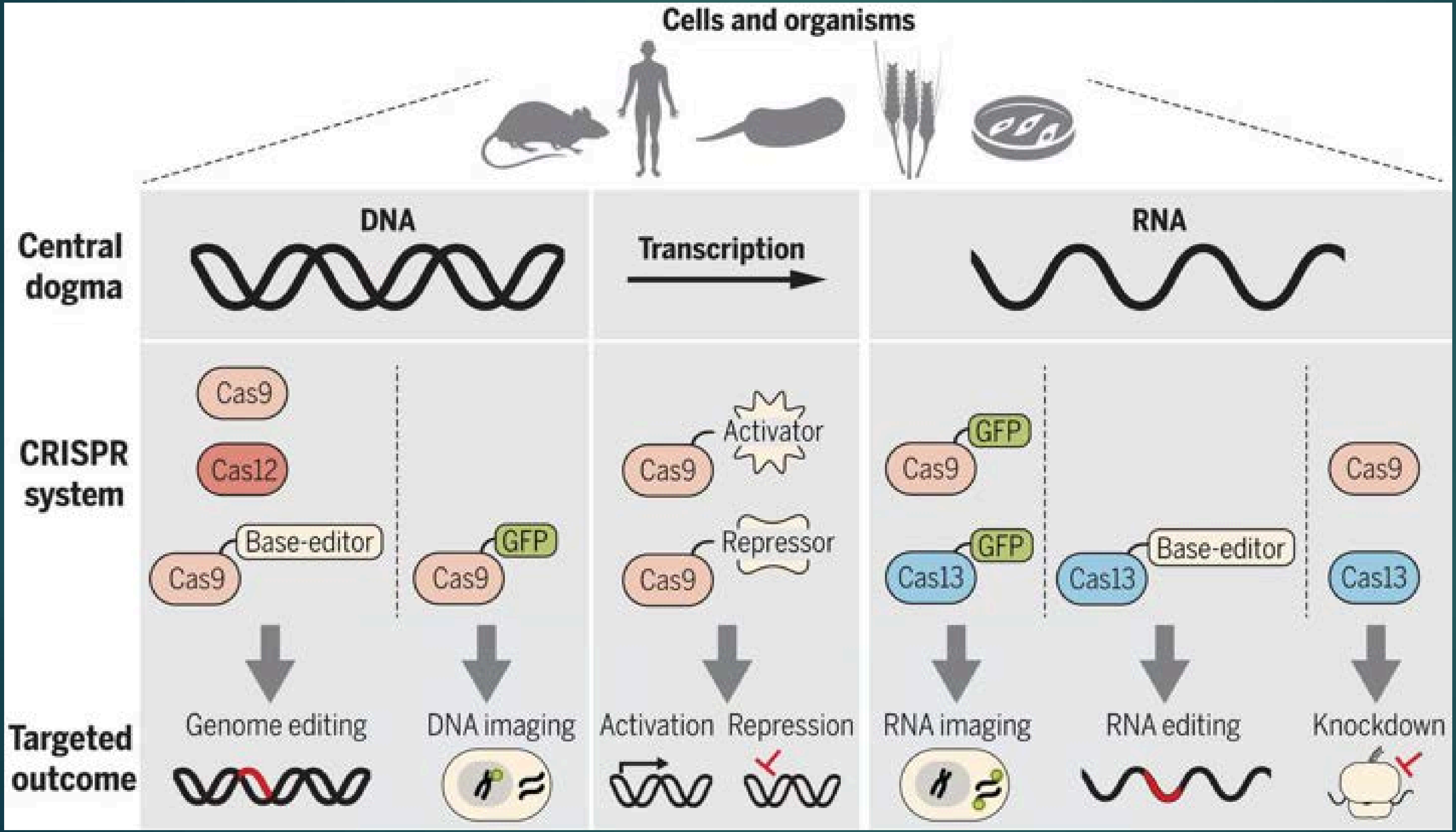
Break is rejoined, often causing small indels that disrupt genes.

06

HDR Pathway

A donor template guides precise gene insertion or correction.

CRISPR-Cas systems allow genetic manipulation across the DNA to protein sequence



CRISPR-Cas12 and Cas13

Cas12 targets DNA with improved specificity; Cas13 targets RNA for gene regulation and viral detection.

Published by AAAS

Gavin J. Knott, and Jennifer A. Doudna Science 2018;361:866-869

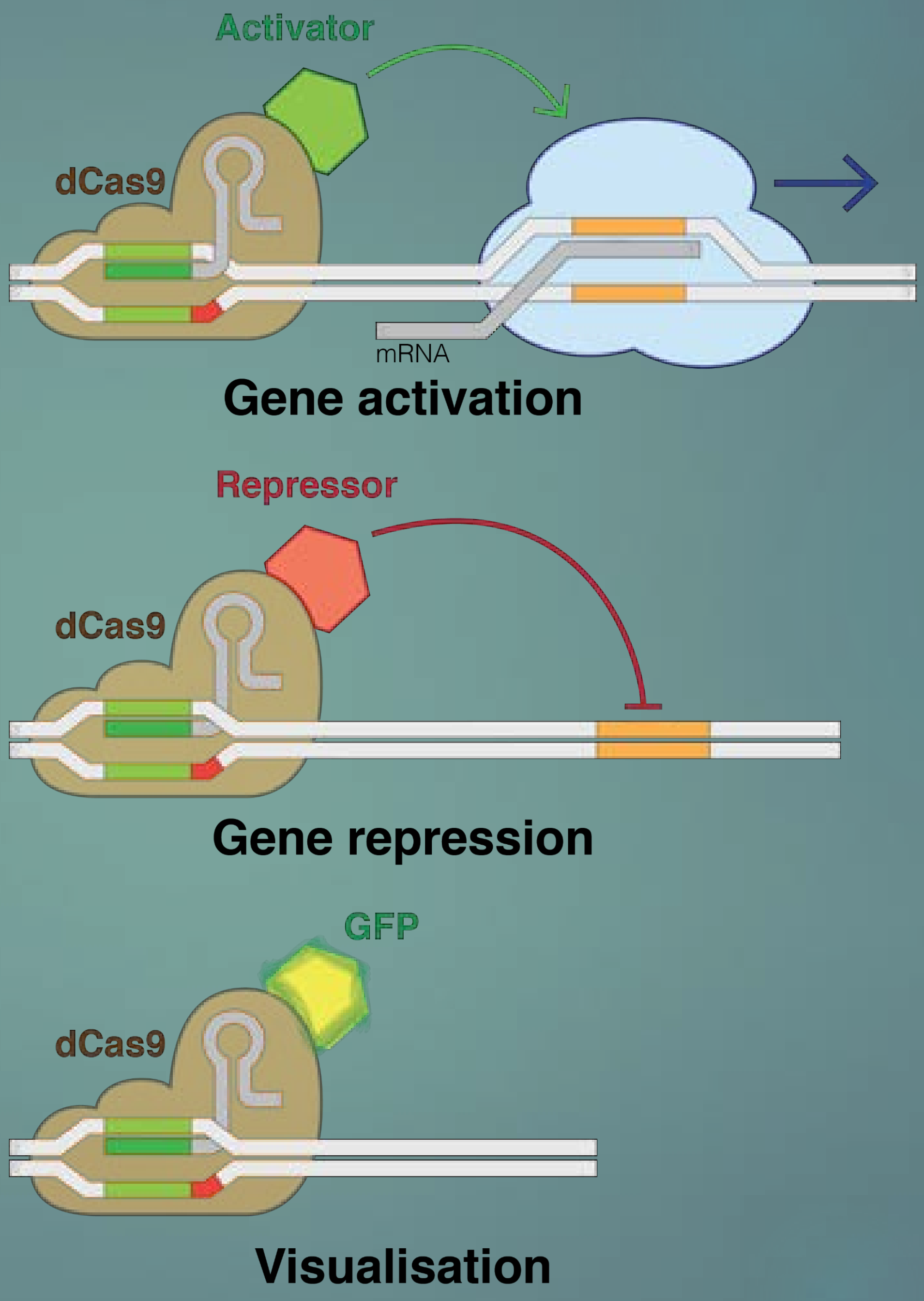
Science AAAS

Challenges and Limitations in Technology

Despite breakthroughs, CRISPR faces challenges including immune responses to Cas proteins, delivery difficulties in certain tissues, mosaicism in edited organisms, and potential off-target mutations.

Some target cells remain difficult to transfect in vivo, limiting therapeutic reach. Ethical concerns about germline editing and variable regulatory environments add layers of complexity.

Some Applications of CRISPR in genetic engineering



Clinical Uses of CRISPR in Medicine

Some Current Applications

- Gene Editing
  - Duchenne muscular dystrophy
    - Editing to induce skipping of defective exon
  - Amyotrophic Lateral Sclerosis (Lou Gehrig disease) and Huntington's
    - Inactivate defective genes
  - Down's syndrome
    - Eliminate extra chromosome in stem cells
  - Endogenous retroviruses in pig cells
    - Make suitable for human transplants
  - Engineered T-cells
    - Activate immune response to cancer cells
  - Sickle-cell anemia

Treatment of Genetic Diseases

Hematologic Disorders: Curative Potential

CRISPR edits patient hematopoietic stem cells to correct mutations in sickle cell disease and beta-thalassemia, yielding sustained gene expression and symptom improvement.

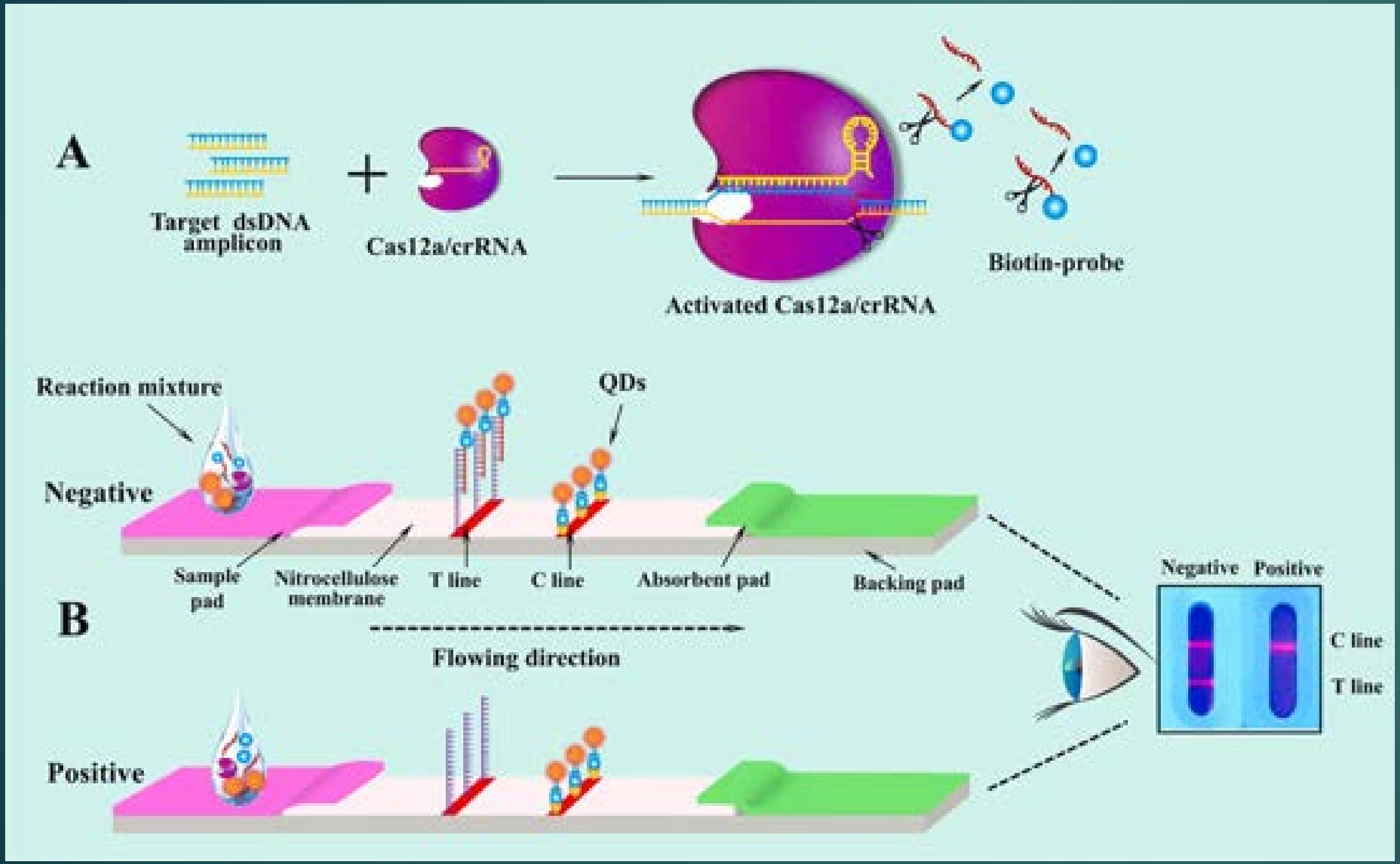
Ocular Targets: Inherited Blindness

Trials aim to restore photoreceptor function by editing pathogenic variants directly in retinal cells.

Pulmonary Targets: Cystic Fibrosis

Emerging approaches edit CFTR mutations in airway tissues, expanding CRISPR's therapeutic horizon across organs.

CRISPR IN LATERAL FLOW DIAGNOSTIC KITS

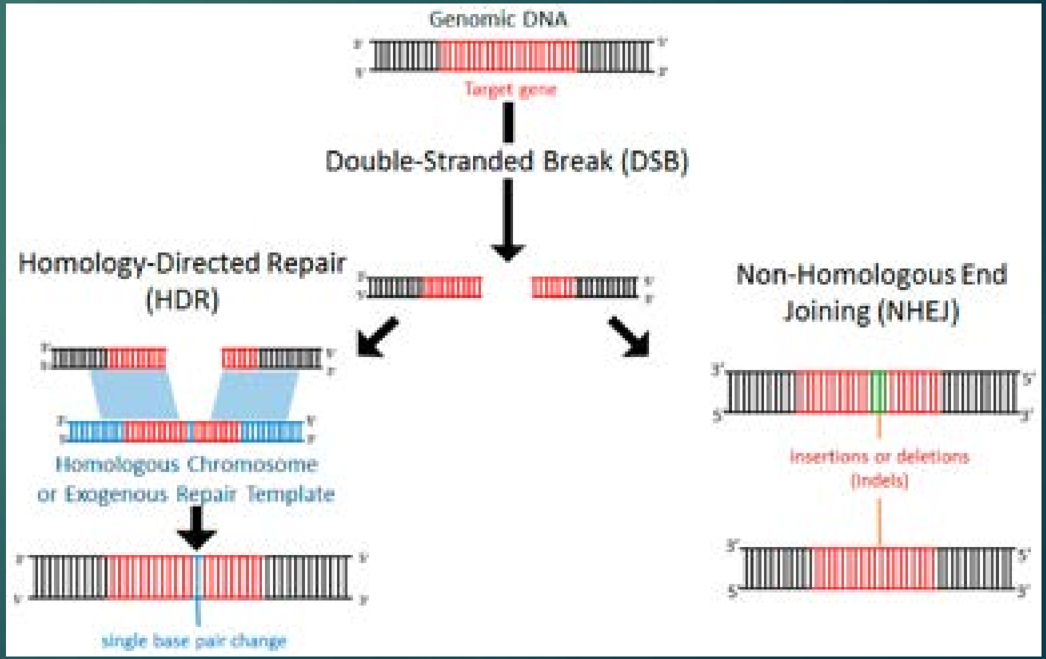


CRISPR-Cas12 is used to recognize DNA (or RNA) sequences specific to the virus and cut and attach probe molecules (biotin here) to the fragments. The labeled fragments are detected on the T-line which is specific for the probe.

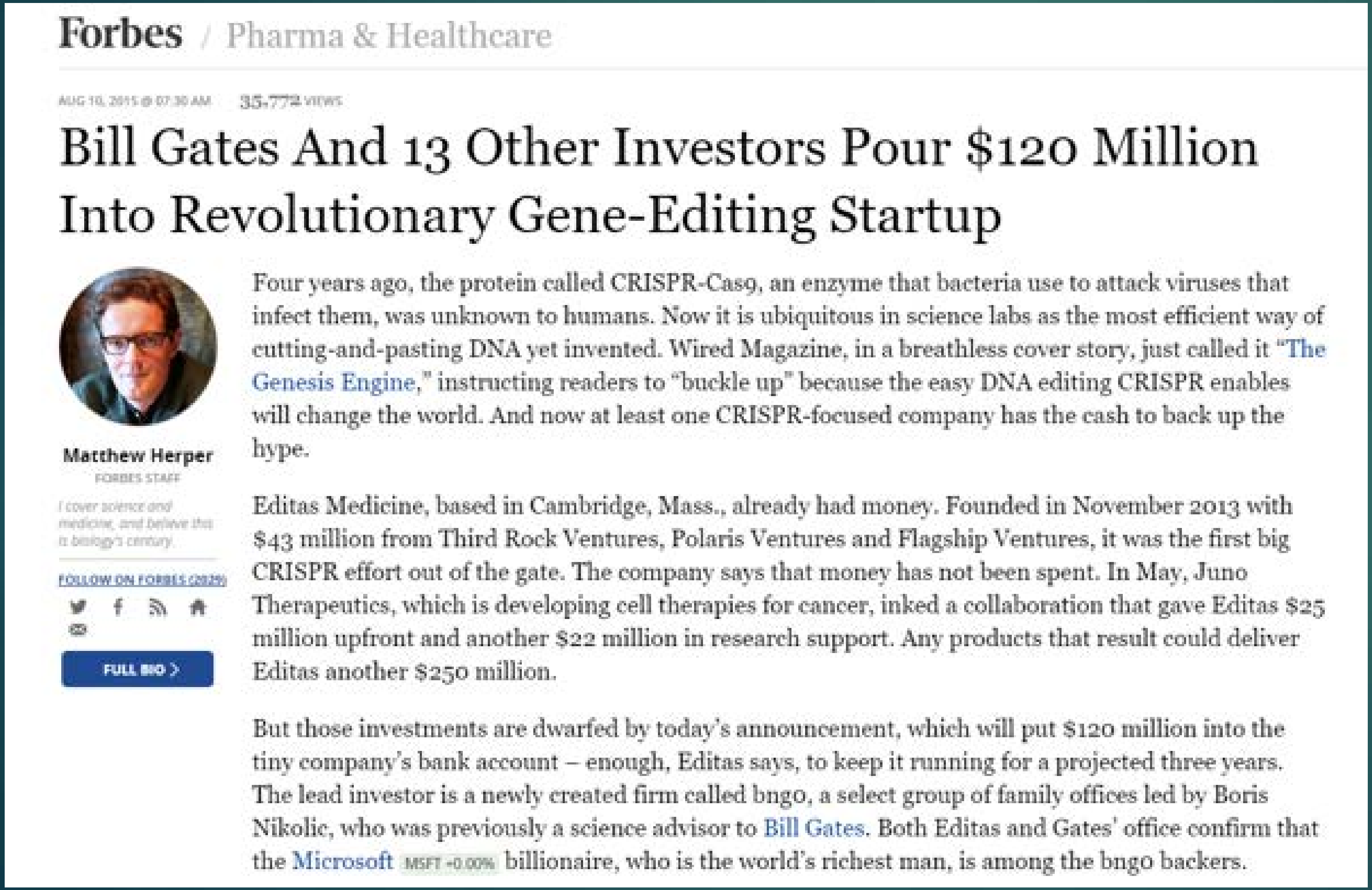
Sherlock CRISPR Lateral Flow Test

Some problems

- How to deliver CRISPR to targets in in-vivo therapy
  - Virus delivery
    - Can be cell-type specific
    - But CRISPR-Cas 9 and associated DNA/RNA editing proteins are too large for most viruses
  - Nanoparticle delivery
    - Not (usually) cell-type specific
- General problems
  - Non-Homologous End Joining vs Homology-Directed Repair
    - NHEJ can produce deleterious mutations before HDR can occur
- Off-target effects
  - Due to shortness of sgRNA



The Future Promise of CRISPR



Forbes / Pharma & Healthcare

AUG 10, 2015 @ 07:30 AM   35,772 VIEWS

Bill Gates And 13 Other Investors Pour $120 Million Into Revolutionary Gene-Editing Startup

Matthew Herper
FORBES STAFF
I cover science and medicine, and believe this is biology's century.
FOLLOW ON FORBES (2029)
FULL BIO >

Four years ago, the protein called CRISPR-Cas9, an enzyme that bacteria use to attack viruses that infect them, was unknown to humans. Now it is ubiquitous in science labs as the most efficient way of cutting-and-pasting DNA yet invented. Wired Magazine, in a breathless cover story, just called it "The Genesis Engine," instructing readers to "buckle up" because the easy DNA editing CRISPR enables will change the world. And now at least one CRISPR-focused company has the cash to back up the hype.

Editas Medicine, based in Cambridge, Mass., already had money. Founded in November 2013 with $43 million from Third Rock Ventures, Polaris Ventures and Flagship Ventures, it was the first big CRISPR effort out of the gate. The company says that money has not been spent. In May, Juno Therapeutics, which is developing cell therapies for cancer, inked a collaboration that gave Editas $25 million upfront and another $22 million in research support. Any products that result could deliver Editas another $250 million.

But those investments are dwarfed by today's announcement, which will put $120 million into the tiny company's bank account – enough, Editas says, to keep it running for a projected three years. The lead investor is a newly created firm called bng0, a select group of family offices led by Boris Nikolic, who was previously a science advisor to Bill Gates. Both Editas and Gates' office confirm that the Microsoft MSFT +0.00% billionaire, who is the world's richest man, is among the bng0 backers.

Top 9 CRISPR Startup Companies Changing the Future of Biotech and Medicine[1]

Inscripta Therapeutics

Plantedit

Beam Therapeutics

eGenesis

Ligandal

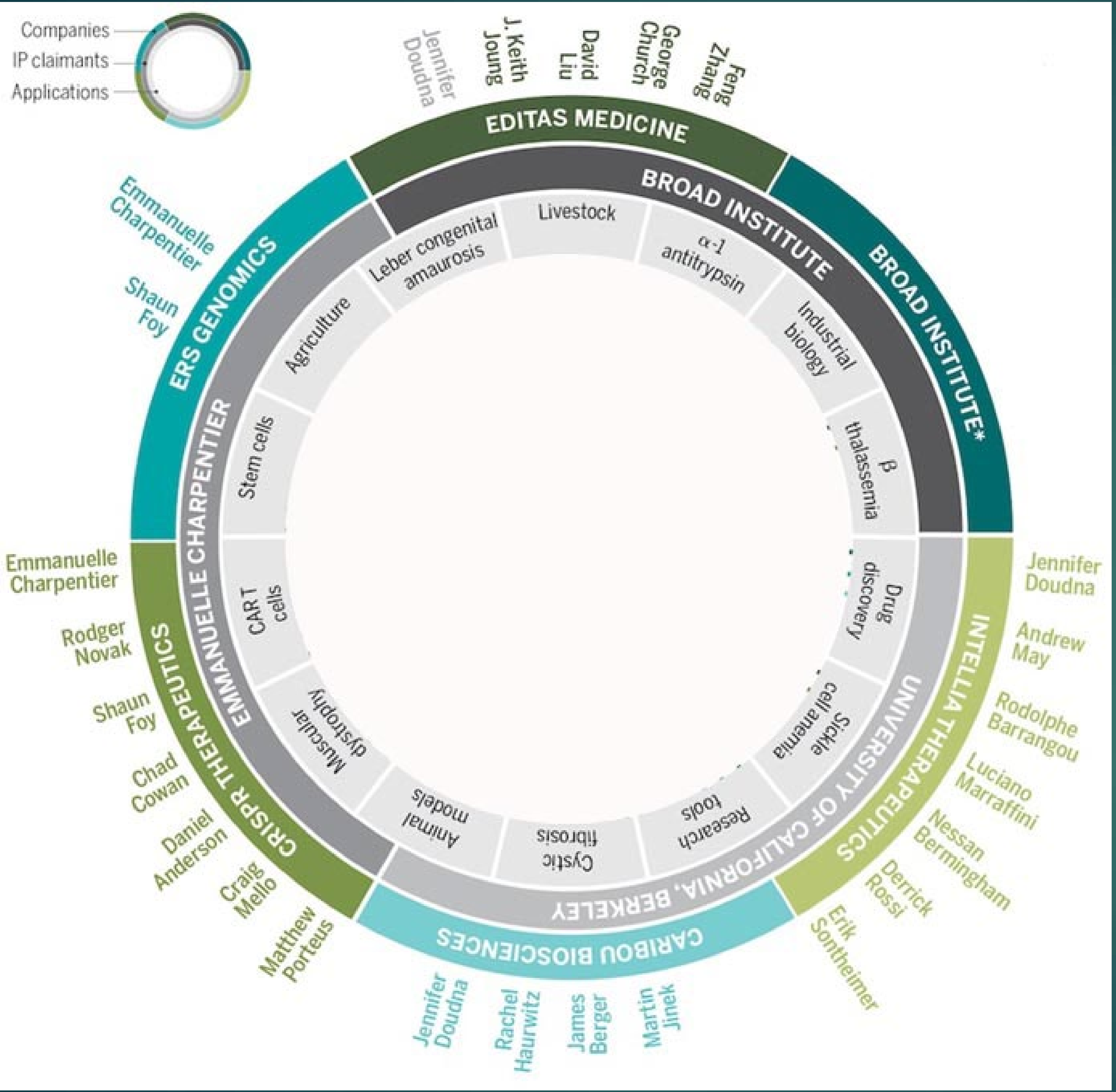
ntrans

Synthetic Genomics

Mammoth Biosciences

Biotech startup companies using CRISPR-Cas9 technology as a main component of their strategy have existed almost since the discovery that CRISPR could be reprogrammed to target essentially any region of any genome. Several CRISPR technology companies, such as CRISPR Therapeutics, Editas Medicine, and Intellia Therapeutics, have already outgrown startup status and are now publicly traded companies.

[1]The Official Synthego Blog where we explore the exciting, rapidly emerging field of CRISPR genome engineering.



Some companies and applications using CRISPR

After https://labiotech.eu/policy-legal-finance/doudna-charpentier-crispr-patent-europe/

Applications in Cancer Therapy

Engineered CAR-T Cells

CRISPR enhances CAR-T cells by knocking out checkpoint genes, improving their ability to target and destroy cancer cells.

Tumor Antigen Recognition

CRISPR-modified immune cells are designed to better recognize and attack tumor-specific antigens, increasing treatment precision.

Clinical Trials

Ongoing trials test CRISPR-edited T cells in both blood cancers and solid tumors, showing promising safety and efficacy.

Personalized Oncology

CRISPR enables tailored immunotherapies, marking a shift toward personalized cancer treatment strategies.

Ethical Implications

- Somatic Gene Therapy
  - Gene replacement therapy
    - Insert “good” gene to replace “bad” one
  - Gene augmentation therapy
    - Insert a functioning gene to provide the protein not produced by a mutated gene.
  - Gene inhibition therapy
    - Inhibits an undesirable gene such as a cancer-causing gene
  - Killing of specific cells
    - Cause cells expressing a certain form of a gene to commit suicide
- Germline Gene Therapy
  - Therapy applied to germ cells and passed on to all subsequent offspring
  - Elimination of inherited diseases

"The power to alter the human germline demands not only scientific rigor but also deep ethical reflection and broad societal consensus."

— International Commission on the Clinical Use of Human Germline Genome Editing

- As of March 2015 at least four labs in the US, labs in China and the UK had announced plans for ongoing research to apply CRISPR to human embryos.

- In April 2015, Chinese scientists reported results of an attempt to alter the DNA of non-viable human embryos using CRISPR to correct a mutation that causes beta thalassemia,. The experiments resulted in changing only some genes, and had off-target effects on other genes. (The study had been rejected by both Nature and Science in part because of ethical concerns.)

- In December, following the Chinese studies, Doudna and others urged a worldwide moratorium on applying CRISPR to the human germline, especially for clinical use. “Scientists should avoid even attempting, in lax jurisdictions, germline genome modification for clinical application in humans until the full implications are discussed among scientific and governmental organizations".

- In April 2016 Chinese scientists were reported to have made a second unsuccessful attempt to alter the DNA of non-viable human embryos using CRISPR - this time to alter a gene to make the embryo HIV resistant.

- On Jan 21, 2018, The Wall Street Journal reported that 86 people in China have had their genes edited using CRISPR.

Long-Term Risks and Societal Implications

Long-term risks

Unforeseen genetic consequences and ecological impacts may arise if edited organisms are released into the environment.

Societal issues

Health disparities could widen, and non-therapeutic enhancements may create ethical divides across populations.

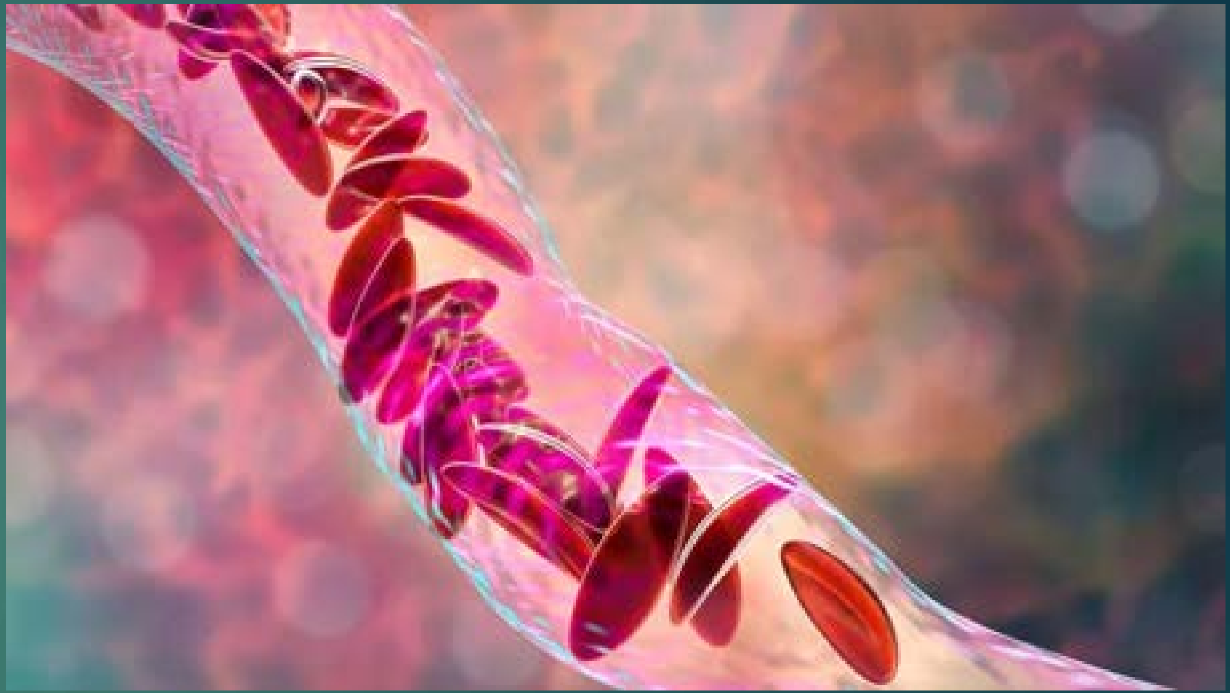
Responsible governance

Ongoing surveillance, public engagement, and adaptive policies are essential to manage risks and societal impacts responsibly.

The world's first treatment that uses CRISPR gene-editing technology has been approved.

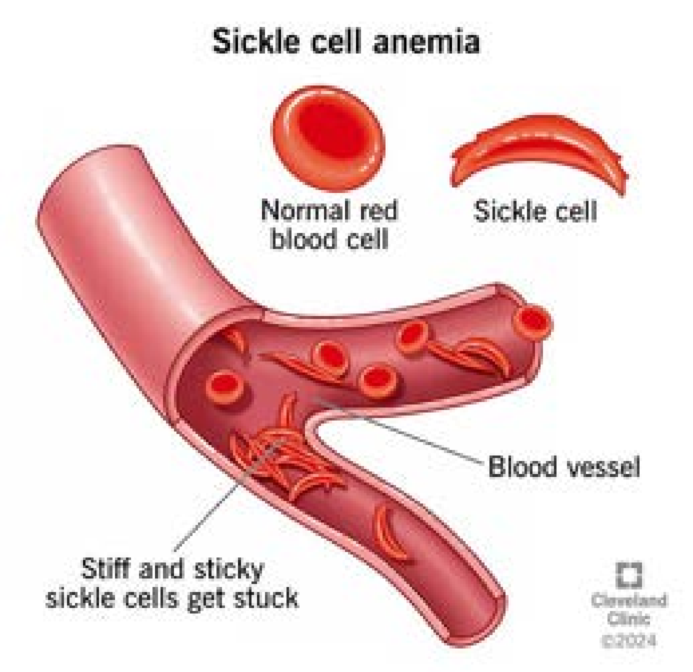
Exa-cel, also known by its brand name Casgevy, received its first regulatory approval on Nov. 16, 2023 from the U.K. Medicines and Healthcare products Regulatory Agency (MHRA) to treat two debilitating blood disorders: sickle-cell disease and transfusion-dependent beta-thalassemia. The U.S. Food and Drug Administration (FDA) later approved the therapy as a treatment for both disorders.

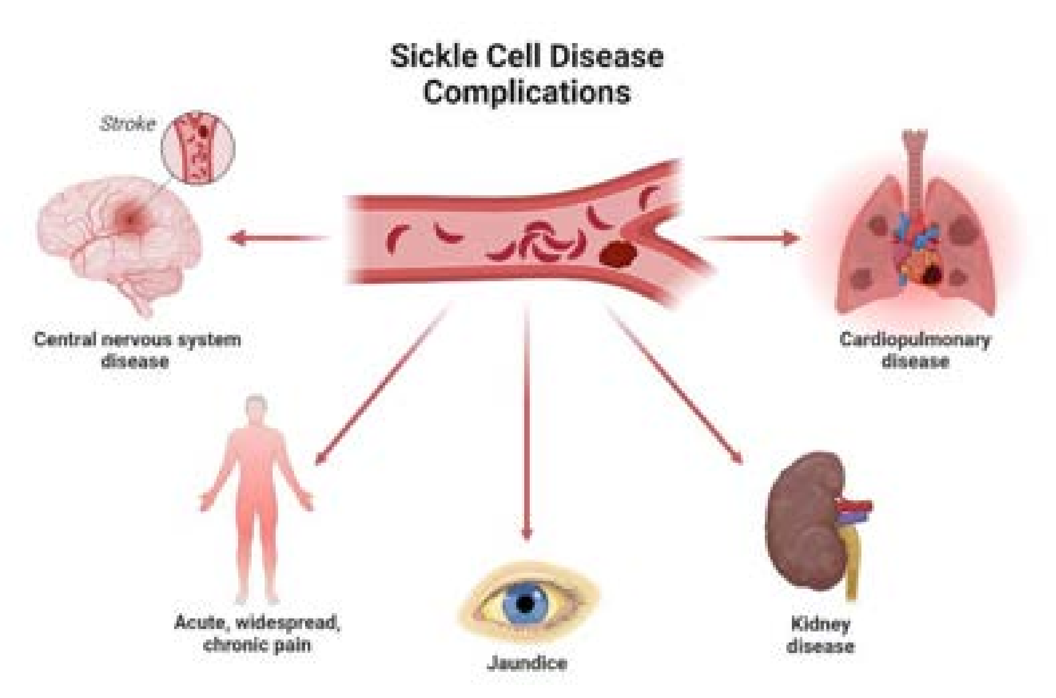
The regulators' historic decision to approve Casgevy may signal the start of a new era of gene therapy. However, questions remain surrounding the treatment's affordability and its long-term safety.

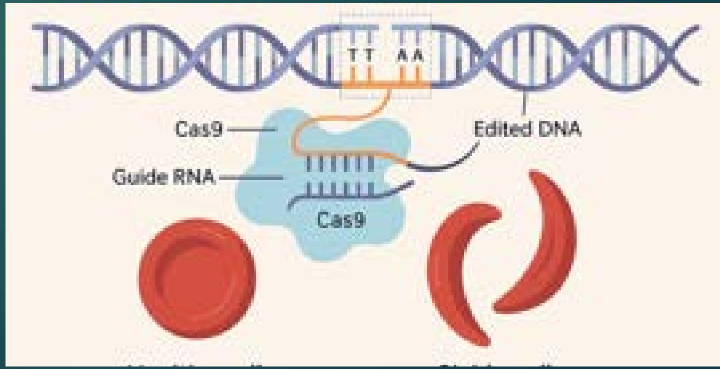


https://www.livescience.com/health/genetics/the-worlds-1st-crispr-therapy-has-just-been-approved-heres-everything-you-need-to-know

CRISPR AND SICKLE-CELL ANEMIA







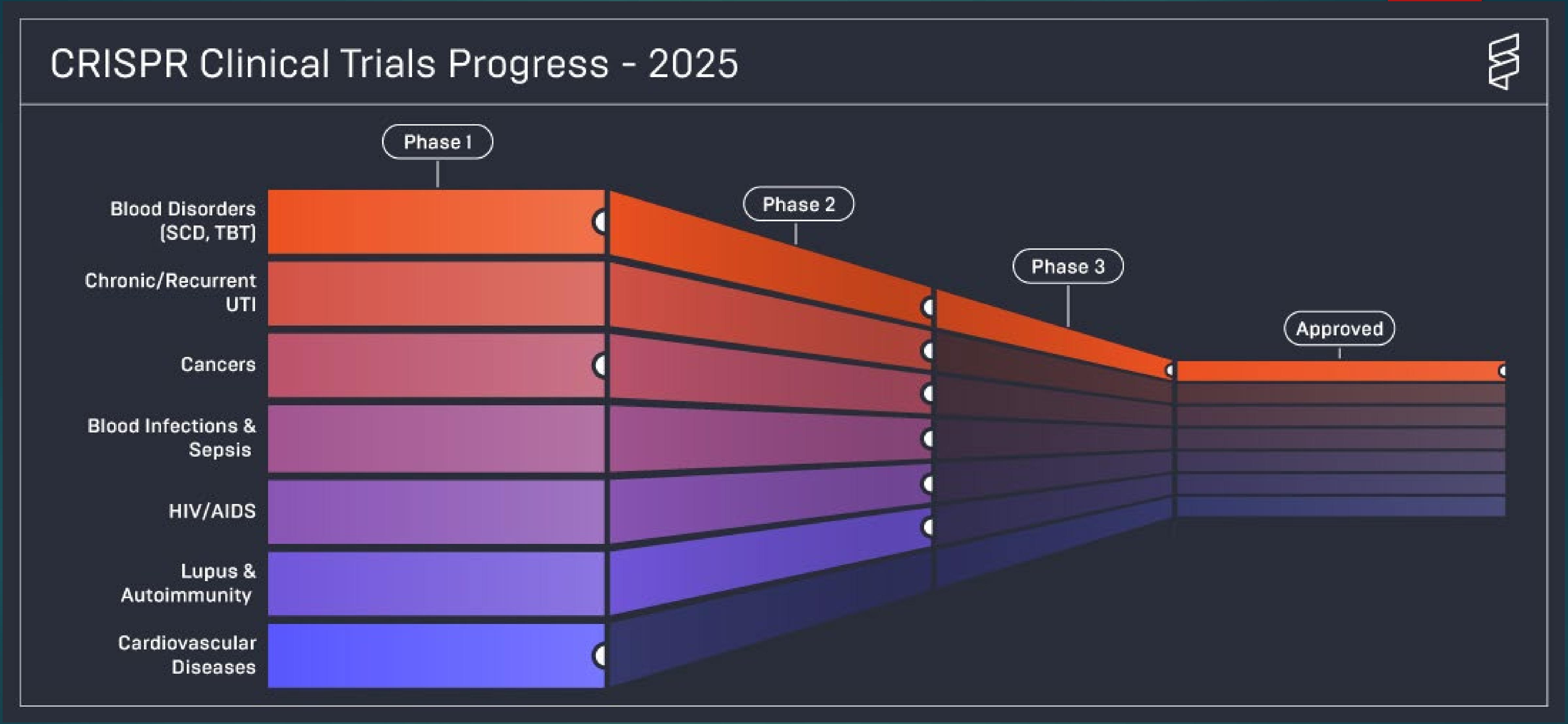
250+

As of 2025, more than 250 CRISPR clinical trials are registered, a sign of the rapid growth in therapeutic research.

As of early 2025, there are approximately 250 clinical trials involving CRISPR or gene-editing therapeutic candidates registered, with over 150 actively listed on platforms like ClinicalTrials.gov. Clinical trials currently registered using CRISPR-based interventions, spanning areas from rare genetic diseases to cancers and viral infections. Innovations focus on in vivo delivery, improved specificity, and combination therapies. Research into CRISPR applications in neurological disorders and organ transplantation is accelerating, heralding expanded therapeutic possibilities.

Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2024 Oct. Report No.: EH0127

Current trends of clinical trials involving CRISPR/Cas systems. Front Med (Lausanne). 2023 Nov 10;10:1292452



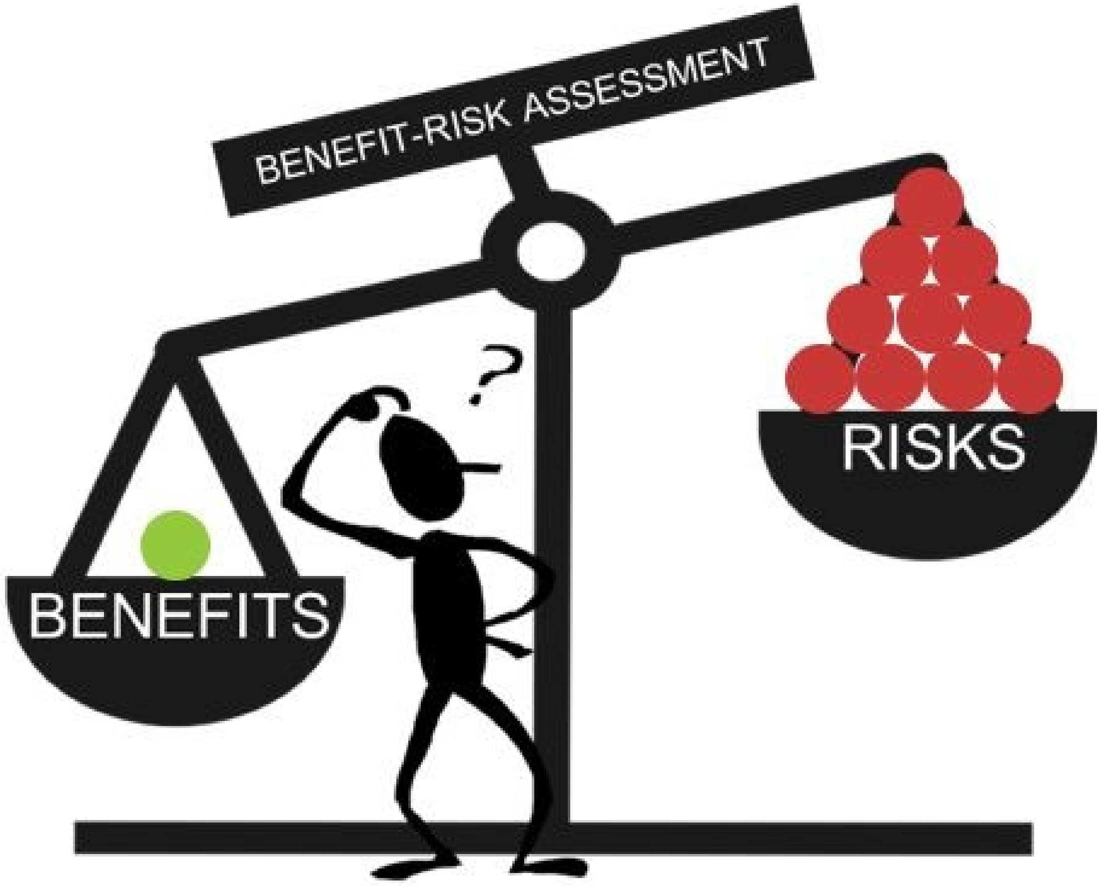
https://innovativegenomics.org/news/crispr-clinical-trials-2025/

INTEGRATION WITH OTHER TECHNOLOGIES

- Gene Drive:
  - Combining CRISPR with gene drives allows for population-level genetic changes, with applications in vector control and ecology.
- Stem Cell Therapies:
  - CRISPR enables precise editing in stem cells facilitating regenerative medicine.
- Synthetic Biology:
  - Integration with synthetic biology expands the creation of novel biological systems and metabolic engineering.
- Artificial Intelligence:
  - AI-driven algorithms optimize gRNA design and predict off-target effects, improving editing accuracy

Where do we stand today???

- Benefits to research into gene functions
- Curing single-gene diseases such as sickle cell anemia
- New agricultural strains, e.g. "golden rice", pest resistance, etc.
- Etc., etc.



- Off-target effects
- Designer babies
- "Frankenveggies" spreading
- Germline editing
- Etc., etc.

Now it's your turn.

THOUGHTS? QUESTIONS?

Open to general feedback and discussion.