NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

A CRISPR Human: Targeted Genome Modification of Disease

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Preparation

CRISPR is an exciting genome modification technique that has become widespread in the field of biology in the last ten years. This technique, which is grounded in molecular biology, has implications for genes, cells, organisms, and even entire ecosystems.

Please scroll through the interactive module on CRISPR from HHMI and watch the TED-Ed video on CRISPR, both linked below. These two resources will provide you with an overview of CRISPR and the potential of this new biotechnology.

- HHMI Biointeractive. *n.d.* CRISPR-Cas9 mechanisms and applications [interactive module]. <https://media.hhmi.org/biointeractive/click/CRISPR/>
- Henle, A. 2019. How CRISPR lets you edit DNA [video]. Running time: 5:09 min. *TED-Ed.* <https://youtu.be/6tw_JVz_IEc>

Please read these two short articles before beginning the case study:

- Charpentier, E., and J.A. Doudna. 2013. Rewriting a genome. *Nature* 495(7439): 50–1. <https://doi.org/10.1038/495050a>
- Hwang, W.Y., *et. al.* 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology* 31(3): 227–9. <https://doi.org/10.1038/nbt.2501> (*Note:* you don't have to be too concerned about the supplementary materials for this article; focus mostly on the article itself.)

Use the information provided in the above articles and online resources to answer the questions below before beginning this case study. Please limit your answers to a single page of typed font.

Questions

1. In what cells were CRISPRs originally discovered and what is their purpose in that type of cell?
2. What is the name of the endonuclease involved with this technology? What molecule is required for this enzyme to direct its cutting function toward a particular sequence of DNA?
3. What are the two types of cellular repair processes that occur after a double-strand break?
4. What was the purpose of the study that Hwang *et al.* (2013) performed?
5. What is the generic format of the genomic sequence that can be targeted by this technology? (*Hint:* Hwang *et al.* mention the format of the sequence in the discussion section of their article.)
6. What are some of the applications of this technology (how will researchers use this in their studies)?
7. What are some of the challenges or concerns associated with this technology?

Part I – An Inherited Disease

Kate and Mike were never going to have biological children. They had settled on this from the start. Kate had cystic fibrosis and Mike was a carrier of the mutation that causes the disease. The thought of intentionally bringing a child into this world that had a high chance of having the disease was not acceptable in their minds. Their suffering and experience with cystic fibrosis was not something they wanted for their child. Additionally, Kate's life would be shorter than most, which likely meant that any child they had might grow up without a mother.

Use the following suggested websites to investigate cystic fibrosis and answer the questions below.

- National Institutes of Health (NIH): <https://www.nhlbi.nih.gov/health-topics/cystic-fibrosis>
- Mayo Clinic: <https://www.mayoclinic.org/>
- Cystic Fibrosis Foundation: <https://www.cff.org/>

Questions

1. Briefly describe the respiratory and digestive signs and symptoms of cystic fibrosis. List any other symptoms that are characteristic of cystic fibrosis.

2. What is the average life expectancy for patients with cystic fibrosis?

3. How common is cystic fibrosis? Are there any ethnic groups affected more than others?

4. Which gene is linked to this disease?

5. Briefly describe the function of the protein encoded by this gene.

6. Briefly describe the type of mutation that occurs in this gene. State the most common mutation found in patients with this disease.

7. What is the manner of inheritance for cystic fibrosis: recessive (must inherit a defective gene from each parent) or dominant (only need to inherit a single copy of the defective gene from either parent)?

8. Is there a treatment for cystic fibrosis?

Part II – A Glimmer of Hope

At a wellness checkup, Kate's physician told her about a new technology that appeared to be a promising treatment for genetic diseases. They discussed research that had successfully replaced defective genes in embryos, and even studies that had replaced defective genes in adult animal models. Dr. White said that this new genetic engineering technology was called CRISPR and that clinical trials for cystic fibrosis and other genetic diseases would probably begin soon. For the first time she could remember, Kate had a glimmer of hope that she might be able to lead a normal life and have healthy children with Mike.

If you were Kate or Mike, would you choose to enroll in a clinical trial to modify your embryo *in vitro*, with the ultimate hope that it would no longer have the genetic mutation associated with cystic fibrosis? Why or why not?

Let's take a quick look at how CRISPR works in this video:

- Genome editing with CRISPR-Cas9. Running time: 4:12 min. Produced by McGovern Institute, 2014. <https://youtu.be/2pp17E4E-O8>

Questions

1. Label the boxes in Figure 1 with the main components of the CRISPR technique for genome editing. Your label choices are: PAM sequence, guide RNA, genomic DNA, donor DNA, matching targeted gene.

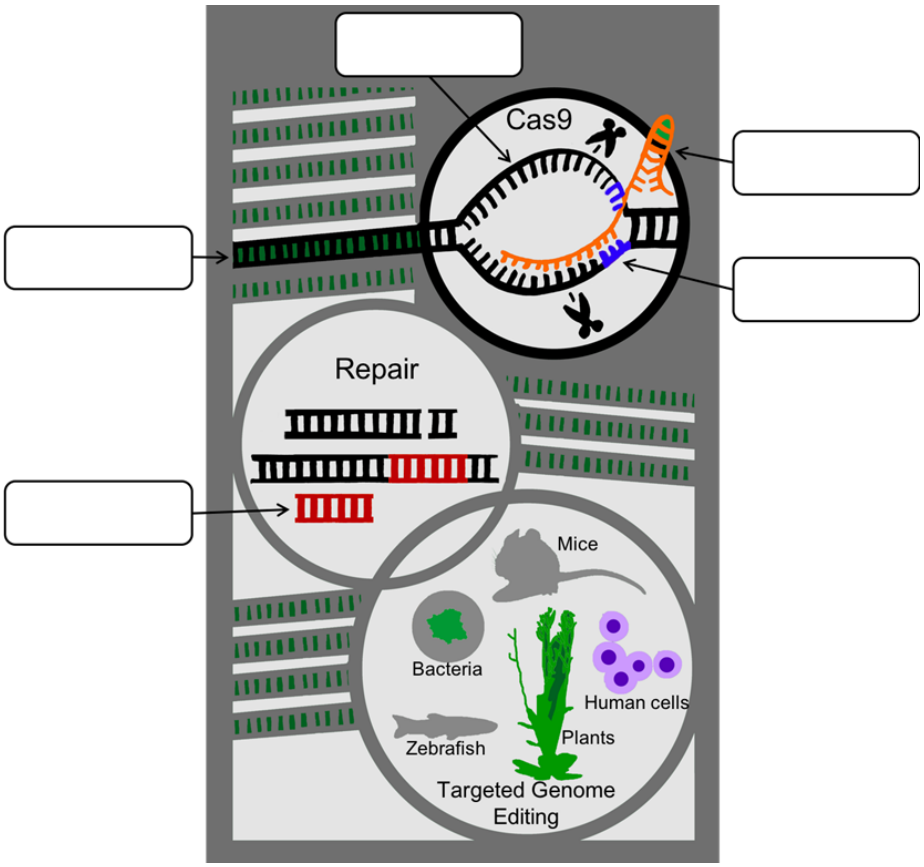

Figure 1. Created by K. Rasmussen and A. Henle.

2. Provide a brief description of how the CRISPR system works that your grandparents could understand.

Part III – A Costly Decision

Later that evening, Mike read all that he could find on the internet about CRISPR and how it had been used to repair genetic mutations. He came across an advertisement for a clinic overseas that claimed to have figured out how to repair the predominant mutation in the *CFTR* gene in embryos, and also in affected children and adults. The clinic was looking for patients to treat immediately. The cost was expensive, well over $200,000 per person and $40,000 for single gene editing in embryos. Kate had recently inherited $260,000 from her deceased grandmother's estate. The couple had planned to use this money for a home and for healthcare. The treatment Mike had found would take most of their money. Mike called to Kate to come take a look at the advertisement.

Questions

1. Should Kate and Mike pay this amount of money for a new technique overseas as opposed to waiting to enroll in a federally funded clinical trial in the United States? Provide at least two pro and two con arguments.

2. Do you think individuals like Mike and Kate should pay out-of-pocket for treatments of this nature? Or should these types of treatments be covered by insurance or universal healthcare that is paid for by taxpayers?

3. Should non-disease genes be considered targets for modification by gene editing with CRISPR (e.g., genes associated with human intelligence, strength, sex or sexual orientation, beauty/appearance, weight, etc.)?

Part IV – Designing the Guide RNA

Mike and Kate decided to speak with a scientist from the overseas clinic to find out more about the specific genetic modifications that would be made to treat Kate's disease and a potential embryo. The scientist discussed recent publications that explained how to create a guide RNA complementary to the area of the genome with the mutated *CFTR* gene, and then insert donor DNA to repair the mutation. Mike and Kate, who had some background in biology from their college days, were intrigued by this process and decided to look at some of the molecular tools themselves.

Use the cDNA sequence of *CFTR* (from March 2006) in the NCBI database <http://www.ncbi.nlm.nih.gov/nuccore/NM_000492.2> to answer the questions below.

Questions

1. What does cDNA represent?

2. What nucleotide numbers comprise the CDS (coding DNA sequence) for this gene?

3. How many amino acids comprise the CFTR protein?

4. In your earlier research for this case study, you may have discovered that nucleotides 1653–1655 (in the cDNA sequence) are deleted in CF patients with the ΔPhe508 mutation. What are these three nucleotides in the cDNA sequence?

5. Write the mRNA sequence (from nucleotides 1651 to 1662) that would be transcribed in this region (if no deletion occurred). Use the mRNA codon table to write the sequence of amino acids that would be translated in this region.

6. Write the mRNA sequence that remains after the deletion. Use the codon table to write the sequence of amino acids that would be translated after the deletion. How does the deletion change the protein that is produced?

7. Assuming Kate has the most common type of mutation found in the *CFTR* gene, what is the sequence of the guide RNA that the scientist might use to direct Cas9 to this region of the genome? *Hint:* Use the cDNA sequence of the gene provided in NCBI and the information about guide RNA sequence requirements from the Hwang *et al*. (2013) article to determine an appropriate guide RNA sequence to treat Kate.

8. What mechanism of repair would use the donor DNA as a template?

9. One criticism of the CRISPR technique is that it is not 100% efficient, meaning that not all of the mutated DNA will be repaired. Do you think this matters in this case? Why or why not? For what types of diseases might this be more of a concern?

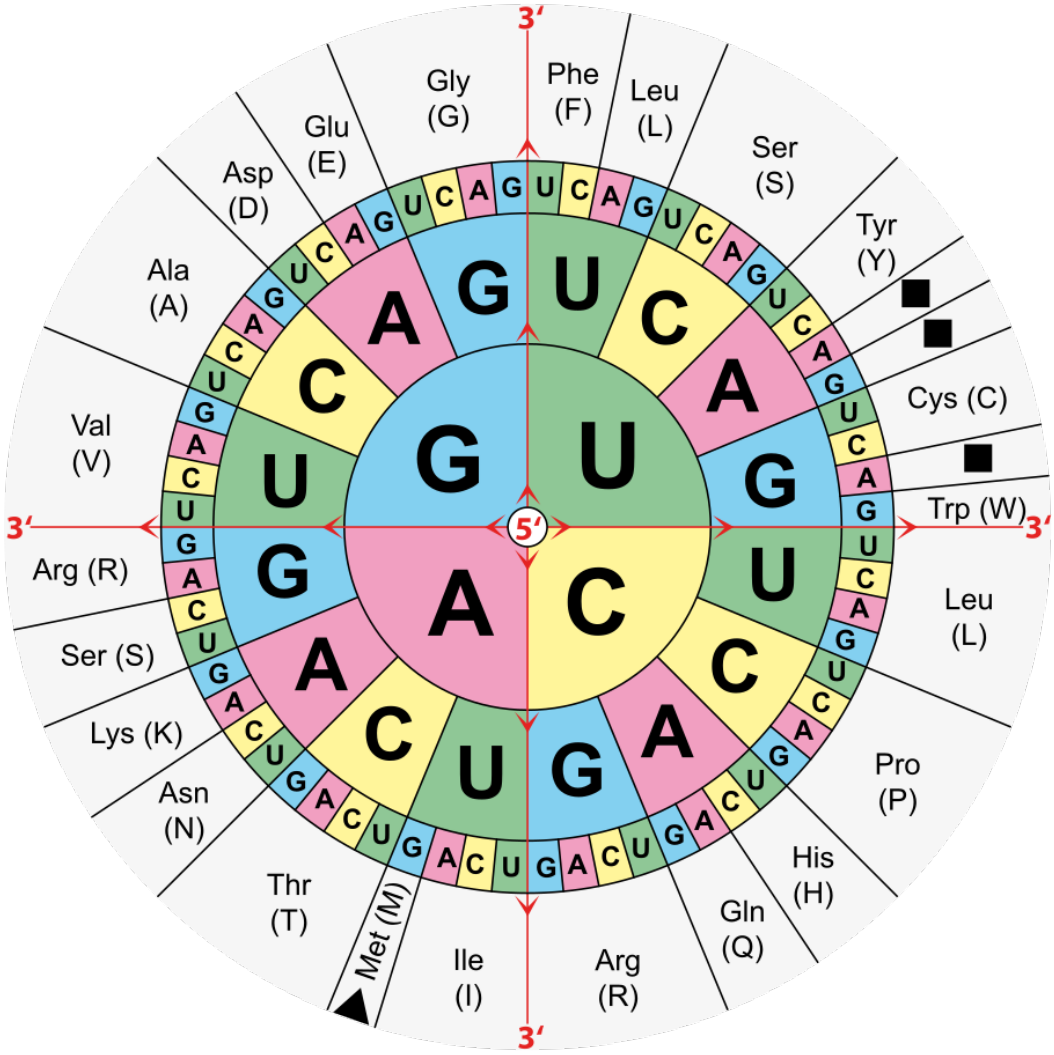

Figure 2. Codons found in mRNA. <https://commons.wikimedia.org/wiki/File:Aminoacids_table.svg>