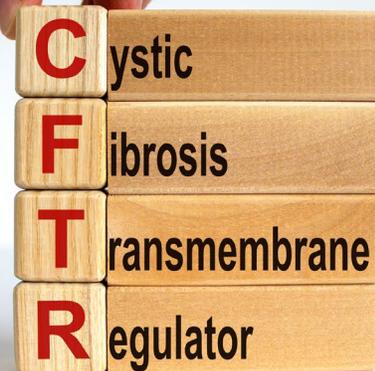


Maggie's Illness:

Protein Structure and Function in Cystic Fibrosis

by

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Part I – Maggie's CFTR Protein

Little seven-month old Maggie Miller had just been admitted to the emergency room at Smithfield Memorial Hospital. Her parents, Mr. and Mrs. Miller, had brought her in because she had been suffering from a chronic, wheezing cough for weeks. The attending ER pediatrician noted that salt crystals were present on Maggie's skin and called Dr. Jackson, a pediatric pulmonologist. Dr. Jackson examined Maggie and suspected that she was suffering from cystic fibrosis (CF).

CF is a genetic disease that affects more than 30,000 children and young adults in the United States, and more than 70,000 worldwide. It is caused by mutations in the CFTR (or cystic fibrosis transmembrane conductance regulator) gene, which disrupt the function of the CFTR protein, leading to disease symptoms. CF can affect multiple organ systems, but the most common are the lungs, pancreas, and gastrointestinal tract, leading to digestive and respiratory problems. Complications from respiratory infections often lead to decreased quality of life and eventual death in these patients.

One of the most common mutations in the CFTR gene to cause CF occurs in exon 10, 1,524 nucleotides downstream of the start site of the gene. In order to determine if Maggie had CF, the doctors ordered a genetic test to look for this mutation in Maggie's DNA. Below is a portion of the normal CFTR gene and a copy of the gene in Maggie's DNA. Since the common mutation occurs over 1000 nucleotides downstream of the start site, we are not looking at the entire gene. The internal "....." in the sequence represents large portions of the DNA sequence that are not relevant to this exercise. The genes shown below have a boxed promoter followed by the start of the CFTR gene (the ... represents the 10 nucleotides between the promoter and the transcriptional start site of the gene). There is one intron depicted by underlined text and the transcriptional terminator is in bold. (*Note:* this intron is for demonstration purposes and is not the true intron sequence of the gene.)

Questions

1. Transcribe and translate the normal gene and Maggie's DNA to determine if she has a mutation that may affect her CFTR protein.

Normal version:

```
5' GCATATAAT...GCGTATGAAAAACAGTTGCAG.....ATCATCTTTGGT.....CGGTAAAGCGTGA 3'
3' CGTTATATTA...CGCATACTTTTTGTCAACGTC.....TAGTAGAAACCA.....GCCATTTCGCACT 5'
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Maggie's DNA sequence:

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5' GCATATAAT...GCGTATGAAAAACAGTTGCAG.....ATCATCGGT.....CGGTAAAGCGTGA 3'
3' CGTTATATTA...CGCATACTTTTTGTCAACGTC.....TAGTAGCCA.....GCCATTTCGCACT 5'
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Normal version (repeated from previous page):

```
5' GCATATAAT...GCGTATGAAAAACAGTTGCAG.....ATCATCTTTGGT.....CGGTAAAGCGTGA 3'
3' CGTATATTA...CGCATACTTTTTGTCAACGTC.....TAGTAGAAACCA.....GCCATTTTCGCACT 5'
```

Primary transcript:

mRNA:

Protein:

Maggie's DNA sequence (repeated from previous page)::

```
5' GCATATAAT...GCGTATGAAAAACAGTTGCAG.....ATCATCGGT.....CGGTAAAGCGTGA 3'
3' CGTATATTA...CGCATACTTTTTGTCAACGTC.....TAGTAGCCA.....GCCATTTTCGCACT 5'
```

Primary transcript:

mRNA:

Protein:

2. Is there a mutation in Maggie's DNA sequence? If so, what type of mutation did you find?

3. What change has occurred in Maggie's CFTR protein?

Part II – The Structure of CFTR Protein

Unfortunately, Maggie has been diagnosed with cystic fibrosis. You are now going to examine how the mutation you have identified is causing her disease symptoms.

Questions

4. Examine the protein sequences you translated on the previous page. Has the primary structure of the CFTR protein changed? Explain your reasoning.

5. You should have identified that the DNA mutation caused a loss of the phenylalanine amino acid from the primary structure of the CFTR protein. Examine the structure of a phenylalanine amino acid in Figure 1.
 - a. Label the carboxyl group, amino group, alpha carbon, and R group on this figure. Be sure to circle all the atoms that make up each group in your labeling.
 - b. Based on its structure, would you predict that phenylalanine is a hydrophobic or hydrophilic amino acid. Why?

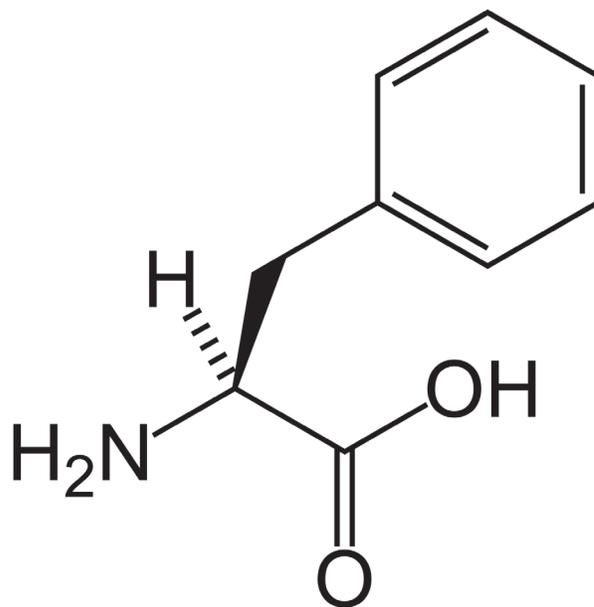


Figure 1. Structure of a phenylalanine amino acid.

6. Based on your prediction above, hypothesize what role a phenylalanine amino acid might have in affecting the protein's tertiary structure formed during protein folding.

To examine if your hypothesis is correct, compare the tertiary structure of a portion of the normal CFTR protein with the same portion of Maggie's mutated CFTR protein. To do this you will follow the directions on the following page.

In order to open protein structure files you will first need to download a specific 3D protein viewer software program.

Directions for Cn3D Protein Viewer

- (a) Go to <https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml> to download the Cn3D Protein Viewer. At least one group member should install the software.
- (b) Open the CFTR normal file provided by your instructor. Resize the screen so that it is easy to view the protein.
- (c) In the Sequence/Alignment Viewer window (the box with the individual amino acid sequence letters) click View, then Find Pattern. Type IIFG in the box that opens. Change the Highlight Mode to Add and then click OK. I, F, and G are the single letter amino acid abbreviations for isoleucine, phenylalanine, and glycine, which you should recognize from your translated protein sequence.
- (d) A portion of the protein will highlight in yellow corresponding to the amino acid sequence you selected. If you cannot see it, you should spin the protein around (click and move the mouse pointer) until you can find it. As you are looking at the protein you will also notice other structures associated with the outer edge of the molecule; these are ATP and magnesium. Many proteins must associate with other molecules in order to function.
- (e) Now open the CFTR mutant file provided by your instructor. Resize the screen so that it is a similar size to the normal protein and position it next to the normal protein window.
- (f) In the Sequence/Alignment Viewer window for the mutant protein, go to View > Find Pattern. Type IIG into the box to highlight the same area in the mutated protein (notice there is no F in this sequence).
- (g) Spin the mutated protein around so the highlighted area is in the same position/location in the screen as it is in the normal protein.
- (h) Compare the two proteins and discuss with your group how the tertiary structure looks similar and different between the two. Can you see secondary structures of alpha helixes and beta sheets? Do they appear to be in the same location/orientation? Are there additional changes in the overall shape of the protein? After discussing these with your group, answer the question below.

Question

7. Does the loss of phenylalanine affect the tertiary structure of the protein? Explain your answer.

Directions (continued)

- (i) On the normal protein window go to Style > Rendering Shortcuts > Ball and Stick. This now shows the structures of all of the amino acids. Spin the structure around; can you identify the ring R group of phenylalanine in the yellow highlighted area?
- (j) As you move the protein around, find the R group from another amino acid that is closest to the phenylalanine ring. *Hint:* the easiest way to view this is to move the structure so the ring is on the outside of the molecule with the black background behind it; you may have to try different views and different angles.
- (k) Once you have found the R-group structure you think is closest, double click on it in the picture. It should turn yellow. If you cannot decide on just one amino acid R group, highlight the two you think are closest.

- (l) Look at the Sequence/Alignment Viewer box, scroll across until you find the yellow highlighted letters (don't click on the letters themselves). One set of highlights is the IIFG sequence you originally chose. What other letter(s) is highlighted (this is the amino acid that you highlighted in the picture)?

Question

8. Look up "single letter amino acid abbreviations" online. What amino acid have you highlighted? Is this a hydrophobic or hydrophilic amino acid?

Directions (continued)

- (m) Now move to the mutant protein. Go to Style > Rendering Shortcuts > Ball and Stick. Then in the Sequence/Alignment Viewer window, scroll until you find the IIG that you highlighted previously. Hold down the shift button and then click on the amino acid letter that you highlighted in Step (d) above for the normal protein. (This will highlight that amino acid in the mutant protein structure for comparison.)

Question

9. Compare the newly highlighted amino acid R group between the mutant and the normal structures. Do the R groups appear in the same place? How does the movement of this second R group(s) relate to the deletion of the phenylalanine in the mutant sequence?

Part III –The Function of CFTR Protein

After Dr. Jackson had explained to Maggie’s parents how the deletion of the TTT nucleotides leads to an overall change in the tertiary structure of the encoded CFTR protein, they wanted to understand how the change in structure led to Maggie’s disease symptoms. To explain this, Dr. Jackson first had to describe what the normal CFTR protein does.

Questions

10. Figure 2 indicates the relationship of the CFTR protein to the cell membrane. The membrane bilayer is represented by the grey block. Is the CFTR protein an integral membrane protein or a peripheral membrane protein? Based on this figure what is the function of the CFTR protein?
11. There is originally a higher concentration of chloride ions inside the cell compared to outside. After transport of chloride ions out of the cell by CFTR, there is now a higher solute concentration outside the cell compared to inside the cell. Would this be considered a hypotonic or hypertonic environment?

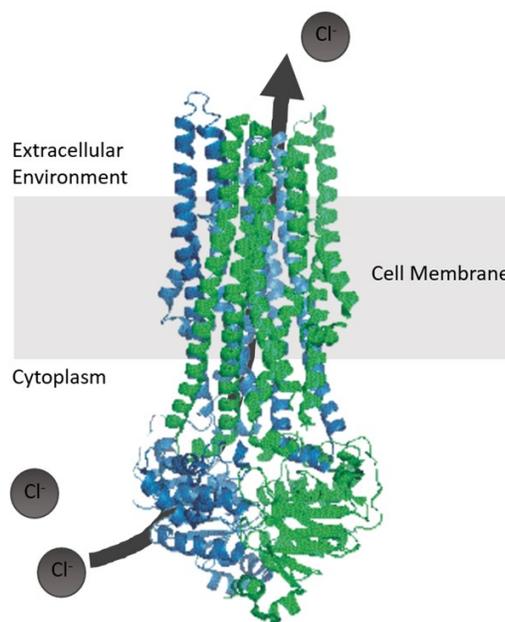


Figure 2. Orientation of CFTR protein to cell membrane.

12. Based on your answer above, what do you predict water does in response to chloride leaving the cell?
13. Cystic fibrosis occurs because the mutated CFTR no longer transports chloride ions out of the cell. Without the transport of chloride ions there is a lower solute concentration outside the cell than there is inside the cell. Would this make the environment hypertonic or hypotonic?
14. Based on your answer above, would water would continue to exit the cells in a person with CF? Explain why or why not.

Conclusion

You should have determined that when chloride is no longer transported, there is a reduction of water leaving the cell. Diminished water causes the mucus layer that lines the respiratory, gastrointestinal, and reproductive tracts to become thick. Maggie was first brought to the doctor because of a chronic cough and wheezing. This is a major symptom and complication of cystic fibrosis because the thick mucus blocks the small airways in the lungs, making breathing very difficult. The mucus also traps respiratory pathogens, causing a large number of lung infections in these patients. The other major symptoms of cystic fibrosis are digestive problems because the thick mucus coats the gastrointestinal tract, which prevents the secretion of digestive enzymes and the absorption of nutrients. As Maggie grows, her parents will need to keep watch for excessive weight loss and vitamin deficiency diseases.

