Cystic Fibrosis
How Mutations Lead to Disease

Goals

- Understand that proteins have different regions or domains responsible for different parts of the function of that protein.
- Understand that mutations in the DNA sequence of a gene encoding a protein can lead to a change in the amino acid sequence of that protein.
- Hypothesize as to how changes in the amino acid sequence in different parts of a protein will have different effects on the function of that protein.
- Explore how knowing a patient's particular mutation can lead to appropriate choice of treatments for that patient.

Summary of Activity

You will work with the case history that you received earlier. First, you will read about the CFTR protein and its structure. Then, you will review the symptoms of the patient described in your case history and locate the position of that patient's mutation on a map of the CFTR protein. You will hypothesize as to how the mutation in the protein leads to the disease symptoms of your patient and present your hypothesis to the class. By comparing the four different cases presented by your classmates you should get a good idea of how different mutations can lead to different molecular defects and different disease consequences.

The CFTR gene and protein

The CFTR gene was cloned in 1989. It encodes a protein, CFTR, which is 1480 amino acids long. This protein is a membrane protein that serves as the primary chloride ion channel in respiratory and intestinal epithelia. Knowing which specific protein is altered in CF disease has been instrumental in designing new therapies for CF.

Below is a linear map of the CFTR protein from the first amino acid (AA) at the amino terminus (N-terminus) on the left to the last amino acid at the carboxyl terminus (C-terminus) on the right. Different regions, or domains, of the protein that have different functions are labeled. The functions of these different domains are discussed later in this exercise.
During and after translation, the protein will fold and assume a three dimensional shape. Below is a diagram showing how the different domains are positioned with respect to the cell membrane. Note that the transmembrane regions are inserted in the membrane, extending across the membrane. The nucleotide binding domains and the regulatory domain are inside the cell, in the cytoplasm.

When viewed three dimensionally from inside the cell looking out, the 12 membrane spanning segments form a hollow ring or channel through which the chloride ions can pass, as shown below.
Below is a side view of the CFTR protein inserted in the membrane. On the left, the chloride channel is open allowing chloride ions to leave the cell. On the right, the regulatory domain has moved up to close the chloride ion channel.

**Functional Domains of the CFTR protein**

- Transmembrane regions cross the membrane and form the walls of the ion channel (above you see only 5 of the 12 regions spanning the membrane, the other 7 are hidden behind.) They also hold the ion channel in place within the membrane.

- Two nucleotide binding domains (NBDs). When ATP binds the NBDs, this will cause phosphates to bind the R domain such that it can no longer fit into the end of the channel. When ATP is bound to the NBDs, the channel is open; when ATP is not bound, the channel is closed. Thus, the NBDs of the protein are required for the chloride ion channel activation by ATP.

- A regulatory (R domain) which actually opens or closes the ion channel. When phosphates are bound to the R domain the channel will be open.

- Note, there are regions of the protein that bind chaperones (unlabeled). These regions are distributed throughout the protein and ensure that the protein is correctly folded, processed, and trafficked. One amino acid known to be important to these processes is the phenylalanine at position 508.
**CFTR Mutations**

To date, more than 700 different mutations in the CFTR gene have been discovered which lead to CF disease. These different mutations occur in different domains of the protein and can lead to different molecular defects. Also, there are mutations leading to stop codons throughout the protein. These mutations result in a truncated protein being produced; only amino acids prior to the mutation will be in the protein. The different molecular defects contribute to the different clinical forms of CF that are observed. Knowing the different molecular defects has allowed researchers to develop treatments that target these specific molecular defects. In this activity you will study how different mutations lead to different clinical symptoms.

**Naming Mutations**

There is a specific way in which scientists name mutations.

- The amino acids in a protein are numbered sequentially from the amino terminus to the carboxyl terminus. CFTR has 1480 amino acids that are numbered from 1 through 1480.
- The number in your mutation name describes the position of the amino acid which is altered in your mutant CFTR protein.
- The letters in the mutation name refer to amino acids. Scientists use a one-letter code to represent each of the 20 amino acids. That code is shown below.
- The letter before the number in the mutation name refers to the amino acid that would be at this position in the normal CFTR protein.
- The letter after the number refers to the amino acid that has been put in place of the normal amino acid in the mutant CFTR protein.
- Delta refers to a deletion of an amino acid.
- X refers to a stop codon.

Example

P574H - The proline (P) usually found at position 574 has been replaced by a histidine (H).

<table>
<thead>
<tr>
<th>Single letter amino acid code</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G Glycine</td>
<td>P Proline</td>
</tr>
<tr>
<td>A Alanine</td>
<td>V Valine</td>
</tr>
<tr>
<td>L Leucine</td>
<td>I Isoleucine</td>
</tr>
<tr>
<td>M Methionine</td>
<td>C Cysteine</td>
</tr>
<tr>
<td>F Phenylalanine</td>
<td>Y Tyrosine</td>
</tr>
<tr>
<td>W Tryptophan</td>
<td>H Histidine</td>
</tr>
<tr>
<td>K Lysine</td>
<td>R Arginine</td>
</tr>
<tr>
<td>Q Glutamine</td>
<td>N Asparagine</td>
</tr>
<tr>
<td>E Glutamic Acid</td>
<td>D Aspartic Acid</td>
</tr>
<tr>
<td>S Serine</td>
<td>T Threonine</td>
</tr>
<tr>
<td>X Stop</td>
<td></td>
</tr>
</tbody>
</table>
**Relationship between genotype and phenotype**

In this exercise you will see that some mutations lead to less severe disease than other mutations. Thus, genotype (the genetic makeup of an individual) can influence phenotype (the symptoms the individual experiences). However, genetic makeup is only one of many factors which influence severity of disease. Individuals with the same mutation can have very different disease characteristics.

One of the factors contributing to the severity of cystic fibrosis symptoms is the percentage of normal CFTR function (see table below). People with two normal CFTR genes will have 100% normal CFTR function. People with one normal and one mutant CFTR gene will have something over 50% normal CFTR function depending on the severity of the mutation. Individuals with two mutant CFTR genes will have between 0 – 50% normal CFTR function depending on their particular mutations and what portion of CFTR is affected. Note that people with levels as low as 10% of normal CFTR function have no known abnormalities.

<table>
<thead>
<tr>
<th>Percentage of normal CFTR function</th>
<th>Manifestations of cystic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>Pancreatic exocrine deficiency</td>
</tr>
<tr>
<td></td>
<td>Plus manifestations listed below</td>
</tr>
<tr>
<td>&lt;4.5</td>
<td>Progressive pulmonary infection</td>
</tr>
<tr>
<td></td>
<td>Plus manifestations listed below</td>
</tr>
<tr>
<td>&lt;5</td>
<td>Positive sweat test result</td>
</tr>
<tr>
<td></td>
<td>Plus manifestations listed below</td>
</tr>
<tr>
<td>&lt;10</td>
<td>Congenital absence of vas deferens</td>
</tr>
<tr>
<td>10-49</td>
<td>No known abnormality</td>
</tr>
<tr>
<td>50-100</td>
<td>No known abnormality (this range represents the levels of asymptomatic carriers and non-carriers.)</td>
</tr>
</tbody>
</table>

Note that the percentages 4.5 and 5 are quite close. There are a very few patients with normal sweat test but severe pulmonary disease. There are even fewer patients with abnormal sweat chloride ion levels but no pulmonary disease.
Activity

Reread your case history and refamiliarize yourself with the symptoms of the patient described in your case history. **Make a short list of the symptoms below.**

Look at the table below showing how what CFTR protein level correlates with the symptoms you are observing (in the relationship between genotype and phenotype section). What percentage of normal CFTR function do you think the patient in your case has? **Write your answer below.**

Find the mutation that your patient has in the table below.

Beth - R117H  
Tom - Delta F508  
Sandy - G551D  
Bill - R553X  

**Write your answer in the space below.**

Note that each person has two copies of the CFTR gene. Assume the individual in your case has two copies of the same mutant gene. In human populations various different combinations of mutations can occur.
Using the rules listed in the naming mutations section – answer the following questions about your mutation?

1. What is the position number of the amino acid that is changed in the case you were given?

2. Is one amino acid substituted for another in the case you were given?

   If yes, which amino acid is found in the normal CFTR?

   Which amino acid is found in the mutant CFTR?

3. Is an amino acid deleted (missing) in the mutant CFTR of the individual in your case?

   If yes, which amino acid is missing?

4. Does your mutation introduce a stop codon?

   If yes, at which amino acid will the CFTR protein end?
Now, find the location of your mutation on the map and **answer the following questions**.

5. In what area of the protein is your mutation found?

6. How might the function of this region be altered by your mutation?

7. Hypothesize as to how the mutation might lead to an alteration in the level of functional CFTR present in the cell membrane. Note this might include either the total protein level or how well the protein works or both. Be prepared to present your results to the class. Be sure to include a brief summary of your patients symptoms, the level of CFTR function you think they have, what mutation they have, where that mutation is located, and how that mutation might explain the symptoms of the patient.