Cystic Fibrosis

I. Overview of cystic fibrosis

Among Caucasians, about one out of twenty people carry the gene for cystic fibrosis (CF), and one of 2,000 to 4,000 people is afflicted with the recessive autosomal disease. CF affects epithelial tissues. These tissues form the barrier between the inside and outside of the body, and include the respiratory, intestinal, and genital systems. In CF, these tissues are impaired in their secretory functions. For example, the respiratory system secretes abnormally viscous mucus which causes problems by not moving out of the lungs, predisposing the patient to infections. On top of that, the pancreas and intestinal systems are only poorly able to secrete the enzymes required for digestion. The disease usually leads to a premature death and furthermore affects the genital tract so that its victims are sterile.

One feature of the disease that can be used for preliminary diagnosis is that the sweat of patients is salty and their skin is often caked with salt. This effect is caused by the fact that people with CF aren’t able to reabsorb the NaCl that comes out in their sweat, so the salt dries on their skin. Everyone’s sweat glands secrete NaCl with the water that forms sweat, but in healthy people the NaCl is reabsorbed and only the water gets out to the surface to evaporate. So, CF patients must have a problem with the secretion of either Na$^+$ or Cl$^-$. It was discovered that the problem lay in the secretion of Cl$^-$. 

II. Ion flow in normal and CF sweat ducts and epithelial cells

The diagram on the left shows part of a sweat duct; the basal side contacts the blood. On the apical side, Na$^+$ and Cl$^-$ readily flow into the sweat duct down their concentration gradients since the intracellular concentrations of the two are kept low. On the basal side, Na$^+$ is pumped out via the Na/K ATPase, and a Cl$^-$ channel opens to allow Cl$^-$ out as well. In CF patients, Na$^+$ could flow into the cell (and could get
pumped out) as usual, but Cl\textsuperscript{−} is unable to enter the cell. Thus, due to the need to balance charge, neither Na\textsuperscript{+} nor Cl\textsuperscript{−} can be reabsorbed into the sweat duct.

(For your interest: In epithelial cell fluid secretion (diagram on the right), ions enter the cell through the basal membrane via a cotransporter, and the Na\textsuperscript{+} pump maintains the inward gradient by pumping Na\textsuperscript{+} out, thus keeping the internal [Na\textsuperscript{+}] down so that Na\textsuperscript{+} continues to be driven in. On the apical side, Cl\textsuperscript{−} normally leaves the cell through channels, while Na\textsuperscript{+} and H\textsubscript{2}O follow paracellularly. In CF, the apical Cl\textsuperscript{−} channel activity is absent, so Cl\textsuperscript{−} can’t get out, and as a result Na\textsuperscript{+} and H\textsubscript{2}O aren’t pulled along, so fluid secretions are thickened or fail.)

III. Measurement of ion flow

A. Patch clamp technique

The flow of ions across a membrane can be measured by a method called patch clamp. In this technique, a glass pipette is held against the outside of a cell to form a tight seal with the plasma membrane. The area inside the pipette is called the patch, and the patch can be pulled off of the cell with the separation between the inside and outside of the membrane maintained by the pipette seal. By placing electrodes on the outside and inside of the patch, it is possible to measure the current produced by the action of even a single channel molecule.

Looking at electrical activity using this technique allows one to see the movement of charge across the membrane. The charges move via channels that consist of multiple transmembrane helices packed so that they are either closed, with no space for anything to get through them, or open for the passage of only certain ions. If the inside is negative, and positive charge is flowing toward the inside, then a negative deflection in the current is seen, as shown in the current trace below.

The negative deflection is the measure of the current that flows through the channel and is usually given in picoamps. The opening and closing of the channel is regulated by factors such as the voltage across the membrane and the binding of
ligands, such as acetylcholine, to the channel. Channels can stay open for a short or long time, or they can flicker between open and closed states.

B. **Cl⁻ conductance in normal cells**

After the patch is excised from a normal cell, no Cl⁻ current is observed; the current trace is flat. In order to activate Cl⁻ conductance, the system requires an increase in [cAMP], which activates PKA. Since the inside of the membrane is the side of the patch exposed to the bathing solution, adding ATP and PKA to the solution provides the necessary stimulation, and small Cl⁻ conductances (downward deflections) appear in the trace. After a waiting period, bigger Cl⁻ conductances appear. When the ATP is used up, the conductances disappear but adding more ATP will cause more conductances. Thus, normal cells exhibit two types of Cl⁻ conductances: an initial small type and a delayed, much bigger type.

C. **CF defect in small conductance**

In a patch taken from the cell of a person with CF, ATP and PKA fail to evoke the small conductance seen in normal cells. Nevertheless, the large conductance can be activated by depolarizing the membrane to +80 mV. From these observations it was concluded that CF patients lack Cl⁻ currents because they lack the ability to activate the channel responsible for the small current. The channel responsible for the large current is intact but cannot be activated by the small current channel.

IV. **Cystic fibrosis transmembrane regulator (CFTR)**

A. **Isolation and structure**

Since CF is inherited, the channel responsible for the small current was found by inheritance analysis, looking for DNA features common to people who have the disease. A region on chromosome 7 was found to exhibit a different pattern in people with CF, and after sequencing 1.5 x 10⁶ base pairs, a coding sequence was found. The gene encodes a 1480 amino acid protein which is predicted to have 6 initial transmembrane domains, 2 blobs in the cytoplasm, 6 more transmembrane domains, and another blob in the cytoplasm. The protein was named the cystic fibrosis transmembrane regulator (CFTR). Two ATP binding regions (Walker sequences) as well as a Ser/Thr-rich regulatory region, containing the PKA phosphorylation motifs RRXS/T, were identified by amino acid sequence analysis. (Since these features are not common to channel proteins, it was initially named a regulator. Also see next section.)
B. **Cl⁻ conductance**

Expression of the CFTR gene verified that the encoded protein does conduct Cl⁻. In untransfected cells (which don't normally have the CFTR protein), activation of adenylylate cyclase (by the drug forskolin) had no effect on the Cl⁻ current. In contrast, transfected cells exhibited some current prior to stimulation and a large current upon stimulation by forskolin. Further, no current could be activated in cells expressing a mutant form of CFTR. These results showed that CFTR causes a Cl⁻ current consistent with the small conductance observed in cells with the endogenous CFTR protein. The channel function of the CFTR is an anomaly for proteins of its structure, which places it in the class of ATP-binding cassette (ABC) proteins, most of which are transporters.

C. **Localization**

Labeling cells with antibodies against CFTR revealed that it is present on the apical surface of epithelial cells, where it faces outward as predicted form the fact that its function is to allow Cl⁻ to go out of the cell.

D. **Regulation by PKA and ATP Hydrolysis**

The CFTR contains PKA phosphorylation sites on the intracellular regulatory domain, as is consistent with the fact that PKA activation is required to activate the current. When these sites are phosphorylated, this domain, which normally occludes the channel pore, comes away from the pore, allowing Cl⁻ to flow through the channel. This regulatory mechanism is known as the **ball and chain mechanism** because the regulatory domain sticks inside the pore like a ball and is released upon phosphorylation, like a ball hanging on a chain. If the CFTR is engineered to lack the PKA regulatory domain, the channel is constitutively open.

ATP is required at another step. As stated above, if ATP and PKA are added to a patch containing CFTR, a current is observed. If these are washed away, no conductance is observed. If we add back just ATP, another wave of current can be monitored. In another experiment, ATPγS, a non-hydrolyzable analog of ATP, is first added together with PKA. No current is observed. When they are washed away and ATP is added, conductance is registered, similar to the previous experiment. However, when we wash away the ATP, and then add ATPγS, conductance cannot be obtained. These observations suggest that the regulatory domain can be phosphorylated by either ATP or ATPγS in the presence of PKA. It remains phosphorylated and does not occlude the channel in the remainder of the experiment. However, ATP hydrolysis is required to actually open the channel. It seems that ATP hydrolysis acts as timing device for this channel. First, ATP binds to the channel and is hydrolyzed to ADP and Pi. Only when Pi falls off will the channel open and it only stays open until the ADP falls off as well. Therefore, if ATPγS is used, the regulatory domain gets phosphorylated, and allows Cl⁻ conductance when ATP is added. But since ATPγS cannot itself be hydrolyzed to release Pi, the channel remains closed when it is added alone.
V. Mechanism of activation of "large" Cl- Channel by CFTR current
How does the small CFTR current lead to the activation of the "large" channel, which carries most of the Cl- current? It turns out that the CFTR acts as a channel for ATP as well.

It appears that when the small Cl- current is activated, ATP also flows out of the cell. When ATP gets out, it can bind to ATP receptors, which then activate Gi. The βγ subunit released from Gi upon receptor activation could then activate the major Cl-channel, evoking the big rectifying conductance. If epithelial cells are exposed to external ATP, the large channel pops open as well.

 VI. Destruction of mutant CFTR
70% of people with CF have a mutation deleting the codon for Phe at position 508 of the protein, referred to as ΔF508. The other 30% have a variety of other mutations. In ΔF508, the CFTR protein is synthesized, but because it contains a mutation, it cannot leave the ER. The unfolded protein response is stimulated as mutant proteins accumulate, but they are ultimately ubiquitinated and destroyed. This fate represents the power of the ER system; just because of a mistake in one amino acid, the CFTR is destroyed and never reaches the plasma membrane, even though if the mutant CFTR is recovered from the ER, it is capable of conducting current in spite of its mutation. (It is not clear why the mutant that contains a deletion of the regulatory region is transported to the cell surface, but ΔF508 is not).

VII. Selective advantage conferred on heterozygotes
The mutant gene appears to have arisen twice in Europe about 50,000 years ago. If CF is lethal, how does the mutant gene stay in the population? Possible explanations include that the gene makes its carriers more fertile, genetic drift, or that heterozygotes for the mutant have a selective advantage similar to the case with sickle cell anemia. The CFTR gene has been knocked out in mice. Studies in these mice support the last explanation. Homozygous knock-out mice have the same disorder as humans with CF. When the heterozygotes were examined as to the amount of fluid secreted into a closed sac in their intestine, less fluid was seen to be secreted in the heterozygotes than in mice with two copies of the wild-type gene. It is thought that secreting less fluid into the intestine could help to protect children from the sometimes lethal effects of diarrhea caused by bacteria such as cholera. Cholera causes diarrhea because it causes the ADP-ribosylation of G proteins, resulting in the constitutive activation of adenylate cyclase and an increase in [cAMP], and ultimately an increased amount of fluid pouring into the intestines.