

Ion Channels and Transporters

Based on their studies of the squid axon with a voltage clamping apparatus, Hodgkin and Huxley concluded that voltage-sensitive conductance mechanisms for sodium and potassium were responsible for the characteristics of the action potential--amplitude, duration, overshoot, undershoot, refractory periods, threshold, etc. They had no way of knowing what physical entities were responsible for these voltage sensitive conductances, however, because relatively little was known in 1950 about membrane structure, except that membranes consisted largely of lipid and protein. The prevailing model at that time was later shown to be wrong and was replaced with the fluid mosaic model in the 1970s. Despite their relative ignorance of the molecules that were responsible for sodium and potassium conductance, Hodgkin and Huxley made several predictions about the characteristics of those molecules, drawn from careful analysis of their data and from their own ingenuity and creativity. First, they predicted that there would be separate transmembrane channels that were selective for Na^+ or K^+ ; that is, because the time course and amplitude of the sodium and potassium conductance were so different, they concluded that there must be two different kinds of molecules, one selective for Na^+ and the other for K^+ that altered their permeability characteristics when the membrane potential changed. Further they concluded that these molecules must form channels or pores in the plasma membrane through which Na^+ and K^+ could pass, rather than being carriers of ions (like the Na^+/K^+ ATPase is--it binds directly to the ions and carries them through the membrane). They drew this conclusion because the rate at which ions flowed through the membrane during an Action Potential was much greater than would be possible for carrier proteins to achieve. They also believed that there were relatively few of these voltage-sensitive ion channels and that there were more for Na^+ than for K^+ .

Because the ion channels changed their permeability characteristics when the membrane potential changed, H & H concluded that the channels must be polar--i.e., they were likely to have a charged region that rearranged itself in space when the electric field caused by the V_m changed. They thought that the movement of charge caused by this channel rearrangement would create a small current (since current is defined as the movement of electrical charges); they predicted that this "gating current" might be detectable with very sensitive electronic gear. Finally, H & H predicted that each ion channel would have only two states--completely open and fully conducting or completely closed. That is, they didn't think the channels could be partially open. Since the current carried by Na^+ or K^+ varied during an Action Potential, they

attributed this variation to the number of channels that were open at any given time, not to the possibility that all the channels were part way open. Needless to say, I wouldn't mention this all to you if H&H weren't right on all counts.

Confirmation of their predictions came in the 1970s and 1980s when new electrophysiological techniques allowed people to measure the characteristics of single ion channels (H&H were looking at the behavior of thousands acting together) and when biochemistry and molecular biology allowed the purification of the proteins that make up the ion channels and cloning of the genes that encode these proteins.

The electrophysiological advance was a modification of the voltage clamp technique called "patch clamping", as described in Box A in Chapter 4 of Purves et al. This technique, developed by two German scientists, Ernst Neher and Bert Sakmann, allows a scientist to pluck a small patch of a cell's membrane into the mouth of a glass micropipet. By controlling the ionic solution inside the pipet and dipping the pipet and attached membrane into another ionic solution, one can isolate the small patch of membrane electrically. The membrane seals tightly to the glass electrode, so that the only ions that enter the electrode are those that pass through ion channels in the membrane. One can alter membrane potential with the microelectrode (hooked up to a voltage clamp apparatus) and then monitor the ionic currents that flow through the small patch of membrane. In some cases the patch may contain a few or even only one ion channel. Thus, this technique allows the scientist to see the movement of ions through a single voltage-sensitive ion channel as it opens and closes. (Not surprisingly, Neher and Sakmann received the Nobel Prize for developing this technique and for their studies of ion channel properties using it).

Patch clamps allowed Neher and Sakmann first to measure the rate of movement of ions through the selective channels in the membrane. They concluded that about 6000 Na^+ ions move through a sodium channel each time it opens, and it's open on average about 1 msec; thus 6 million ions pass through per second. A carrier protein like the Na^+/K^+ ATPase cannot move ions that fast (their maximum rate is about 10,000 ions/sec). This means that there must be an actual hole or pore in the membrane created by the sodium "channel" protein that allows Na^+ ions to flow down their electrochemical gradient into the cell.

Second, they could observe the time course of opening and closing of single channels; examples of these kinds of data for voltage dependent sodium channels are shown in Fig. 4.1 of Purves et al. As Fig. 4.1B shows, each sodium channel opens up more or less instantaneously, and stays fully open until it

suddenly closes, just as Hodgkin and Huxley predicted. When the membrane potential is changed by the voltage clamp, this greatly increases the probability of the channels opening up, but eventually all the channels close. When one adds up the behavior of all the individual channels one gets the time course of current flow in Fig. 4.1C, which mimics what H & H saw in squid axon (Fig. 4.1D). In other words what changes over time is the probability of a particular sodium channel's being completely open or not, but single channels don't open and then slowly close to cause the time course seen in 4.1D. Potassium channels behave similarly (Fig. 4.2B), except that they generally remain open as long as the V_m is held at a depolarized level.

One thing that Hodgkin and Huxley didn't predict (aha! They were human after all) was the existence of voltage dependent Ca^{2+} channels in nerve cells. However, it's clear that such channels exist, and their properties have been extensively studied by many scientists. Calcium is present in the extracellular fluid of most animals in concentrations around 10^{-3} M. Inside cells the free calcium concentration is between 10^{-7} and 10^{-6} M. Thus, calcium is between 1000 and 10,000 times more concentrated outside the cell the inside. Since Ca^{2+} is, like Na^+ , positively charged, there is an enormous electrochemical tendency for Ca^{2+} to flow into cells, and opening a voltage-dependent Ca^{2+} channel will allow Ca^{2+} to rush in and depolarize the cell, just as Na^+ does. In most organisms some of the positive charge carried into the cell during an action potential is calcium and most is sodium, but wide variation can occur. In squid giant axon, almost no Ca^{2+} ions flow in during an AP, and in a few cases, the action potential is caused only by Ca^{2+} flowing in, and there are no voltage-dependent sodium channels. Voltage-dependent Ca^{2+} channels are particularly important in synaptic transmission between cells, as we'll soon see.

These results indicate that the sodium and potassium (and calcium) channels are separate molecular entities, a prediction that was confirmed when the proteins were purified, their genes cloned, and their amino acid sequences determined.

Without going into the details of how it was done, let me just tell you that it was possible to purify a protein from the electric organ of electric eel and from rat brain. The protein consisted of one very large polypeptide (250,000 MW) and two smaller ones of about 35 kD. Reconstitution experiments, in which the protein was incorporated into a pure lipid membrane showed that one can get a voltage dependent Na^+ channel using only the large polypeptide; the function of the two small polypeptides is uncertain. Using a similar approach it was possible to purify voltage-dependent Ca^{2+} channels--they consist of a large

polypeptide of 170 kD and 4-5 accessory polypeptides. When these purified proteins were reincorporated into artificial membranes, one can show that the membranes acquire a selective, V-dependent conductance for Na or Ca, showing conclusively that these are the ion channel proteins.

Once the protein for Na⁺ and Ca²⁺ channels was purified and sequenced, it was possible to clone the genes that encoded them. A somewhat different approach was used to clone K⁺ channel proteins but now many genes that encode Na⁺, K⁺ and Ca²⁺ channel proteins have been cloned and sequenced from many different organisms from *Drosophila* to humans. One of the most interesting findings is that there are many different Na⁺ channels, K⁺ channels, and Ca²⁺ channels, not just one. Indeed there seem to be dozens of genes that encode similar, but not identical, channel proteins. These groupings of genes with similar structure that encode proteins of similar functions are called “gene families”. It’s thought that gene families arise in evolution by duplication of some ancestral gene with mutations over time, causing the sequences of the genes to drift apart. Indeed it appears that the Na⁺, K⁺ and Ca²⁺ channel genes are part of the same gene family, because they have amino acid sequences that are more similar to each other than would be expected by chance.

Once people clone a gene and obtain its nucleotide sequence, they can deduce the sequence of the amino acids in the protein that the gene codes for from the genetic code. This has, of course, been done for the Na⁺, Ca²⁺ and K⁺ channels whose genes have been sequenced. If you know the amino acid sequence and something about the properties of the individual amino acids, you can make predictions about the way that the protein will fold up in three dimensions.

When kind of analysis was carried out on the predicted amino acid sequence of voltage-dependent Na⁺ channels, it is predicted that there is a repeating *motif* of 6 mostly hydrophobic sequences that are predicted to form alpha helices. There are four repeats of this motif in each channel protein. Each of the motifs is homologous to the others which implies that there was an ancestral gene that contained only one set of the six sequences and that this got duplicated 3 times. It is thought that each of the hydrophobic, helical segments is embedded in the lipid bilayer (see Fig. 4.6A). Since there are 4 sets of 6 transmembrane segments, it’s thought that there are 24 transmembrane segments that make up the lining of a membrane pore that is the sodium channel. The Ca²⁺ channel seems to function similarly--there are 24 transmembrane segments that seem to make the Ca²⁺ pore (Fig. 4.6B). K⁺ channel proteins appear to be much smaller than Na⁺ and Ca²⁺ channel proteins—many are about 70 kD, some even smaller, and they have only 6 predicted

transmembrane segments (these are somewhat homologous to the individual 6-transmembrane motifs in Na⁺ and Ca²⁺ proteins); see Fig. 4.6C and E. However, it's thought that the garden-variety voltage-gated K⁺ channel in the membrane actually consists of 4 of these peptides combined into a tetrameric protein. That is, there are 4 x 6 transmembrane segments for K⁺ channels as well.

K⁺ channels are enormously diverse--nearly 100 different K⁺ channel proteins have been detected. This is not only because there are lots of different genes that encode K⁺ channels, but because there is alternative splicing of the introns and exons in the mRNA, giving the possibility that several different protein sequences can be generated from one gene. In addition because 4 different peptides can make up one K⁺ channel, the 4 can be all the same or can be mixtures of different kinds of channel peptides, which also increases the diversity of structures that are possible.

To analyze the function of these proteins, people often use a system whereby the DNA or RNA that encodes the ion channel is injected into a cell that normally doesn't make these kinds of channels. One can then use patch clamping to determine whether the cell acquires some new channel property as a result of the injection. One of the most popular cells for this kind of work is the frog (*Xenopus*) oocyte because these are large cells (roughly a millimeter in diameter) into which it's fairly easy to inject RNA or DNA and because they're fairly easy to patch clamp (see Box B in chapter 4). So if you inject an oocyte with the gene for what you think is a Na⁺ channel, you can wait a while and then see if the oocyte develops a voltage-dependent Na⁺ conductance. If so, you can conclude that the gene you injected encoded the voltage-dependent Na⁺ channel protein.

But this is only the start, because the techniques of molecular genetics allow one to modify the sequence of DNA in the gene at will, thus changing the amino acid sequence of the protein that will be made, allowing the scientist to ask how these changes affect the function of the channel protein. The idea here is to do a molecular dissection of the protein and assign particular functions (such as voltage sensor, ion selectivity filter, inactivation mechanism) to specific regions of the protein.

Consider the model of Na channel function $gNa^+ \text{ closed} \leftrightarrow gNa^+ \text{ open} \rightarrow gNa^+ \text{ inactive}$ (a similar model seems to apply to many Ca²⁺ and even K⁺ channels; that is, there is an irreversible inactivation of the channels after they open). Experiments suggest the channel structure contains a mobile section of protein on the cytoplasmic side of the membrane that can "plug up" the channel and prevent ions from passing.

This ball and chain model predicts that a chunk of the intracellular portion of the channel protein actually swings into place and blocks the channel during inactivation; a number of experiments and crystallographic studies of the protein structure are consistent with this model. For example, proteolytic (protein-digesting) enzymes that are applied to the cytosolic side of the membrane eliminate the inactivation process, but the same enzymes applied extracellularly do not. By changing the amino acid sequence of the K⁺ (Shaker) channel, it was possible to show that what makes up this ball and chain inactivation gate was actually the N-terminal 80 or so amino acids of the protein. Slowly but surely then, people hope to use a combination of molecular genetics, to vary the sequence of amino acids in channel proteins, oocyte expression systems to make the proteins and insert them into membranes, and patch clamp analysis to measure the electrophysiological properties of the altered proteins as a way of teasing apart the functions of the various parts of these amazing and complex proteins. One of the leaders in taking this approach, combined with X-ray crystallography of ion channel structure, is Roderick MacKinnon, who received a Nobel Prize in 2003.

Finally, a brief word about the gating current that Hodgkin and Huxley predicted. H&H predicted that the channel protein would change configuration in the membrane when the V_m changed; it was the only way they could think of that the Na⁺ and K⁺ channels would open up when the membrane potential changed. In fact they figured that some parts of the channel proteins were electrically charged themselves --i.e, amino acids like lysine, arginine, aspartate, glutamate--so that when the electric field across the membrane changed these sections of the proteins would realign themselves. If they're right, then there should be a small charge movement associated with the channels opening up--called the "gating current". That is, they thought that 3 currents would be caused by depolarizing the cell with a voltage-clamp--an ionic current, a capacitive current and a gating current, and that all three would comprise the total membrane current measured by the voltage clamp. I.e., $I_{\text{membrane}} = I_{\text{ions}} + I_{\text{cap}} + I_{\text{gate}}$; and that $I_{\text{gate}} \ll I_{\text{cap}} \ll I_{\text{ionic}}$, so I_{gate} would be hard to measure. In order to see if this was true, in the 1970s two groups of biophysicists did the following experiments. They blocked the ionic currents through the membrane using toxins or drugs that specifically bind to ion channels and inhibit ion flow through them (tetrodotoxin to block Na⁺ and tetraethylammonium to block K⁺). They reasoned that there would only be a gating current when the channels opened up, which only occurs when the membrane is depolarized; hyperpolarizing the membrane potential by the same amount won't cause a gating current because the channels don't open when V_m becomes more negative than the resting value. Now, if the ion channels are blocked, then $I_{\text{membrane}} = I_{\text{cap}} + I_{\text{gate}}$. While I_{cap} can't be eliminated, it turns out that I_{cap} is

dependent only on the amount of change in V_m , not on the polarity of the change. In other words you see a brief I_{cap} when you depolarize the membrane by 20 mV, and a brief I_{cap} of the opposite sign but same magnitude when you hyperpolarize by 20 mV. So they did this, and discovered that $I_{membrane}$ was bigger when the membrane was depolarized than when it was hyperpolarized. They concluded that the difference is I_{gate} .

From the size of I_{gate} measured in this way, one can calculate how much charge must move when the channels are opening--for Na^+ channels it seems to be the equivalent of 3-4 charges moving the width of the membrane (from one side to another), or 6-8 charges moving halfway across the membrane, etc. One possible way this can be achieved is the corkscrew model in which the whole protein moves slightly within the membrane when V_m changes, as shown in Fig. 4.7.

It is possible to measure the gating current for sodium channels in this way, but difficult to measure a gating current for the Hodgkin-Huxley style potassium channels. That's because the sodium channels all open rapidly and simultaneously, so the charges on different channel proteins are moving together to create a current, but potassium channels open more slowly and their movements are slower and less coordinated, so it's hard to detect the current that's associated with their reorganization during the action potential. A recent model for potassium channel activation by MacKinnon and colleagues, shown in Fig. 4.9A, is very controversial because it requires highly charged regions of the protein to move through the lipid bilayer, which many scientists find to be unlikely because of the considerable energy barriers to moving a charged substance through the hydrophobic core of the bilayer.

Thus, while we know a lot more about the molecules involved in creating the action potential than Hodgkin and Huxley did over 50 years ago, much of what we have discovered has confirmed their predictions, and some has revealed features of the nervous system they didn't anticipate, such as the significance of voltage-gated Ca^{2+} channels in nervous system function.

Finally, I want to consider the other kinds of proteins that facilitate the movement of ions across membranes, the ion transporters or "pumps". Typically these are also transmembrane proteins, like channels, but they do not form a hole or pore in the membrane, Rather they bind to ions on one side of the membrane, alter their conformation (which requires the input of energy in some form), and then release the ions on the opposite side of the membrane. Most of these transporters are "antiporters"--that is, they transport two different kinds of ions in opposite directions across the membrane, like the Na^-/K^+ ATPase—or "symporters" that transport two different kinds of ions in the same direction across the

membrane. Mostly these transporters are electrically neutral, in that they transport equal numbers of charges in each direction or carry equal numbers of positive and negative charges in the same direction. Sometimes, however, they are “electrogenic”, moving unequal numbers of charges in opposite directions across the membrane, with the result that they have a direct effect on the membrane potential. (Of course all ion transporters have an indirect effect on the membrane potential, because the concentration gradients that they establish are one of the two main causes of membrane potential difference, the relative permeability of the membrane to the various ionic species being the other factor). The Na^+/K^+ ATPase is an electrogenic pump in most cells, because it exports 3 Na^+ ions for every 2 K^+ ions that it imports. Hence, it tends to make the cell’s interior more negative, by removing more positive charges than it imports. In some cell types, blocking this “sodium pump” will raise the resting potential by a few millivolts, showing that the pump does make a detectable contribution to the resting potential, but in most neurons, the magnitude of this effect is negligible because the rate at which the pump is exporting sodium ions is low.

Although the exact mechanism by which pumps work is still not fully understood, most of the basic features are known. In the 1950s Richard Keynes, first collaborating with Alan Hodgkin and later with others, established the importance of the sodium pump to producing the membrane potential and to understanding the basic features of its operation. He and his coworkers showed that the pump required the presence of K^+ ions and a source of intracellular ATP in order to function. (See Fig. 4.11B for the results of a typical experiment using the squid giant axon; the ordinate gives the rate of radioactive sodium accumulation outside the cell per unit time; as the concentration of radioactive sodium inside the cell falls, so does the rate at which it is exported). Based on these and many other experiments since, Fig. 4-11B shows the current model of how the pump works. It’s thought to oscillate between two forms with an opening exposed alternately on the cytosolic and extracellular side of the membrane, and the addition of a phosphate group (from ATP) or its removal provides the “motive force” that switches the channel between these two configurations. There’s an ATP-driven Ca^{2+} pump that is thought to operate in a similar manner.

Some other transporters are ion exchangers. They derive the energy necessary to function from the energy stored in the concentration gradient of one ion, which can be converted into energy stored in the concentration gradient of another ion. For example, since sodium has a strong thermodynamic energy to enter cells, the movement of sodium into the cell can be coupled to the export of another ion, such as a proton or calcium ion out of the cell against it’s own electrochemical gradient. As long as the free energy released

by the entry of sodium exceeds the free energy cost of exporting the other ion, the overall process will have a negative free energy, and thus be thermodynamically favorable. You should be familiar with another situation in which the free energy stored in an ion gradient can be used to drive an energetically unfavorable process, the synthesis of ATP in mitochondria and chloroplasts from the energy stored in proton gradients. The thermodynamic principles behind the workings of ion exchangers are similar (See Figs. 4-10C).

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