ION CHANNELS FOR BEGINNERS

This is a website produced by the Cystic Fibrosis research team at Montana
University. You may find it interesting, particularly the pages on what ion
channels do.

http://opal.msu.montana.edu/cftr/IonChannelPrimers/beginners.htm

ION CHANNELS FOR BEGINNERS

The ABCs of Ion Channels

How Ion Channels Work

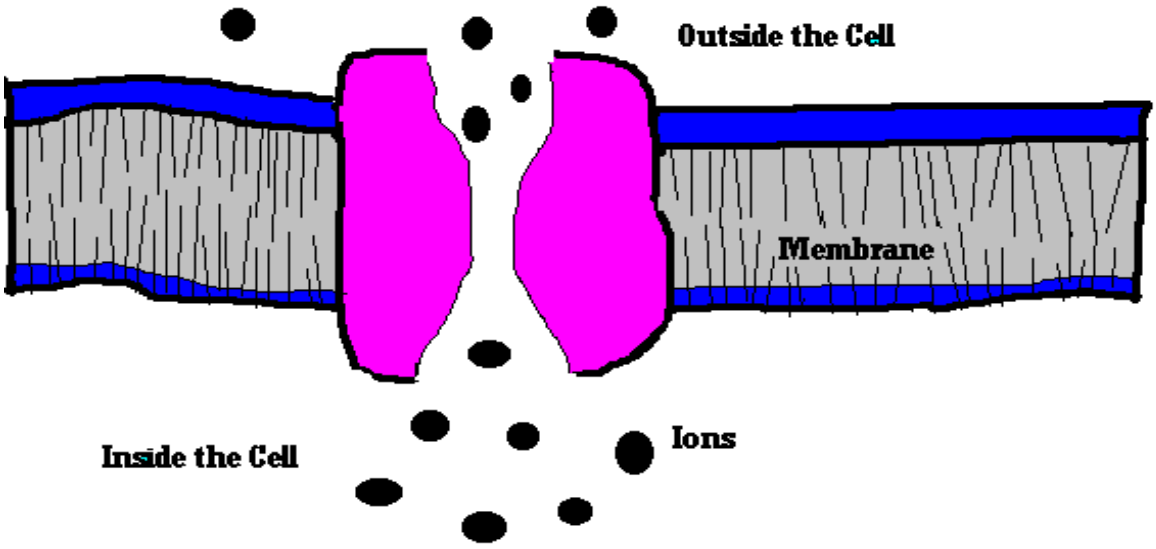
At some time in Earth's early history, perhaps when the planet was less than a billion years old, the ancestor of all present day living cells acquired a protective membrane in which to surround itself in. Made out of oily lipid, the advantages of this membrane barrier for the cell would have been considerable. Protection of the valuable interior contents of the cell from the harsh primordial environment probably was the major driving force. A membrane also provided this first cell with a higher concentration of needed metabolites and other "food" molecules by the simple act of confining them

inside a small volume which is the cell's interior. However, cellular membranes create problems of their own for living systems, due to the same reason that made them so beneficial in the first place: they are very effective barriers to the transfer of large molecules as well as small negatively and positively charged molecules (ions) which are needed by the cell for various activities including growth and reproduction. As just about anyone who has ever tried to wash grease from their hands using water without soap knows, some types of chemicals, like oil and water, do not want to mix with each other. Such is the case for water-loving ions in the environment and the oily lipid membranes which guard the contents of all cells. The problem is that ions prefer to remain in water, but in order to pass from the watery exterior of the cell to the also-watery interior of the cell's cytoplasm where they are needed, they must first find a way to traverse the cell's oily membrane barrier. And they have evolved ion channels to perform this task. It is true that the first cells may not have needed ion channels because the first lipid membranes may have been inherently leaky on their own. It's also possible that small hydrophobic peptides evolved along with the membrane itself, and only later became the more complex ion channels we see today. However ion channels came about, nearly all types of cells we see today are highly dependent on the actions of these ion channels to transfer ions in and out of the cell. In fact, if it wasn't for mitochondrial membranes and ion channels, the use of ion gradients which are necessary for the production of chemical energy (ATP) and therefore life itself would be impossible.

One hundred years of work with cells by scientists in the 19th Century had provided the first hints of the prominent place **proteins** would soon assume in the search for clues as to how life works. By the mid-1800s, proteins in the form of "*enzymes*" were discovered by the chemist Bertholet and others to be necessary for the long sought explanation as to how the process of fermentation leads to the formation of alcohol from sugar, which is just one of many of the useful transformations enzymes perform. And up until the middle of the 20th Century, it was even assumed that proteins were responsible for carrying genetic information; due in large part to their ability for providing endless diversity; simply by varying the number and arrangement of their constituent amino acids (note: DNA would turn out to be genetic material by the early 1950s, not proteins). By the early 1960s, it was becoming clear that ion channels were composed of protein; and it came as little surprise to anyone. This is because it seemed reasonable to assume that in much the same way that a protein in the form of an enzymes was known to "help" chemical reactions along the chemical reaction pathway from substrate to product, protein ion channels could perhaps help ions back and forth thru the cell membrane by lowering the energy barrier inherent in this process.
And as we shall see, this today is the generally accepted explanation for the mechanism of ion transfer from one side of the cell membrane to the other.
Ion channels provide a pathway for the ion which is of lower energy, and

therefore more favorable. This is not unlike the way in which water will always choose the path of least resistance when flowing from one place to another.

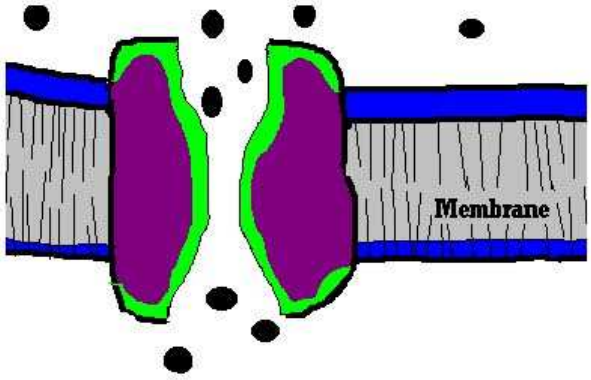
Below: Cartoon drawing of part of a cell membrane with an ion channel (purple) embedded in the membrane. On either side of the membrane is a watery environment. Ions, which are water-loving (hydrophilic) must pass thru the ion channel to go from one side of the cell to the other. This is because the cell membrane is hydrophobic (or "water-fearing").



By the 1830s, it had been determined by chemists that nearly all of the chemicals that make up living tissue were simply combinations of just a few non-metallic elements: carbon, hydrogen, oxygen, and nitrogen. Some proteins (proteins were actually first called "albuminous substances") also were found to have a small amount of phosphorus and sulfur thrown into their formulas for good measure. By the end of the 1800s, Emil Fisher was able to show that proteins were made up of various amino acids strung together like pearls on a necklace. He proved this simply by creating small proteins (called "peptides") in a test tube starting out with only a few types of amino acids and joining them together until they started to resemble proteins in the way they proteins were known to react to the "biuret" test. These artificial peptides he created looked and acted just like the albuminous substances, the

proteins. By the 1920s, nearly all of the chemical structures of the 20 various kinds of amino acids which make up proteins were known, which meant that chemists could predict what kinds of reactions they could undergo, and whether they preferred being in water or in oily situations. Since the advent of x-ray crystallography in the 1960s, when it became possible to actually look at the individual atoms in a single type of protein molecule, it has been considered more accurate to think of the vast majority of proteins as being like strings of amino acids which fold up into a highly ordered "balls of yarn". Many of the 20 kinds of amino acids which have a tendency, chemically speaking, to be in oily hydrophobic environments were found on the insides of proteins more often than the outsides, and the rest of the amino acids were found to be in watery hydrophilic environments and tend to be found on the outside of proteins. It is also now known that the "domains" of ion channels that are embedded in and in contact with the oily hydrophobic lipids of the cellular membrane tend to be made out of the kinds of amino acids that tend to be found in oily kinds of environments. Some examples of hydrophobic amino acids are phenylalanine and valine. There are about 10 all together like this.

Amino acids which form the *pore* of the ion channel, the part of the ion channel where ions and water travel thru the membrane, because they are required to be in contact with water-loving ions, will of course tend to be the hydrophilic water-loving kinds of amino acids. Some examples of these are asparagine (first isolated from asparagus!) and serine. Like their hydrophobic amino acid cousins, there are also about 10 hydrophilic amino acids. An important question by the 1960s was: do the ions get any kind of help from the protein's amino acids as they travel thru the membrane? After all, ion channels have been shown time and again to be able to tell the difference between the different types of ions they let thru. For example, sodium channels let only sodium ions thru and potassium channels let only potassium ions thru. This means therefore that ions most probably are making some kind of physical and chemical contact with the protein ion channel as it traverses the membrane. But how? And does this have anything to do with "*gating*" of ion channels? In other words, how a channel opens and closes its pore?

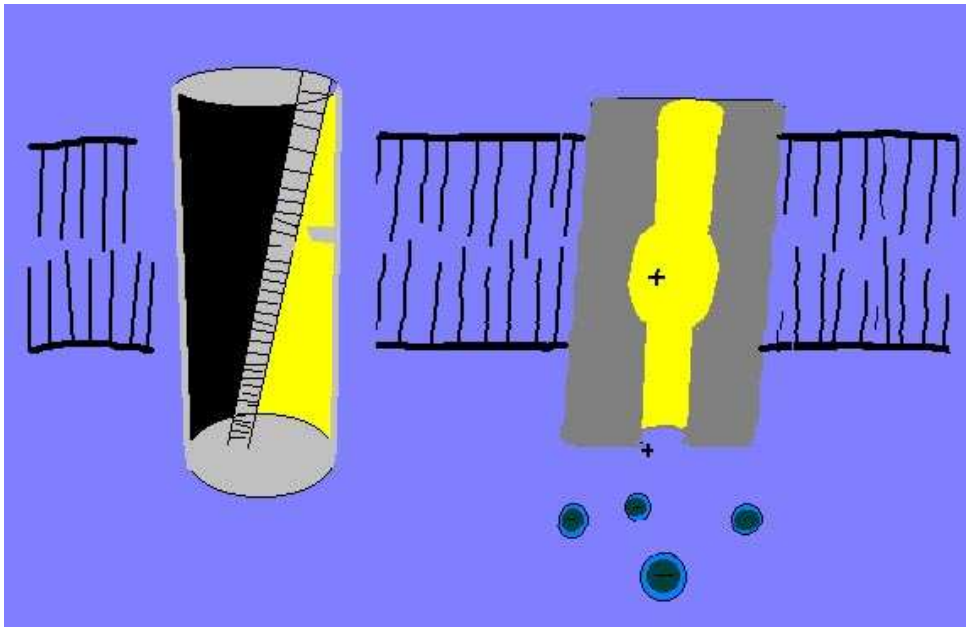


Distribution of amino acids making up an ion channel in a membrane (above): The lipid membrane bilayer is hydrophobic in the center (gray) and hydrophilic on the surfaces (blue). Ions (black) can only pass thru the pore of the ion channel because this is the only part with ***hydrophilic*** *amino acids lining the pore (green = area of ion channel with hydrophilic water-loving amino acids). The rest of the ion channel mostly consists of* ***hydrophobic*** *amino acids (purple).*

What forces might come together for the purpose of guiding an ion up to and thru the pore of its respective type of ion channel? The answer appears to involve a special kind of chemical and physical interplay, almost like a dance which takes place on the sub-molecular level, between the ion and the pore of its channel; where the distances are measured in terms of millionths of a millimeter, rather than everyday macroscopic distances we tend to be used to. Consider the following admittedly rough analogy: Imagine that you have just fallen to the bottom of a deep well, and at first it appears that the only way you can climb out of this well is by scaling up the sides of the walls to the surface. Since it is dark down there, you find you must first feel your way around the sides of the well, grasping at anything that might attract your attention. It is at this point that you touch what feels like the bottom rung of a ladder; and you hold tightly onto it. Your actions would be similar to the way in which a negatively charged ion is attracted to a positively charged amino acid (for example, lysine) on the exterior of the ion channel near the opening of the pore. This type of interaction is electrical (negative attracted to positive charge) in nature and it serves to increase the "*effective concentration*" of these ions near the outside of the pore (don't worry if you didn't understand that completely. Freshman chemistry would help a lot here, though).

Next, you guide your feet onto the first rung of the ladder and you place your hands onto the next rung above in it order to pull yourself up towards the

surface. But the hole becomes smaller as you go up and you find you need to shed the heavy jacket you are wearing in order to squeeze thru. In much the same way, an ion must often times shed the water molecules which surround it before it enters the pore (note that these dozen or so water molecules are always there surrounding the ion whenever the ion is in the watery liquid environment outside or inside the cell). It is unfavorable, energetically speaking, for an ion to lose its water molecules, so as the ion sheds itself of the water molecules, what needs to take place at the same time is for the amino acids composing the ion channel pore to make up for this energy cost by forming the same type of bonds (called "*hydrogen bonds*") with the now naked water-less ion just as if these amino acids were themselves the water molecules. Amino acids such as serine and threonine "look" enough like water, chemically, to take water's place in some proteins. In the same way, the oxygen-studded peptide backbone of the protein can help to take the place of water somewhat as well since water is also composed of oxygen.



Anyway, you continue your climb up the ladder, rung by rung, until you start to tire out. As for the ion in the channel, it is using "chemical rungs" formed by the ion channel's amino acids and the peptide backbone of the channel in order to guide it and keep it moving in the right direction. Together, they form what are called "*noncovalent bonds*" which are much weaker in nature than the kinds of bonds that hold atoms together as in a molecule (i.e. "covalent bonds"), but added together these hydrogen bonds are of considerable influence on the now dry ion. As you continue climbing, you notice that the diameter of the hole you are climbing out of would preclude someone larger or smaller than yourself from fitting thru, because someone too big could not fit thru the hole, and someone too small wouldn't be able to navigate the rungs of the ladder correctly. Similarly, the ion channel is able

to select the proper type of ion it needs to let thru it (called the "selectivity filter"). For example, it isn't unusual for a potassium ion channel to let thru 10,000 potassium ions before it will let a single sodium ion thru, even though both might be present in the same amount near the outside of the pore.

By the time you reach the halfway point, you are tired and need a rest. Very hard to do on a ladder. Fortunately, the hole you are climbing out of has a small platform for you to rest on at the halfway point. This would be analogous to the "*energy well*" that an ion falls into in order to keep it moving thru the channel and in the right direction. An example of an energy well for a negatively charged ion would be the presence of a positively charged lysine amino acid, just like the one on the outside of the pore, placed this time strategically in the middle of the pore by the ion channel protein (this kind of ion platform is sometimes called the "*vestibule*" of the ion channel. CFTR is believed to have one just on the intracellular side of the middle). This vestibule acts like insurance so that once inside the pore, the ion will keep moving thru in the right direction, and not get stuck in the middle or diffuse back out the way it came.

Back to the analogy. At this point, following your rest, you sense the presence of a second climber beneath you. Not wanting to hold the second climber up, you keep moving towards the surface. This second person therefore has the effect of keeping you traveling out of the pit. So too, with some ion channels a second ion is often allowed into the pore behind the first one and essentially gives the first ion enough of a nudge (electostatically speaking) to keep it moving in the right direction. This is because both ions are of the same charge (positive or negative) and these like charges repel, or push on each other. Both you and the ion are now all the way thru to the other side and diffuse into the solvent, or walk away, depending.

In Summary then, ion channels are proteins (or more often groups of proteins made up of individual "*subunits*") which reside imbedded within the lipid bilayer membranes of cells and some viruses, where their job is to regulate the passage of small charged molecules (ions) in and out of the cell or it's various organelles. Because it takes approximately 20 times more energy for an ion to pass thru a lipid membrane bilayer compared to an uncharged water molecule, ion channels and pumps are necessary for transport of all charged molecules thru cell membranes. During the latter half of the 20th Century, it has even been conclusively demonstrated by biochemists and structural biologists that ion channels have an even finer level of structure than first imagined, and are composed of distinct regions which act as a kind of molecular "division of labor". For example, one part of the protein forms the pore where ions pass, while other parts (also called "*domains*") are responsible for opening and closing of the pore. There are also domains which have the ability to interact with the lipid membrane in an energetically favorable way

as well as domains which may bind certain regulator molecules (called "*ligands*") and have the effect of influencing the activity of their specific ion channels. Next we will look at some of the physiological reasons ion channels exist in the first place, and try to understand why their absence or failure to operate properly can lead to diseases like cystic fibrosis.

THINGS ION CHANNELS DO

A dramatic example of the importance ions and ion channels have for living cells is often demonstrated by a classic experiment in freshman biology. The students are first instructed to withdraw a small amount of blood from the end of their lab partner's finger with a sterile needle by stabbing it very quickly. A single drop of blood is milked from the spot by massaging the tip of the finger and the drop is then placed gently on a glass microscope slide. After several minutes watching hundreds or thousands of red disk-shaped cells (which are mostly red blood cells) streaming back and forth across the field of view of the microscope, the students are next told to add to the drop enough pure water to "swamp" the sample several times over in excess water. When the students next look for the cells, many are surprised to find just how few of the cells are left intact on the slide. Nearly complete devastation has taken place on a cellular level in a matter of minutes simply by adding pure water. The explanation given to the students as to why all they now see are "chunks" of lysed, broken cells is because ions (mainly potassium, sodium and bicarbonate ions) could not escape the confines of the cell fast enough thru their respective ion channels to make up for the inrush of water which has an easier time moving thru the cell due to specialized water channels in the red blood cell membranes. These water channels tend to always be wide open. But the real reason that the water flows into the cell's interior is because of the presence of the trapped ions inside. And only a limited amount of water can be added to the cell's interior before the membrane gives way and the cell bursts wide open.

One of the principles of osmosis this demonstration clearly shows is that wherever ions are, water will tend to want to be also. And this is the reason often cited by CF researchers as to how the loss of a chloride ion channel (CFTR) is able to cause the mucus lining the lungs of patients with this condition to become very sticky. No functional CFTR means no ions moving thru the cell membranes of the lung epithelial cells from the cells and bloodstream to the lumen of the lung, which means no water can accompany the ions from the bloodstream thru the epithelium and do what the water is supposed to: help hydrate the mucus lining the lumen of the lungs. The

mucus therefore becomes very viscous, hard to remove, and an ideal environment for bacteria to colonize.

Some ion channels in plant leaves have assumed the responsibility for the regulation of the volume of the cell and are able to open and close ("gate") their pores according to how much tension is placed on the cell membrane due to swelling or shrinking. These channels, like most are grouped according to how they are regulated, and are therefore called "*volume-regulated*" ion channels. One reason they are needed is because if too much water enters a particular cell there is a danger the cell might burst. Therefore volume-regulated ion channels will open and let their respective ions out of the cell when the cell expands in volume, which will have the combined effect of reducing cell volume back to normal. This is because water from inside the cell will tend to want to travel to the outside of the cell right along with the ions, thus decreasing cell volume back to normal. If the red blood cells in the above experiment had had volume-regulated ion channels which respond in this way by opening when there was an increase in cell volume due to water rushing into the cell, they wouldn't have exploded. Specialized cells on the outsides of plant leaves called "guard cells" depend on these kinds of changes in cell volume to control whether the stoma (a pore in the leaf designed to let in carbon dioxide) is open or closed. This regulation is important because when the stoma is open for carbon dioxide, it also inadvertently lets water vapor leave the plant. Here's how it works: when the plant has plenty of water, ions travel into the guard cells because specialized sodium and potassium ion channels will open and these ions will travel down their concentration gradient and into the cells. This causes the cells to swell because water also flows in. This causes the stomata to open and let carbon dioxide into the leaf. It's interesting to consider the following fundamental difference between animal and plant cells: Since animal cells have no rigid cell walls like plants have to help keep their cells from bursting (they can't have them for the simple reason that endocytosis is necessary for animal cells to eat), our cells must regulate water passage very carefully by more control and use of ion channels.

One of the single most important processes all cells must carry out is called "intracellular messaging". Intracellular messaging refers to the way in which a single cell is able to relay information from the environment around it to the cell's interior thereby leading to some kind of a change in either the metabolism or behavior of the cell. An important signal that takes place inside many cells is an increase or decrease in the intracellular calcium ion concentration (Ca^{++}). Muscle cells, for example, depend on an increase in calcium ions in the cytoplasm which travel thru calcium ion channels in order to know when to start muscular contraction. Changes in calcium concentration also is able to trigger an increase the process of "exocytosis", which is how many cells release material into their surroundings. Exocytosis

is crucial to the proper function of nerve synapse. Nerve synapses allow the electrical signal from one neuron to travel to the next neuron. This is a highly regulated phenomena and must take place quickly and at the right moment. Another example of the importance of calcium ion channels in intracellular signaling is when a blood clot causes a stroke in the brain. The cell death caused by the stroke is now believed to be due to the neurons in the brain "working themselves to death". They appear to go into a kind of "metabolic overdrive" when intracellular calcium ion levels inside the cell increases at inappropriate times. As the blood supply becomes unavailable to the brain during the stroke, it causes a loss of cell membrane potential across the neurons. This presents a problem because neurons require a constant supply of oxygen in order to produce enough energy (in the form of ATP) to meet their needs, which is in turn needed to maintain ion gradients across the neuron cell membrane and keep the cell membrane potential high. When these cells die therefore, they inadvertently release neurotransmitters such as glutamate, which then travels by simple diffusion to the nearby neurons and binds to their glutamate receptors. Next, the cells respond to this high level of glutamate signal by releasing large amounts of calcium ions from within it's intracellular calcium stores by opening up the calcium ion channels in these membraneous organelles which reside inside the cell. Since these type of neurons are "hardwired" to respond to high levels of calcium in their cytoplasm by increasing their metabolism, they eventually die probably of oxidative by-products which damage its cell membrane. And still another important function of intracellular calcium ion stores is in vesicle fusion. Vesicles are small spherical packets of membrane encapsulated cargos which are transported around in the cell's cytoplasmic interior. At times, such as during a neurotransmission event, exocytosis of neurotransmitter-loaded vesicles is triggered by inrush of calcium thru channels.

The first ion channels to be discovered and characterized were the *voltage-gated ion channels*, found in nerve and muscle tissue (see: *history of ion channels*). These ion channels open and close (gate) in response to changes in the membrane potential, or voltage. In order for neurons to conduct an electrical impulse, it is necessary for sodium, potassium, and calcium ion channels to open and close at the precisely the right times. When the so called "*action potential*" travels along the neuron's axon, the membrane potential (voltage), which is itself the actual signal, jumps quickly along it's length from a -70 mV inside the cell (compared to 0mV outside of the cell) to +50 mV inside the cell and then back to a normal resting potential of -70 mV again. Another spectacular example of voltage-gated ion channel function comes from the electric eel. This marine animal is capable of delivering several hundred volts of electrical energy at one time because of the specialized arrangement of the excitatory cells in its electric organ. This unusual arrangement allows for amplification of the resulting ion currents across the cell membranes, not unlike the way a series of electric batteries

connected together is able to deliver a large amount of electrical energy at one time simply by the turn of a switch. This type of voltage potential in the electric eel is not restricted to electric fish. For example, it has been calculated that just 20 human neurons connected end to end could deliver enough voltage to light up a small flashlight bulb (i.e. ~ 1.5 Volts).

Ion channels are usually found on the surfaces of cells and are therefore easily accessible to most small molecules such as toxins injected into the blood and lymphatic fluid by venomous creatures like some kinds of snakes, scorpions, bees, among many others. It is not surprising then, that most animal venoms, tetrodotoxin from puffer fish for example, which are "designed" as protection for the animal, bind to and specifically inhibit ion channel function in higher animals. This inhibition can have dramatic and immediate effects on the functioning of muscle or nerve tissue and quickly render a perceived attacker unable to move or respond any further. They have been used as natural anestetics for centuries by indigenous peoples. Protein chemists have taken advantage of this high degree of specificity various toxins have to their respective ion channels in order to purify them from tissue sources. The nAChR sodium ion channel was the first ion channel ever purified (in 1982). This was accomplished by attaching the specific toxin for this ion channel to a stationary "matrix" on a chromatography column and then adding all of the thousands of different membrane proteins from the electric organ of *Torpedo*, a marine ray onto the column. Since only the nAChR molecules will bind to the toxin on the column, it was possible to "wash thru" all the other proteins. All that was left was the now-purified nAChR channels stuck to the column. These nAChR ion channel proteins were then "eluted" (washed) from the column and in this way obtained in extremely purified form.

The time-scale in which ion channels operate are very fast compared everyday human experiences. Many ion channels are capable of allowing ions thru at the rate of 100 million ions each second, and many of these channels stay open less than a millisecond at a time before closing again. This rapid rate of change which ion channels are capable of allows the cells, and consequently the organism itself, a fine level of control over what goes in and out of a nerve or muscle cell, and how fast such exchange events occur. Speed is often a very important factor for survival in nature because the amount of time it takes to respond to danger in the environment (i.e. the reflexes; which are muscles movements responding to nerve stimuli) can often mean the difference between survival or the alternative: death. Since the proper functioning of nerve and muscle tissue is dependent on exchange of ions thru ion channels in the cell membranes, ion channel "kinetics" has evolved over millions of years to be very rapid indeed.

The *Ligand-gated ion channels* are channels which bind to small molecules responsible for other types of regulation. These ion channels open or close

depending on the presence of the type of ligand they bind to. For example, there are sodium ion channels which bind to the neurotransmitter ligand acetylcholine and open; while at the same time, other sodium ion channels may close upon binding the same type of ligand. In this way, a single ligand can regulate ion channels differently depending on where they are found in the body. The nAChR ion channel is a sodium channel which gets its name because it binds to the ligand nicotine (n) as well as the ligand acetylcholine (ACh) and is therefore functions as a receptor (R) as well as an ion channel. There are also a host of ion channels which are gated by ligands found only in the insides of cells. These are therefore named "intracellularly ligand-gated ion channels". G-coupled protein receptors are capable of opening or closing certain ion channels indirectly by causing enzymatic cascades which result in ligand-formation (like cyclic-GMP) to take place within the cell. These various ligands are then able to change the function of the ion channel directly by either opening or closing the channel. Mechanical forces such as those which initiate the sensations of touch and sound can be converted directly into electrical signals when ion channel are activated directly by these signals, whereas ion channels which gate in response to light (vision) and smell (olfaction) must be activated indirectly by way of GPCRs. It has been calculated that the ion channels in hair cells in the inner ear are capable of opening and closing in response sounds by movement of the hair cell's cilia hair by a distance equivalent to that of a single atom's diameter. This would be equivalent to the entire Eiffel tower in Paris swaying a distance equal to only the width of a person's thumb. The first and so far only mechanosensitive ion channel of this type that has been identified and cloned is from the fruit fly Drosophila's sensory brittle neuron.

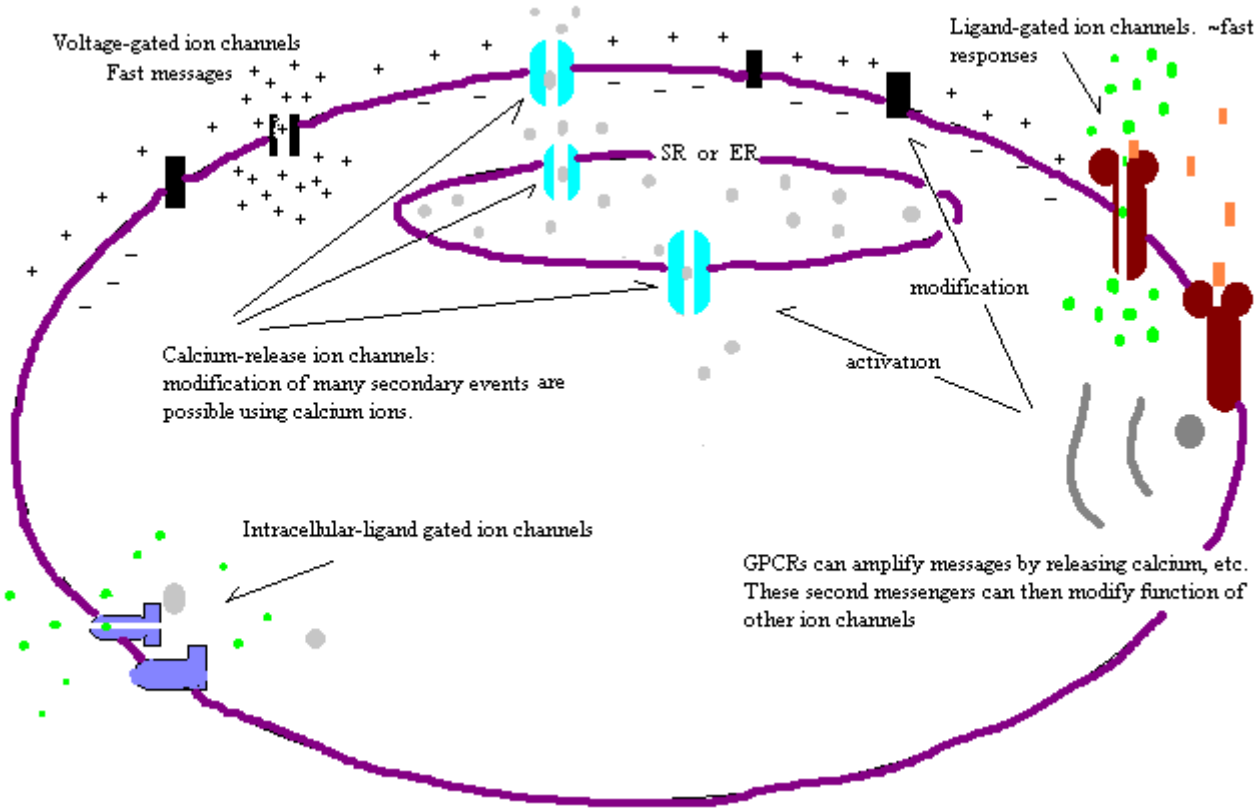
ATP-synthase is the protein complex which lets hydrogen ions into the mitochondria and makes ATP in the process. No life is known to exist on this planet without the ATP manufactured by these protein/enzyme/ion channels.

Given the importance of maintaining a constant chemical environment within the cell, it is not too surprising that ion channels have been shown to be involved in host defenses. Defensins are small molecular peptide ion channels (peptides are very small proteins often less than 100 amino acids in length) modified to "punch holes" in cell membranes of bacteria and other pathogens and are found in extracellular fluids of mammals and other animals. Bacteria and even plants also produce them to attack microbes. Recent experimental evidence has shown that the protein Bcl-2, dubbed by some in the media as the immortality protein may form ion channels under certain circumstances in purified lipid membrane bilayers (liposomes). Along these same lines, the Beta-amyloid plaque protein which plays some as yet unknown part in Alzeheimer's Disease has been shown effectively form a

calcium ion channel and could provide the long sought after mechanism for the death of neurons in the brains of patients with Alzeheimers Disease.

Eosinophil cells of the human immune system are able to express on their surfaces a wide variety of ion channels which play an important role in the regulation of cellular activity during protection from microbial invaders. During eosinophil respiratory burst, for example, where the eosinophil attempts to kill an invading cell directly, the efflux of protons through its proton (H+) ion channels provides an efficient mechanism of proton release and charge compensation.

After certain egg cells are fertilized by a sperm cell, the egg uses ion channels (potassium, for example) to set up a change in the electrical polarization of the cell membrane. This, in turn, somehow keeps other sperm which come along later from fusing with the egg and therefore protects it.



Above: a hypothetical eukaryotic cell, showing the outer cell membrane (purple) as well as an intracellular organelle membrane (endoplasmic or sarcoplasmic reticulum) (also purple). Note that the calcium ions (gray) have a special place within the ion

milieu of the cell. Calcium ions are actually second messenger ions and have the dual ability to traverse ion channels as well as deliver a message to other ion channels, usually involving activation.

TYPES OF ION CHANNELS KNOWN

(Still Incomplete)

CLASSIFICATION OF ION CHANNELS

The three main groups of ion channels are 1) the voltage-gated channels such as the sodium and potassium channels of the nerve axons and nerve terminals, 2) the extracellular ligand-activated channels which includes channels such as GABA and glycine receptor channels, most of which are regulated by ligands that are "neurotransmitters". These channels are often named according to the ligand they bind to. 3) Intracellular ligand-gated ion channels. These include CFTR and some other ABC family members as well as ion channels involved in sense perception. These are often activated indirectly by GCPRs. Other common intracellular ligands which activate these kinds of channels include calcium ions, ATP, cyclic AMP and GMP as well as phosphadidyl inositol (PI). There are additional systems of nomenclature which have joined the second and third groups into the "chemically activated" or just simply "ligand gated" ion channels. It has been shown by sequence comparison that ion channels within the above groups will also show the greatest sequence similarity and are therefore most likely all be descended from a common ancestor. The mechanosensory and volume-regulated channels have their own grouping, but they are still in the process of being classified. We have made a fifth "catch-all" group, miscellaneous 2, which includes any ion channels not included in the above there. This group includes the GAP junctions, peptide ion channels like Gramicidin, and various venomous insect toxins like the conus toxins from cone shells. At the end is a section on Recent Discoveries.

A few words regarding the classification and naming of ion channels: Of the several possible ways of arranging ion channels into groups, so far the most successful seems to be a system based on how ion channels are regulated. It should be remembered that no method known is without drawbacks. For example, it is possible to arrange ion channels according to the ion that they conduct thru their pores rather then regulation mechanisms, however this method has the problem that very few other characteristics will be shared in common among the channels. For instance, the nAChR channel conducts sodium ions, as does the voltage-gated sodium channels of the neurons. But other important features are very obviously not be shared between these two very

different ion channels, including gating mechanisms, as well as the sequence of amino acids making up the channels themselves (i.e. they are not related evolutionarily). Many researchers believe that until there is a classification system based solely on sequences and molecular structures, as well as evolutionary history of all ion channels at the level of the genome, the method of classification (even the one used here based on activation mechanisms) will have to suffice. A good example which exemplifies a need for this new kind of system is given by the finding that certain cyclic nucleotide gated channels share enough sequence similarity with voltage-gated ion channels that they may in fact belong to the voltage-gated channel superfamily rather than the ligand-gated superfamily they are presently placed in. Another problem with the present system is that while the glutamate ion channel, which is activated by the ligand glutamate, is often relegated to the family of ligand-gated ion channels, it shares no sequence similarity and therefore is probably unrelated evolutionarily to any other ligand-gated ion channels in this group.

EXTRACELLULAR LIGAND-GATED ION CHANNELS

nAChR: The "nicotine acetylcholine receptor" has the distinction of being the first ion channel sequenced: in 1983. It functions as a multimer of 5 subunits which form the channel (2 alpha, 1 beta, 1 gamma, 1 delta). It is found in nerve and muscle cells and is not to be confused with other nAChR receptors, some of which are not ion channels at all. The ion channel nAChRs include those found in nerves (nerves release acetylcholine which binds to nAChR in muscle cells). When the channels open, they let pass just about all cations, including sodium, potassium, and calcium; and this depolarizes the cell membrane which can in turn trigger voltage-gated channels which in turn causes muscle contraction. nAChR is considered to be a model ion channel mainly because of its historical importance due to its high abundance from natural sources which made it easier to study especially before molecular biology techniques became more refined. nAChR is used in many detailed studies on ion channel kinetics and allosterism and was first isolated and characterized from the fish *Torpedo* (tissue source was its electric organ. *Torpedo* is a marine ray). There are many different "variations" of the nAChR channel. For example, mammals have several just in the nervous system. The poison curare is known to shut down (and therefore act as an "antagonist" of) nAChR. Nicotine is an alkaloid drug from tobacco which exerts its physical effects on the body in part because it also binds to nAChR. However instead of simply shutting it down like curare did, nicotine activates it instead. Chlorpromazine, a tranquillizer, is able to block the channel pore. It is quite possible that general anesthetics exert their effects via direct binding to the transmembrane helices of ion channels such as nAChRs. The nAChR family, together with the GABA and Glycine chloride channel families forms a superfamily of ligand-gated ion channels which is based on strong sequence similarity. These neurotransmitter ligand-gated channels are not related to voltage-gated channels or to the glutamate ligand-gated ion channel. They all have 4 distinct transmembrane segments.

GABA and **Glycine** Receptors (these are both families of chloride channels). GABA(A) and glycine chloride channels have relatively complex gating characteristics and are made up of 5 subunits, with each containing 4 transmembrane helices (TMs), and are characterized as having multiple states of conductance apparently due to them having

more than one possible open state. TM2 has been found to contribute to pore (i.e. the five TM2 segments from the 5 subunits come together to line pore). Chloride channels in excitable tissues function in the same way as the potassium channel in that they serve to "dampen" the electrical excitability of the neuron. GABA(A) and glycine receptor chloride channels function in the postsynaptic neuron as well as skeletal tissue. GABA(A) is so named because it binds to gamma-aminobuteryic acid. GABA and Glycine neurotransmitters act mostly on inhibitory neurons. Like glutamate, they are small molecules and therefore act as fast messengers in neurons. GABA inhibits ability of neurons to fire action potentials, and should not be confused with the unrelated GABA(B) receptor, which couples to the intracellular second messenger systems. As many as 1/3 of brain neurons use GABA. GABA is manufactured by the body from glutamate, however vitamin B6 is a necessary component. Lack of it can cause seizures. Alcohol and barbituates like Valium on the other hand act on GABA(A) receptors as agonists (activators), by increasing burst time of the channels. They bind at sites where GABA doesn't normally bind to, and potentiates the action of GABA when it binds at it's own site. This often makes these types of drugs beneficial for epilepsy. Glycine Receptors tend to be more localized in brain, but can be found in spinal cord and other places. Some molecules bind GABA(A) Receptors but do not open them, thereby blocking them from activation by the endogenous neurotransmitter itself. Diversity of GABA(A) receptors can be increased due to the fact that each channel is made up of distinct amounts of various individual protein subunits (each subunit is a distinct protein). There are 6 alpha subunit isoforms for GABA(A) receptors (3 beta and 3 gamma and one delta). All subunits have a GABA ligand-binding site, unlike nAChR in which only a single subunit is able to bind its ligand, ACh. Their mRNA transcripts can also be alternatively spliced to provide further diversity. Note: the majority of all known ion channels consist of more than one protein subunit, as with GABA(A) and Glycine Receptors. Glycine Receptor is also chloride channel, but binds glycine instead of GABA. The glycine and GABA(A) Receptors are both found in the post-synaptic membranes of neurons and are both composed of oligomers consisting of several homologous subunits (each subunit is about 50 KDa) with the amino terminus facing out of the cell and the second transmembrane helix lining the pore (like with nAChR). Each subunit most likely consists of 4 membrane-spanning helices, not unlike the nAChR subunits. The glycine receptor has 4 different alpha subunits and one beta subunit. Both GABA(A) and Glycine Receptors are involved in producing inhibitory responses and serve to dampen the action potential of neurons and show amino acid sequence similarities. All of the subunits are of similar size. Note: the various glutamate receptors are in a different family completely due to differences in sequences. Blocking inhibitory receptors causes convulsions. The GABA(A) Receptor is widely found in brain. The disease called "Startles Disease" results from a mutant form of the glycine receptor alpha-1 subunit. Patients with this disorder are subject to muscle rigidity in response to external stimuli believed to be due to a lowered affinity of the ion channel to its ligand, glycine, which results ultimately in a lowered chloride conductance into neurons. This reduces the inhibitory effects of chloride on certain neurons. Strychnine acts as a competitive antagonist. It binds the alpha-subunits of the Glycine Receptor. These channels, like the GABA(A) ion channel, form mostly heteromeric pentamers. Both GABA(A) and glycine receptors conduct chloride in the range of 10 to 90 pS. Both conduct bicarbonate anions as well. Glycine receptors can be found throughout the CNS, not unlike GABA(A). Note: GABA and Glycine neurotransmitters act mostly on the inhibitory type of neurons As many as 1/3 of the neurons in the brain have them.

There are 3 types of chloride channels: voltage-gated, ligand gated (like GABA and Glycine receptors), and CFTR. . There is no sequence homology between the three families. Chloride channels contribute to processes as diverse as membrane excitability, transepithelial transport, regulation of cell volume and regulation of pH of intracellular organelles.

5-HT, (includes)**MOD-1** (Serotonin-gated ion channel) : 5-HT is a cation selective ion channel and so named because it is activated by 5-hydroxytryptamine. Note that the ligand-gated channels all seem to be related to each other (GABA, 5-HT, glycine, acetylcholine) except for the glutamate ligand-gated ion channel. Note: the neurotransmitters GABA, 5-HT, and glutamate can also produce much slower responses by binding receptors that are not ion channels, such as GCPR's. A serotonin-gated ion channel, MOD-1 was recently been discovered in C. elegans. (Note: 5-HT is serotonin). This channel helps control locamotory behavior. (Note: serotonin generally binds to either GPCR's like 5-HT, to mediate slow responses or the ionotropic 5-HT(3) receptor, a "non-selective cation channel which mediates fast membrane depolarizations.) MOD-1 has a predicted structure similar to nAChR family of ligand-gated channels like glycine and GABA receptors. MOD-1 has a reversal potential dependent on chloride concentration, but not cations. It is not blocked by calcium ions or 5-HT(3a)-specific antagonists. It is inhibited by metabotropic 5-HT receptor antagonists mianerin and methiothepin. MOD-1 functions as a 5-HT receptor in vivo.

P(2X) Is an ion channel known to respond to ATP. It has different structure than other ion channels and is in the same family as P(2) Purinogenic receptors. They are sodium and calcium channels and are made up of multiple subunits. They are known to be involved in neurotransmission.

Glutamate: **NMDA, AMPA, Kainate Receptors, GluR-B** Unlike the nAChR and glycine receptors which were cloned after using affinity chromatography, the glutamate receptors were found using expression cloning with cDNA and therefore without any protein chemistry involved. The majority of synapses important for fast excitatory transmission confined to the vertebrate CNS use glutamate as a neurotransmitter. The NMDA type of glutamate receptor ion channel selectively passes calcium ions over sodium or potassium and therefore can affect intracellular messaging. It is possible that its main role may not be in the generation or dampening of electrical signals (which they do since many pass sodium and potassium monovalent ions), but rather in signal transduction perhaps ultimately affecting learning. NMDA types are found in almost all neurons. Almost none are known to exist in the peripheral nervous system, which instead use ACh. Glutamate Receptors are relatively diverse due to the "mix-and-match" strategy using multiple subunits, as well as alternate splicing and are involved in long-lasting changes for example in learning and memory. High glutamate levels cause death of neurons and are perhaps involved directly or indirectly in causing neurological diseases such as ALS, Alzheimer's Disease, and Huntington's. There are at least three main types of glutamate receptors. Some glutamate receptor channels include NMDA, AMPA (quisqualate), and kainate receptors, so named because AMPA binds to AMPA receptors, kainic acid kainate receptors, etc. The subunits composing these ion channels are longer than nAChR family and very diverse. Some AMPA receptors may have their messenger RNA transcripts edited (called "RNA editing") which has been shown to

make their channels become more permeable to calcium. Some animal venoms (spider, wasp) are known to affect Glutamate Receptors directly. Unlike the neurotransmitter ACh, transmitters like glutamate, glycine and GABA have no enzyme which breaks them down. It is therefore necessary for them to be removed from the cleft by diffusion or by sodium-coupled transporters. GluR0 is a recently discovered channel from prokaryotes. GluR-B is a glutamate-sensitive ion channel expressed in neurons of the CNS. It's pre-mRNA has recently been shown to undergo RNA-editing which results in an A to I (note: an I is similar to a G as far as hydrogen-bonding during translation) via deamination. This causes a change in protein sequence from a glutamine to an arginine, making the channel less permeable to calcium. GluR-B double knockout mice suffer from epileptic seizures and die soon after weaning. It still is not known if this example of RNA-editing is to "correct" for an DNA mistake, or to generate more diversity. There is an NMDA ion channel which binds glutamate. It acts as a calcium channel in synapses. ATP and serotonin have been found on rare occasions to act as fast excitatory transmitters in vertebrates.

ORCC : uncloned chloride channel. Activated by extracellular ATP perhaps via the purinergic receptor (P2u) ORCC: outwardly rectifying chloride channel. Found in epithelial tissues, it is probably the main contributor to chloride currents thru the apical cell membrane (30-70 pS) with an anion selectivity of I > Cl > Br. ORCC was discovered by patch clamp before CFTR was cloned and was assumed by many to be the "CF chloride channel". . Also called ICOR or ORDIC, it is activated in the presence of active CFTR (when there is no cAMP stimulation, ORCC remains inactive) or extracellular ATP. Perhaps ATP is released from CFTR. ORCC is activated by PKA indirectly and this is probably due to CFTR activation by its NBDs. It is likely CFTR releases ATP somehow into the extracellular environment and the ATP binds to purinergic receptors (P2u subtype?) and this activates ORCC.

P2X3: Receptor ion channels that bind extracellular ATP which in turn contributes to pain sensation resulting from tissue destruction and bladder discomfort (due to stretching) and sensations of warmth. (Note: the sensation of touch is the least understood of all the senses on the molecular level) The P2X3 receptor is localized to the apical cell membrane and opens when ATP binds it. It is believed that cytosolic ATP is released when tissues are damaged, which in turn excites these pain-sensing neurons, also called nociceptors. Knockout mice lacking this channel have been developed.

Inward Rectifying Channels: ***ROMK** (requires internal ATP), ROMK2, **IRK** (a potassium channel), **BIR** (potassium channel widely expressed in pancreas and heart), **RACTK** (a pH sensitive potassium channel found in kidney. Probably involves potassium removal in urine), **Kir**6.2, Kir1.1a, Kir6.1 (note: the sulfonylurea receptor SUR is an ABC protein found to heterodimerize with Kir6.1 and Kir6.2 Some theorize CFTR can take it's place at times.

Ligand-gated channels have subunits arranged as pentamers (5 subunits per ion channel)

VOLTAGE-GATED ION CHANNELS

Nearly all eukaryotic cells have some amount of voltage-sensitive ionic permeability, some more complex than others. Voltage-sensitive ion channels are diverse in the kinetics of their gating. Some are modulated by neurotransmitters or intracellular messengers.

Sodium Voltage-Gated Ion Channels: In 1978, these channels were first purified (Agnew et al.) from electric eel electric organs. Found to be a single peptide of almost 2000 amino acids in length (but with internal repeats corresponding to the equivelant of subunits). However, in other tissues such as mammalian skeletal muscle or brain, it can be found as subunits (4 subunits in the case of voltage-gated channels, but 5 in the fast ligand-gated receptors. GAP junctions have 6. It seems that the more subunits an ion channel is composed of, the less selective it is for its respective ions. This may be because the pore is correspondingly larger the more subunits it is composed of). The channel from electric eel was also found to have 30% of its weight in carbohydrates (500 sugars of mostly sialic acid and N-acetylglucosamine) and 6% as attached fatty acids. Some Sodium Voltage-gated channels may have as many as 6 different kinds of neurotoxins which bind and inhibit them to various degrees and each toxin appears to bind at a different site, which is unusual. Some of these toxins are classified as peptides, while others are alkaloids, cyclic polyethers, esters, and heterocycles. Most peptide neurotoxins are 60-100 amino acids in length, which allows them to assume a defined shape, but curiously, the peptide toxins made from cone shells are often only between 10 and 30 amino acids long. They accomplish their inhibitory task by forming disulfide bonds with each other. Usually 2 or 3 come together and form these larger structures. Voltage-gated Sodium channels are responsible for the action potential of neurons while the voltage-gated potassium channels help to re-establish the membrane potential back to normal. Pore sizes are estimated to be ~3x5A for the selectivity filter region. Potassium channels are more diverse, and yet it is also true that sufficient diversity exists among sodium channels for different monoclonal antibodies to distinguish sodium channels from different tissues (axons from muscle, etc). Sodium channels deactivate quickly compared to calcium channels. This is the reason calcium ions are used by the cell for more of a sustained response to external stimuli. Some other members of this family: **mH1, mH2, SCN4A** (skeletal muscle), **PN1, PN3, SkM1, RSMK, Kat1, EAG, ELK, Drk1,**

Potassium Voltage-Gated Ion Channels: Potassium ion channels are easily the most diverse of all ion channels; more so than even chloride and calcium channels. This diversity can make identification difficult during whole-cell recordings. Like chloride ion channels, they serve to 'dampen' excitation potentials in excitable cells. Also called "delayed rectifiers" in axons, these channels are composed of 4 membrane-spanning, pore-forming alpha subunits and 4 cytoplasmic beta subunits and function as inwardly rectifying potassium channels. Voltage-gated Potassium channels are often multi-subunit channels like nAChr and others, but sodium and calcium voltage-gated channels share the distinction of being composed of single subunits all around 2000 amino acids in length. It should be noted that the more subunits an ion channel is made out of, the less selective it tends to be to the passage of its respective ion. Cells use potassium channels to regulate pacemaker potentials, as well as regulating their overall

excitability. Potassium channels have been worked with more than most channels and for that reason much is known about them. Inwardly rectifying potassium channel family members often differ widely in gating kinetics. There are many different types of the voltage-gated potassium channels (many more than there are sodium ion channel types) and all seem to be related to each other. Often, it is possible to find several different types within the same cell! Some of the types of K+ channels are: "A channels", which are fast but brief acting, "and others that differ in voltage dependence, sensitivity to modulators. KvLQT is a recently discovered potassium channel found in basolateral membrane of epithelial cells and is responsible for keeping potassium at right concentration to allow chloride to flow out of apical epithelia in airway thru CFTR. It is also involved in the syndrome "long QT". It is voltage-dependent. First cloned from mouse heart. T-cells of the immune system express **Kv1.3** and **IKCa1** channels, and blockade of these channels may be a possible treatment in autoimmune diseases like EAE (similar to MS). The hot pepper ingredient capsaicin selectively blocks K+ channels in the tongue. Some more members of this sub-family: **HERG** (cardiac), **HCN, Kv**, Kd (delayed rectifier), Kf (fast transient), KCa (calcium-activated), **MaxiK** (activated by Ca++)**,** **TASK-1** is a continuously activated potassium channel which serves to dampen cellular excitability in certain neurons in the brain. A list of more potassium voltage-gated channels: **Shaker, Shal, Shab, Shaw, minK** (miniature potassium channel, or **IsK**), **KvLQT, KCNK channels** Note: recent evidence suggests **MaxiK** channels may be involved in transepithelial chloride transport in lungs: *Pflugers Arch 2001 Apr;442(1):1-11*

Calcium Voltage-Gated Ion Channels: **L (HVA), T (LVA), and N types** found in skeletal muscle and heart muscle, these channels can best be described functionally as very diverse (in ion selectivity, metabolic regulation, pharmacology, and single-channel conductance). There is now a fourth type called "P type", found in Purkinje cells. First isolated in 1984 from transverse tubules of skeletal muscle, it is used primarily as a voltage sensor for excitation-contraction coupling in muscle. Voltage-gated calcium channels are found in protazoans like Paramecium as well as in almost every excitable cell in animals. They have a unique role in that they are involved in taking electrical signals and making chemical signals out of them. Some are involved in excitation while others in regulation of secretion, contraction (muscle) and gating (other ion channels). For example, intracellular calcium activates calmodulin, troponin and other proteins, which in turn activate enzymes that increase cAMP and phosphorylation, triggering muscle contraction or ciliary and flagellar motions. Voltage-gated Na+, K+, Ca++ channels have a basic design: a set of 6 transmembrane segments (S1-6) flanked by cytoplasmic hydrophilic segments plus an H5 sequence between S5 and S6. S4 has basic residues every 3rd or 4th position and is probably a voltage sensor. H5 helps control ion selectivity. Segments S2 and S3 of Na+ and K+ channels have a pattern of conserved charged residues also exhibited by calcium channels. There is sequence similarity among 5 classes of channels: Voltage-gated, calcium activated potassium channel, cyclic nucleotide-gated channels, a calcium channel for PI-mediated Calcium entry, and a potassium plant channel/transporter. Voltage-gated calcium channels in excitable cells are, like other K+ and Na+ channels, structurally homologus but phenotypically diverse. They are electrophysiologically characterized into L (long-lasting), T (transient), and N (for their involvement in norepinepherine). The L-type is found in Skeletal muscle and heart muscle. They are all very structurally homologus to voltage-gated Na+ channels. L-type voltage-gated calcium channels in the cell membrane of heart muscle (cells have action potentials which last relatively long time

compared to nerve cells) let in calcium to cytoplasm. This causes more calcium to be released from the SR, which ultimately causes the contraction of muscle and a heartbeat. Some drugs act to increase contraction by controlling the amount of calcium inside the muscle cell. The disease HypoPP (hypokalemic periodic paralysis) is a skeletal muscle disorder caused by mutations in the S4 regions of L-type Calcium channels. Neuronal voltage-dependent calcium channels of the N and P/Q type can be modulated by activated G-proteins. Calmodulin is a dominant calcium sensor in the calcium-dependent inactivation of Ca(v)1.2 calcium channels. Calcium channels were discovered by Fatt and Katz in 1953 in crab muscle. They found sodium ions were not needed for the weak action potentials of muscle cells, but calcium was being used instead. They therefore described "calcium spike", an action potential based on the inflow of calcium ions, for the first time. Vertebrate smooth muscle cells use only calcium ions to generate the action potential. These channels are also used to a degree in conjunction with sodium channels in many other excitable cell types to help generate action potentials. Some voltage-gated calcium channels are deactivated by sustained intracellular calcium concentrations and are therefore self-limiting. Calcium ion channels tend to have conductance rates and open probabilities lower than that of sodium or potassium ion channels. Most single-channel measurements are therefore done at unphysiological concentrations of calcium ions.

Voltage-Gated Proton Ion Channels: Found in eosinophils and other phagocytic cells and are activated by the generation of reactive oxygen species by NADPH oxidase. Efflux of electrons causes a build-up of positive charge which these channels are responsible for offsetting.

Slowly-activated voltage-gated channels are new members which have only 1 transmembrane segment.

Voltage-gated channels have their subunits arranged as tetramers (4 subunits make up each channel)

The channel shaker from Drosophila has been found to have homologues (i.e. are related) in the plant Arabidopsis.

Anion Voltage-Gated Ion Channels: **VDAC** (voltage-dependent anion channel) This ion channel has a high conductance (over 600 pS) for anions. It is slightly selective for anions over cations and is somewhat voltage-dependent. These voltage-dependant Anion Channels have been found in the mitochondrial outer membranes. They have high conductances and beta-barrel secondary structures.

A class of voltage-activated channels that let pass both sodium and potassium have been found in cardiac pacemaker and Purkinje fibers. **I(h), I(f), I(Q),** and **I(AR)**, are all names for the same channel. It is similar to K(ir) channels in that it is activated by negative potentials and close at positive potentials. They pass an inward current when open.

INTRACELLULAR LIGAND-GATED ION CHANNELS

Unlike the axons of neurons, which function as carriers of messages only, cells from tissues such as smooth muscle, secretory glands, and the parts of the neuron like dentrites and somota need to be able to modulate messages based on the state of the individual. They adjust the incoming messages by changing the intracellular concentrations of various second messengers as well as alteration of various coupling proteins and cofactors. While this method of regulation of ion channels is definitely slower (from seconds to minutes), it can be longer-lasting than more simple types of regulation like voltage-gated and extracellular ligand-gating.

ATP-sensitive Potassium Channels (ATP-K), includes **ROMK2, IRK, BIR, RACTK, Kir** These channels are regulated by ATP, and appear to be similar to CFTR in this way. Their pharmacology is controlled by the sulfonylureas glibenclamide and tolbutamide. These molecules also inhibit CFTR chloride currents, with a half-maximal concentration of ~20 and 150 uM for each. The **ADAC** (ATP depletion-activated cation channel encoded by the gene LTRPC7) and **ATP-regulated Potassium Channel** are also regulated by changes in intracellular levels of ATP. Some can be found in mitochondria, and may be involved in changes in volume of this organelle and therefore may indirectly control oxidative phorphorylation. It has been found that opening these channels during ischemia/reperfusion seems to provide cardioprotective effects.

CFTR CFTR: (also designated as ABCC7) is a chloride channel and a whole lot more. It was the first chloride channel to be cloned (1989, by chromosome walking). Expressed in epithelial tissues along with other chloride channels like the calcium-activated chloride channel, volume-regulated chloride channel, and a calcium-dependent channel activated by extracellular ATP and UTP. Evidence suggests CFTR, in addition to chloride, transports bicarbonate ions, as well as water. CFTR is predicted to help regulate the following channels: ORCC, ROMKK+, ENaC, and Cl-/HCO3- exchanger. CFTR is different from all other epithelial chloride channels in that it prefers chloride over iodide ions. It is possible iodide blocks the channel. No other halide blocks the pore. CFTR belongs to the ABC transporter family, a family most of whose members are transporters, not channels. Members of the ABC family expressed as a single protein tend to be found at cell surfaces, while ABC subunits in multi-subunit channels tend to be found in membranes of intracellular organelles like the ER. CFTR is activated both by phosphorylation of its R-domain by PKA (protein kinase A) as well as ATP binding at its nucleotide binding domains, namely NBD1.

Note: It is not unusual to refer to all anion channels found in living cells as "chloride channels" regardless of their selectivity for various anions, due to the fact that there are much more chloride ions than there are any other type of anion inside or outside of the cell. Anion channels tend to be less selective than cation channels overall. Some anion-selective channels also let cations thru if there happens to be an anion present during passage.

Calcium-activated Chloride Channels (ClCas): Found in epithelia and may be the reason initial attempts at CFTR mouse knockouts failed to develop lung pathology similar to CF lungs. These channels may augment chloride transport when CFTR is not present. ClCas play several important roles in different types of cells.

ENaC is an amiloride-sensitive sodium channel probably made up of 4 subunits, 2 alpha, one Beta and one gamma. Each subunit consists of 2 transmembrane helices. Found in all epithelia CFTR is found in, ENaC is involved in absorbing water. It has been suggested that ENaC is also a receptor because it has an unusually large extracellular domain region (with cysteine-rich-boxes). It may be activated by an extracellular protease. It is involved in sodium and water absorption in kidney as well as lung, bladder and colon. Shut down by the diuretic amiloride reagents, they are activated in the body by hormones like vasopressin and oxytocin. Current treatment for CF involves attempts to inhibit this channel, and hence water absorption in the lung lumen, using amiloride. Members of the **DEG/ENaC** channel family are involved in sensation of temperature (tongue) in mammals.

ASIC family:

Calcium-activated Potassium Channels: (SK, BK) First one was reported by Gardos in 1958 in red blood cells. Potassium channels with small conductances expressed in non-excititory tissues like kidney. Subtypes include SK1, SK2, SK3. SK3 is expressed in all parts of the kidney and is probably the renal apamin receptor. Meech in 1974 found calcium-activated potassium channels in molluscan neurons. Driving force for the release of potassium is also encouraged by the increase in positive cell potential due to the increase in calcium concentration. BK channels have a voltage-dependence for activation. Calcium binds directly to BK channels. BK channels have the highest strongly-selective cation conductance of any known mammalian ion channel.

K(ATP) is a potassium channel activated by intracellular ATP binding. Inhibition of these channels leads to increased insulin secretion. **S-type** potassium channels are voltage-insensitive. **M-channels** are a class of potassium channels found in sympathetic neurons that shut off indirectly by the neurotransmitter ACh, which binds muscarinic ACh receptors. There are reports to a potassium channel activated by millimolar concentrations of sodium in vertebrate and invertebrate neurons.

cGMP Channel (CNG Channels) These tetrameric sodium and calcium channels are found in rod photoreceptor cells and are similar to the voltage-activated potassium channel, shaker. They are likely to be formed by the assembly of at least two different subunit types, alpha and beta. Second messenger-gated channels such as these have the same overall design as voltage-gated channels, with binding sites for second messengers in C-terminal region. But while the voltage-gated Na and Ca channel alpha-subunit has 4 internal repeats, it is present only once in the K+ channel (ex: shaker), and the cGMP-gated channel and newer ones. This means these potassium channels probably resemble more closely the ancestral ion channel, which is likely to be a tetramer of similar subunits. These channels are involved in light perception and bind cGMP and cause depolarization of the cell membrane upon photon reception. It is a photoreceptor cGMP-gated cation channel found vertebrates. Also a member of the voltage-gated channel family. CNGs are also found in sensory neurons, the brain, and many other tissues and are important in directly linking changes in intracellular cAMP and cGMP to electrical excitability. They may be among the targets of local anesthetics.

G-Protein Activated Inwardly Rectifying Potassium Channels: GIRK2 (Kir 3.2) unlike its close relative IRK1 (Kir 2.1) which is constitutively active, GIRK2 is activated directly by a G-protein, and inactivated by mechanical stretch. They can be found in atrial myocytes. The sulfonylurea receptor (SUR) regulates Kir6.2 channel activity by enhancing surface expression.

PI-mediated Calcium Channel: IP(3) Receptor. Is a calcium ion channel responsible for phototransduction (i.e. vision) in invertebrates like Drosophila and is probably mediated by activation of PLC (phospho-lipase-c). This channel causes an increase in intracellular calcium levels (both from inside and outside cell). It is possible that a light-activated calcium channel may be the "transient receptor potential", or TRP gene. It shows sequence similarity to voltage-gated calcium channels. In neurons such as the Purkinje cerebellar cell, release of calcium due to InsP3 is involved in learning. Some IP3 Receptors include: **IP(3)R-1**,

TRP-PLIK (transient receptor potential-phospholipase C interacting kinase) is a recently characterized ion channel that is also a protein kinase. It has a wide tissue distribution and apparently is a non-selective calcium-permeant channel. It has a zinc finger alpha-kinase domain. Long TRP channels (LTRPs) include **melastatin**, **MTR1** and TRP-PLIK. Melastatin has been linked to melanocytic tumor progression. MTR1 is associated with Beckwith-Wiedemann syndrome and a predisposition to neoplasias. It is unknown how they are gated. *Runnels, et al., Science 2/9/01 Vol 291 pgs 1043-1047*

Calcium-activated Chloride Channels: These channels open upon an increase of cytoplasmic concentrations of calcium and are often grouped according to rate of conductance. SK channels are potassium channels also activated by calcium. They can be found in many organisms as well as many excitable cell types. Discovered by Bader in 1982. There are several subtypes with unknown roles. Mice over-expressing SK3 lead to an altered response in respiratory rhythm to hypoxic challenges (similar to sleep apnea and sudden infant death syndrome) and it causes problems during delivery of mouse pups. Lack of SK3 in mice resulted in "no macroscopically altered phenotype", however. SK potassium channels are found in excitable cells and are responsible for the slow after-hyperplolarization that often follows the action potential. Note: intracellular calcium increases during the action potential, which is what is responsible for SK activation. The channel's alpha-subunits interact with calmodulin-calcium. IP(3) Receptors are also found in the ER (endoplasmic reticulum) and binds inositol 1.4.5-triphosphate (IP3) inside the cell. The calcium IP(3) channel therefore helps control intracellular calcium concentrations (calcium in turn is regulated by amounts of IP(3). Drugs (like the Cardizem line from Avenis) which treat high blood pressure do so by blocking calcium channels.

Calcium Release Channels: calcium channels are required for voltage-gated depolarizations in most muscles, as well as control of intracellular processes and are therefore widely distributed in vertebrate tissues. Calcium channels have the distinction of being the most selective of all ion channels. **P/Q type calcium channels** involved in neurotransmission have been found to be the targets of autoantibodies produced by LES (Lambert-Eaton syndrome) patients. When a nerve cell signals to a muscle cell

to contract, or a cell to release a neurotransmitter, etc, it does so by causing a calcium channel either in the cell membrane or in the ER or SR to open and release calcium into the cytoplasm. The increase in calcium ions into the cytoplasm controls many other processes, including cell death, protein secretion, cell metabolism, and development. It is also able to control the gating of still more ion channels as well as the activity of enzymes. Calcium channels were first discovered by Fatt and Ginsborg in 1958 in crab muscles. **L-type** calcium channels in the heart muscle are responsible for strength of contraction during heartbeat. These channels are phosphorylated by PKA as a result of an increase in cAMP production. This second message is produced when norepinephrine is released from nerve terminals and binds receptors in the heart muscle and provides for a stronger heartbeat during critical times known as 'flight-or-fight'. When the channel is phosphorylated, it increases the open probability of the channel, and hence the beating strength. **CRAC** are calcium-release activated calcium channels. Their identity remains unknown, and they are among the most selective of known calcium channels. They have been shown to be regulated by changes in cytoplasmic concentrations of calcium. They also exhibit dramatic changes in selectivity and conductivity in the presence of divalent cations.

Ryanodine Receptor (RyR) : Ryanodine is a very large alkaloid of ~5000 molecular weight. Ryanidine receptors are found in SR (sarcoplasmic reticulum) in the interior of the cell and is closely associated physically and functionally with a voltage-gated calcium channel in cell membrane. These receptors are widespread in the CNS and therefore probably play critical roles in intracellular calcium dynamical changes. They are regulated at least in part by phosphorylation. The skeletal muscle ryanodine receptor (RyR1) is one of largest channels known. RyRs are associated with FK506 binding proteins (FKBPs) which play a role in intracellular calcium signaling. So large, in fact that its gross structure can be resolved by cryo-electron microscopy. A tetramer, it is a ligand-gated calcium channel. Each monomer is 5,037 aa. (565kda). In smooth muscle, the SR is located just 200 A from the cell membrane, which allows these channels to be activated by calcium flowing into cell via voltage-sensitive calcium channels in cell membrane. These channels are activated by an increase in positive cell potential. This is an example of how a "global" change in calcium can set off a "local" change (also called a "calcium spark") in calcium concentration inside the cell by the ryanodine receptor calcium channel localized in the SR. This "calcium spark" has the net effect of activating a third channel, the potassium channel called **Big K+** (also abbreviated as **BK**) located in the cell membrane. The increase in potassium flow out of the cell due to this channel causes the cell potential to become negative once again (which deactivates the voltage-activated calcium channel) and therefore resets the whole process so it can begin again when the voltage-gated calcium channel is reactivated. Big K+, or BK, is a potassium channel localized to the cell membranes of smooth muscle cells. It lets potassium thru at a high rate when the channel is activated by a localized "calcium spark" produced by the ryanodine-sensitive calcium channel in the SR. BK is the channel responsible for resetting the cell membrane potential back to the negative resting state. BK channels consist of 4 alpha subunits (which form the pore) and an unknown number of beta subunits, of which there are 4 kinds, B1-B4. It is believed that the difference in beta subunit type gives each tissue it's own specificity. For example, the B1 subunit increases the channel's sensitivity to calcium and slows the kinetics of opening and closing of the channel. BK B1 knockout mice have severe constriction of arteries and therefore constant high blood pressure. BK channels are found in neurons and are near cell membrane calcium channels and respond to local

calcium concentrations there. They are also found in the kidney and secretory cells. Each tissue's BK channels have different gating kinetics.

Calcium-Release Activated Channel (Icrac) is a highly calcium selective ion channel activated upon depletion of intracellular calcium levels and depletion of intracellular calcium stores. **CaT1** (and a close relative called **ECaC**) is a relative and member of the now extensive **TRP family** found in mammalian cells (see below). **SOC** (store operated calcium channels; also called "capacitive calcium entry") are found in arteriolar smooth muscle. **TRP** channels are found in Drosophila (TRP5 is activated by extracellular calcium). Calcium ion concentrations inside cells are triggered by hormones and neurotransmitter molecules. A longtime mystery has involved the exact mechanism in which a fall in the concentration of calcium in the endoplasmic reticulum is able to cause an increase in calcium conductance thru the cell membrane and into the cell.

TRP Family: 20 known so far (July, 2001). TRP channels are unusual because some seem to have enzymatic activity in addition to being ion channels. These enzymatic activities may serve to couple to signalling and metabolic pathways within the cell. There appear to be 3 subfamilies, based on sequence identity. Each has 6 transmembrane helices which form a cationic channel. The two ends, acid and amino, have other functions. TRP proteins are believed to form tetramers in order to become an ion channel. This could provide for extensive diversity. One subfamily, the "long TRP channel" (LTRPC) appear to have extensive N and C terminal ends, with the acid end containing the enzymatic activity. Specifically, LTRPC7 is known to have a protein kinase domain, and can phosphorylate other proteins as well as itself. LTRPC2 has enzymatic ability to remove the terminal ribose-5-phosphate group from ADP-ribose. TRP4 and CaT1 may be the long-sought after store-operated calcium channels. There is evidence that TRP channels influence diseases such as cancer. The levels of some LTRPC-family members have been shown to change during progression of tumors like melanoma and prostate cancer. *Nature 411 5/31/01 pg 542-3*

Calmodulin (CaM) mediates Calcium modulation of several ion channels including those involved in calcium-induced calcium release (CICR). CaM in the heart has been found ligated to L-type calcium channels and is needed for calcium-induced inactivation and facilitation of the channel.

ENaC: is an amiloride-sensitive sodium channel and may be activated by an intracellular protease.

GORK is a delayed outwardly rectified potassium channel expressed in guard cells of Arabidopsis thaliana, and is also able to sense potassium

Note: intracellular-ligand gated channels are often controlled by GPCRs (G-protein coupled receptors). This is the case when regulation of ion channels can be measured in seconds as opposed to milliseconds. A well-known example of an ion channel being controlled indirectly by a GPCR via a G-protein includes the regulation of the heartbeat in cardiac muscle, here the G-protein is first activated by receptor and then binds to the ion channel and directly increases or decreases it's gating. Sometimes, the G-protein may bind an enzyme like adenylyl cyclase or phospholipase C which may

trigger cAMP or calcium ion build-up in cytoplasm which can then affect the ion channel. G-protein responses are slower and longer than other means of gating, such as voltage-gating of ion channels in neurons.

Aquaporins: AQP1 not only mediates water flux but is also a cGMP-gated ion channel. While most aquaporin channels passage water, some only allow specific molecules like glycerol thru.

Sensation of Taste: The exact mechanism by which taste molecules activate ion channels in the tongue depends a great deal on the molecule itself. Sodium ions (salty) travel directly thru the ion channel in the taste cell and therefore activate it directly. Protons (sour) actually block the channel directly, while other taste molecules act as ligands and open the channel by binding it. Molecules like sucrose (sweet) exert their action by binding a non-ion channel receptor, which sets off a series of enzymatic events culminating in phosphorylation and closing of a potassium channel. Molecules that taste bitter raise internal calcium concentrations which also shuts off the potassium channel.

MECHANOSENSITIVE AND CELL VOLUME-REGULATED ION CHANNELS

Miscellaneous 1

Mechanosensitive Ion Channels : also called **SACs** (stretch-activated ion channels) (ex: **MscL** from E.coli, has been solved to 3.5A) open and close in neurons in response to mechanical stimuli which cause sense of touch and hearing. They respond to membrane tension by opening a large water-filled pore. A mechanosensitive ion channel has been identified in the sensory brittle neuron of the fly Drosophila. The ion channel **minK** (short for "miniature potassium channel"), also called **IsK**, is found in kidney and upon depolarization of the cell membrane, slowly induces a potassium current from this channel. Swelling-activated (i.e. volume-regulated) chloride channels have been found in all cells so far studied. There is some evidence CFTR is able to control the ones that are found in epithelial cells. Some swelling-activated chloride channels may also be voltage-gated. This is a good example of how a classification system based only on how ion channels are regulated can be misleading. **VRAC** (volume-regulated anion channels) found in epithelial cells may be regulated by tyrosine kinases. A putative yeast calcium channel, **CCH1**, may be involved in increasing cytosolic calcium upon hypotonic shock. **Mid1** is also found in yeast. It has been postulated that tension-driven gating may be due to an increase in the tilt angles of the transmembrane helices, which would enable the pore to open in a way analogous to the iris. Nonselective cationic SACs let pass calcium as well as sodium and potassium, while others are selective for potassium and perhaps chloride. SACs may be responsible for generating fast arrhythmias in the atrium of the heart.

Note about Mechanosensitive Ion Channels: While vision, olfaction, and taste all use G-protein coupled receptors (GPCRs) to stimulate a signal to the brain, the senses of touch and sound use mechanical stimuli which regulates ion channels. They do so by directly converting mechanical forces into electrical signals.

CLC group of chloride channels: some possibly activated by strong hyperpolarizaiton as well as changes in cell volume. Not ligand gated.

In 1997 there were just over 12 different types of chloride channels known. Generally, chloride channels have been neglected as subjects of study compared to sodium, potassium, and calcium channels due in part to their once thought relative insignificance in cellular and organismal functions. CLC-O was the first and chloride channel cloned from the CLC family. The CLC family is the largest known chloride channel family. The CLC Group is present in many tissues and organisms, from the electric eel electric organ to vertebrae skeletal muscle and is widely expressed in most mammalian cells. CLC-O is also found in the electric ray Torpedo. It may be involved in cell volume regulation and is also found in the kidneys. CLC-1 is expressed in skeletal muscle SR and has a conductance of 1 pS (undetectable by patch clamp methods). CLC-2 has been discovered by Northern blotting to be ubiquitously expressed, including epithelia of the lung. It is activated by cell-swelling or strong hyperpolarization. One of the types of diseases caused by defects in a chloride channel are the "myotonias". CLC-1 mutations result in electrically hyperexcitable muscle membranes.. In 1992, a mutant form of the channel CLC-1 was cloned and determined to be the cause of a type of myotonia, a disease causing muscle stiffness and which exhibits both recessive and dominant forms. 3 separate point mutations were discovered to cause the dominant form, while the rest of the characterized mutations caused the recessive forms. The dominant form is called "Thompson's Disease, or myotonia congenita, and results in a lowered conductance of chloride in and out of muscle cells. The CLC family members are all conserved in sequence, and have 12 transmembrane domains. The yeast has scCLC as its single CLC chloride channel called GEF1. It was discovered by mutant yeast which lack ability to grow without high iron concentrations. A plant CLC named AtClC-d is able to compensate for this defect in yeast. CLC-5 is mainly expressed in the kidneys and is involved in Dent's disease, a rare X-linked form of nephrolithiasis, which results in hypercalciuria and proteinuria. The mutations which cause loss-of-function in CLC-5 cause the disease. As of 1996, the CLC channels CLC-4, CLC-6, and CLC-7, have not been expressed in functional form, and therefore are not proven to be chloride channels yet. It's possible that expression systems like xenopus do not have the correct accessory factors, or subunits necessary. It's also possible that their required function is as intracellular chloride channels and therefore harder to determine experimentally. CLC-K1 and CLC-K2 as well as CLC-3 have been reported to be slightly outwardly rectifying chloride channels. **EriC** is a CLC channel from E.coli and functions as a dimer which selectively conducts anions. **CLH-5** is a CLC channel involved in touch sensation in nematode neurons. It may be volume-regulated.

GEF1,

TRP is a superfamily of ion channels required for production of mechanoreceptor currents by insect bristles (which are mechanosensory organs). It is probably a mechanically-gated ion channel.

Acid-sensing ion channels (ASICs): A subfamily of the sodium selective ENaC/DEG channels, these channels are gated by a decrease in extracellular pH. ASIC3 has been shown in neurons when overexpressed to most likely be involved in sensing cardiac ischemia and perhaps triggers angina pain or heart attack. It may be that acid does not directly activate the channels, but release of a calcium ion from a binding site on the

channel. *Immke and McCleskey (Vollum Institure, Portland, OR).* **KCNK3** is a potassium-selective leak channel sensitive to protons in a potassium dependent manner.

MISCELLANEOUS 2

KCNK potassium ion channels: they are highly regulated, potassium-selective leak channels, discovered around 1996. Even though they are new to the potassium ion channel family, they already outnumber all other potassium ion channels combined. KCNK channels are easy to identify because of their unique structure--they possess two pore-forming domains in each subunit. Although leak currents are fundamental to the function of nerves and muscles, the molecular basis for this type of conductance had been a mystery. For a review, see: Goldstein, et al. *Nat Rev Neurosci 2001 Mar;2(3):175-84*

GAP Junctions GAP Junctions were first detected by electrophysilogical measurements in 1959 by Furshpan and Potter in giant synapses. They are the reason synapses are now distinguished as either "electrical synapses" (like these) or "chemical synapses" (as with ACh, etc). These electrical synapses have been found in most multicellular tissues. Note: GAP Junctions are not to be confused with "tight junctions", where cells are ~0A apart. Here, cells are 20-30A apart. Most cells are around 200A apart, but have large pore diameters. GAP junctions are nonselective channels which are hexameric in structure. Connexins are the name of its subunits and are often found in close array. 16 are known so far in vertebrates. GAP junctions allow both anions and cations thru with equal frequency. Molecules up to 1000 A in diameter can pass thru. Fluorescent molecules pass easily and can be followed spectroscopically. Gap junction channels are present across both membranes of the 2 cells they connect. Gap Junctions and water pores tend to be arranged as hexamers (6 subunits per channel). Heart muscle cells are connected by gap junctions in order to sense signals from each other to begin contracting quickly.

Guard Cell Slow Anion Channel is important for the basis of water loss control in plants. It allows prolonged osmolite efflux which is necessary for stomatal closure. Curiously, a functional antibody raised against CFTR was found by N. Leonhardt, et al to be able to inhibit this process, as well as an ability to immunoprecipitate a polypeptide from guard cell protein extracts. The antibody also immunolabeled stomata in Vicia faba leaf sections.

MIP Family The MIP Family consists of major intrinsic proteins of the mammalian lens. At least 18 family members. Large variety of functions. Glycerol uptake in E. Coli, Drosophila brains, and aquaporins.

Intracellular Chloride Channels: A new family called **CLICs** found recently in the nuclear membrane due to sequence similarity with the microsomal chloride channel p64. Some have been shown to exist in soluble form inside the cell. These include **p64, CLIC-1,** and **HuH1**. Conductance of CLIC-1 has been measured at 162 pS. CLIC3 is found in the nucleus and binds MAP kinase ERK7, which implies it may be involved in regulation of cell growth. Another nuclear chloride ion channel, **NCC27,**

is one of only a few cloned nuclear ion channels. It is a relatively small, 27,000D transmembrane protein with sequence similarity to p64. These channels are highly conserved across species and therefore could be involved in cell cycle regulation.

Eicosanoid-Modulated Chloride Channel: appears to be gated directly by EET.

Gramicidin A peptide antibiotic effective against some gram + bacteria. They consist of 15 amino acids that alternate between D and L forms. These structurally simple and easy to work with ion channels were the first to have their single currents measured. Gramicidin must form a dimer with another gramicidin molecule in order to form a working ion channel. One half of the dimer situates itself on one side of the membrane bilayer while the other half of the dimer is joined to it while sitting in the other half of the membrane bilayer. **alamethicin channels** are an antibiotic anion channel like gramicidin but are 20 amino acids long.

Influenza M(2) Protein The flu virus has an ion channel (4 single transmembrane tetramer) called M2. It is a proton channel. (H+ or H3O+, it isn't known for sure). It lets protons into viral membrane during endocytosis to activate HA. It is an integral membrane protein and is highly expressed in infected cells. It is only 97 amino acids long.

Alpha Toxin An ion channel toxin secreted from Staphlococcus bacteria which attacks red blood cells of the host. It binds to cholesterol in the membrane. It's structure has been determined to a resolution of 1.9A The central fluid-filled pore is 14-46 A in diameter. Other toxins known to influence membrane permeabilization include: Colicins, diphtheria toxin, aerolysin, tetanus toxin, mellitin from bee venom and margainin, a toxin from xenopus skin.

VPU HIV, the AIDS virus, has an ion channel called VPU which is weakly selective for cations (mRNA in oocytes exp and lipid bilayer exp). These channels are not found in viral membranes, however and are probably used to help virus bud.

Porins are found in gram negative bacterial outer membranes. They are generally nonselective. In some bacteria they may select slightly for cations. Porins have a high conductance rate for high molecular weight solutes up to 600 Da, They usually form tetrameric structures, Beta-sheets and have pore diameters of around 10 A in diameter; large enough to let cell wall constituents like oligosaccharides thru.

More Information About Ion Channels:

The human genome probably codes for at least 50 different kinds of ion channels. A single excitable cell membrane probably has between 5 and 10 different ones.

Some More Possible Chloride Channels?: note: all are unrelated. **pIcln, phospholemman, p64, Ca-CC.**

Expression and Distribution of Ion Channels:

Flies have ~50 ligand-gated ion channels; C. elegans around 100 out of its 19,000 total genes. C. elegans has 42 nAChR channels and 37 GABA(A)-like subunits. Drosophila has a number of families: 3 voltage-dependant chloride channels, 14 Trp-like channels, 24 amilioride-sensitive sodium channels, 2 porins, one ryanodine receptor, one IP(3) receptor, and 8 innexins. C. elegans has over 80 potassium channel genes and 90 neurotransmitter-gated ion channels, all of which are in the nervous system. Drosophila only 30. No voltage-activated sodium channels are found in C. elegans.

More Facts: Ion channels are more likely to be homologus (related) if they share the same method of regulation; for example, all voltage-gated ion channels are thought to have arisen from a common ancestor gene. To date, no disease-causing mutation has been found in the promoter region of an ion channel. Murine myotonia and hyperekplexia are ion channel diseases caused by insertion of transposons. ATP-sensitive potassium channels can often be impaired as a secondary effect of diabetes in pancreatic beta-cells.

Chloride channels are believed to be found in nearly every type of cell known, including yeast and bacteria. They are involved in volume regulation, transport across epithelia, acidification of intracellular organelles, stabilization of cell membrane potential and signal transduction. Chloride anions are the most abundant anions in both plant and animal tissues. Chloride is unusual in that it is one of the only anions found in equilibrium concentrations across cells. As of yet, there is no known pump that uses ATP to change chloride gradients, but chloride can be pumped to form gradients using electrical gradients.

References:

Ion Channels: Molecules in Action Aidley 1996

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More Information About Recently Discovered Ion Channels:

From the October 11th issue of the journal Nature: "Calcium and cyclic nucleotides control sperm motility..... Here, we describe the cloning and functional characterization of the of an unusual sperm cation channel (CatSper).....Several voltage-dependent calcium channel (Ca_v) mRNAs and cyclic nucleotide-gated (CNG) proteins have been detected in sperm cell precursors...Furthermore, low-voltage-activated, dihydropyridine-sensitive 'T-type' channels and pharmacologically defined N- and R-type currents have been measured in spermatogenic cells." The CatSper gene is unique because it codes for a single, 6-transmembrane-spanning repeat (like voltage-dependent K+ channels such as Kv, and yet its pore region and overall homology is closest to a single domain of the much larger 4-repeat Cav channels. It was noted that

CatSper protein has not yet been shown using heterologous expression systems to allow transport of calcium ions. Sperm lacking CatSper are poorly motile and are unable to fertilize eggs with intact zona pellucida. *Dejian Ren et al., "a sperm ion channel required for sperm motility and male fertility" Nature Vol 413 603-609*

In the journal Nature (2/2001) Hanno Tan et al. reported finding a mutation in the cardiac voltage-gated sodium channel gene, **SCN5A**, responsible for causing isolated cardiac conduction disease. They found that a missense mutation (G514C) changes the voltage-dependent gating behavior such that the ion channel deactivates more quickly when open. The channel also had a modified voltage dependence of activation. These effects combined to produce an overall sodium current that was lower than the wild-type ion channel produces, thus slowing cardiac impulse conduction. *Nature Vol 409 2/22/01 pgs 143-147*

KcsA is a potassium channel recently crystallized and solved to atomic resolution. It appears to use its selectivity filter as a gate. When in the closed state, it traps K+ ions by binding them with high affinity. When the channel is open, the affinity for K+ actually decreases and K+ flows thru the channel. VanDongen and Chapman (from Duke University) state "The cytoplasmic constriction seen in KcsA is not a universal gate, since it is not found in inward rectifying K channels and glutamate receptors.....Affinity switching allows the channel to be both highly selective and permeate efficiently." *Biophysical Society Meeting 2/2001*

"A vivid example of [the discovery of anion channels] has unfolded in the past few years with the identification of the genes causing diastrophic dysplasia, congenital chloride diarrhoea and Pendred syndrome. While these three disorders are clinically distinct, the associated genes (**DTDST, CLD** and **PDS**, respectively) emanate from a well conserved family of genes that all encode anion transporters." *Everett LA; Green ED Hum Mol Genet 1999;8(10):1883-91.*

In the journal Cell, Komak et al. speculate that the chloride channel CLC-7 is responsible for the bone condition osteopetrosis, a disease where not enough bone is resorbed (the opposite of osteoporosis). In order for bone-digesting enzymes secreted from bone cells called osteoclasts to function, protons need to be pumped into the bone matrix. Chloride flow thru CLC-7 is apparently necessary for this process becaues it maintains electrical balance. Mice deficent in this ion channel had all the hallmarks of osteopetrosis. CLC-7 was found to be expressed in lysosomal membranes in normal cells. *Cell 104:205-215 2001*