Overview- Function and Three dimensional structures of ion channels

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Introduction: Actions speak louder than words. The molecular roots of our actions and the thoughts and feelings that drive us to act are ion channels, proteins that form macromolecular pores in cell membranes. These transmembrane proteins generate and propagate the electrical signals that allow us to sense our surroundings, process information, make decisions, and move.

Ion channel proteins act as gates that span the lipid bilayer that surrounds all cells where they open and close to allow the flow of ions down their electrochemical gradients (Figure 1). The ion flux through a channel pore can be extremely high, ~10^6 ions per second [1]. Because of their central role in the function of the excitable tissues like heart, brain, muscles, and nervous system there has been a long quest to understand ion channel properties from a molecular perspective. Decades of biophysical measurements and functional studies have been devoted to understanding ion channel function [1]. Yet, the very nature of these molecules, transmembrane proteins that are difficult to obtain in the large quantities and high purity necessary for structural investigation, has impeded attempts at obtaining the most essential information for understanding their functions, a high-resolution, three-dimensional description of their molecular architectures. In the past five years, the once impregnable fortress separating biophysicists and neuroscientists from this essential information has been breached. The first high-resolution structures of ion channels and ion channel associated proteins are providing the substrates for sophisticated tests of the mechanisms of channel gating and permeation. This chapter briefly touches on these pioneering studies and the questions they raise.

Ion channels perform two basic functions. They open and close to control the passage of ions across the cell membrane (Hille chapter) and they sense and respond to signals that drive them between open and closed states (Isacoff, Karlin, Siegelbaum, Adelman, and Meyer chapters). The response times of channels to these inputs can be very fast, on the order of tens of microseconds to a few milliseconds [1]. Different classes of ion channels have been designed by nature to respond to the three types of signals one can imagine sensing in a membrane environment: extracellular signals like neurotransmitters

(ex. Acetylcholine and Glutamate receptors, Karlin and Meyer chapters), transmembrane voltage changes (typified by voltage-sensitive cation channels, Isacoff chapter), and intracellular signals like calcium and cyclic nucleotides (Adelman and Siegelbaum chapters). While channels are generally classified based on the primary signal that opens them, many channels serve as integrators and respond to some combination of signals.

The pore forming domains of most ion channels are multimeric assemblies possessing cyclic symmetry that creates a general architecture known as 'barrel-stave' (Figure 2). A fixed number of subunits assemble around the axis of the ion conduction pore. The number of subunits is roughly related to the size and selectivity characteristics of the channel. For example, the most selective channels like voltage-gated sodium channels and voltage-gated potassium channels are tetramers where four identical or highly-homologous subunits are arranged around the pore. Pentameric channels like the nicotinic acetylcholine receptor (nAChR) have larger pores and generally discriminate between positive and negative ions but not among ions within these general classes. Hexameric channels like gap junctions allow ions and small solutes to pass [2]. This 'barrel-stave' channel arrangement has been a boon to structure-function studies as the channel symmetry imposes strong constraints on the likely location of amino acids close to the pore. However, Nature does not always follow this plan when constructing ion channels. Voltage-gated chloride channels have two pores that are formed from a dimer of subunits wherein each subunit makes its own ion passageway (Figure 2c) [3].

Studies of full-length ion channels X-ray crystallographic and nuclear magnetic resonance experiments are the most powerful tools for obtaining information about the atomic structure of macromolecules. Unfortunately, it is still extremely difficult to use these methods to study membrane proteins like ion channels. Ion channels have domains that reside in the hydrophobic environment of the cell membrane as well as domains that reside in the aqueous intra- and extracellular spaces. To keep the transmembrane domains soluble

upon removal from the cell membrane, reagents like detergents or lipids must be used in the purification and handling of full-length channels. The search for the precise detergent or lipid that will work for a given channel complicates purification attempts as well as the search for conditions that produce diffraction quality protein crystals, the necessary prerequisite for any X-ray crystallographic study. The large size of most ion channel proteins places them outside what is currently possible with the most sophisticated NMR methods. Furthermore, good overexpression systems for producing eukaryotic membrane proteins in the quantities required for high-resolution studies are not currently available. Solving this technical problem is one of the major necessities for routine high-resolution investigation of membrane protein structure.

Electron microscopy studies have proven particularly useful in obtaining low to medium resolution descriptions of eukaryotic ion channels and the conformational changes that accompany ion channel opening. Studies of ion channels found in high abundance in the electric organs of electric rays and electric eels like the nicotinic acetylcholine receptor (nAChR) and the voltage-gated sodium channel [4,5] reveal the general cyclic symmetric architecture of both of these channels (Figure 3). While difficult, these studies require much less protein than other structural methods and information can be obtained from two-dimensional crystals, tubular membrane crystals, and even single particles.

General pore features revealed by bacterial channels The problems with obtaining material for ion channel structural studies can be overcome by turning to bacterial ion channels. These molecules can be more readily expressed and purified in large quantities than their eukaryotic counterparts. The atomic details of the inner workings of an ion channel were first seen in the X-ray crystallographic structures of the bacterial potassium channel, KcsA (Figure 4a) [6-9]. The KcsA structure revealed many of the general features of ion channel pores that had been anticipated from careful biophysical studies coupled with structural reasoning (Hille chapter). For instance, many channels seem to be

made on a funnel-shaped plan with a large entryway that tapers to a narrow constriction that can serve as a selectivity filter that allow only particular types of ions to pass.

Potassium channels are remarkable for their ability to discriminate between potassium and sodium ions with very high precision preferring potassium by a factor of ~10,000:1 [1]. Both ions are monovalent cations. A sodium ion has a radius of 0.95Å while potassium has a radius of 1.33Å. How does the larger potassium ion pass through the potassium channel selectivity filter while the smaller sodium ion does not? Chemistry. All ions have shells of closely associated water molecules in solution [1]. For an ion to enter the filter it must shed its waters of hydration. The selectivity filter of potassium channels is arranged in a way that displays rings of carbonyl oxygen atoms from the protein backbone at the exact diameter of a potassium ion. Thus, the waters of hydration surrounding a potassium ion are exactly replaced by oxygen atoms from the protein creating a perfect chemical and energetic match as the ion enters the selectivity filter (Figure 4b) [7,9]. Although the smaller sodium ion can pass through the filter, it is much more energetically costly since fewer of its lost water ligands can be replaced by the channel. Other selectivity filters may work in similar ways in which the protein makes intimate contact with the permeant ion.

Pore-helices: electrostatic aids to permeation A second feature that is common in the high-resolution ion channel structures (bacterial potassium and bacterial chloride channels) is the use of the N- or C-terminal ends of short α-helices (known as pore helices) to stabilize the ion as it passes through or near the points of narrow constriction in the channel pore. For example, the pore helices of KcsA have their C-terminal ends aimed at the ion conduction pathway (Figure 5, left). It is thought that the negative end of the helix dipole (the C-terminal end of helices bear a small net negative charge while the N-terminal ends bear a small net positive charge [10]) stabilizes the potassium ion on its journey through the channel [11]. Likewise, the recent structure of a bacterial

chloride channel shows that the N-terminal ends (positive end) of two helices that form the narrowest part of the ion conduction pathway form a binding site for the negatively charged chloride ion (Figure 5, right) [3].

Open channels: The key thing that ion channels do is open and close. Structural studies are beginning to reveal the general rearrangements that occur when channels are prompted to move between closed and open states. Electron microscopy studies of the nAChR show that ligand binding to the extracellular domain causes a twisting of the subunits that is propagated some 60Å away to the narrowest part of the channel pore embedded deep in the membrane. This conformational change widens the narrow constriction or 'gate' that prevents ion flow in the closed state [4] (Figure 6).

Potassium channels also have a portion of the protein that acts as a gate to prevent ion flow in the closed state. X-ray crystallographic comparisons of the homologous pore regions of open [12] and closed [6] bacterial potassium channels suggest that the lower part of the inner helix moves during gating to widen the narrow constriction formed by the bundle crossing of the inner helices [13] (Figure 6). These conformational changes occur below the selectivity filter which remains largely unchanged. This mechanism of opening is likely to be conserved among many diverse types of potassium channels.

Eukaryotic ion channels at high resolution: divide and conquer Bacterial channels have provided insight into the guts of ion channel permeation machineries showing the intimate details of permeation pathways that are likely to be conserved and recapitulated in their larger eukaryotic cousins. In contrast to prokaryotic membrane proteins (which are difficult to obtain in their own right), eukaryotic membrane proteins are presently extremely difficult to obtain in the quantities required for high-resolution study. Eukaryotic channels often contain a host of extramembranous regulatory domains and subunits that are essential for their activity, signal sensing, and gating. These domains have proven a tractable entry point for the study of eukaryotic ion channel structure and function.

A number of groups have successfully 'liberated' extramembranous domains from the membrane spanning part of a variety of ion channels so that they can be expressed, purified, crystallized, and treated like soluble proteins. This divide and conquer approach has proven particularly powerful for illuminating channel gating mechanisms when the high-resolution information about these domains is incorporated into structure-function studies of the intact channel. For example, studies of an assembly domain from eukaryotic voltage-gated potassium channels lead to the discovery of a new role for this domain in channel gating [14,15]. The structure of a soluble homolog of the extracellular domain of the nicotinic acetylcholine receptor found in snail glial cells has provided molecular landmarks for interpreting decades of study by chemical modification, mutagenesis, and electron microscopy of the intact receptor [16]. Similarly, structures of the ligand binding domains of glutamate receptors [17] and calmodulin-activated potassium channels [18] have lead to detailed models of channel gating and ligand recognition. This 'divide and conquer' approach is likely to remain a fruitful endeavor in the near future while better methods for purifying ion channels from native sources and new means for expressing full-length ion channels are developed.

Ion channel accessory subunits: soluble and transmembrane Many eukaryotic ion channels have soluble subunits that associate with and regulate the properties of the channel *in vivo*. For example, some voltage-gated potassium channels associate with soluble β-subunits that affect their ability to rapidly inactivate. Curiously, the structure of the Kvβ subunit reveals a structure that is common to oxidoreductase enzymes [19] complete with a firmly-bound NADP molecule. This structural observation suggests that Kvβ may act as some sort of enzyme that depends on the activity of the channel. However, to date, there are no functional data to support this hypothesis.

Many other channel subunits exist. Some are soluble proteins like the calcium channel β subunit and many are transmembrane proteins that bear intra- and

extramembranous domains that affect the function of the pore forming subunit [1]. Little is known about the structure of any of these molecules.

The future: Ion channels as electrosomes: Beyond the classical pore-forming subunits and auxillary subunits that comprise ion channels, it is becoming ever more clear that in real biological settings, ion channels are part of large protein networks. These networks include cytoskeletal components, signaling proteins like protein kinases and phosphatases, and channel-associated proteins that recruit these signaling molecules to the channel to modify its function. To understand the biological structure of ion channels it will be necessary to move from thinking about channels as proteins that simply form ion conduction pores to thinking about them as electrical signaling centers ('electrosomes'), large, multi-protein macromolecular complexes that not only generate electrical signals or changes in the membrane potential but also generate and respond to other chemical signals within a cell.

Perhaps the best example of a channel as an electrosome is the voltage-gated calcium channel [1]. When these channels open, they provide a means to depolarize the cell by allowing calcium entry. Calcium influx through the channel pore interacts with a channel-resident calcium sensing protein (calmodulin) that accelerates channel inactivation [20] and also causes the activation of signals that lead to alterations in transcription in the cell nucleus [21]. Together, these actions affect both the immediate electrical properties of the neuron as well as its long-term adaptation to activity. Understanding the activity of channels, the systems that regulate their action like G-protein coupled receptors, and the complex interplay between chemical and electrical signaling pathways in cells will be essential for developing an molecular understanding of complex processes like the regulation of heartbeat and the molecular basis of learning and memory.

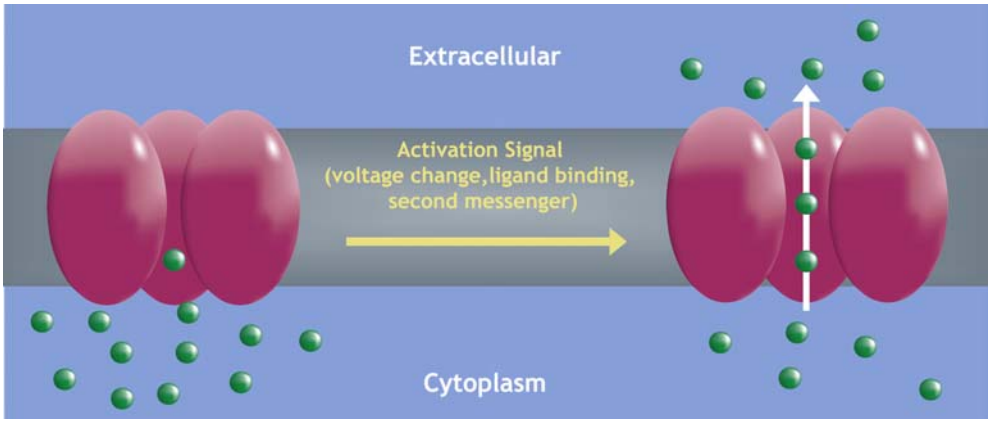

Figure 1 Schematic of ion channel function as viewed from the plane of the membrane. Three subunits of an ion channel are shown in magenta. Ions are shown as green spheres. Upon activation by a stimulus like a transmembrane voltage change or ligand binding the channel undergoes a conformational change that opens a pore, formed by the protein. Ions flow through the open pore in a direction that is determined by the electrochemical gradient.

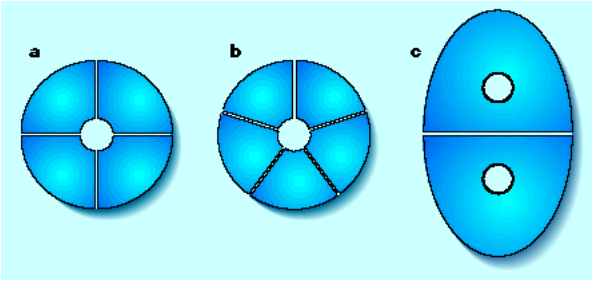

Figure 2 General architecture of ion channels. 'a' and 'b' show the 'Barrel-stave' plans of the voltage-gated cation channel family and the nicotinic acetylcholine receptor family. In each of these the channel subunits are arranged around the pore through which the ions flow. 'c' shows the general architecture of voltage-gated chloride channels. These channels are dimers in which each subunit makes its own pore. (Figure adapted from [22])

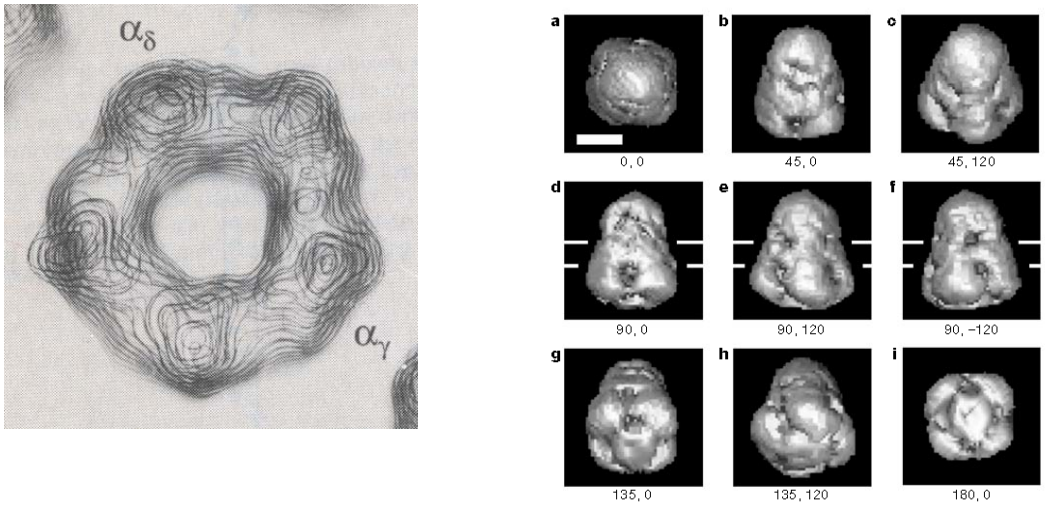

Figure 3 Electron microscopy reveals the general features of the nictotinic acetylcholine receptor seen from the extracellular space at 9 Å resolution, left, and the voltage-gated sodium channel at 25Å, right. The panels for the sodium channel show successive rotations of a surface representation of the channel and start from the extracellular side (0,0) through the intracellular side (180,0). The pairs of numbers indicate the degrees of rotation around the x and y axes. Figure adapted from [4] and [5].

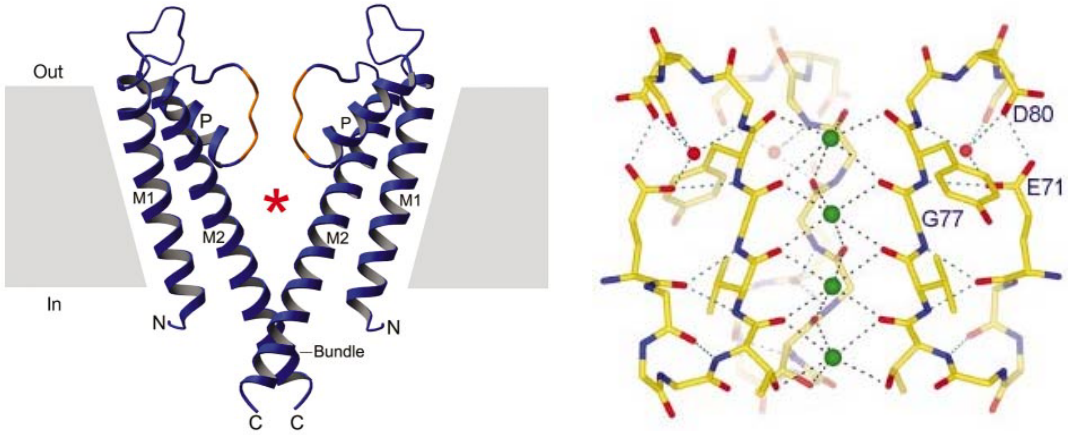

Figure 4 Structural elements of a potassium channel pore. Left, Two subunits from the KcsA potassium channel are shown. The transmembrane segments are labeled M1 and M2. M2 subunits cross at the region marked ‘bundle’ and restrict access to the channel pore. The pore helix is indicated by ‘P’ and the selectivity filter is shown in yellow. The red star marks the inner cavity of the channel. Right, Close-up view of the intimate contacts between the KcsA selectivity filter oxygens (red) and potassium ions (green spheres). Figure adapted from [23] and [9].

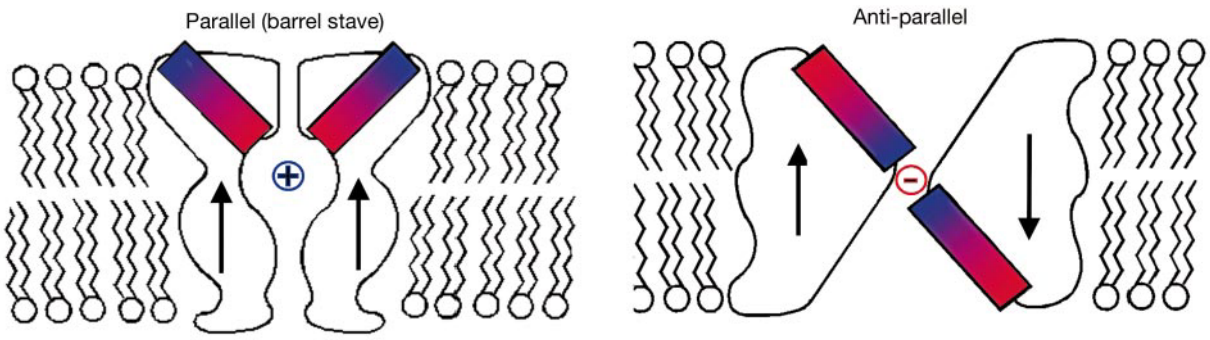

Figure 5 Examples of how pore helices stabilize permeant ions for cation channels, left and anion channels, right. The negative ends of the helices are shown in red and the positive ends in blue. Figure adapted from [3].

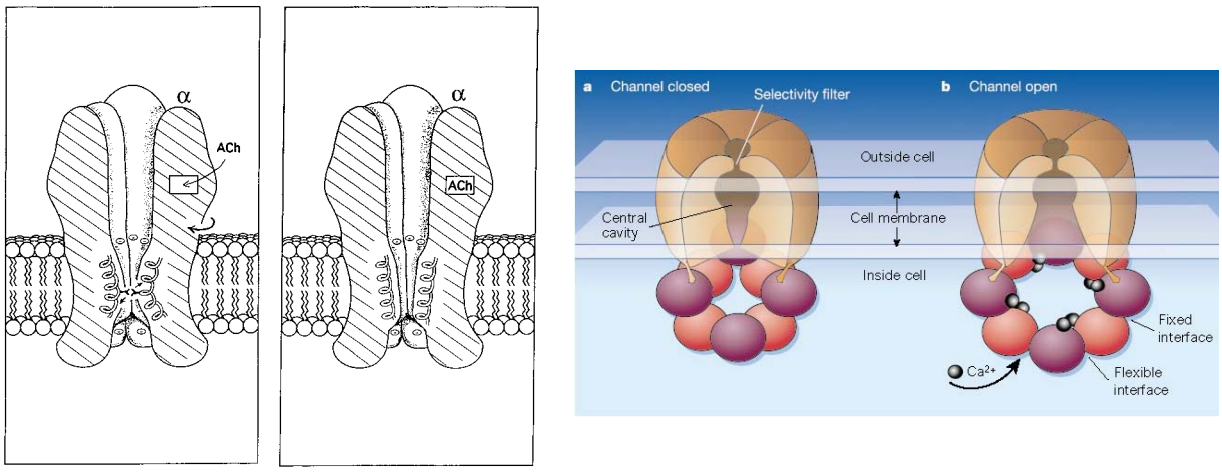

Figure 6 Opening mechanisms of ion channels. Left, simplified diagram of the opening mechanism of the nAChR. Acetylcholine (Ach) binds to the extracellular domain of the receptor initiates a rotation that causes the closest approach of the ring of pore-lining helices to widen creating a pathway for the ions. Right, Schematic model for the opening of a bacterial calcium-gated potassium channel. Calcium binding to the cytoplasmic domains causes a conformational change that is propagated to the inner pore-lining helices. This opens the pathway for the ions to enter the channel. Figure adapted from [24] and [25].

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