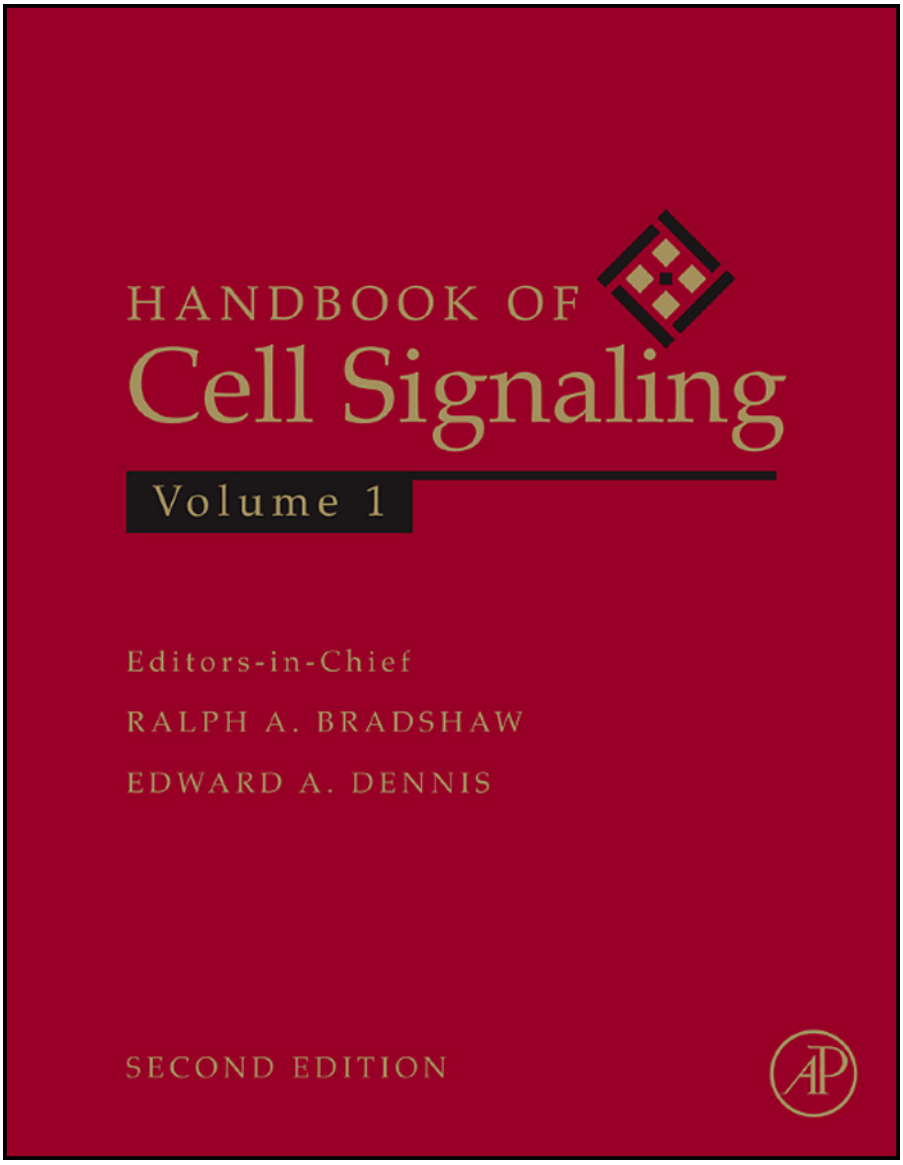





From Daniel L. Minor, An Overview of Ion Channel Structure. In: Ralph A. Bradshaw and Edward A. Dennis, editors, Handbook of Cell Signaling 2nd edition. Oxford: Academic Press, 2009, pp. 201-207.
ISBN: 978-0-12-374145-5

Chapter 30

# An Overview of Ion Channel Structure

**Daniel L. Minor, Jr**

*Cardiovascular Research Institute, Departments of Biochemistry & Biophysics and Cellular & Molecular Pharmacology, California Institute for Quantitative Biosciences, University of California, San Francisco*

## INTRODUCTION

The physical and mental processes you are using to pick up this book, feel its heft or scroll through an electronic copy, read the text, and understand the ideas presented within, rely on electrical signals in your muscles, eyes, and brain. The molecular bases of these bioelectric signals are ion channel proteins, transmembrane proteins that form rapidly activating and inactivating pores that permit ions to flow down their electrochemical gradients and across cell membranes.

Ion channel proteins make macromolecular pores in cell membranes that allow ions to move in response to a range of signals mediated by chemical and protein ligands, membrane potential changes, temperature, and mechanical force [1]. These changes in ion distribution can cause changes in the membrane potential and, in the case of calcium ions [2], directly activate a variety of intracellular signaling cascades.

The signals generated by ion channels are among the fastest found in biological systems, and occur on the timescale of tens of microseconds to hundreds of milliseconds. The flux through a channel pore can be as high as $10^{8–9}$ ions per second [1]. Because of their central role in nervous system function, channels have been the subjects of intensive biophysical studies for more than 50 years. The past few decades have witnessed the transformation of ion channels from a biophysical idea, into molecules identified by molecular biology and gene cloning approaches, to, finally, three-dimensional structures. The very nature of channels, membrane proteins that have segments within the lipid bilayer as well as large domains on the extracellular and cytoplasmic sides, has been the largest impediment to understanding their architectures at high resolution. Transmembrane proteins remain among the most difficult classes of proteins to obtain in the large quantities (milligram) and high levels of purity that are required for structural studies. The last edition of this chapter was written at the dawn of ion channel structural biology, just a few years after the first high-resolution structures of ion channels, ion channel regulatory domains, and ion channel associated proteins were being uncovered. Since then, our understanding of ion channel structure has progressed rapidly. We now have high-resolution frameworks for the pore domains, regulatory domains, and, in a few cases, complete channels, for examples of many of the well-studied ion channel types. A recent survey of channel structures can be found in Minor, 2007 [3]. Because of the many advances, it is impossible to cover the breadth of this swiftly growing field in a brief chapter. Thus, the focus here will be to present an overview of some key concepts, highlights of important advances, and an accounting of a few of the many outstanding questions.

Ion channels do two basic things. They open and close to regulate the passage of specific ions across the cell membrane (see Chapter 32), and they sense and respond to signals that shift the equilibrium between closed and open states (see Chapter 19; also Chapters 31, 33, and 34). Their main task is to provide passageway for ions, which are charged, to traverse the formidable hydrophobic barrier presented by the hydrophobic core of the cell membrane. There is a wide variety of ion channel types that respond to a range of signals and that have varied types of ion selectivity. In the human genome, ion channel pore-forming subunit genes are abundant. For example, genes for the pore forming subunits of members of the voltage-gated ion channel family number around 150 [4], placing this class just behind G-protein-coupled receptors, and kinases in terms of the most abundant human genes. Occurrences of genes for other ion channel types (ligand-gated ion channels, ionotropic glutamate receptors, and chloride channels) each number in the 10–30 range. Thus, it is clear that a significant number of gene products in our genome are dedicated to generating electrical signals. As many ion channels are composed

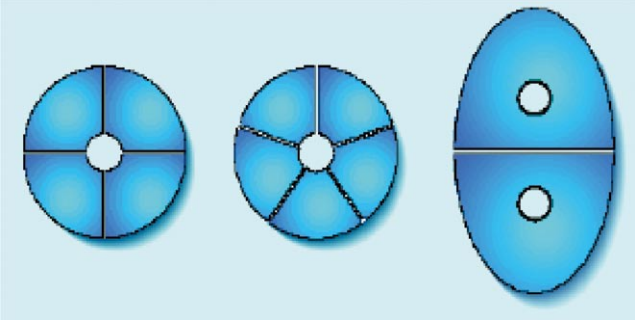
**Handbook of Cell Signaling, Three-Volume Set 2 ed.**

of multiple pore-forming subunits, and heteromeric channels often have different functional properties than do homomeric ones, the functional diversity available from this genetic complement is even greater than one might imagine from simply counting up the ion channel genes.

The general principle of ion channel architecture is that nearly all are multimeric assemblies of three to six pore-forming subunits that possess cyclic symmetry (Figure 30.1a, b). In this barrel-stave architecture, a fixed number of subunits are arranged around the central axis that forms the pathway for ion conduction. The number of subunits is roughly related to the size and selectivity properties of the pore. For example, potassium channels, which have an exquisite selectivity for potassium over sodium, are tetramers in which four identical or very homologous subunits are arranged around the pore [5]. Pentameric channels, typified by the nicotinic acetylcholine receptor (nAChR) family (see Chapter 33), have larger pores and generally are only able to discriminate between positive and negative ions, but not among the ions in these general classes. Hexameric channels, such as gap junctions, allow ions and even small solutes to pass between cells [6]. Despite the prevalence of the barrel-stave architecture among diverse types of ion channels that have different numbers of subunits and different numbers of transmembrane domains, this plan is not the only one used by nature. Voltage-gated chloride channels have two pores that are formed from a dimer of subunits in which each subunit makes its own ion conduction pathway [7] (Figure 30.1c).

## OBTAINING THREE-DIMENSIONAL STRUCTURES OF CHANNELS: METHODS AND CHALLENGES

The main goals for understanding ion channel architecture can be simply stated. One would like to see the structures of the same channel in each of its functional states (ex. closed, opened, and inactivated) to understand the molecular rearrangements that underlie function. Nevertheless, these goals remain unattained for any ion channel. Although the full battery of structural approaches, including electron microscopy, nuclear magnetic resonance spectroscopy, and X-ray crystallography has been employed to reveal features of ion channel structure. X-ray crystallography remains the most powerful tool in structural biology studies, as it can yield atomic-level resolution of macromolecules of any size [3]. The challenge for all of these structural methods with respect to ion channels is that the objects of study are membrane proteins that have transmembrane portions that reside in a very hydrophobic environment as well as domains that reside in the aqueous intra- and extracellular spaces. To keep channels soluble upon extraction from the bilayer, a necessary step for purification, reagents such as detergents, lipids, or both, must be used in sample purification and preparation. There is a multitude of detergents and lipids, and it is impossible to predict which combination will work for any particular membrane protein. This complicates purification attempts and the search for conditions that yield diffraction-quality crystals. Further, trapping the channels in some state, be it open or closed, can prove difficult, as pharmacological reagents that are capable of binding a single state are not available for every channel type.



(a) (b) (c)

**FIGURE 30.1 Cartoons showing the general architectural plans of ion channels.**

(a, b) The "barrel-stave" plans of the voltage-gated cation channel family and nicotinic acetylcholine receptor family, respectively. In each, the channel subunits are arranged around a central pore through which the ions pass. (c) The general achitecture of voltage-gated chloride channels. These channels are dimers in which each subunit contains a pore. Figure adapted from [31] (Jentsch, 2002).

The size of most ion channels has kept them outside the realm of what can be studied by NMR. There have been a number of notable advances in both solution and solid state NMR methods that are beginning to allow study of some ion channels [3], but such efforts remain at the edge of the possible for the moment. Electron microscopy has proven very useful for studying ion channels that can be isolated from high-abundance natural sources. The greatest insights have come from landmark studies of the nAChR from the Torpedo electric organ [8, 9]. While difficult, electron microscopy studies require much less protein than other structural methods, and information can be obtained from two-dimensional crystals, tubular membrane crystals, and single particle analysis.

All of the structural methods require highly purified samples. The principal challenge to all ion channel structural studies is the difficulty in obtaining milligram quantities of pure material. There are two sources of such material; specific organs in which a particular channel is highly expressed (such as the nAChR in the Torpedo electric organ), and heterologously expressed sources. Very few ion channels are found in natively enriched sources. The main advances over the past five years have come from the identification and development of expression systems for archaeal and bacterial ion channels, and from the identification of expression systems for eukaryotic ion channel domains and a few full-length channels. Routine overexpression of prokaryotic or eukaryotic membrane proteins remains a major challenge, and the focus of much current research [3].

## PROKARYOTIC ION CHANNELS: GATEWAYS TO FULL LENGTH CHANNEL STRUCTURE

One of the most remarkable discoveries of the past 10 years has been the abundance of ion channels in single-celled organisms [10, 11]. Once thought to be only in the purview of higher organisms, ion channels have been found throughout the tree of life. Although the functions of channels in microorganisms remain poorly understood, the existence of such molecules has been a boon to structural biologists. Prokaryotic channels can often be expressed more readily than their eukaryotic counterparts, and have been the first ion channels to be crystallized and have their structures determined by high-resolution X-ray crystallography. Even though many of the bacterial channels are less complex than in their eukaryotic cousins, the ones that have been characterized structurally contain the core elements that are required for function, and have proven exceptionally instructive for understanding fundamental mechanisms of selectivity and gating. Potassium channels [11], cyclic nucleotide-gated channels [12], voltage-gated chloride channels [7], and ligand-gated ion channels from the nAChR family [13] all have counterparts from the microbial world that have been successfully used for crystallographic structure determination.

One of the most impressive properties of potassium channels is their ability to discriminate potassium ions from sodium ions. Ion channels, including those selective for potassium, pass $\sim 10^{8-9}$ ions per second, leaving only tens of nanoseconds for the channel to interact with each ion. Despite the brief interaction, potassium channels allow potassium ions to pass with a fidelity of ~10,000:1 versus sodium (see Chapter 32) [1]. The reason this level of discrimination is so impressive is that sodium is the smaller ion and thus, based on size considerations alone, any pore through which potassium fits should also pass sodium. The way the potassium channels accomplish this amazing feat is through 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 organized in such a way as to display rings of carbonyl oxygen atoms from the protein backbone that are arranged to coordinate potassium ions. Thus, the waters of hydration surrounding a potassium ion are replaced by oxygen atoms from the protein, creating a perfect chemical and energetic match as the ion enters the selectivity filter (Figure 30.2) [14, 15]. 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, where the protein makes intimate contact with the permeant ion [16].

## 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 60 Å 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 [9] (Figure 30.3).

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 [17] and closed [18] 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 [19]

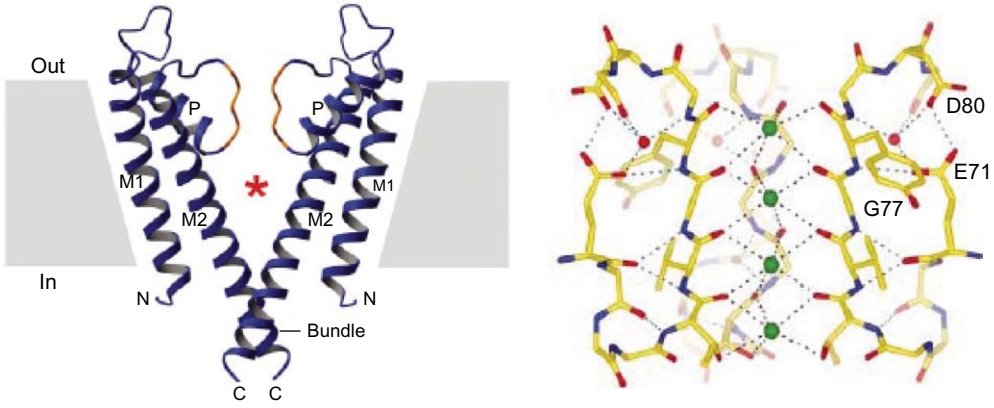



**FIGURE 30.2 Structural elements of a potassium channel pore.**
Left: two subunits from the bacterial KcsA potassium channel are shown. The transmembrane segments are labeled M1 and M2, and the pore helix is labeled P. M2 subunits cross at the region marked "bundle" and are thought to restrict access to the channel pore. The red star (color shown in online version) marks the inner cavity. Right: close-up view of the network of contacts made between the KcsA selectivity filter oxygens (red) and potassium ions (green spheres). Figure adapted from (19) and (15).

(Figure 30.3). These conformational changes occur below the selectivity filter, which remains largely unchanged. A similar idea has been proposed for how voltage-gated channels work. Instead of movements of the extramembranous domains driving pore rearrangements, the linker segment that connects the transmembane voltage-sensing domain to the pore domain is thought to play a key role [20] (see Chapter 111). The details of voltage-sensing [21] as well as ion channel gating mechanisms are far from resolved. The field has yet to see high-resolution structures of the same channel trapped in different states. Such information will be essential for understanding exactly how accurate the current inferences made about gating are, and for elucidating how the movement of domains that sense the signals to which the channel responds (ligand binding domains or voltage sensors) couple to the pore domain.

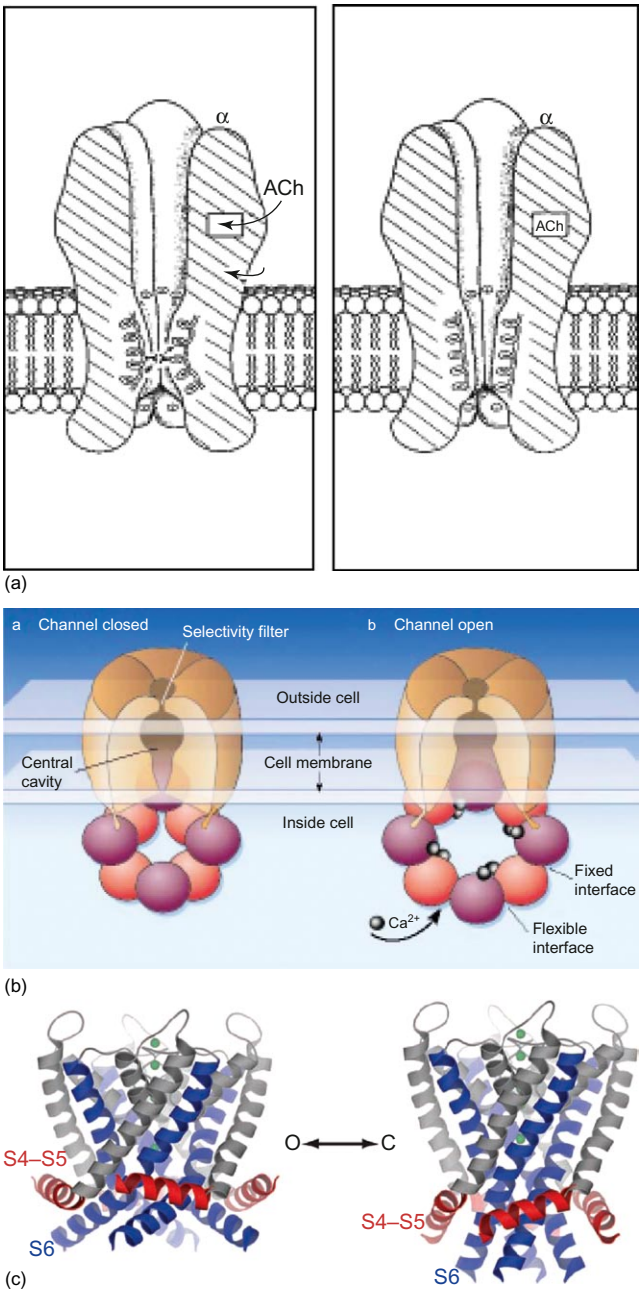



**FIGURE 30.3 Opening mechanisms of ion channels.**
(a) Simplified opening mechanism of the nAChR. Acetylcholine (ACh) binds to the extracellular domain of the receptor and initiates a conformation change in the membrane domain that opens the pathway for the permeant ions. (b) Schematic model for the opening of a bacterial calcium-gated potassium channel. Calcium binding to the cytoplasmic domains causes a conformation change that is propagated to the pore-lining helices. (c) Electromechanical model for how movements of voltage-sensing domains drive conformational change in the pore region of voltage-gated ion channels through the movement of the S4–S5 segment. Figure adapted from [32] (Unwin, 1998), [33] (Schuhmacher and Adelman, 2002), and [20] (Long *et al*., 2005).

## EUKARYOTIC ION CHANNELS AT HIGH RESOLUTION: WHOLE CHANNELS AND EXPLOITATION OF MODULAR STRUCTURE TO DIVIDE AND CONQUER

While working with bacterial and archaeal membrane proteins has been difficult, the challenges are even greater for eukaryotic membrane proteins. One of the major challenges is the development of robust methods for the overexpression and isolation of eukaryotic membrane proteins [3]. There are only three eukaryotic ion channels that have had their architectures resolved at high resolution (Figures 30.4, 30.5). The first is the Torpedo nicotinic acetylcholine receptor [8]. Unlike in most ion channels, the Torpedo nAChR can be isolated in large quantities from a highly enriched native source. Two channels have been crystallized and had their structures solved from recombinant material; the Kv1.2/Kvβ complex [22, 23] and the Acid Sensing Ion Channel, ASCI-1 [24]. In all cases, the structures show a modular architecture in which defined extramembranous domains are linked to the transmembrane domains that constitute the transmembrane pore.

It is not surprising that channels are built from specific modules such as a transmembrane pore and an extracellular ligand binding domain. What is surprising, though, is the degree to which such modularity appears to be present in the membrane. Structures of the mammalian Kv1.2 channel have revealed two striking features. The first is that the pore domain, formed from the last two transmembrane segments (S5 and S6) of each subunit, and the voltage sensing domain, formed from the first four transmembrane segments (S1–S4), are really two separate domains (Figure 30.5b, c). Further, the Kv1.2 structure revealed an unexpected interlocking of subunits, which may contribute to the highly cooperative nature of channel opening. A similar arrangement has been seen in the structure of a bacterial cyclic nucleotide-gated channel [12], suggesting that the interlocked arrangement is a general feature of the voltage-gated channel family. The consequences for such an architecture arrangement in terms of function and intersubunit cooperativity remain to be explored. Seen schematically, members of the voltage-gated

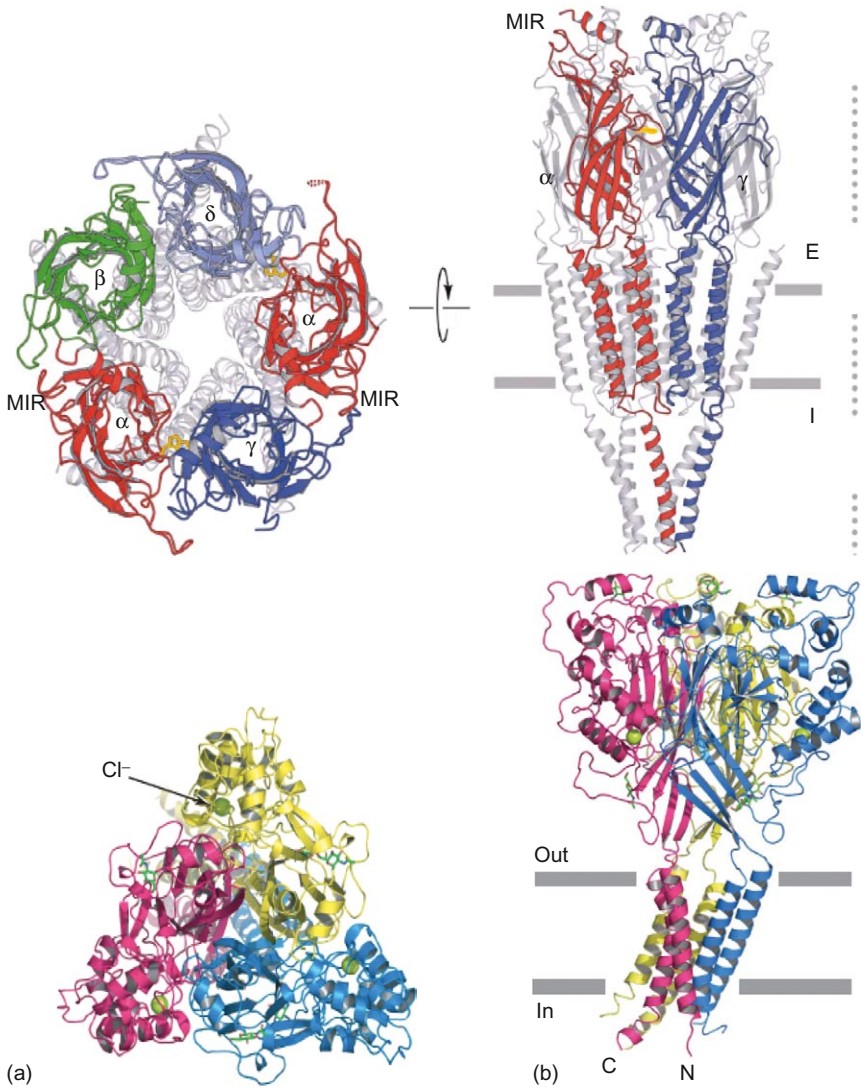



**FIGURE 30.4 Structures of nAChR (top) and ASIC (bottom) channels. In each panel, the extracellular view is shown on the left.** Figures are from [34] (Unwin, 2005) and [24] (Jasti *et al*., 2007).

ion channel superfamily are put together from three domains that each have specific roles (Figure 30.5c): a pore-forming domain; a transmembrane module that can be used for voltage sensing; and intracellular domains that may be used to direct assembly, bind modulatory subunits, and serve as a sensor for detecting intracellular signals that modify function such as changes in calcium concentration or phorphorylation. Understanding how channels integrate into cellular signaling networks remains an important future goal, with implications for both basic science and the understanding of disease.

## DIVIDE AND CONQUER: EXPLOITATION OF THE MODULAR NATURE OF ION CHANNEL STRUCTURE

In the absence of suitable full-length material for structural work, a second very fruitful approach has been to exploit the modular nature of many ion channels. Thus, one can focus on obtaining structures of isolated extramembranous regions that are known to have important roles in assembly or modulation, and use this information to inform functional studies.

The list of channels that have been successfully dissected is now very long [3]. The most notable success is for the ionotropic glutamate receptors, in which there are now more than 60 structures of the agonist binding domains from various isoforms alone and in complexes with a range of pharmacological agents [25]. The combination of these structures with biochemical and electrophysiological studies has given a deep insight into the likely mechanism of glutamate receptor action, even though high-resolution structure of the entire channel has not been attained. Voltage-gated calcium channels are also yielding structural information as a result of this dissection approach [26].

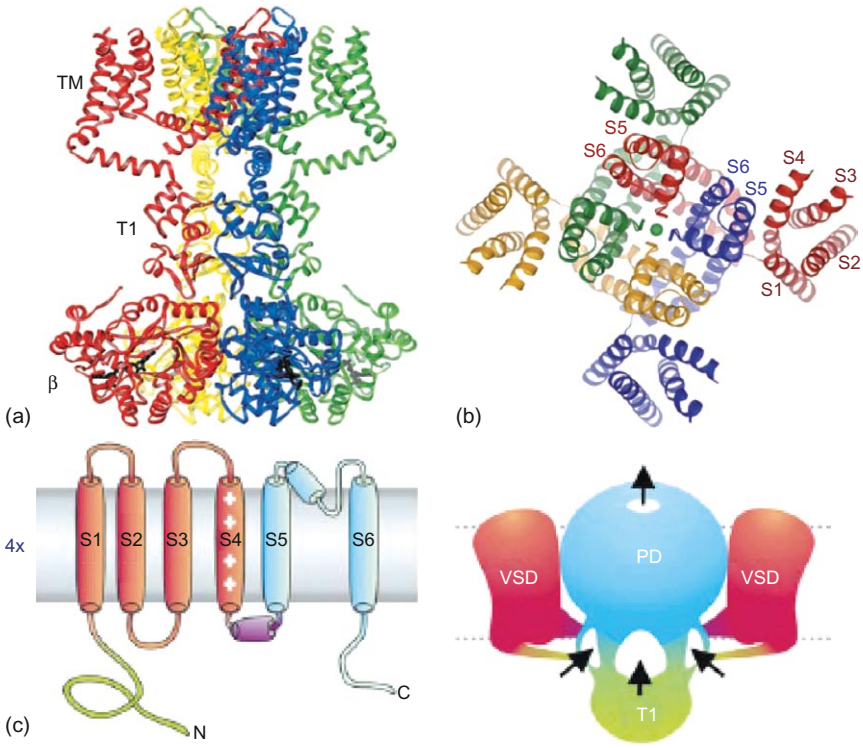



**FIGURE 30.5 Voltage-gated potassium channel structure.**
(a) Side view of the Kv1.2/Kvβ complex. The transmembrane domains, T1 assembly domain, and Kvβ are indicated by TM, T1, and β, respectively. (b) Extracellular view of Kv1.2 Note that the voltage sensor domain (labeled S1–S4) is adjacent to the pore forming domain (S5–S6) from a different subunit. (c) Cartoon diagram of a voltage-gated channel subunit (left) and the modular architecture of voltage-gated potassium channels (right). VSD, PD, and T1 correspond to the voltage-sensing domain, pore domain, and T1 assembly domain. Figures adapted from [22] (Long *et al*., 2005) and [35] (Tombola *et al*., 2005).

One class of eukaryotic channels that has engendered much excitement because of their prominent roles in sensory processes and pain consists of the TRP channels [27]. These channels are gated by a diverse range of stimuli that include pungent compounds such as capsaicin and menthol, and temperature. As close relatives of the voltage-gated ion channels, TRP channels share the six-transmembrane, four-subunit property [28, 29]. Thus far, structural understanding of TRP channels has largely come from the "divide and conquer" approach [30]. In terms of topology, TRP channels look very much like voltage-gated ion channels. Just how far the similarly goes on the structural and mechanistic level is unknown, and provides a fertile area of current research and speculation.

## ION CHANNEL COMPLEXES

It is clear that efforts to understand ion channel function at high resolution now have a great deal of momentum. We can anticipate that the coming years will see the determination of more high-resolution structures of a variety of ion channels and ion channel regulatory proteins. As the field matures, it will be ever more important to place the structural efforts in the context of the protein complexes that form around ion channels in cells that allow them to interface with cellular signaling pathways and to understand, from a structural perspective, how the dysregulation of such interactions leads to human diseases.

## REFERENCES

1. Hille B. *Ion channels of excitable membranes*. Sunderland, MA: Sinauer Associates, Inc; 2001.
2. Clapham DE. Calcium signaling. *Cell* 2007;**131**:1047–58.
3. Minor Jr DL. The neurobiologist's guide to structural biology: a primer on why macromolecular structure matters and how to evaluate structural data. *Neuron* 2007;**54**:511–33.
4. Yu FH, Catterall WA. The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci STKE* 2004;**2004**:re15.
5. Gouaux E, Mackinnon R. Principles of selective ion transport in channels and pumps. *Science* 2005;**310**:1461–5.
6. Kovacs JA, Baker KA, Altenberg GA, Abagyan R, Yeager M. Molecular modeling and mutagenesis of gap junction channels. *Prog Biophys Mol Biol* 2007;**94**:15–28.
7. Dutzler R. A structural perspective on ClC channel and transporter function. *FEBS Letts* 2007;**581**:2839–44.
8. Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* 2003;**423**:949–55.

9. Unwin N. Acetylcholine receptor channel imaged in the open state. *Nature* 1995;**373**:37–43.
10. Kung C, Blount P. Channels in microbes: so many holes to fill. *Mol Microbiol* 2004;**53**:373–80.
11. Kuo MM, Haynes WJ, Loukin SH, Kung C, Saimi Y. Prokaryotic K(+) channels: from crystal structures to diversity. *FEMS Microbiol Rev* 2005;**29**:961–85.
12. Clayton GM, Altieri S, Heginbotham L, Unger VM, Morais-Cabral JH. Structure of the transmembrane regions of a bacterial cyclic nucleotide-regulated channel. *Proc Natl Acad Sci USA* 2008;**105**:1511–15.
13. Hilf RJ, Dutzler R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* 2008;**452**:375–9.
14. Morais-Cabral JH, Zhou Y, MacKinnon R. Energetic optimization of ion conduction rate by the $K^+$ selectivity filter. *Nature* 2001;**414**:37–42.
15. Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R. Chemistry of ion coordination and hydration revealed by a $K^+$ channel-Fab complex at 2.0-Å resolution. *Nature* 2001;**414**:43–8.
16. Shi N, Ye S, Alam A, Chen L, Jiang Y. Atomic structure of a $Na^+$- and $K^+$-conducting channel. *Nature* 2006;**440**:570–4.
17. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* 2002;**417**:515–22.
18. Doyle DA, Morais Cabral J, Pfuetzner RA, et al. The structure of the potassium channel: molecular basis of $K^+$ conduction and selectivity. *Science* 1998;**280**:69–77.
19. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. *Nature* 2002;**417**:523–6.
20. Long SB, Campbell EB, Mackinnon R. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. *Science* 2005;**309**:903–8.
21. Tombola F, Pathak MM, Isacoff EY. How does voltage open an ion channel? *Annu Rev Cell Dev Biol* 2006;**22**:23–52.
22. Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family $K^+$ channel. *Science* 2005;**309**:897–903.
23. Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent $K^+$ channel in a lipid membrane–like environment. *Nature* 2007;**450**:376–82.
24. Jasti J, Furukawa H, Gonzales EB, Gouaux E. Structure of acid-sensing ion channel 1 at 1.9-Å resolution and low pH. *Nature* 2007;**449**:316–23.
25. Mayer ML. Glutamate receptor ion channels. *Curr Opin Neurobiol* 2005;**15**:282–8.
26. Van Petegem F, Minor DL. The structural biology of voltage-gated calcium channel function and regulation. *Biochem Soc Trans* 2006;**34**:887–93.
27. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* 2007;**76**:387–417.
28. Moiseenkova-Bell VY, Stanciu LA, Serysheva, II, Tobe BJ, Wensel TG. Structure of TRPV1 channel revealed by electron cryomicroscopy. *Proc Natl Acad Sci USA* 2008;**105**:7451–5.
29. Tsuruda PR, Julius D, Minor Jr DL. Coiled coils direct assembly of a cold-activated TRP channel. *Neuron* 2006;**51**:201–12.
30. Gaudet R. TRP channels entering the structural era. *J Physiol* 2008;**586**:3565–75.
31. Jentsch TJ. Chloride channels are different. *Nature* 2002;**415**:276–7.
32. Unwin N. The nicotinic acetylcholine receptor of the Torpedo electric ray. *J Struct Biol* 1998;**121**:181–90.
33. Schumacher M, Adelman JP. Ion channels: an open and shut case. *Nature* 2002;**417**:501–2.
34. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J Mol Biol* 2005;**346**:967–89.
35. Tombola F, Pathak MM, Isacoff EY. How far will you go to sense voltage? *Neuron* 2005;**48**:719–25.