Chapter 14

3D Structures and Molecular Evolution of Ion Channels

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73665

Abstract

Ion channels mediate selective passive transport of ions across biomembranes. They participate in diverse physiological processes and belong to distinct protein families. Understanding specific roles of different channels in physiology, pathology, and pharmacology requires knowledge of their origin and evolution. Traditional approaches include experimental physiological studies and analysis of sequences and genomes. In the last two decades, availability of 3D structures of many ion channel proteins revolutionized ion channel studies, including their evolution. In this chapter, we consider examples of how 3D structures provided clues for understanding evolutionary aspects of multi-domain organization, domain folding, and roles of highly conserved and variable residues. Such achievements are important for addressing practical problems including drug design, channelopathies, and acquired resistance to insecticides.

Keywords: evolution physiology, ion channels, sequence alignment, folding, domains

1. Introduction

The human genome encodes hundreds of proteins that form ion channels. These proteins create transmembrane pores through which inorganic ions, mainly Na^+, K^+, Ca^{2+} or Cl^-, permeate according to their electrochemical gradient. Selectivity of the channels for particular ions, mechanisms of their activation and kinetic characteristics are greatly variable. This variability underlines involvement of ion channels in very diverse fundamental physiological processes such as generation of the membrane potential, regulation of cell electrical excitability, propagation of the action potential along neurons and muscle cells, synaptic release of neurotransmitters, generation of postsynaptic response, and calcium signaling. Ion channels are

indispensable in multicellular organisms. They are also found in various bacteria and even some viruses [1]. Ion channels are targets for numerous endogenous ligands including hormones and neurotransmitters, as well as for multiple exogenous toxins and medically important and illicit drugs. Due to these roles, ion channels are key objects in pharmacology and toxicology.

This functional importance of ion channels motivates their intensive studies in academia and industry. A large body of experimental data is accumulated on selectivity and permeability characteristics of different channels, physiological mechanisms of their activation by different factors, including membrane voltage, various endogenous and exogenous ligands, metal ions, temperature, pH, and membrane tension. Selective sensitivity of ion channels to various pharmacological agents allowed to reveal their presence in various organs and tissues, cellular localization, and specific roles in physiological and pathological processes. The next big step was understanding molecular characteristics of ion channels due to methodological advancements in molecular genetics and molecular biology. Currently we know sequences of many channels, their subunit composition, stoichiometry, and transmembrane topology. These studies have demonstrated that, unlike many other classes of proteins, which are involved in fundamental physiological functions (e.g. G protein coupled receptors), ion channels do not have a common ancestor. Surprisingly, there is no correlation between functional properties and molecular organization of ion channels. For example, proton-activated channels belong to families of trimetric ASIC channels and tetrameric TRPV channels. Chloride selectivity is observed in voltage-gated ClC channels, which are homodimers with a gated pore in each subunit [2], and in pentameric glycine-gated and GABA-gated channels. On the other hand, close relatives of the latter channels, nAChR receptors, are selective for cations. Besides pentameric nAChRs, cationic selectivity is a fundamental property of tetrameric glutamate-gated channels, trimetric ATP-gated P2X channels, ASIC channels, and certain mechanosensitive channels. Both tetrameric cation-selective channels and pentameric Cys-loop channels are gated by glutamate, the major neurotransmitter in the central nervous system. The potassium selectivity is observed in five clades of potassium channels superfamily, which includes voltage-gated channels, calcium-activated channels, Kir channels, mechanosensitive two-pore (K2P) channels, and CNG/HCN channels [3]. Thus, among ion channels we can see examples of divergent evolution and examples of evolutionary homology and functional analogy. Therefore, understanding of the evolutionary history is crucially important in studies of ion channels. For example, the presence of proton-activated currents in different types of cells does not necessarily mean that these currents are mediated by the same or even evolutionary related channels. And *vice versa*, expression in a certain cell type of ion channels with close sequences does not necessarily mean that these channels have the same or even similar functional properties.

Until the pioneering publication of the first X-ray structure of a prokaryotic potassium channel, KcsA [4], 3D structures of ion channels were unknown and only indirect evidences about some features of their spatial structures were available. The lack of experimental high-resolution 3D structures greatly hindered research in the field of ion channels, including analysis of their origin and evolution. The reason for rather late appearance of 3D structures of ion channels is problems of crystallization of proteins that have both membrane-spanning and water-exposed parts. For example, MacKinnon and colleagues used bioinformatics tools to find extremely

stable potassium channel in thermophilic bacteria and removed flexible cytoplasmic segments [4]. For this and subsequent seminal crystallographic studies of ion channels Roderick MacKinnon was awarded the 2003 Nobel Prize in chemistry.

Since then, many 3D structures of ion channels have been published. Most of the structures were obtained by the X-ray crystallography, but recently the amazing progress in the Cryo-EM methodology has provided high-resolution structures of different channels including open and closed states of the TRPV1 channel [5] and glycine receptor [6]. A complex of heteromeric eukaryotic calcium channel with ancillary subunits is now available [7]. The ongoing revolution in structural studies of ion channels advances research in different fields, in particular, molecular evolution of ion channels. Below we describe several representative examples.

2. Multi-domain organization of ion channels

The basic ion-conducting function of ion channels dictates that they have a transmembrane pore-forming domain. This domain is usually assembled from different subunits, which surround the central ion-conducting pore. In most of the channels the pore is lined by alpha-helical segments, but some channels have the beta-barrel structures. 3D structures show different organization of the pore-forming domains (**Figure 1**). The big variations in the number of transmembrane subunits, transmembrane topology and other structural peculiarities indicate that the domains have different evolutionary origins. In other words, various pore domains are examples of analogies rather than homologies. This conclusion is obvious in view of high-resolution structures, but before such structures become available, discrimination between analogies and homologies was by far not a trivial task.

An instructive example is the discovery of evolutionary origin of ionotropic receptors of glutamate (for review see [8]), which is the most widespread excitatory neurotransmitter in the central nervous system of vertebrates. The physiological characteristics of the glutamate receptors are similar to those of nicotinic acetylcholine receptors. Both receptor classes are ligand-gated channels, which are permeable to potassium, sodium, and, to some extent, calcium. Both receptor classes are involved in the fast synaptic transmission. In vertebrates glutamate is responsible mainly for excitation in the CNS and acetylcholine mainly provides excitation in skeletal muscles. In contrast, insects have cholinergic transmission in ganglia and glutamatergic neuromuscular transmission. These indirect evidences suggested a common origin for the superfamily of ligand-gated ion channels, including acetylcholine receptors and glutamate receptors. Comparison of the amino acid sequences of ionotropic acetylcholine and glutamate receptors reinforced this view. Indeed, in both types of receptors the neurotransmitter molecules interact with the N-terminal parts of the proteins, which are located extracellularly. Analysis of the hydrophobicity profiles revealed four putative transmembrane segments in both channel proteins. Furthermore, according to mutational data the ion selectivity and interactions with channel blockers in both channel types are controlled by residues belonging to the second potentially transmembrane segment. Thus, the idea of evolutionary homology between ionotropic receptors of glutamate and acetylcholine was supported by solid evidences [8].

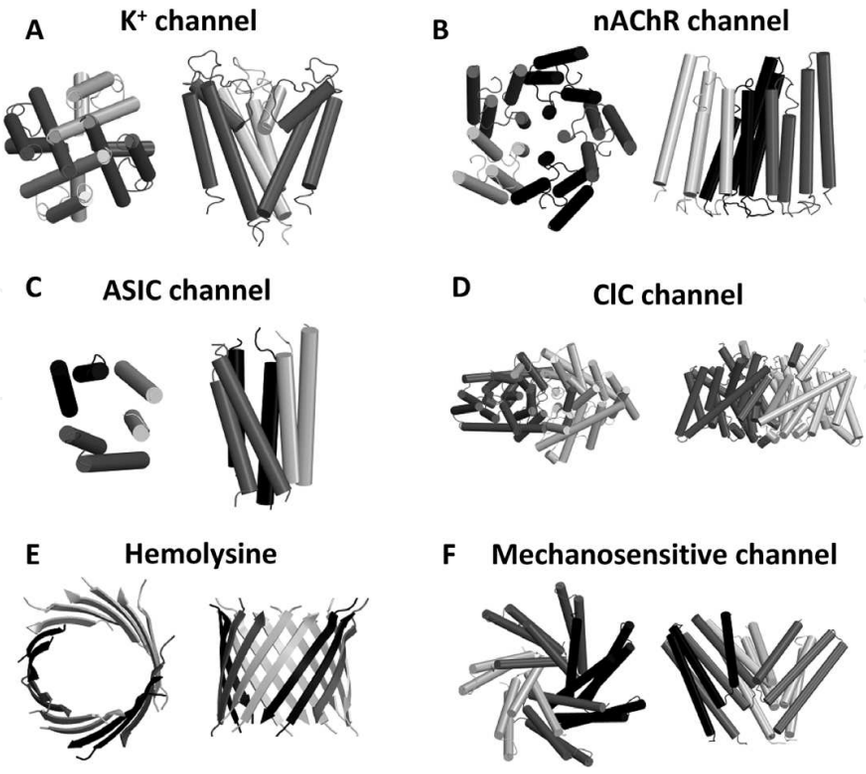

Figure 1. Diversity of pore-forming domains in ion channels. Extracellular (left) and side (right) views. (A) A potassium channel representing the family of tetrameric P-loop channels. (B) $GABA_A$ receptor channel representing pentameric channels. (C) Trimeric acid-sensing channel. (D) Dimeric chloride channel. (E) Beta-barrel structure of the hemolysin channel-forming toxin. (F) A mechanosensitive channel.

However, this idea turned out to be wrong. Increasing data on location of individual amino acid residues provided evidence that the M2 segment of the glutamate receptor does not span the entire membrane, but forms a membrane-reentrant loop both ends of which are exposed to the cytoplasm [9]. Since the transmembrane topology is one of the most conserved characteristics of membrane proteins, the hypothesis on homology between ionotropic receptors of acetylcholine and glutamate was rejected [10]. On the other hand, it is well known that the voltage-gated potassium, sodium, and potassium channels have extracellular membrane-diving loops (P-loops). Another critical series of studies demonstrated that, unlike pentameric receptors of acetylcholine, GABA and glycine, glutamate receptors have four subunits, and by this characteristic they are close to tetrameric potassium channels [11]. The final proofs were provided by the discovery an evolutionary transitional channel type in prokaryotes, which are usually difficult to find among presently existing organisms [12]. The discovery was a potassium channel activated by glutamate. This protein (named GluR0) has a ligand-binding domain, which is highly homologous to the ligand-binding domains of eukaryotic glutamate receptors. The selectivity filter of the GluR0 channel has the TVGYG sequence, which is a fingerprint of potassium channels. Importantly, the GluR0 receptor was first found by searching the database of protein sequences and then was studied experimentally [12]. Thus, glutamate receptors and voltage-gated channels inherited the pore domains from a common ancestor. This conclusion is absolutely clear from comparing 3D structures, which are available now (**Figure 2**).

However, large efforts were required to draw this conclusion before 3D structures become available.

The ligand-binding domain of glutamate receptors also has homologs among bacterial proteins (**Figure 2B**), which are glutamate-binding periplasmic proteins. Their crystal structure

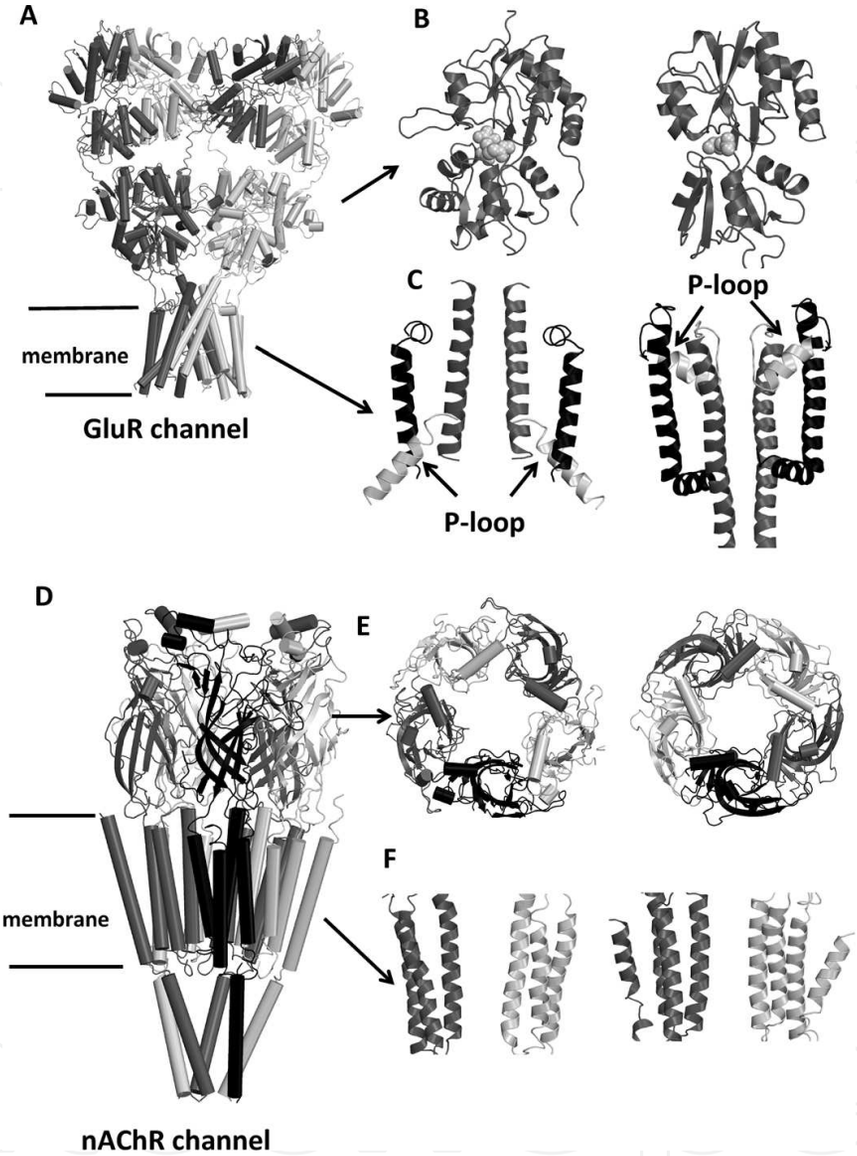

Figure 2. Different organization of glutamate (A–C) and acetylcholine (D–F) receptors. (A) In the ionotropic glutamate receptor four extracellular N-terminal domains (top), four extracellular glutamate-binding domains (middle) and a single transmembrane pore-forming domain (bottom) are connected by flexible linkers. (B) Glutamate binding domains in an ionotropic receptor (left) and in a bacterial non-channel glutamine-binding protein (right) are structurally similar. Ligands are space-filled. (C) Architecture of the pore-forming domains (only two subunits are shown for clarity) is similar in a glutamate-gated channel (left) and a voltage-gated channel (right). Note the opposite orientation of the domains: The P-loop dives into membrane from the cytoplasm in glutamate receptor and from the extracellular space in the voltage-gated channel. (D) In the nicotinic acetylcholine receptor the extracellular ligand-binding domain (top), transmembrane pore-forming domain (middle) and intracellular domain (bottom) are connected by flexible linkers. (E) Ligand-binding domains in the nicotinic acetylcholine receptor (left) and in a non-channel acetylcholine binding protein (right) are structurally similar. (F) Pore-forming domains of the cation-selective nicotinic acetylcholine receptor (right) and in the anion-selective $GABA_A$ receptor (left) are similar.

has been determined [13] and found to be remarkably similar to the glutamate binding domain in eukaryotic ionotropic receptors [14]. Probably, it is the extracellular localization of the ligand-binding domain that determines the "inverted" transmembrane topology of glutamate receptors as compared to voltage-gated channels. Nicotinic acetylcholine receptors belong to the structurally different family of so-called Cys-loop pentameric receptors, which also include channels gated by GABA, glycine and serotonin (**Figure 2**). Pore forming domains of these channels are markedly similar [15–17]. Neurotransmitter-binding domain of nicotinic acetylcholine receptors also originated from proteins that are not ion channels [18] (**Figure 2E**).

Like other ion channels of modular architecture, voltage-gated ion channels have the pore domain and four voltage-sending domains, which are believed to be of independent evolutionary origin. Indeed, some voltage-dependent enzymes (phosphatases) have voltage-sensing domains that are similar to those in ion channels. Interestingly, fusion of the voltage-sensing domains from the evolutionary unrelated phosphatase (a marine invertebrate) and a simple viral potassium channel that lacks any voltage-sensing or ligand-binding domains produced a functional voltage-gated potassium channel, which combines the pore properties of the contributing channel and the voltage dependence of the contributing phosphatase [19].

Besides the pore-forming domains and domains, which are responsible for the channel activation by specific stimuli (e.g., voltage or ligands), there are other domains involved in the channel regulation (**Figure 2A** and **D**). Regulatory functions may be performed not only by domains, but also by ancillary subunits, which are tightly associated with the channel proteins. The regulatory domains or subunits are typical characteristic of eukaryotic channels, which are involved in complex physiological systems and multiple interactions with other proteins.

Thus, various ion-channel proteins of modular organization may be assembled from domains, which originated from evolutionary and functionally distant protein families. Growing knowledge on the domain organization of ion channels shows that existent classifications of ion channels (e.g. according to the gating mechanism or the principle permeant ion) does not reflect their evolutionary history. Evolutionary studies would benefit from classification that also involves domain architecture, which is evolutionary much more conserved than physiological, biochemical or pharmacological properties.

3. Evolutionary changes in domains can govern specific channel properties

Certainly, evolutionary changes are not limited to domain organization. Homologous ionotropic receptors diverge the sensitivities to specific ligands as a result of local changes in the ligand-recognition domains. An interesting example is TRPV channels, which share with voltage-sensing P-loop channels folding of the pore domain and four "voltage-sensing" domains with apparently very similar structural organization. However, TRPV channels are not sensitive to voltage and can be activated by various stimuli, including temperature, pH, and ligands that bind to distinct sites [20]. Unexpectedly, these specific features appeared in evolution without incorporation of specific domains. Capsaicin, an active component of chili pepper, and related compounds bind in the interface between the "voltage-sensing" domain and the pore domain. Sensitivity of TRPV channels to pH is due to protonation of several residues in the extracellular

loops, which have nothing in common with proton-binding domains in proton-gated ASIC channels. Thus, TRPV channels are an example, where specific properties appeared without incorporation of additional domains.

One of the most important features of pore-forming domains is their ion selectivity. Usually the selectivity is governed by a local region in the narrow part of the pore, which is named the selectivity filter. This rather small part of the channel allows very fast passing of specific types of ions and rejects other ions. Concrete mechanisms of selectivity are different, but in any case, they involve specific interactions of the permeant ions with the pore-facing amino acids.

Classical example is opposite selectivity (cationic or anionic) within the family of Cys-loop channels. The 3D structures of the pore domain are very similar (**Figure 2F**), but acetylcholine and 5-hydroxytryptamine receptors are cationic channels, whereas Glycine and $GABA_A$ receptors are anionic channels. Experiments demonstrated that just few mutations can convert the cationic selectivity to the anionic one and *vice versa*. In these channels, which discriminate anions and cations by their charge, the selectivity mechanism is realized mainly through electrostatic interactions with the rings of amino-acids in the internal and external ends of pore-lining helices.

The big and diverse family of P-loop channels includes transmembrane proteins, which permeate different types of cations. The group includes potassium, sodium, calcium channels and less selective glutamate-gated and TRP channels. Multiple studies strongly suggest that the pore-forming domains of all these channels have a common ancestor from which the folding is inherited (see above section), while specific families evolved through gene duplication and gene divergence [21]. According to 3D structures, in all these channels the ion selectivity is governed by rather small number of pore-lining residues downstream from the P-loop turns (**Figure 3**). Potassium selectivity is governed by the highly conserved VGYG motif found in various organisms including prokaryotes (**Figure 3A**). Four potassium binding sites are present in the selectivity filter (**Figure 3B**). Unlike potassium channels, in which high selectivity is achieved due to tight binding of potassium ions to the backbone carbonyls, in sodium and calcium channels the selectivity is achieved by side chains of residues, which are located in the same region. Such evolutionary divergence from a common ancestor, which was driven by the necessity of more complex electrophysiological signaling, is accompanied by local refolding of the selectivity-filter region, which is seen in 3D structures as the appearance of so-called P2 helices in sodium and calcium channels (**Figure 3C**). However, evolution of eukaryotic sodium channels from their prokaryotic ancestors includes not only replacements in the selectivity filter. Although experimental high-resolution structures of eukaryotic sodium channels are still lacking, analysis of various data suggests deletions in non-matching positions of the four repeats in the selectivity-filter region [22, 23] (**Figure 3A**).

The structure of the selectivity filter of glutamate receptors, which include NMDA-, AMPA-, and kainate-gated channels, is still unavailable, but mutational analysis suggests that selectivity is governed by the ring of Asn, Gln, or Arg residues [24]. AMPA channels, which have glutamine residues in the selectivity filter, are the most ancient among this group of channels; they are permeable for monovalent cations and calcium. NMDA channels, which have asparagine residues in the selectivity filter, are more selective for calcium ions and are blocked by Mg^{2+}. Both these properties determine physiological roles of NMDA receptors in synaptic regulation and plasticity in the glutamatergic system. On the other hand, in the GluR2 subunit

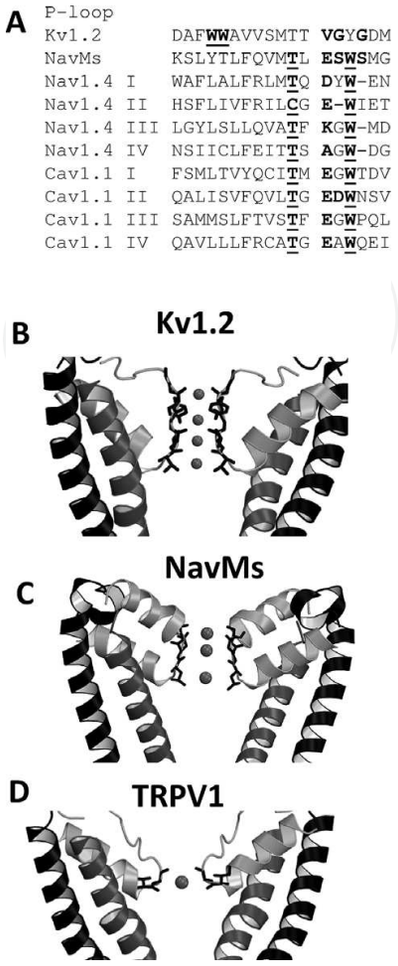

Figure 3. Selectivity in P-loop channels. (A) Sequence alignment of P-loops with the selectivity filter residues highlighted. (B–D) Available 3D structures with the ion binding sites and the selectivity filter highlighted.

of AMPA receptors Gln is replaced by Arg. This substitution completely eliminates calcium permeation. Thus, existence of three types of glutamate receptors enables different degree of coupling between electrical synaptic signaling due to permeability for sodium and potassium ions and calcium signaling, which regulates various biochemical processes within the neuron.

4. Importance of 3D structures for phylogenetic studies

Phylogenetic studies involving protein sequences are broadly used to understand molecular evolution. The first and most critical step in these studies is multiple sequence alignment of proteins. Regrettably, in the field of ion channels the standard sequence alignment tools work only within families of closely related channels, e.g., voltage-gated potassium channels [21]. However, attempts to align structurally conserved elements of the pore domains of potassium, calcium, sodium, glutamate-gated and TRP channels (all of which are P-loop channels) yielded contradictory results. Correct alignments, which later have been confirmed by comparing

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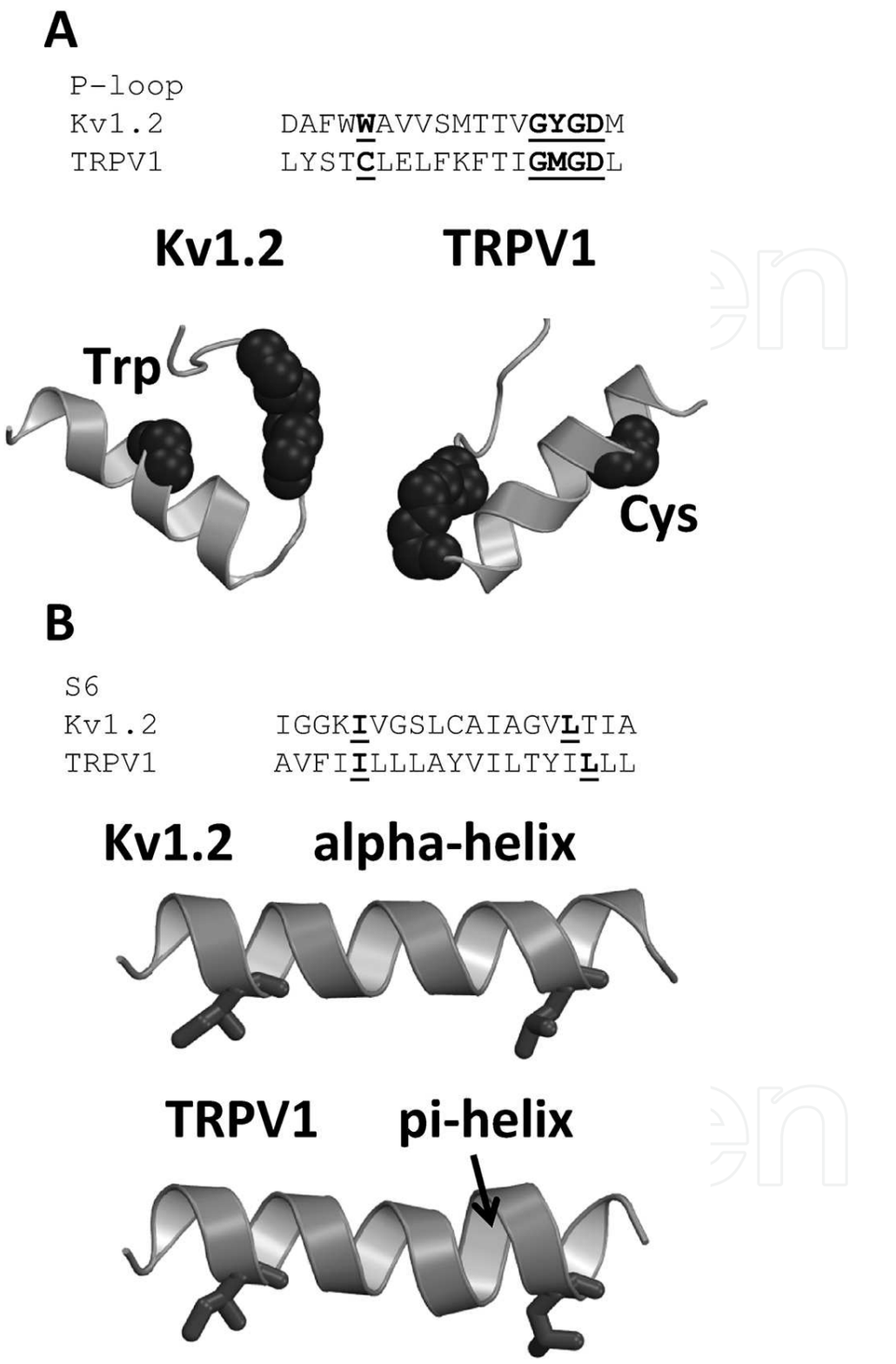

Figure 4. Mismatched sequence and 3D alignments of Kv1.2 and TRPV1 channels. (A) Alignment mismatch in P-loops. Underlined residues in matching positions have different localization in 3D structures and thus different functions. (B) Alignment mismatch in S6 segments. Underlined Leu residues in mismatching positions have the same spatial orientation due to the presence of pi-helix element.

experimental 3D structures, were elaborated by careful analysis of a large body of data on individual residues. When X-ray structures of various P-loop channels become available, the channels were found to have rather conserved folding despite vastly different sequences. Superposition of 3D structures allowed to adjust the sequence alignments, which provide much better basis for phylogenetic studies.

Alignments of residues in sequences and respective 3D structures do not necessarily coincide. For example, the sequence alignment of P-loops unambiguously shows the TVGYGDL motif of potassium channels and TVGMGDL motif of the TRPV channel in the matching positions and other positions within the P-loops do not suggest any alternative alignment (**Figure 4A**). However, in the superposed 3D structures of the TRPV and potassium channels the residues, which are in the matching positions of P-helix sequence alignment, occur at the opposite faces of the helices and thus would play different roles in the protein folding and function. In contrast, the 3D alignment, which is proposed to maximize the 3D similarity of the P-loops, shows the above residues in mismatching positions [25]. Thus, during evolution homologous residues may have changed their role and disposition, whereas the general domain folding did not change. The evolutionary mechanisms of such changes are unclear.

Transmembrane segments of ion channels are usually alpha-helices because in this secondary structure the polar groups of the backbones are hidden from the lipid environment. On the other hand, probability of possible evolutionary changes within alpha-helical structures is relatively small. Indeed, any insertion/deletion within a helical structure results in a big reorientation of other helical residues. Such reorientations would affect folding-stabilizing intersegment contacts and the exposure of functionally important groups into the aqueous pore or lipids. Therefore, the chances of acceptance of respective insertions/deletions during evolution would be small because so dramatic structural changes would result in the loss of the channel function. However, there are structural mechanisms that may provide tolerance of helical structures to insertions/deletions. The most common helical structure is an alpha-helix, which has H-bonds between residues in positions i and $i + 4$, but there are other types of helices, in which H-bonds are formed between positions i and $i + 3$ or i and $i + 5$. These secondary structures, which are called 3–10 helices and π-helices, respectively, are energetically less stable than alpha-helices and therefore are found predominantly as short segments. An insertion in an alpha-helix, which is involved in multiple intersegment contacts, would typically result in the appearance of a π-helix element (local bulging) without dramatic reorientation of residues at both sides of the insertion. An example of such a change is seen in the pore-lining S6 segment of the TRPV channel (**Figure 4B**). It was the analysis of 3D structures that allowed to reveal such changes. Analogously, deletion may results in local changes with appearance of 3–10 helices.

5. Importance of residue conservation in view of 3D structures

Multiple sequence alignments reveal conserved and variable residues. Conserved residues may be indispensable for protein folding or have crucial roles in the protein function. But it is only the 3D view that allows to really understand specific role of conserved residues. An

appealing example is exceptionally conserved tryptophans in the selectivity filter region in sodium and calcium channels. It was proposed that the tryptophans are involved in the folding stabilization [26], but it is the X-ray structure of a bacterial sodium channel [27], which has revealed atomic details of H-bonds that stabilize the folding (**Figure 5B**). Tryptophan is an amino acid with the largest side chain, which can also donate an H-bond. These two factors allow tryptophan residues to simultaneously participate in a large number of specific interactions. That is why tryptophans are often found to have important structural roles as folding stabilizers. For example, in the pore helix of potassium channels side chains of two adjacent tryptophans are oriented toward neighboring segments and form multiple contacts, thus forming cyclic motifs (**Figure 5A**) in the 3D structure [25].

Asparagine residues in the middle of the pore-lining helices are highly conserved in TRPV, sodium and calcium channels. Engineered substitutions of the asparagines in sodium and calcium channels affect gating properties as well as interactions with pore-targeting ligands. Available 3D structures of sodium and calcium channels show involvement of the asparagines in inter-segment contacts, but do not allow to make an unambiguous conclusion about their functional roles. A modeling study suggests that these asparagines stabilize the open channel state [28]. Another example of highly conserved residues is positively charged arginines or lysines in every third sequential position of some transmembrane helices in voltage-gated ion channels. These positively charged residues are "signatures" of the S4 helices within the voltage-sensing domains of ion channels. At the negative resting membrane potential, the positively charged S4 helices are attracted to the cytoplasmic side of the membrane and the

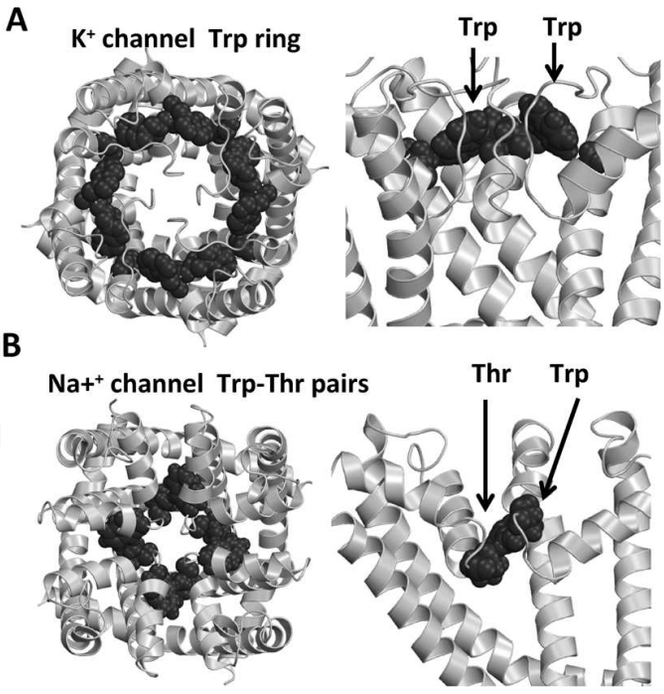

Figure 5. Conserved tryptophan residues as folding stabilizers. (A) Two Trp residues in the pore helix of potassium channel (see **Figure 3A**) form multiple contacts with neighboring segments and create the ring stabilizing the tetrameric structure. (B) Conserved Trp residues in the selectivity filter of sodium and calcium channels (see **Figure 3A**) form intersubunit contacts with Thr residues in the pore helix and provide spatial stabilization of the selectivity filter folding.

channels are closed. Upon membrane depolarization, the S4 helices shift in the extracellular direction thus initiating the process of the channel activation (opening).

The structural and/or functional importance of conserved residues is obvious. Non-conserved residues may be not critical for folding or function, but sometimes such residues play key roles in determining specific properties of the proteins. For example, variable hydrophilic residues, such as lysines, arginines, glutamines or asparagines in a matching position may indicate exposure of respective position in the aqueous environment rather than specific functional roles. However, sometimes this conclusion may be wrong. For example, asparagine, glutamine, or arginine (N/Q/R) residues determine permeability of glutamate receptor subtypes for specific cations [24]. The mechanism of selectivity was suggested in a modeling study [29], but experimental 3D structures of the N/Q/R site are still unavailable.

6. Local adaptive changes of toxin-targeted ion channels

Evolutionary changes are observable in phylogenetic studies, but driving forces for these changes remain largely unclear. Local adaptive changes of animal ion channels, which are exposed to toxins, are easy to reveal. An interesting example of the constrained convergence during evolution is resistance of snake species around the globe, which feed on newts that possess sodium channel blocker, tetrodotoxin [30]. Some garter snake populations around the globe have evolved resistance to extremely high levels of TTX [31]. Amino acid substitutions are observed in the selectivity-filter region of sodium channels and structural explanation for the acquired resistance has been proposed [26]. Interestingly, such changes are seen in the sodium channel paralogs, which are expressed in the skeletal muscle and peripheral nervous system of snakes and thus should be exposed to the ingested tetrodotoxin. In contrast, sodium channel paralogs, which are expressed solely in the central nervous system, showed no evidence of the TTX resistance, indicating that the blood-brain barrier protects from the toxin [32]. The observed genetic changes represent only a small fraction of the experimentally validated mutations known to increase the sodium channels resistance to TTX. These results suggest that evolutionary convergence at the molecular level results from the compromise between ion channel function and resistance to toxin. Thus, the natural selection may be constrained to produce similar genetic outcomes even in independent lineages.

An example of genetic adaptation of large practical importance is acquired resistance of insects to sodium channel-targeting insecticides [33]. Well known insecticides such as DDT and pyrethroids are sodium channel activators, which disturb the normal processes in the nervous system. Many insect populations worldwide mutated to elaborate residence to DDT and the earlier generation of pyrethroids. Such adaptive genetic changes are called kdr (knock-down resistance) mutations. In various inset populations many kdr mutations within sodium channel are found. Additional studies involving molecular modeling, mutational analysis and electrophysiology led to discovery of two pyrethroid binding sites in insect sodium channels [34, 35]. The receptors are located within the pore domain, in the lipid-exposed interfaces between individual channel segments whose mutual disposition changes upon the channel

gating. This fast adaptive evolution of ion channels presents a big economical problem and requires development of new insecticides whose action would not be abolished by known kdr mutations. Structure-based design of new insecticides is hardly possible without knowledge of the sodium channels 3D structures.

7. Conclusions

A goal of research in the fields of molecular evolution in general and ion channels in particular is to understand how functional diversity of proteins is related to genetic changes. A traditional approach uses amino acid sequences to build phylogenetic trees and relate these with known functional characteristics of ion channels. Obviously, the amino acid sequence determines the 3D structure and functional characteristics of a protein. Nowadays, properties of small molecules are reliably predictable from their chemical structures. However, a general approach to predict 3D structures and properties of proteins just from their amino acid sequences is still lacking. Successful predictions are based on the knowledge of proteins that have similar sequences. For example, the presence of the VGYGD motif allows to predict that respective protein is a potassium channel, but such predictions are based on the fact that many channels that have this "signature sequence" have been previously studied experimentally and were found to have the potassium selectivity. It is the knowledge of 3D structures of proteins that helps to link genetic and functional data. In this work we presented examples of how the rapidly growing body of experimental data on 3D structures of ion channels influences progress in understanding their molecular evolution.

Obviously, besides molecular evolution, knowledge of 3D structures of ion channels is important in other fields. Ion channels are among the prime targets for many different drugs and toxins. This determines large demand for practical applications of knowledge on ion channels in chemistry, medicine, toxicology, and pharmacology. Structure-based analysis of action of existent drugs and toxins and design of new channel-targeting molecules requires knowledge of 3D structures of the target proteins. Available experimental 3D structures still do not represent the large diversity of ion channels and multiplicity of their functional states (open, close, inactivated, etc.). Even nowadays any new experimental 3D structure of an ion channels is an important event in the field, which is usually reported in a high-impact journal. Comparative (homology) molecular modeling is an approach to fill the gap between the numbers of desired and available 3D structures of ion channels. A key assumption of homology modeling is that the evolutionary close ion channels have similar 3D structures. Homology modeling of an ion channel involves selections of structural templates (available 3D structures of relative proteins), sequence alignment between the templates and the query protein, computer-assisted building and optimization of the model 3D structure, and analysis of possible structural deviations of the model from the templates. The choice of an incorrect template or even one-position misalignment between the template and the query protein may result in entirely incorrect model. Understanding mechanisms of molecular evolution of ion channels is necessary to avoid such errors. This is an example of how basic evolutionary studies can be translated to practical applications.

Acknowledgements

This work was supported by State budget funding by Federal Agency of Scientific Organizations Russia (Neurophysiological regulatory mechanisms and their evolution).

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [receptor]
ASIC	acid-sensing ion channel
Cryo-EM	cryo-electron microscopy
ClC	chloride channel
CNG	cyclic nucleotide-gated [channels]
GABA	gamma-aminobutyric acid
HCN	hyperpolarization-activated cyclic nucleotide-modulated [channels]
Kir	inward-rectifying potassium [channels]
nAChR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate [receptor]
TRPV	transient receptor potential vanilloid [channel]
KcsA	prokaryotic potassium channel from the soil bacteria *Streptomyces lividans*
TTX	tetrodotoxin

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