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Structural and Regulatory Evolution of Cellular Electrophysiological Systems

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Cellular electrophysiological systems have been studied intensively since the time of Galvani (1791) and are amongst the best understood of any physiological systems. There is a detailed understanding of electrophysiological systems at the molecular (MacKinnon, 2004; Yu and Catterall, 2004; Hille, 2001) and cellular level that extends to complex models of cellular function (Koch, 1999; Rudy and Silva, 2006). Because of the relative depth of understanding of these systems, it is of some interest to consider the ways in which electrophysiological systems evolve. This is particularly true in light of the recent suggestion (Hoekstra and Coyne, 2007) that physiological systems evolve predominantly by structural evolution, the modification of protein structure, rather than by regulatory evolution, changes in gene regulatory function, which has been proposed to be the predominant mechanism underlying the evolution of developmental systems (Wilson, 1985; Carroll, 2005; Wray, 2007).

In this review we will focus on the intrinsic electrophysiological properties of electrically excitable cells, which are largely determined by the voltage-gated ion channels that these cells express. In this context, the term 'structural evolution' will refer to changes in protein sequence that lead to substantive changes in ion channel function, which can generally be measured very accurately using electrophysiological techniques. It does not refer to changes in either cellular or animal morphology, both of which are largely determined by developmental rather than physiological processes. Studies on the evolution of developmental systems have focused primarily on *cis*-regulatory evolution because this mechanism appears most likely to produce discrete changes in gene expression. For the purposes of this review, however, the term 'regulatory evolution' will refer to any substantive change in ion channel expression, in large part because the analysis of ion channel gene regulation has been relatively limited, whereas channel expression can be measured accurately using functional assays.

Early Evolution of Ion Channels

Voltage-gated ion channels (Fig. 1) were, until relatively recently, primarily thought to be associated with the electrically excitable cells found in multicellular animals, such as neurons and muscle cells. It was not widely anticipated, therefore, that most of the major structural motifs that are characteristic of voltage-gated ion channels would be found in prokaryotes. These include the potassium ion selectivity filter (MacKinnon, 2004), the signature sequence of the calcium ion selectivity filter (Yue et al, 2002; Shafrir et al, 2008),

the voltage sensor (Jiang et al, 2003; Ren et al, 2001b), the calcium sensor (Jiang et al, 2002) and the cyclic nucleotide binding domain (Nimigean et al, 2004; Clayton et al, 2008).

The main forms of potassium channels found in animals, the voltage-gated, calcium activated and inward rectifier potassium channels, appear in essentially modern form in prokaryotes (Jiang et al, 2003; Jiang et al, 2002; Kuo et al, 2005). Precursors of eukaryote calcium and sodium channels are found in prokaryotes in the form of voltage-gated sodium channels (Durell and Guy, 2001; Ren et al, 2001b; Koishi et al, 2004) and possibly calcium-selective channels (Koishi et al, 2004; Shafrir et al, 2008). A key structural difference is that the prokaryote channels assemble from four independent subunits whereas eukaryote calcium and sodium channels are comprised of a single subunit with four homologous domains. Four-domain voltage-gated calcium channels first appear in yeast (Paidhungat and Garrett, 1997) and four-domain sodium channels appear to be restricted to multicellular animals (Anderson et al, 1993).

Although there has been considerable expansion in the number of voltage-gated ion channel α -subunit genes in the genome of animals and an elaboration on some basic themes (Yu and Catterall, 2004; Jegla et al, 2009), the solution for most of the key structural problems associated with the production of electrically excitable cells was achieved during the course of prokaryote evolution (Loukin et al, 2005) and essentially all major problems were solved soon after the emergence of multicellular animals.

In addition to the primary or α -subunits, which catalyze the movement of ions across the membrane, many channel assemblies also include one or more auxiliary subunits that can modify channel expression or gating function. Unlike α -subunits, auxiliary subunits are structurally very diverse, with at least 10 completely unrelated gene families contributing to this group in mammals (Yu and Catterall, 2004). In many cases auxiliary subunits appear to have been co-opted from other functions and some auxiliary subunits retain multiple functions (Yu and Catterall, 2004). Not surprisingly, auxiliary subunits have diverse evolutionary histories. Some auxiliary subunit families are evolutionarily ancient, with clear prokaryotic antecedents (Mangubat et al, 2003; Cai et al, 2006), whereas others are relatively recent innovations with, in some cases, different gene families performing analogous roles in different animal lineages (Chopra et al, 2007; Tseng et al, 2007).

Evolution of Cellular Electrophysiological Systems

Cellular electrophysiological systems are the lowest level physiological unit within which voltage-gated ion channels typically function. Ion channels mediate the flux of ions across the cell membrane and the mix of channels that a given cell expresses determines its electrical properties, which can be quite variable between different cell types (Bean, 2007). The evolution of two cellular electrophysiological systems has been studied in some detail and the key findings will be described.

Squid Giant Axon

The giant axon of the squid is a key component of the neural network underlying predator escape behavior (Young, 1939; Otis and Gilly, 1990) as well as other motor behaviors (Otis and Gilly, 1990). The large size of the giant axon helps maximize the conduction velocity of an action potential along the axon, thereby minimizing the time required to initiate muscle contraction and effect an escape. The giant axon is a relatively simple electrophysiological system with two main types of channels (Fig. 2): voltage-gated sodium and potassium channels (Hodgkin and Huxley, 1952).

The gating function of voltage-dependent channels is strongly dependent on temperature (Hille, 2001) and squid that have adapted to different ocean temperature habitats would be expected to compensate for this dependence. One change seen in the giant axon of four species of squid adapted to different temperature environments was a systematic change in action potential duration, with colder environments being associated with shorter action potential durations (Rosenthal and Bezanilla, 2002). Because the kinetic properties of ion channels are very sensitive to changes in amino acid sequence (Keating and Sanguinetti, 2001; George, 2005; Bidaud et al, 2006), *a priori* it would seem most likely that changes in channel function would be the primary mechanism underlying these changes. Unexpectedly, however, no differences in the kinetic properties of the sodium and potassium channels were found. Instead, there were relatively large changes in potassium channel expression in the axon, which account for the observed differences in action potential duration (Rosenthal and Bezanilla, 2002). Thus the adaptation of electrophysiological function in the squid giant axon occurs as a result of regulatory evolution, with no structural evolution of the key components, the voltage-gated ion channels.

Mammalian Cardiac Myocytes

Another intensively studied electrophysiological system is the mammalian ventricular myocyte, which comprises the bulk of heart tissue and is the cell type responsible for pumping blood throughout the body. There is an enormous variation in the body size of mammals and appropriate scaling of cardiac function is absolutely required for this morphological diversity (Schmidt-Nielsen, 1984; Elzinga and Westerhof, 1991). The ventricular myocyte is a complex electrophysiological system (Rudy and Silva, 2006) and the properties of a large number of different ion channels and calcium handling proteins must change in a coordinated fashion in order to scale the action potential and calcium transient duration over an approximately 10-fold range in terrestrial mammals (Rosati et al, 2008).

In this system there is no evidence for significant structural evolution (Rosati et al, 2008). Key channels that control action potential duration such as the L-type calcium channel and the rapid and slow delayed rectifier potassium channels show no, or very limited, functional differences in comparisons between different mammalian species. In contrast, there are large changes in the expression of a number of ion channels and transporters including four different potassium channels, the L-type calcium channel and the sarcoplasmic reticulum Ca^{2+} -ATPase pump (Lu et al, 2001; Su et al, 2003; Rosati et al, 2008).

For the two potassium channels that have been studied most intensively, there are corresponding changes in the *cis*-regulatory function of the genes encoding their α -subunits (Rosati et al, 2008). The mechanism by which the changes in the expression of other channels and transporters in this system is mediated has not been studied in as great a detail, however, and could reflect mechanisms other than *cis*-regulatory evolution.

In such a complex system, it is difficult to entirely exclude the possibility that the structural evolution of some component has been important for the scaling of electrophysiological function. Nonetheless, the role of structural evolution appears to be very limited. The changes in expression levels of the various ion channels and transporters are large and appear to fully account for the differences in action potential and calcium transient duration.

Reasons for the Dominance of Regulatory Evolution in Electrophysiological Systems

Although limited in number, studies on the evolution of cellular electrophysiological systems paint a strikingly different picture to that suggested for the evolution of many other physiological systems (Hoekstra and Coyne, 2007). Electrophysiological systems appear to evolve in a similar way to that proposed for developmental systems (Wilson, 1985; Carroll, 2005; Wray, 2007). It is worthwhile considering, therefore, what these systems may have in common. In addition to their primary dependence on regulatory evolution, it is notable that electrophysiological systems and the gene regulatory systems the control development share two other key features; for both systems structural evolution has the potential to create significant problems of pleiotropy and both systems are predominantly computational in nature.

Pleiotropy

The importance of pleiotropy as a constraint favoring regulatory evolution in developmental systems has been discussed at length (Stern, 2000; Carroll, 2005) and essentially identical arguments can be applied to electrophysiological systems. In brief, both systems depend on proteins that are broadly expressed in a large number of highly differentiated cell types. As a consequence, structural evolution, by changing the properties of a protein that is important for the function of a range of differentiated cells, has a high probability of producing pleiotropic effects. In contrast, regulatory evolution, particularly the evolution of *cis*-regulatory modules, can in principle, produce cell type specific effects, thereby avoiding, or limiting, pleiotropic effects.

Ion channels form a large gene family in most animals (Moulton et al, 2003; Yu and Catterall, 2004). There are at least 143 members of the voltage-gated ion channel superfamily in mammals, 49 genes in *Drosophila* and 101 genes in *C. elegans* (Yu and Catterall, 2004). In addition to these main, α -subunit genes there are a large number of auxiliary subunit genes. The structural diversity of these auxiliary proteins makes it difficult to provide an exact number. There are, however, relatively few ion channel genes compared to the very large number of distinct types of excitable cells in a typical animal. In particular, there is an enormous number of phenotypically distinct neurons (Stevens, 1998; Bota et al, 2003). Most electrically excitable cells express a significant fraction of the total number of ion channel genes (Dixon and McKinnon, 1996; Gaborit et al, 2007). There are distinct cell-type specific expression patterns for different ion channel genes and important quantitative differences in gene expression levels, both of which can significantly modify electrophysiological function. Almost no members of the ion channel gene family are specific to a single type of cell.

In effect, ion channels function as general purpose proteins in electrically excitable cells, and ion channel expression levels are varied in a highly flexible fashion to produce a wide variety of electrophysiological phenotypes (Bean 2007; Rosati et al, 2008). Changing the functional properties of any one channel would be expected to have broad pleiotropic effects.

One interesting exception to this general trend is the CatSper channel, a Ca^{2+} ion selective channel. This channel has a extremely restricted expression pattern, it is found only in a subsection of the tail of mammalian sperm cells (Ren et al, 2001a; Qi et al, 2007; Liu et al, 2007) and is absolutely required for male fertility in mammals (Ren et al, 2001a; Qi et al, 2007). Many of the subunits that comprise this channel show unusually high rates of sequence change (Liu et al, 2007), suggesting that the restricted expression pattern removes

a constraint on protein evolution. In addition, complete loss of this gene family occurs in several metazoan lineages (Cai and Clapham, 2008), which is unusual for an ion channel gene family.

Biological Computation

The other similarity between the two systems arises from the fact that electrophysiological systems must solve two sets of problems, one primarily physical and the other primarily computational. The physical tasks that ion channels perform are conceptually relatively simple, requiring the transport of common cellular ions in a specific fashion across the cell membrane in response to one or more stimuli (Fig. 1).

Assessment of the computational tasks that electrophysiological systems perform is more complex. No single description that can encompass the entire scope of biological computation exists. It has been argued that computation in different biological systems will have few shared principles of operation and different systems will have to be considered on a case by case basis (Marr, 1975). In addition, computational function in a given system can be analyzed at multiple levels of organization, and no simple relationship between those levels may be readily apparent (Marr and Poggio, 1977). As a consequence, identification of the computational tasks that a given physiological system performs is more ambiguous than determining the physical tasks that the system performs. This ambiguity does not, however, diminish the importance of computational tasks. Computation is the primary function of the nervous system and most physiological systems are inconceivable without a significant computational component to regulate function.

The computational nature of individual electrically excitable cells was first explicitly recognized by Hodgkin and Huxley (1952) in their study of action potential propagation in the squid giant axon. At the lowest level, the computational task performed by the axon requires the summation of two ion currents, with the balance of inward and outward currents determining the direction of change of the membrane voltage at any given moment (Fig. 2). In addition, the axon is embedded in a neural network that performs multiple higher level computations regulating squid motor function (Otis and Gilly, 1990).

The priority of the computational task in this system is demonstrated by considering what is achieved by firing a single action potential in the squid axon. Following completion of the action potential, the inward flow of sodium ions has been exactly matched by the outward flow of potassium ions (Fig. 2). Not only is no useful physical task performed by this operation, but the net effect on the physical system is to partially deplete the gradient of sodium and potassium ions across the cell membrane, which was created at considerable metabolic cost by the Na,K-ATPase. Clearly, the physical tasks performed by the ion channels are subservient to a computational imperative. The only rationale for the squid to fire an action potential is a computational one, in this case to transfer information from one part of its body to another.

Like electrophysiological systems, gene regulatory systems also have a large computational component (Brenner, 1998; Istrail et al, 2007) and similar mathematical formulations can be used to describe gene regulation (Bolouri and Davidson, 2002) as have been used to described electrophysiological systems. A very simple analogue for both systems is the summing junction of electrical circuit theory, in which positive and negative inputs are combined to produce a single output (Fig. 3). In the case of electrophysiological systems, this output is the membrane potential. For gene regulation, the output is the rate of gene transcription. Figure 3 represents a great simplification, however, and both systems are capable of performing much more complex computations than simple addition (Koch and Segev, 2000; Istrail et al, 2007).

Differences in the Balance of Regulatory and Structural Evolution for Developmental and Physiological Systems

The preceding arguments suggest that the relative balance of physical and computational tasks that a biological system has to perform combined with the probability that these tasks may have to change significantly during the course of evolution will be major factors in determining the relative mix of regulatory and structural evolution that will be observed in a given system.

Clearly, developmental systems perform a large number of physical tasks while building a new organism. However, development, at least in more complex animals, proceeds in a largely self contained environment that is buffered from the physical environment. As a consequence, these physical tasks remain relatively constant across large phylogenetic distances and it is primarily the computational component of development that will evolve over time. This is a situation where regulatory evolution seems likely to predominate.

For physiological systems, more variability is to be expected. Physiological systems that directly interface with the environment will almost always perform some kind of physical task. In the majority of cases, this will require structural evolution in order for the tasks themselves to evolve. As physiological systems become more complex and control structures form an increasing component of the overall system, a greater fraction of their function will be computational in nature. This trend reaches an apogee in the central nervous system, where the vast majority of tasks are purely computational and often quite abstract. In these latter cases it seems likely that regulatory evolution will also predominate.

The squid giant axon and the ventricular myocyte described above are unusual in that their computational function is relatively simple and well understood. Typically, neurons are embedded within neural circuits that perform multiple computations, and in most cases the algorithms used for these computations are not well understood. Nor is it generally known how the performance of these circuits might be optimized during the course of evolution by modifying the electrophysiological properties of the underlying neurons (see Meyrand and Moulins (1988) for one attempt). As a consequence, for most cellular electrophysiological systems, it is considerably more difficult to study the evolution of their computational function rather than the physical function of their molecular components. This is likely to be more generally true for most physiological systems. Consequently, attempts to draw general inferences about the evolution of developmental systems from what is known about the evolution of physiological systems (Hoekstra and Coyne, 2007) are likely to be particularly susceptible to sampling bias. A fair comparison would require the systems to have similar computational complexity, a difficult criteria to meet given the current level of understanding.

Constraints Determining the Mechanism of Evolution in Electrophysiological Systems

For the two electrophysiological systems described in this review the evolution of physiological function could in principle be mediated either by structural or by regulatory evolution.

Regulatory evolution, by increasing or decreasing the expression of different ion channels, naturally lends itself to the evolution of the computational function of cellular electrophysiological systems. For example, to solve the problem of matching ventricular action potential duration to body size (Rosati et al, 2008), a vector of ion channel expression levels can be essentially hardwired into the genome to provide an appropriate solution for

different sized mammals. No structural innovation is absolutely required to solve this kind of problem, assuming that an adequate level of complexity in the number and kinds of channels is already encoded in the genome (see below).

Alternatively, structural evolution, by adjusting the kinetic properties of different channels, could generate a wide range of electrophysiological phenotypes in a given electrophysiological system without changing channel expression. Even modest changes in the kinetic properties of the ion channels found in both the squid axon and the ventricular myocytes could, by themselves, produce large changes in action potential duration in these systems.

The fact that the evolution of these two systems largely relies upon regulatory evolution suggests that there are strong constraints on channel functional properties in multicellular animals. The relative stability of channel function is particularly striking because channel kinetic properties are very sensitive to a wide range of mutations (Keating and Sanguinetti, 2001; George, 2005; Bidaud et al, 2006). Avoidance of pleiotropy is likely to be a major constraint on the evolution of channel function in these systems. There may, of course, be other constraints on channel function associated with the specific physiological function of particular classes of channels. For example, the properties of most voltage-gated sodium channels are likely to be highly constrained in order to generate the rapidly activating and inactivating currents required to efficiently generate action potentials (Angelino and Brenner, 2007).

Requirement for an Adequately Complex Tool Kit of Channel Genes for Regulatory Evolution

For the relatively subtle changes in squid axon function required for temperature adaptation, only adjustments in the expression levels of channels that were already expressed in the axon were necessary (Rosenthal and Bezanilla, 2002). For the large changes in action potential duration required for the scaling of cardiac function the changes were more complex. In order to produce the shortest action potential durations in the smallest mammals, expression of rapidly activating potassium channels that are not normally expressed in the hearts of most mammals was required (Rosati et al, 2008). Clearly, for regulatory evolution to work in this system, a pre-existing tool kit of ion channels with a range of kinetic properties was necessary. In this particular case, the channel genes that were recruited for expression in the ventricular myocytes of the smallest animals already had important functions in other excitable cell types, including many neurons. As a general principle, it would seem that the practicality for a given electrophysiological system to evolve by regulatory evolution will be determined in large part by the complexity of the channel genome that the animal possesses.

The ion channel α -subunit gene family in animals is relatively large (Lopreato et al, 2001; Yu and Catterall, 2004; Hoegg and Meyer, 2007; Novak et al, 2006; Jegla et al, 2009), and encodes channels with a wide variety of functional properties. Within the mammalian lineage, voltage-gated ion channel gene number is quite stable, with no known duplications of ion channel α -subunit genes (Demuth et al, 2006) and very limited loss of channel genes (1 or 2 genes) in different species (Yu and Catterall, 2004; Jegla et al, 2009). There are, however, large differences in the number of channel genes and the relative representation of different ion channel subfamilies between different animal lineages (Moulton et al, 2003), indicating substantial losses and gains of channel genes during animal evolution.

Exactly what drives the expansion or contraction of the ion channel gene family in different animal lineages is not well defined at present. In principle, the computational requirements

of a given electrically excitable cell can be satisfied by expression of a large number of alternative combinations of channels. As a consequence, the exact complement of genes that becomes fixed in a particular animal lineage may be somewhat arbitrary, as long as it is sufficiently complex. What this minimum level of complexity might be for a given lineage is not known and is a difficult issue to address. Channel gene number by itself seems to be a very inexact reflection of electrophysiological complexity. *Paramecium*, a single cell protist, appears to have more than three times the number of K⁺ channel genes found in the human genome (Haynes et al, 2003).

Evolution of Physical Function in Electrophysiological Systems

There are some obvious exceptions to our suggestion that the evolution of cellular electrophysiological systems will primarily require the solution of computational problems. Most notable are sensory systems, which form the interface between the predominantly computational nervous system and the physical world. Not surprisingly, some of the best known examples of structural evolution are found in sensory systems, including the evolution of different light sensitive rhodopsins (Yokoyama, 2002) and the expansion and contraction of olfactory receptor gene number (Niimura and Nei, 2007). These are examples of structural evolution to solve purely physical problems, the transduction of physical stimuli. The problem of pleiotropy is moot in these instances because the transduction proteins generally have no or very limited roles outside of the sensory cell type, in marked contrast to the general purpose nature of most ion channels.

A clear example of structural evolution in broadly expressed ion channels is the acquisition of resistance to natural and synthetic toxins (Soong and Ventkatesch 2006; Ffrench-Constant et al, 1998). Acquired resistance to natural toxins targeting ion channels can have a significant impact on predator-prey interactions (Hanifin et al, 2008) but usually has limited effects on physiological function. In general, the acquisition of toxin insensitivity involves changes in a very small set of amino acid locations that affect the contours of the toxin binding site. The selected changes usually have limited effects on the function properties of the channel, presumably because of purifying selection acting to maintain channel function unchanged (Jost et al, 2008).

There is likely to be a complex interplay between gene duplication, regulatory and structural evolution and the pleiotropy constraint during the evolution of new physiological functions. One example of this, in the context of the acquisition of a new sensory modality, is the evolution of the Nav1.4 gene in electric fish. Two copies of this gene, Nav1.4a and Nav1.4b, arose early in teleost evolution, probably as a result of a genome duplication event (Novak et al, 2006). In non-electric teleosts, both the Nav1.4a and Nav1.4b genes are expressed broadly in skeletal muscle and appear to have overlapping functions. In electric fish, expression of the Nav1.4a gene is restricted to the electric organ, which is a specialized sensory organ derived from skeletal muscle that generates an electric signal for communication and electrolocation (Zakon et al, 2006). In electric fish, the overall rate of amino acid replacements in the Nav1.4a channel is much higher than in non-electric fish and the changes appear to contribute to diversity in the duration of the electrical signal in different species (Zakon et al, 2006). There is no difference in the rate of sequence evolution for the Nav1.4b gene in electric and non-electric fish (Zakon et al, 2008), consistent with the retention of a broader expression pattern for this gene.

It seems likely that the regulatory changes resulting in the restriction of Nav1.4a gene expression to the electric organ largely predated the changes in channel function, because restricted expression was required in order to relieve the pleiotropy constraint on Nav1.4a channel function. This conclusion is supported by the convergent evolution of electric

organ-specific Nav1.4a expression in two independently derived lineages of electric fish (Zakon et al, 2006).

An example of a physical problem that could only be solved by structural evolution is seen in the evolution of the Na,K-ATPase (Colina et al, 2007), which establishes the gradient of Na⁺ and K⁺ ions across the cell membrane. Efficient function of the pump is important because maintenance of ion gradients consumes a major fraction of the total cellular energy budget in excitable cells (Attwell and Laughlin, 2001). The shift from a marine to a land environment resulted in a large change in the concentration of Na⁺ ions in the extracellular fluid of animals (from approximately 450 to 150 mM). To maintain efficient pump function following this change in operating conditions, the electrostatic charge on the outer surface of the pump was modified in order to alter the local Na⁺ ion concentration at the entrance to the pump (Colina et al, 2007). No conceivable form of regulatory evolution could produce a satisfactory solution to this particular problem. It is notable, however, that this protein only creates the appropriate conditions for the computational function of the cell and is generally not a key participant in that computational process.

Conversely, there are likely to be some physical problems that are resolvable by regulatory evolution. A possible example in an electrophysiological system is the minimization of ion fluxes and consequent energy utilization during action potential propagation by the selection of optimal levels of ion channel expression (Crotty et al, 2006).

Conclusions

Most complex biological systems have both a physical and a computational component. The relative importance of these two components will vary considerably among different systems and this will be an important factor in determining whether a particular system can evolve primarily by regulatory or structural evolution. For biological systems that can call upon a sufficiently large and complex complement of pre-existing structural components encoded in their genome, it seems likely that many of the challenges to computational function that arise during the course of evolution may be most readily solved by some form of regulatory evolution. In contrast, purely physical problems are more likely to require novel structural solutions.

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Figure 1.

Ion channels have two key functional properties:

1. ion selectivity - the ability to discriminate between the various common ions found in the cell. Voltage-gated channels are typically selective for K^+ , Na^+ or Ca^{2+} ions.
2. channel gating – mechanisms that control the opening and closing of the channel

Typically there are two functional domains, the channel core, which contains the ion selectivity sequence (S) and the channel gate (G), and a gating module (GM), which is physically linked to the gate. The channel core is relatively small and is embedded in the cell membrane. It provides a pathway for ion movement between the inside and outside of the cell. The most constricted region of this pathway corresponds to the selectivity sequence (S), the region of the pore that allows one type of ion to pass while excluding all the other ions. The selectivity sequence is very highly conserved for a given type of ion selectivity. For potassium channels the gating module shows significant variation between different types of channels resulting in potassium channels with a wide range of gating properties. The two most common gating modules confer voltage sensitivity or sensitivity to the binding of internal Ca^{2+} ions. Movement of the gating module moves the gate to open or close the channel to the passage of ions. In this cartoon a voltage sensing module is shown, which is located within the cell membrane. Modules responsible for Ca^{2+} or cyclic nucleotide gating are located close to the cytoplasmic surface of the channel.

Figure 2.

The squid giant axon expresses two types of voltage-gated channels: a Na⁺ channel and a K⁺ channel. The sum of the currents flowing through these two types of channels ($I_{Na} + I_K$) determines the current flow across the cell membrane (I_M) and this determines the electrical potential across the membrane, known as the membrane potential (V_M). The two channels have different kinetic properties, of particular importance is that the Na⁺ channel opens more rapidly than the K⁺ channel. During an action potential, the Na⁺ channels open first and current flow through these channels moves the membrane potential to more positive potentials. Then, the K⁺ channels open and the Na⁺ channels inactivate, which moves the membrane potential back to the resting value. By the end of the action potential, the amount of Na⁺ ions flowing into the cell through the Na⁺ channels is matched by an equal and opposite flow of K⁺ ions flowing out of the cell through K⁺ channels. The system is maintained in steady-state by the Na,K-ATPase ion pump, which expends metabolic energy to maintain the normal internal concentrations of Na⁺ and K⁺ ions. Current flow into the cell is defined as negative and outward current flow as positive. More complex systems simply add more channel types, each with different kinetic properties and/or ion selectivity.

Figure 3.

At their simplest, electrophysiological systems and gene regulatory system work like summing junctions from electrical circuit theory. The summing junction can have multiple inputs but produces a single output. In the case of a electrophysiological system there are multiple inward (excitatory) and outward (inhibitory) currents that contribute to a single output, the membrane voltage. In the case of gene regulation there are multiple transcription factors that can bind to the regulatory region to have positive or negative effects on a single output, the level of gene transcription.