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Making sense of channel diversity

When the eminent British geneticist J.B.S. Haldane was asked what God had revealed about himself through his works, Haldane is said to have replied "an inordinate fondness for beetles". Were he alive today, Haldane might instead have cited ion channels; although their diversity may no longer be absorbing the creative energies of the Almighty, they did at least attract several hundred people to a three-day meeting in New York last month. The event was organized by the New York Academy of Sciences, and it provided an excellent overview of recent progress in understanding the molecular basis of ionic conductances, including ionotropic receptors as well as voltage-gated and other channels. Although the advances have been impressive, it was also clear that the field faces a formidable challenge in making sense of what has already been discovered.

An unprecedentedly comprehensive picture of channel and receptor diversity is now emerging from large-scale genome sequencing projects, notably of the nematode worm *Caenorhabditis elegans* (the sequence of which is now around 80% complete). As discussed by Larry Salkoff (St Louis), the worm sequencing project has already led to the identification of large numbers of new channel and receptor subunits. In particular, at least 80 potassium channel subunits have been found, a remarkable number considering that the nervous system of *C. elegans* contains only 302 neurons, which have been classified into 118 types. About 50 of these genes belong to a new class, distinguished both by their four transmembrane domains and by the lack of knowledge about their function (the best guess is that they are leak channels that regulate cell excitability). By tagging the coding sequences with green fluorescent protein, it is possible to visualize their expression patterns; many of the subunits are restricted to single cell types, and at least one is expressed only in a single interneuron. If this can be extrapolated to the mammalian brain (which is not yet clear), not only does this imply a very large number of channels, but some may be so restricted in expression that they are unlikely to be discovered except by genomic sequencing. The mammalian sequencing projects are far less advanced, the available evidence suggests that any given channel family will contain many more members in mammals than in worms. For example, *C. elegans* has a single voltage-gated K^+ channel of the Shaker class, whereas at least eight have already been identified in humans.

In addition to the large number of genes encoding channel and receptor subunits, there are several other levels at which diversity can arise. One is alternative splicing; some of the new K^+ channel genes discovered in *C. elegans*, for instance, can give rise to six or seven different isoforms. Another is RNA editing, a remarkable process by which single base changes (and hence changes in the encoded protein) can be introduced into an already-transcribed mRNA. Editing has been described in the mammalian brain for both AMPA- and kainate-type glutamate receptors, where it is known to regulate ion selectivity and channel kinetics (Rolf Sprengel, Heidelberg; Steve Heinemann, Salk Institute). Robert Reenen (University of Connecticut) has now found that the *Drosophila* Na^+ channel encoded by the *paralytic* gene is also edited at several different sites, and that like the glutamate receptors, *para* editing is under tight developmental regulation. Why RNA editing has been exploited by the nervous system in this way, and in such diverse species, is still unclear; one possibility is that it may allow the expression of two almost identical sequences without the risk of gene conversion.

The greatest source of diversity, however, arises from the fact that most channels and receptors are composed of multiple subunits, which can be assembled in different combinations. In many cases, a given channel can show profoundly different behavior depending on which modulatory subunits are present. Many examples were presented, including K^+ and Ca^{2+} channels as well as all the major classes of ionotropic receptors; to cite just one, Terry Snutch (Vancouver) showed how P- and Q-type calcium currents, long thought to be distinct (both are voltage-gated but they differ in their ability to undergo spontaneous inactivation), can both arise from the same pore-encoding α1a subunit. Moreover, this difference can be caused not only by differential association with regulatory β subunits, but also by alternative splicing of the α1a subunit itself. The properties of cloned channels must generally be studied in heterologous expression systems, but just because a particular subunit combination can form *in vitro* does not necessarily mean that it occurs in the brain. The process of determining which subunits associate with which others *in vivo* is long and laborious, yet essential if the lessons from recombinant channels *in vitro* are to be extended to real neurons.

The number of channels and receptors that can be encoded by the genome is thus enormous, and although this may be good news for pharmaceutical companies, it presents a daunting prospect for any attempt to understand the underlying principles of brain organization. The challenge, of course, is to determine what this prodigious molecular diversity might signify in functional terms. The easy answer is that the brain is very complex and that it needs a correspondingly vast number of molecules to perform its diverse functions; but although this may be true, it is hardly satisfying.

It is possible, of course, that not all the observed diversity has any adaptive significance. Gould and Lewontin have warned against the uncritical acceptance of adaptive explanations in biology, and it is at least possible that some of the diversity that has arisen among different families of channels and receptors has no purpose – genes may duplicate and diverge in evolution simply because they can, in other words because there is no selective disadvantage to doing so and because the process is not easily reversed once it has occurred. To take one simple scenario, imagine that a gene encoding a particular channel undergoes duplication, and that the two sequences drift apart and acquire differences in their promoters; although they may at first be mutually redundant, if certain populations of neurons lose

the ability to express one or the other gene, they both become essential even if they differ very little in their functional properties.

One way to address the question of adaptive significance is to ask whether individual members of gene families show conservation in evolution. Broadly, the answer so far seems to be that they do. For instance, Salkoff noted that the major classes of potassium channels that exist in humans all have recognizable homologs in *C. elegans*, suggesting that the evolution of more complex nervous systems has not been accompanied by the appearance of new types of channels, but rather by diversification of pre-existing types. Moreover, even the individual family members often show high conservation; the human Slo1 gene (which encodes a high-conductance calcium-gated K^+ channel), for example, is much closer to nematode Slo1 than to human (or nematode) Slo2, and the same principle holds for other classes of K channels. Perhaps the most striking demonstration of functional conservation was the worm homolog of one of the Long QT-type K^+ channels, mutations of which lead to abnormal heart rhythms in humans. The worm KQT homolog is expressed in the pharynx, which like the heart generates a rhythmic pumping action. Moreover, when the human mutation is introduced into the worm coding sequence, the mutant animals show a defect analogous to the human condition, a 'long pharyngeal pump syndrome', as it were. Examples like this offer hope that comparing model systems will reveal some general principles of how different patterns of channel expression determine the properties of different classes of neurons.

The current favorite method for determining the function of a channel is to knock it out genetically, but it was clear from many of the presentations that this approach has serious limitations. Although gene knockouts avoid the problems associated with lack of specificity in pharmacological blocking agents, they raise interpretational problems of their own. Often, mutant phenotypes are either nonexistent or too subtle to be recognized using the available techniques. Even in cases where a mutant phenotype is found, it is often difficult to rule out the possibility that the absence of the gene during development has led to compensatory changes that complicate the interpretation of the result. The ideal gene-knockout method would be cell-type specific and under tight temporal control. But despite several apparently encouraging reports in the literature, such techniques are far from robust, as Peter Seeberg (Heidelberg) emphasized. At present, the technology does not exist to inactivate specific genes in specific parts of the mammalian nervous system with high efficiency and specificity, let alone in a rapidly inducible or reversible manner that would eliminate concerns about developmental compensation. A reliable method for doing this would be invaluable, but it does not yet seem to be close to realization.

The challenge in understanding ion channel function may be reduced to two broad questions: what do specific channels contribute to the behavior of the cells in which they are expressed, and how does the behavior of these cells contribute to the working of the system as a whole? Although certain mutations have given interpretable and interesting phenotypes, there are major obstacles to be overcome before this can be achieved on a routine basis. For one thing, the sheer effort of descriptive analysis will be considerable. It will be essential to correlate ion channel expression with single-cell properties, and this is far from trivial *in vivo*. In situ hybridization or antibody staining can provide information about expression patterns, but it is also necessary to correlate this with cellular physiology. One powerful approach, discussed by Hannah Monyer (Heidelberg), is to record from single neurons via a whole-cell patch pipette and then to aspirate the contents of the cell into the pipette, so that mRNA expression can be analyzed by PCR. Monyer has used this technique to show that principal neurons and interneurons in the primary visual cortex show different patterns of AMPA receptor expression, and she hopes to determine how specific patterns of channel and receptor expression can be related to cortical information processing.

Ultimately, it seems clear that to understand how channels and receptors determine neuronal behavior, the field will have to go beyond the level of molecular description and adopt a more quantitative and biophysical approach. This is perhaps the greatest challenge for the years ahead. To explain the electrical properties of a neuron, it is not sufficient merely to specify the types of channels it expresses; one must also know their densities and distributions, as they relate to the fine structure and cable properties of axons and dendrites. Such an analysis would seem essential for any serious attempt at understanding channel function at the cellular level, but surprisingly the question hardly came up in the meeting.

Consider, for instance, how a neuron might achieve the appropriate number and distribution of each of the channels it expresses. To obtain the desired pattern of excitability, there must presumably be some form of feedback from activity to channel expression. Yet how this might occur is almost entirely mysterious. To what extent is channel density regulated by activity, and if so by what feedback pathways? At what level is control exerted? It could be transcription, or at post-transcriptional levels such as protein synthesis, degradation, trafficking or association with modulatory proteins. In muscle fibers, the distribution and turnover times for different types of acetylcholine receptors are regulated with great precision, both during development and in response to changing patterns of electrical activity, but whether this is also true for neuronal receptors and ion channels is still very unclear.

Not only the number but also the precise localization of different molecules must in some cases be specified. Efforts to understand this were exemplified by the presentation from Ole Ottersen (Oslo), who has used immunogold labeling to study the fine structure of cerebellar Purkinje cells. He has shown that different molecules are targeted to different sites; the δ2 glutamate-like receptor, for instance, is present at the postsynaptic sites formed with parallel fibers but not with climbing fibers. Another molecule that is precisely localized in these cells is the glutamate transporter EAAT4, which is known to play a role in clearing glutamate and shortening the EPSC. How it does so is unclear; Ottersen has shown that the main site of EAAT4 expression is at the base of dendritic spines, close to the site of contact with glial cells and several microns away from the postsynaptic membrane where ionotropic glutamate receptors are concentrated. How EAAT4 can affect synaptic activation given its exclusion from the site of transduction remains to be determined, but the results highlight the importance of precise molecular descriptions of synaptic structure if the details of synaptic transmission are to be understood in quantitative terms.

The molecular basis of this structural specificity is even less clear, but some details are starting to emerge. Morgan Sheng (Massachusetts General Hospital), Mary Kennedy (Caltech) Heinrich Betz (Frankfurt) and Nat Heintz (Rockefeller University) each discussed molecular components of postsynaptic sites, and have identified various molecules that may govern how receptors and channels become localized. An important goal now is to determine how these various components interact, and how the appropriate density and distribution of synaptic signaling components is achieved and maintained. In the longer term, it will also be important to find out whether similar mechanisms regulate channel distribution elsewhere on the membrane, and thus whether they play a more general role in regulating neuronal excitability.

New York Academy of Sciences conference: *Molecular and functional diversity of ion channels and receptors*. New York, May 14-17, 1998. See http://www.nyas.org/brochion.html for program details.