Ion channels: **Structure of a molecular brake**

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A combination of crystallographic and mutagenesis studies on the HERG K^+ channel, a key determinant of cardiac excitability, has suggested how the protein's extramembraneous amino-terminal domain might act as a 'molecular brake' that slows down channel deactivation.

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Voltage-gated K^+ channels, known as Kv channels, play a key role in the physiology of excitable cells. In particular, they are responsible for the repolarising phase of action potentials, in which the membrane potential is restored to its 'resting' value. The activation and conductance properties of the various different Kv channels in the membrane of an excitable cell help to determine the overall shape of its action potentials. The past year has seen considerable progress in our understanding of the three-dimensional structure of K^+ channels. Kv channels are tetrameric, and each of the four subunits contains six transmembrane helices, named S1 to S6 (Figure 1a). S4 is the voltage sensor, initiating the voltage-induced conformational changes that result in channel opening or 'activation'. Helices S5 and S6 plus the intervening P-loop form the pore-lining domain of each subunit.

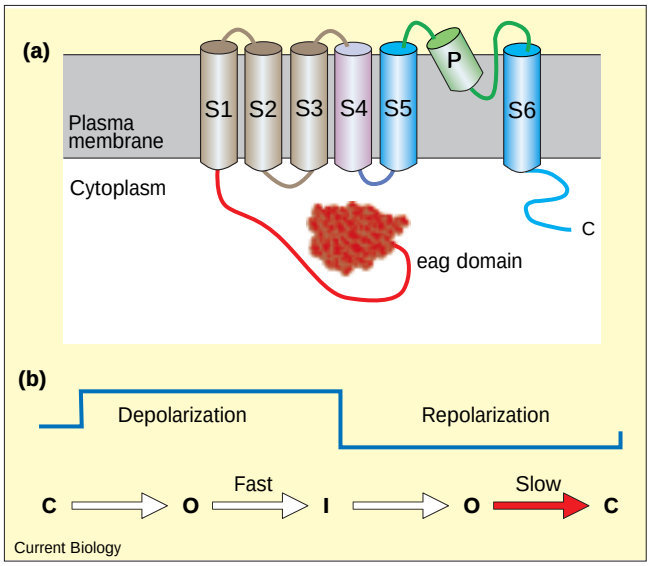
As reviewed in these pages [1], last year saw reports of atomic resolution structures for the pore domain of a bacterial K^+ channel [2] — equivalent to the S5–P–S6 region of a mammalian Kv channel — and for the amino-terminal domain that determines the pattern of subunit association in the tetrameric Shaker K^+ channel [3]. Now MacKinnon and colleagues [4] have determined the structure of a very different K^+ channel amino-terminal domain. This so-called eag domain controls the gating kinetics of the HERG channel (Figure 1a), a member of a distinct family of Kv channels. This provides us with a structural insight into some of the complex dynamics of these integral membrane proteins.

HERG is a K^+ channel found in human cardiac muscle and neurons [5]. Defects in HERG are associated with LQT2, one form of the 'long QT syndrome', a genetic disorder that leads to cardiac arrhythmia and sudden death [6]. The biomedical importance of this is evident given that cardiac arrhythmias cause about a third of a million deaths each year in the USA alone. A detailed understanding of the molecular mechanism(s) of LQT2-inducing defects will be required before any rationally designed therapy for the syndrome can be attempted. The structure reported by MacKinnon and colleagues [4] is a key step towards understanding the gating properties of HERG channels at the molecular level. This structure enables rationalisation of the effects of mutations that alter the gating properties of HERG channels. Curiously, the HERG channel's eag domain turns out to have a similar fold to the 'PAS' domain that is characteristic of a family of apparently unrelated 'sensor' proteins.

Function of the eag domain

One of the key properties of the HERG channel is that of slow deactivation [7] (Figure 1b). On depolarisation, a change in membrane potential from about –80 mV to +10 mV, HERG channels open transiently and then rapidly inactivate to a non-conducting state. On repolarisation, when the membrane potential returns to to the

Figure 1



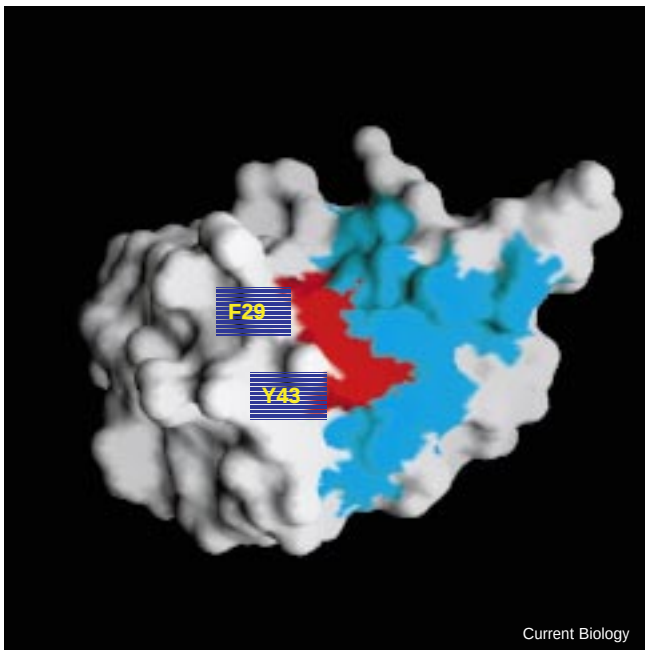
(a) Topology of a HERG channel subunit, showing the six transmembrane helices S1–S6 (bronze, purple and cyan), the P-loop (green), and the amino-terminal eag domain (red). The functional channel is formed by four such subunits, the pore being formed by association of four P-loops. (b) The cycle of conformational changes that the HERG channel undergoes during membrane depolarisation and subsequent repolarisation. The blue line indicates the changes in membrane potential. The conformational states of the channel are: C, closed channel; O, open channel; I, inactive channel. The eag domain regulates the rate of open-to-closed transition (red arrow).

'resting' value of –80 mV, HERG channels have to pass back through the open (conducting) state before closing. The open-to-closed transition, referred to as deactivation, is relatively slow. The slow deactivation kinetics determine the length of action potentials involving HERG and thus control cardiac excitability.

The amino-terminal eag domain controls the rate of deactivation of the HERG channel. This protein's first transmembrane helix, S1, starts at about residue 390, and its cytoplasmic eag domain is formed by the amino-terminal 135 residues. The eag domain contains the 'signature' sequence that relates HERG to the subfamily of Kv channels defined by the *Drosophila* channel 'ether-a-go-go', from which domain's name is derived. This region does appear to form a distinct folding unit — and so is a domain *sensu stricto* — as evidenced by its expression in *Escherichia coli* and subsequent crystallisation and structure solution. When the eag domain is deleted, the resulting mutant channel undergoes faster deactivation than the wild-type protein. When the eag domain is coexpressed with the deletion mutant in *Xenopus* oocytes, wild-type deactivation kinetics are slowly recovered.

These observations suggest that the eag domain is responsible for the slow deactivation kinetics of HERG channels — in other words, that the eag domain acts as a 'molecular brake' on the conformational change that underlies deactivation. The slow recovery of native deactivation kinetics in the coexpression experiment suggests that the eag domain is rather tightly associated with the remainder of the protein. Experiments on rat eag channels [8] have shown that deletion of their amino-terminal domain results in slow (HERG-like) deactivation, which can in turn be reversed by a point mutation in the S4 transmembrane helix. This suggests that, in channels of this type, the S4 segment — the voltage sensor of Kv channels — interacts with the amino-terminal domain in a way that controls the rate of deactivation. The eag domain thus modulates HERG gating kinetics in a physiologically significant fashion.

Figure 2



A representation of the molecular surface of the eag domain, generated from the domain's recently determined crystal structure [4] using the program GRASP. The hydrophobic surface patch is shown in cyan, with the two key residues identified by mutational analysis – phenylalanine 29 and tyrosine 43 – in red (see text for details).

Relating structure to function

The crystal structure reported by Cabral *et al.* [4] shows that the eag domain of the HERG channel protein forms a five-stranded, anti-parallel β sheet flanked on either side by two α helices, with a single turn of a 3_{10} helix stacked against the sheet. The first 25 residues of the domain are disordered within the crystal so that their structure cannot be seen; interestingly, this is the most highly conserved region of the domain and is of great functional significance (see below). For this reason, at least, the crystal structure may not be able to answer all of our questions about the mechanism of regulation of HERG deactivation.

Cabral *et al.* [4] examined the effects on the HERG channel deactivation kinetics of 15 point mutations scattered across the eag domain surface, as well as of a number of deletion mutations. Most mutations had little effect, but deletions of residues 2–9, 2–23 or 2–26 were found to cause accelerated deactivation. Two point mutations also caused accelerated channel deactivation: substitution of either phenylalanine 29 or tyrosine 43 by alanine. Both of these point mutations affect residues within a distinct hydrophobic patch on the surface of the eag domain (Figure 2). It seems likely that this patch is involved in binding the eag domain to the rest of the channel protein — possibly via interactions with the S4 helix — and that this binding normally slows down channel deactivation. Notably, deletions 2–23 or 2–26 had a greater effect on channel deactivation than either of the point mutations. However, as mentioned above, the first 25 residues of the eag domain are highly conserved but disordered in the crystal. Perhaps these key residues do adopt a defined conformation when they interact with the remainder of the protein, in particular the S4 helix.

One curious aspect of the eag domain's fold is its close similarity to that of photoactive yellow protein, a bacterial light-sensing protein. Photoactive yellow protein is a member of the PAS domain family, to which the HERG domain had been suggested to belong on the basis of sequence analysis [9]. The work of Cabral *et al.* [4] on the

eag domain of the HERG channel protein has thus provided the first structure of a eukaryotic PAS domain. PAS domain proteins have a bewilderingly diverse range of functions. In prokaryotes they play a sensory role, for example in the detection of light levels or redox potentials. In eukaryotic cells PAS domain proteins are involved, for example, in the control of circadian rhythms. How the action of the PAS domain in these various contexts relates to that of the HERG eag domain is far from obvious. It is possible that evolution has recycled the PAS domain fold for a very different biological role in the HERG channel.

The new work on the eag domain of the HERG channel protein [4] is significant in two respects. Firstly, it has revealed the structure of an extramembraneous channel domain which helps to shape the cardiac action potential and is essential for normal cardiac excitability. Secondly, it illustrates how structural biology and molecular physiology may be used in a concerted fashion to dissect structure–function relationships of channel proteins in a manner that would have been barely conceivable a few years ago. The resulting picture is incomplete, however. We still do not understand the nature of the structural transitions involved in channel gating. To complete the picture, we will need not only the structure of an entire Kv channel, but also snapshots of such a channel in its various conformational states.

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