*Commentary*

# Mutations, Molecules, and Myotonia

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The linkages between a protein's primary sequence, three-dimensional structure, and the detailed manifestations of its function are well appreciated. Exploring and understanding these linkages have proven remarkably complex, however. While some functions may be localized exclusively to one protein region, others may be distributed or even diffusely coded in the structure.

The kinetics of the tetrodotoxin-sensitive $Na^+$ channel and its voltage-gated siblings provide many examples of such complex and distributed coupling. The channel consists of four domains, each a repeat of the fundamental unit that is the primary motif of the voltage-dependent $K^+$ channel. In each domain, six membrane-spanning regions (S1–S6) give the channel its essential form, with charged groups embedded within these regions, presumably imparting the channel's characteristic sensitivity to membrane potential. Two sets of hydrophilic loops are important for function: One set of loops (the S5-S6 linkers in each domain) on the extracellular side line the pore region; another such loop, between domains III and IV on the cytoplasmic side, is critical for fast inactivation. Clearly, the various elements of this channel's gating and permeability are linked to its detailed structure; alteration of charge in the S4 segments changes activation (Stühmer et al., 1989), and alteration of hydrophobicity in the cytoplasmic linker between domains III and IV slows inactivation (West et al., 1992). Neither observation, however, is unambiguous regarding mechanism. Not all S4 charges give the same functional alteration in channel activation, and noncharged residues in these and nearby regions also affect the voltage dependence of gating. This observation is indeed expected if the underlying conformational changes involve sliding or rotational motions of domains, which would be sensitive to a wide variety of interactions at their interfaces.

Inactivation properties, too, are determined by a broad variety of structures, which may be physically and functionally removed from the III-IV linker itself, as exemplified by a set of naturally occurring $Na^+$ channel mutations. Familial skeletal muscle disorders such as hyperkalemic periodic paralysis and paramyotonia congenita have been linked to alterations in the $Na^+$ channel gene (Fontaine et al., 1990; see Rüdel et al., 1993, for review) and subsequently localized to diverse sites including the cytoplasmic end of the S6 segments of domains II and IV, the S5 segments in domains II and III, as well as to changes in the extracellular ends of domain IV's S3 and S4 segments (see Fig. 1 of Hayward et al., 1996, in this issue). How could such diverse changes give rise to altered inactivation, and concomitant disease? This question is examined in this (Hayward et al., 1996) and the February (Ji et al., 1996) issues of *The Journal of General Physiology*.

One hypothesis to explain such distributed participation in inactivation is that this reaction is analogous to a pharmacological blocking reaction, as originally proposed by Armstrong (1970). While $Na^+$ channels may not have a ball at the end of a chain, binding of the III-IV linker to the inner channel vestibule may serve the same function. Such binding would involve a specific reaction that would ultimately depend, like a jigsaw piece fitting into its designated spot, on the detailed structures of both the linker and its binding site. Mutations, both proximal and distant, that altered either structure would impact on the channels' inactivation kinetics, while spontaneous rearrangements of their tertiary or quaternary structure might cause transient and reversible changes in reaction rates (so-called "mode" shifts). Moreover, inactivation is in turn linked to movement of one or more activation gates, so mutations or modes in these gates or in regions that affect their kinetics would indirectly influence inactivation properties.

Building a more complete understanding in the face of such complex linkages is one of the major challenges one faces in these studies. Several strategies have now emerged to aid our understanding of structure–function links. One approach, pioneered by Horn and others (e.g., Yang et al., 1996), probes cysteine availability in S4 and other channel regions as a function of membrane potential. Such studies provide new, intimate views of the inner workings of the target channel by showing how certain portions of the S4 shift their accessibility from the cytoplasmic to the extracellular side of the channel with depolarization.

Another important approach seeks to pair specific mutations in a manner that allows them to compensate

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for each other, providing a much higher degree of certainty regarding points and conditions for intramolecular interactions (Carter et al., 1984). The pioneering work of Miller and co-workers (Goldstein et al., 1994; Miller, 1995) in pairing mutations in $K^+$ channels and their toxins, and of Papazian et al. (1995) on intragenic suppression/rescue mutagenesis in gating regions of *Shaker* K channels have illustrated the power and specificity of such approaches in ion channel research. The recent report by Depp and Goldin (1996) of functional coupling of the inactivation gates' "latch" to an appropriate binding site illustrates as well the power of such studies in $Na^+$ channels and helps to confirm the inactivation hypothesis described above.

Ji et al. (1996) use a similar approach to cast light on the functional coupling of activation to inactivation in $Na^+$ channels. They studied the kinetic alteration of two naturally occurring paramyotonia congenita mutation sites of the human skeletal muscle gene. An earlier report (Chahine et al., 1994) detailed the changes seen when the outermost arginine-1448 of domain IV-S4 was mutated to remove its charge. Inactivation was slowed, recovery was enhanced, and voltage dependence was partly lost, presumably because of the functional linkage of IV-S4 to the movement of the distant inactivation gate itself. In their most recent report, these investigators show that altering the closely apposed IV-S3 leucine-1433 also prominently affects inactivation. Here hydrophobicity plays the dominant role. Most interesting, however, was the result of combining the two mutations. In this case they were neither additive nor compensatory, but instead mimicked one or the other single mutation, depending on conditions. IV-S4 appears critical for inactivation of the open channel, while IV-S3 is more important for the closed to inactivated reaction.

Although such ingeniously designed pairs of mutations give us a clearer appreciation for linkage within channels, nature is much more capricious in generating mutations in our own channels. Here it is far more critical how such random alterations link to cell and tissue function in the phenotypic appearance of genetic disease. In this issue, Hayward et al. (1996) report major steps in elucidating how subtle variations in inactivation lead to distinctly different disease states. They examine two naturally occurring mutation sites on the III-IV linker and describe in detail how these mutations alter inactivation onset, steady state, recovery, voltage dependence, and $K^+$ or temperature sensitivity. By using their experimental observations in a cellular model system, they show that these distinct structural changes produce behaviors that distinguish the mutations' clinical manifestations. Mutations that shift the steady inactivation curve to more depolarized potentials, like the G1306A/V/E mutations studied here, cause a destabilization of the membrane potential, repetitive firing, and the concomitant stiffness of myotonia. Conversely, those disorders that cause substantial persistent $Na^+$ current lead to a prominent symptom of muscle weakness or paralysis via depolarization and extensive channel inactivation. This latter category includes the T1313M also described here, which has symptoms of both myotonia and weakness.

The sequence–function linkages developed in these and other studies advance our understanding of both myotonia and of the inner workings of the $Na^+$ channel. These studies, however, also highlight the degree of complexity and the subtleties in channel gating, especially that one cannot pinpoint a given function to a very delimited part of the channel structure. The "normal" $Na^+$ channel inactivation depends, ultimately, on the movement of one or more activation gates, possibly structural rearrangement(s) of the channel pore or the III-IV linker region itself, and docking of the linker region to functionally close the channel. Reaction pathways may be different for open and closed channel inactivation, and particular steps may be dominant depending on voltage, temperature, or mutagenic status. Thus, each of these new studies is a jigsaw piece, linked with the work of many other labs, building a functional picture of $Na^+$ and other channels.

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