Commentary

ClC Channels: Reading Eukaryotic Function through Prokaryotic Spectacles

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It's a truism that structures are helpful for mechanistic understanding of protein function, and nowhere is this dictum better illustrated than with ClC-type Cl^- channels, a large molecular family found in virtually every type of cell in every biological niche. These channels, first observed over 20 yr ago (White and Miller, 1979; Miller, 1982), are now known from a barrage of knockout studies (Jentsch et al., 2002) to maintain the smooth operation of many varied physiological systems: skeletal muscle excitability, inhibitory interneuron responses, renal control of blood pressure, endosome acidification, and, well, the list goes on and on. Although the first ClC channel was cloned nearly 15 yr ago (Jentsch et al., 1990), our physical and mechanistic picture of these proteins at the molecular level languished at a frustrating level of murkiness until the first high-resolution structure of a ClC homologue burst onto the scene two years ago (Dutzler et al., 2002). Before this, we knew nearly nothing worthwhile about the molecular determinants of channel gating or selectivity; even the number of Cl^--permeation pores in the homodimeric channel was in contention (Maduke et al., 2000). This situation changed abruptly after the identification, overexpression, and functional reconstitution of a bacterial ClC channel (Maduke et al., 1999), followed by its crystallization and high-resolution structure determination (Dutzler et al., 2002, 2003). Suddenly, the pore became visible, with two Cl^- ions sitting in it close to each other, mostly dehydrated, and engulfed by specific coordinating groups. Also evident was the fact that these ions are completely buried within the protein, i.e., that the structure is of a closed conformation. This structural work led to the proposal that the gate is merely a single glutamate sidechain located on the extracellular side of the two buried ions; in the closed channel, the glutamate occludes the pore, and when the channel opens, this sidechain rotates out of the way, according to this idea.

In this issue, T.-Y. Chen and coworkers (Chen and Chen, 2003; Lin and Chen, 2003) attack the first question naturally arising from the structural work: how well does the bacterial channel structure help us understand the function of eukaryotic ClC channels? Can we read the electrophysiological behavior of eukaryotic ClCs in terms of the structure? This is a pertinent question since prokaryotic ClCs are stripped-down versions of their eukaryotic relatives; they are about half the mass and lack the large COOH-terminal intracellular domain common to all eukaryotic members of the family. We know that the bacterial channel is activated by low pH (Iyer et al., 2002), but we do not know if it shares with the eukaryotic channels features such as voltage dependence and Cl^--activation. An unfortunate problem here is that up until now, no electrophysiological recordings of the bacterial channel have been accomplished, and only a single eukaryotic ClC channel, ClC-0, has a unitary conductance large enough to be amenable to full electrophysiological analysis. So Chen and coworkers plunged into a detailed study of ClC-0 to see if its conduction and gating properties respond to mutations as anticipated from the bacterial channel structure.

These two papers are separately aimed at Cl^- permeation and channel gating. The bottom line is that on both of these fronts, ClC-0 seems to behave in harmony with the bacterial channel structure, at least in broad outline. The first paper (Chen and Chen, 2003) describes a series of electrostatic mutations in the region of the selectivity filter, i.e., at positions equivalent to those in the bacterial channel close to the two bound Cl^- ions. We can crudely visualize this region, located toward the intracellular side of the protein, as a rather narrow kitchen-funnel held upside down, in which the completely dehydrated "central" ion is bound in the neck, and the partially hydrated "internal" ion resides right at the point where the funnel begins to open toward the cytoplasm. The wall of the widening funnel is decorated with several charged and polar groups known to influence Cl^- permeation, and the influence of these groups on single-channel currents are examined in great detail here. In particular, Chen and Chen (2003) take an enzymological approach by heroically measuring single-channel conductance over a very wide range of Cl^- concentrations, from 20 mM to >1.5 M, a range over which conductance saturates in a simple Michaelis-Menten fashion. This allows K_m and V_{max} effects of mutations to be distinguished. To my mind it is remarkable that these experiments could be done at all; five residues within this intricate selectivity region could be mutated singly and in pairs while preserving

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familiar ClC gating and selectivity and producing only small (<10-fold) effects on absolute unitary conductance. (You cannot do this to K^+ channels without killing or severely debilitating them; I reckon that the Cl^- channel's mutagenic acquiescence reflects ionic coordination largely by flexible sidechains, rather than the backbone coordination of K^+ channels.) A remarkable result emerging from this study is the behavior of Lys519, a residue recognized early on (Pusch et al., 1995; Middleton et al., 1996) to influence permeation. This position, which lies near the cytoplasmic entrance to the funnel, exerts a simple charge effect on conductance, which is high for any positively charged group, intermediate for any neutral residue be it large or small, polar or nonpolar, and small for negative residues. This smells like a through-space electrostatic effect (or, in alternative language, an effect of "local concentration" of the conducting ion), and Chen and Chen (2003) find that mutations here strongly alter K_m but leave V_{max} untouched, as expected from such a picture. But they discover a perplexing fly in the ointment: these electrostatic effects are completely unaffected by raising ionic strength with a nonconducting ion like $SO_4^=$. That is an unprecedented result in any ion channels, and its explanation remains elusive. Even more puzzling is the dramatic result that the strong electrostatic influence of position 519 is completely lost in a mutant channel in which nearby glutamate residue is neutralized. Nevertheless, the overall picture arising from these structure-guided mutations is that the selectivity region of ClC-0 is an electropositive confined space, as in the bacterial homologue.

The second paper (Lin and Chen, 2003) asks whether gating of ClC-0 can really be as simple as proposed for the bacterial channel—the movement of a glutamate sidechain out of its extracellular pore–blocking position. A first guess is that opening of ClC-0 can't be this minimalistic, since it is known to be elaborately linked to voltage and the movement of Cl^- ions within the channel's preopen states (Chen and Miller, 1996). This doubt provides the motivation for testing whether channel opening might also involve deocclusion of the pore on the cytoplasmic side. In the spirit of much previous work on other channels (Yellen, 2002), the authors substitute cysteine residues at various pore positions and measure the rate of chemical modification (reported by functional changes) to probe the accessibility of this region. The results are roughly consistent with expectations of the bacterial channel structure, with residue equivalents projecting into the pore more chemically reactive than buried ones, and with negatively charged reagents more reactive than positively charged ones. Most importantly, a cysteine-substituted selectivity-filter residue located between the two Cl^- ions is equally reactive to intracellular reagents in open and closed states. This result suggests that whatever structural changes may occur in ClC-0 upon opening, these do not involve a gate located intracellular to the selectivity filter, in accord with the simple gating model proposed on the basis of the bacterial channel structure.

I'll conclude with a bit of editorializing. There is a massive amount of hard-won experimental data here pointing clearly to the basic conclusion drawn: that the structure of the bacterial ClC channel does indeed provide a helpful roadmap toward understanding electrophysiological behaviors of its larger and more complex eukaryotic congeners. But it is likely that gating in ClC-0 goes beyond the local movement of an extracellular-facing glutamate, as in the bacterial channel. As Chen himself has noted (Chen, 2003), voltage dependence in ClC-0 arises from an extracellular Cl^- ion entering deeply into the pore in the channel's closed state, after which the opening reaction occurs. In addition, in a paper to be published next month, Accardi and Pusch (2003) argue from their finding that certain hydrophobic pore blockers bind with strong preference to the closed conformation that a substantial conformational change must occur upon opening of ClC-0. So I think that there will be a lot of surprises ahead in our efforts to understand the delightful and unprecedented mechanisms of operation of ClC channels.

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