Cell biology

# Chloride channels are different

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Proteins that conduct chloride ions are vital for a range of cellular processes. The long-awaited crystal structure of a chloride channel shows what these proteins look like, and gives hints about how they work.

Ion channels are proteins with a seemingly simple task — to allow the passive flow of ions across biological membranes. But this process requires more sophistication than one would imagine. Channels must be highly selective for a particular type of ion, yet must also maintain high transport rates and be able to regulate ion flow by switching it on or off (a process called 'gating'). Most attention has been directed towards cation channels, probably because of their role in generating electrical signals in nerve cells. Three years ago Roderick MacKinnon and colleagues revealed the crystal structure of a bacterial potassium channel[1] — a representative of a large family of cation channels. In another spectacular breakthrough, described on page 287 of this issue[2], the same group now reports the crystal structures of two bacterial chloride channels. They prove to be amazingly different to cation channels.

These new structures[2] are very welcome, in part because surprisingly little is known about how chloride ($Cl^-$) channels work. We do know that they come from several gene families[3], and that many are gated by voltage (electrical differences across the membrane in which the channel sits). On the basis of elegant biophysical studies by Miller and White[4], who showed that the electric ray *Torpedo* contains large amounts of a peculiar anion channel, the first voltage-gated $Cl^-$ channel was cloned by my group[5] in 1990. We named it ClC-0, as we assumed that it would found a family of $Cl^-$ channels. This turned out to be true: CLC channels are found in all kingdoms of life, with humans alone having nine different CLC genes[3].

Some mammalian CLC channels are found in the outer (plasma) cell membrane, whereas others reside in membranes of intracellular organelles. Plasma-membrane CLC channels may stabilize the voltage of excitable cells such as muscle cells, or transport salts across cell layers; for example, mutations in ClC-Kb or its associated subunit[6] impair salt reabsorption in the kidneys[3]. Intracellular $Cl^-$ channels may counterbalance the electric current produced by proton pumps, and mutations in ClC-5, for example, impair the transport of normally acidic vesicles in a kidney disease[7]. The function of bacterial CLCs such as those now crystallized[2] is not yet clear.

What about the structure and mechanism of the CLC channels? Initial insight came from biophysical analyses of mutated channels; such studies suggested that CLCs consist of two identical proteins (or subunits), each with its own pore[8–10]. But, as might be expected, this architecture has made it hard to identify specific amino acids that contribute to the pores — mutations that changed the ion-permeation properties of CLC channels were scattered over much of the amino-acid sequence, and their effects were not very pronounced. (This contrasts with tetrameric $K^+$ channels, in which four identical segments from each subunit come together to build a single pore.) Another complication was that almost all mutations that changed the properties of the CLC pore also affected gating[11].

No wonder, then, that the ion-channel community has been eagerly awaiting a high-resolution crystal structure of a CLC channel. A first step was made last year[12], with a cryo-electron microscopic analysis of two-dimensional crystals of the bacterial EcClC protein at 6.5-Å resolution. The study confirmed that CLC channels are homodimers, and suggested that two areas of lower electron density off the central axis represented two pores.

Dutzler *et al.*[2] now reveal the three-dimensional crystal structure of two bacterial CLC proteins in unprecedented detail. Their high-resolution (3.0 Å) X-ray analysis allows peptide helices, amino-acid side chains and, amazingly, even the $Cl^-$ ion within the pore to be identified. The crystal structure should convince remaining sceptics[13] that CLC channels are double-barrelled: two proteins contact each other at a broad interface formed by four helices from each protein. The two pores are not found at this interface, but are completely contained within each subunit, exactly as deduced from the mutational analyses[8,10]. This contrasts starkly with many other channels, in which four or five identical or structurally related subunits jointly form one pore (Fig. 1). But channels with several pores are not as exotic as they might seem; for instance, aquaporin water channels are quadruple-barrelled. The physiological advantage of having several pores is not clear in either case.

The structure[2] also helps to explain why previous biochemical analyses of CLC-channel topology produced confusing results. If one looks at the intermingling of helical protein segments in the structure, it's no wonder. These segments often do not cross the entire width of the membrane, and are often severely tilted rather than perpendicular to the membrane (Fig. 2). Each subunit has an internally repeated pattern, not recognized previously, with the two parts having opposite orientations in the membrane (an 'antiparallel' architecture).

At the narrowest part of the pore, there is no positively charged amino-acid side chain that could lead to strong electrostatic binding of $Cl^-$ ions. Otherwise, $Cl^-$ would stay put instead of moving on through the pore. Instead, $Cl^-$ is coordinated by amino acids at the ends of several helices ('D', 'N' and 'R') that, if electrically polarized, would interact with it favourably, but not too strongly. In $K^+$ channels, the ion is coordinated by amino acids at the ends of four parallel helices coming from the extracellular side. But in the CLC channels[2] the helices are antiparallel, coming from both sides of the membrane to coordinate $Cl^-$ next to the central plane of the membrane.

Surprisingly, just above the chloride-binding site there is a negatively charged side chain of a glutamate amino acid (Fig. 2), which would be expected to hinder ion transport severely. Dutzler *et al.*[2] speculate that this side chain acts as a gate, swinging out to open the channel. Conceivably, this movement could be facilitated by electrostatic repulsion when a $Cl^-$ ion enters the pore. This provides an attractive, though speculative, explanation for the observed gating of ClC-0 by anions[11].

Many residues that turn out to line the pore[2] were in fact mutated in earlier studies. For instance, subtle mutations[8,14] of the key

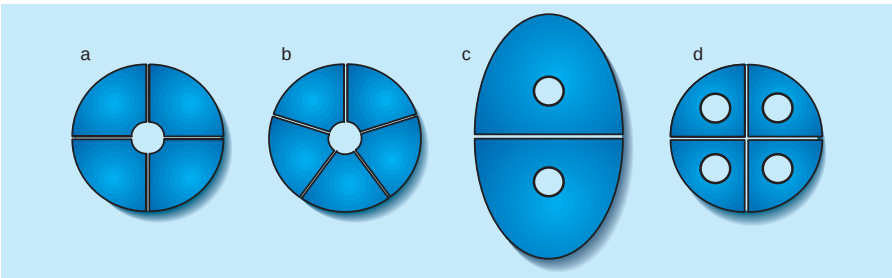


Figure 1 **The different ways in which ion channels composed of more than one protein can form pores.** a, **In tetrameric potassium channels, a single pore is formed by four identical or structurally similar proteins (subunits).** b, **In 'ligand-gated' cation or anion channels (such as channels that detect the neurotransmitters acetylcholine or GABA), the single pore is formed by five identical or structurally similar subunits.** c, **Chloride channels from the CLC family are dimers, in which each subunit has its own pore.** d, **Aquaporin water channels are tetramers, again with one pore per subunit.**

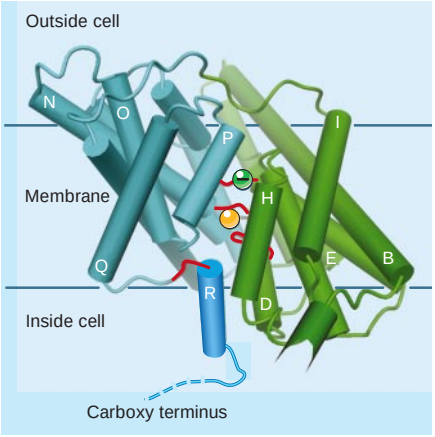


Figure 2 **Chloride-channel structure, as revealed by Dutzler *et al.*[2]. This is a side-on view of one of the two subunits of a CLC channel, looking at the side that interacts with the other subunit. α-Helical structures are indicated by cylinders, coloured in green and cyan to indicate the two 'repeated' units. Regions directly involved in formation of the ion-permeation pore are in red. A negative charge that might be involved in gating the channel is shown as a green circle; it is just above where the chloride ion (shown in yellow) is coordinated. The amino terminus of helix R (bright blue) contains a tyrosine amino acid, which helps to coordinate the $Cl^-$ ion; the carboxy terminus emerges into the cell. In mammalian CLCs, helix R is connected to a large intracellular region, indicated by the dashed line. Figure modified from ref. 2.**

chloride-coordinating amino acids at the ends of helices D and R changed the channel's ion selectivity, conductance and gating. (But, perhaps surprisingly, the mutant channels were still selective for anions rather than cations.) Intriguingly, the carboxy-terminal end of helix R sticks out into the cell[2] and, in all CLCs from higher organisms, is connected to a large intracellular segment; some mutations in this segment affect gating drastically[15]. So it is tempting to speculate — as Dutzler *et al.*[2] do — that helix R can transduce intracellular events into channel gating. If so, by pure coincidence, 'R' might stand for 'regulatory'.

Finally, CLC channels — unlike $K^+$ channels — do not have a large aqueous cavity at one side of the pore. Instead, the CLC pore has a more symmetrical, hourglass shape, whose internal surfaces (except in the narrowest point) have positive charges to attract $Cl^-$ ions. So the crystal structure of CLC channels has revealed many delightful contrasts to cation channels.

What next? The details of the ion-permeation process, including whether or not several ions occupy the pore region simultaneously[11], remain to be worked out. And the gating mechanism is difficult to understand from the static snapshots provided by crystals, although the negative charge mentioned above is an intriguing candidate for a gate at a single pore. In addition, in at least two non-bacterial members of the CLC family, ClC-0 and ClC-1, there is another gate that closes both pores of the dimeric channel at the same time[4,8,9,16]. This process may require both subunits to change in a way that depends on their extensive intramembrane contacts. Alternatively, intracellular portions of ClC-0 and ClC-1 that are not present in the bacterial channels now crystallized may come into play. Studies of single bacterial CLC channels might be crucial in answering such questions. ■

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Molecular neurobiology

# Priming plasticity

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Nerve cells communicate by using chemical messengers, which are released from neurons after a 'priming' step. It seems that priming may be key to controlling the strength of chemical transmission.

The roots of cognition, behaviour, learning and memory are embedded in the brain's intricate network of nerve cells and their specialized points of contact, the synapses. Synapses can convert electrical impulses into chemical signals and back again, as well as modulate the strength of the transmitted signals. This ability to modify the strength of transmission — known as synaptic plasticity — is thought to be the cellular basis of the brain's ability to compute, learn and remember. A goal of many neurobiologists is to understand the molecular basis of synaptic plasticity. On pages 321 and 327 of this issue, two papers from the same groups provide some answers. Schoch *et al.*[1] and Castillo *et al.*[2] have carried out a series of elegant analyses of a synaptic protein called RIM. In so doing, they show that a key regulatory step in some forms of synaptic plasticity involves 'priming' — preparing the chemical-messenger carriers to release their cargo.

Synapses are made up of the 'bouton' found on the end of the signal-transmitting (presynaptic) neuron; the signal-detecting apparatus on the receiving (postsynaptic) neuron; and the small gap in between (the synaptic cleft; Fig. 1, overleaf). The presynaptic bouton is filled with synaptic vesicles, small membranous vehicles that contain the chemical messengers (neurotransmitters). After a priming step, these vesicles dock at a specialized region of the presynaptic plasma membrane known as the active zone. Electrical impulses arriving at the active zone trigger the fusion of synaptic vesicles with the plasma membrane, releasing their neurotransmitter into the synaptic cleft (Fig. 1). The neurotransmitter then diffuses across the cleft and activates receptors clustered in the postsynaptic plasma membrane, initiating a new wave of electrical signals in the postsynaptic neuron.

This process of synaptic transmission can be enhanced or weakened by changing the probability that neurotransmitter is released in response to electrical activity in the presynaptic bouton, or by altering the size of the postsynaptic response. These processes together determine the overall strength of synaptic transmission. Synaptic strength is activity dependent — it is modulated by the frequency of electric impulses arriving in the presynaptic bouton and the activity of the postsynaptic neuron. Changes in synaptic strength can be transient, lasting milliseconds to minutes (short-term plasticity[3]), or long-lasting, persisting for hours, days or months (long-term plasticity[4]).

The molecular underpinnings of synaptic plasticity, in particular long-term plasticity, are thought to involve changes in the composition or activity of presynaptic and/or postsynaptic proteins. Several forms of long-term plasticity involve postsynaptic changes, whereas many forms of short-term plasticity and at least one form of long-term plasticity require presynaptic changes. Presynaptic changes — alterations in the probability of neurotransmitter release — could theoretically occur at several stages in the release process, such as the docking, priming or fusion of synaptic vesicles. Docking involves tethering synaptic vesicles to the plasma membrane at the active zone[5]. Priming prepares docked