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Commentary

Cyclic nucleotide-gated channels: An expanding new family of ion channels

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Cyclic nucleotide-gated ion channels are relatively new entries in the world of ion channels. Their discovery began with the surprising finding in the mid-1980s that the cation conductance (the "light-sensitive" conductance) mediating visual transduction in retinal rod photoreceptors is directly activated by guanosine 3′,5′-cyclic monophosphate (cGMP) (1, 2). Before then, cyclic nucleotides had been known to affect the functional characteristics of some ion channels, but these were indirect effects mediated by cyclic nucleotide-dependent protein kinases and channel phosphorylations—i.e., along the conventional course of action of cyclic nucleotides (for a recent review, see ref. 3). The discovery of an ion channel directly activated by a cyclic nucleotide thus represented a departure from this rule. When the nature of the phototransduction channel first came to light, it was viewed to some extent as a curiosity, considered perhaps unique to visual transduction. However, a similar channel was subsequently identified in olfactory transduction (4), followed by the same in the pineal gland (5). Furthermore, molecular cloning has provided a powerful way to search for homologous channels in other tissues. For example, a protein apparently identical to the olfactory channel was recently cloned from the aorta (6). In this issue of the *Proceedings*, Biel *et al.* (7) report the cloning of a homologous but distinct channel from the kidney. Concurrently, and reported elsewhere, Weyand *et al.* (8) have cloned the same gene from testis. Thus, this new family of ion channels continues to expand.

To appreciate the functional roles of these channels, one might start with its archetype, the rod phototransduction channel. Retinal rod photoreceptors are known to respond to light with a membrane hyperpolarization, caused by the closure of a cation conductance that is open in darkness and was thought to be selective for Na^+ (for review, see ref. 9). At the same time, light is known to trigger a G protein-mediated signaling cascade that leads to the activation of a cGMP phosphodiesterase and the lowering of cytoplasmic cGMP level (for review, see ref. 10). The exact connection between the light-sensitive conductance and cGMP, however, was unclear for a long time until Fesenko *et al.* (1) found, with an excised patch of rod plasma membrane, that cGMP directly activates a conductance on the membrane. The same conductance was also identified by using ion-flux measurements (11). Soon afterwards, it was established that this conductance and the light-sensitive conductance are indeed identical (2). One surprising feature of this channel that turns out to be of great importance is its high permeability to Ca^{2+}. The inward membrane current through the open channel in darkness is ≈80% Na^+ and 15% Ca^{2+} (with the rest apparently carried by Mg^{2+}); however, because Na^+ is ≈100 times more concentrated than Ca^{2+} extracellularly, the channel really prefers Ca^{2+} over Na^+ by ≈10 to one (12). This permeability to Ca^{2+} is what underlies the ability of the rod cell to adapt to steady illumination (13, 14), a fundamental property of visual function. Briefly, in darkness, there is a balance between the Ca^{2+} influx through these channels and a Ca^{2+} efflux through a $Na^{2+}/Ca^+,K^+$ exchange carrier (15, 16); in the light, closure of the channels stops the Ca^{2+} influx, but the efflux continues, thus causing a decline in the cytoplasmic Ca^{2+} concentration (15). This Ca^{2+} decrease then activates a rather elaborate negative-feedback mechanism to produce light adaptation (for the most recent review, see ref. 17). Shortly after the discovery of the cGMP-activated channel in rods, a similar channel was found in retinal cones, which are the photoreceptor cells responsible for vision in bright light (18, 19). There is, however, a subtle difference in electrical properties between the rod and the cone channels (18), suggesting that the two may be distinct proteins. On the other hand, the Ca^{2+} permeability and its involvement in light adaptation still hold true for the cone channel (14, 20, 21). For both the rod and the cone channels, cGMP is the most effective activating ligand among the various cyclic nucleotides (22), consistent with its being the second messenger in phototransduction. cAMP can also open the channels, but an ≈50-fold higher concentration is necessary (22).

Remarkably, a similar channel turns out to be involved in olfactory transduction (4). In this process, at least some odorants are known to activate a G protein-mediated signaling cascade leading to activation of an adenylate cyclase and production of cAMP, which in turn opens a cyclic nucleotide-activated channel to generate a membrane depolarization (for review, see ref. 23). In this manner, visual and olfactory transductions have a similar motif, although they differ with respect to the cyclic nucleotide involved and the polarity of its concentration change resulting from sensory stimulation. Like the photoreceptor channels, the olfactory channel is highly permeable to Ca^{2+}, a feature again important for sensory adaptation, which involves, in this case, a rise in cytoplasmic Ca^{2+} upon odorant-induced channel opening (24). Unlike the photoreceptor channels, however, the olfactory channel requires much lower ligand concentrations (4). Even though cAMP is the second messenger in olfactory transduction, it is actually slightly less effective than cGMP as the activating ligand (4); the significance of this peculiarity is unclear at present.

Molecular cloning of these channels first became possible when the rod channel protein was successfully purified from bovine retina (25). The deduced amino acid sequence of this channel based on the cloned cDNA (26) shows a domain on the cytoplasmic C-terminal segment with homology to the cyclic nucleotide-binding domains in cGMP- and cAMP-dependent protein kinases. Closer inspection of the sequence has also identified a region resembling the voltage-sensing S4 domain in voltage-gated channels (27), even though the rod cGMP-gated channel cannot be activated by voltage in the absence of cyclic nucleotide. Moreover, it has a region homologous to the pore of voltage-gated K^+ channels (28). Unlike K^+ channels, however, the rod channel does not discriminate between Na^+ and K^+, and it is partially blocked by (as well as, of course, being permeant to) divalent cations (9). From mutagenesis studies, both of these differences appear to arise from two amino acid residues, tyrosine and

glycine, present in the pore of K^+ channels but not in that of the rod channel (29; see also refs. 53 and 54). Voltage-gated Ca^{2+} channels likewise lack these two amino acid residues in the pore region; at the same time, Ca^{2+} channels are nonselectively permeable to monovalent cations in the absence of divalent cations, but this conductivity to monovalent cations is blocked by divalent cations. These resemblances, together with tentatively similar folding patterns in the membrane (for example, see ref. 30), strongly suggest a common ancestry between cyclic nucleotide-gated and voltage-gated channels (27, 29).

The olfactory channel was subsequently cloned by homology to the rod channel (31–33). The olfactory channel bears ≈60% amino acid identity to the rod channel and has the characteristic domains described above (see also refs. 34 and 35). Subsequent cloning studies have also suggested that this channel is in the aorta (6), whereas the rod channel is present in the kidney (36). These findings provide the first strong evidence that the sensory channels may be more widespread than previously thought. Finally, distinct rod and cone channel genes were cloned from the chicken retina (30), confirming the suggestion from electrophysiological experiments.

Biel *et al.* (7) now report the cloning of another member of this channel family from the bovine kidney. This protein shows ≈60% sequence identity to the rod and olfactory channels, but it is closer in physiological properties to the rod channel. Analysis by Northern blotting and PCR indicated that, besides the kidney, it is also present in the testis and the heart (7). Independently, the same gene was cloned by Weyand *et al.* (8) from the bovine testis, where the channel protein is apparently present on the sperm membrane, as revealed by direct patch-clamp recordings from excised membrane (8). Remarkably, Weyand *et al.* (8) also found, using immunocytochemistry, expression of this gene in bovine retinal cone photoreceptors. Thus, increasingly, the tissue distribution of each of these channel proteins appears rather broad. The functions of these channels in tissues other than the retina and the olfactory epithelium are still not completely clear. The evidence so far is that they provide, as in sensory tissue, a second-messenger-regulated pathway for Ca^{2+} influx (7, 8), either to bring about a cytoplasmic Ca^{2+} rise (8) or to possibly reload depleted intracellular Ca^{2+} stores (see refs. 7 and 37). In sperm, for example, there is evidence that chemotaxis involves activation of a membrane-bound guanylate cyclase (38) and an internal Ca^{2+} rise. A cGMP-activated channel could provide the link between the rises in cGMP and Ca^{2+} (8).

For a while, it was thought that cyclic nucleotide-activated channels are perhaps homo-oligomers. However, this is now known not to be the case. Thus, the native rod channel has at least two distinct subunits, one of which (subunit 1 or α) being the originally purified and cloned channel protein (26) and the other (subunit 2 or β) being a homologous protein that cannot form functional channels by itself but nonetheless imparts specific properties to the heteromeric channel complex (39). A heteromeric nature also appears to be true for the native olfactory channel (40, 41). By extension, this rule is likely to apply to other members in the family and is consistent with what is known about ligand-gated channels in general, such as those activated by neurotransmitters. Different combinations of the various subunit species in different locations can provide a way to increase functional diversity.

The family of cyclic nucleotide-activated channels is likely to expand further with future work. For example, the vertebrate homolog of the recently established cGMP-activated channel in *Limulus* photoreceptors (42) may or may not be the same as the rod or cone channel. The same can be said for the channel in pinealocytes (5). Most recently, a cAMP-activated channel has also been demonstrated in molluscan neurons (43). Finally, there is some suggestion of a cGMP-activated channel in retinal ON-bipolar cells, which receive input from photoreceptors (44, 45), and perhaps also in skeletal muscle (46). The molecular identities of these channels have to await the cloning of their genes.

One might also include in this family a number of recently characterized ion channels that do not obligatorily require cyclic nucleotide binding to open but are modulated by it. For example, the open probability of a cation channel in the renal inner medullary collecting duct has been shown to be reduced by cGMP, with part of the effect apparently arising from a direct interaction between cGMP and the channel (47). Whether this channel bears a relation to the cloned kidney channel mentioned above remains to be seen. In the sino-atrial node of the heart, there is a voltage-gated, pacemaker current (I_f) that shows a voltage shift in its activation curve when cAMP supposedly binds to it (48). In larval *Drosophila* muscle, there is a voltage-independent K^+ channel with a low basal open probability, but this probability is increased directly by cAMP (49). Finally, cloning of several K^+ channels, including the *eag* channel in *Drosophila* (26, 50) and the KAT1 and AKT1 channels in plant (51, 52) have, surprisingly, revealed a consensus cyclic nucleotide-binding site in their sequences. These channels are expected to be dually controlled by voltage and cyclic nucleotide binding (see, for example, ref. 55), like the I_f channel. It appears that nature has a way of repeating a useful design feature, in this case a cyclic nucleotide-binding domain, in different contexts.

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