Commentary

Ca^{2+}-activated Chloride Channels Go Molecular

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With the sequencing of the human genome it turned out that many of the genetically identified genes have an unknown function and one of the major problems of biology is the "annotation" of the genome. But the reverse problem persists, i.e., that for many functionally well-defined proteins the coding gene has not been identified. This reverse problem is difficult to tackle because the molecular tools that help for the annotation (for example knock-out, heterologous expression, localization) cannot be applied. For ion channels in particular, biochemical approaches are also hampered by the low protein abundance. These are well-known problems for two different classes of Cl^- ion channels: "swelling"-activated and Ca^{2+}-activated channels. The latter type of Cl^- channel is involved in epithelial salt transport, in the regulation of the membrane potential of smooth and heart muscle cells, in the block of polyspermi in oocytes, in the amplification of the receptor potential in odorant receptor cells, and probably many other physiological processes (for review see Jentsch et al., 2002). Despite considerable efforts in the past years these physiologically important ion channels have been notoriously difficult to identify at the molecular level. In this issue of the *Journal of General Physiology*, Criss Hartzell's group (Qu et al., 2004) makes a significant step forward to fill this knowledge gap for Ca^{2+}-activated Cl^- channels. In short, the authors demonstrate that the mouse bestrophin 2 protein (mBest2) by itself forms a Ca^{2+}-dependent Cl^- channel in heterologous expression systems and they analyze in great detail the functional properties of this channel.

Bestrophin 1 was identified 6 yr ago as the gene product of the *VMD2* gene, mutations of which cause Best vitelliform macular dystrophy (or Best disease) (Marquardt et al., 1998; Petrukhin et al., 1998). The disease is characterized by a dominant, early onset macular degeneration associated with an accumulation of lipofuscin-like material at the level of the retinal pigment epithelium (RPE). A total of four homologues have been identified in humans and similar proteins are also found in many other species like, e.g., *Xenopus laevis* (Qu et al., 2003). In *Caenorhabditis elegans*, 24 members of the family are found. Initially, their function was completely unknown, but the presence of several hydrophobic segments suggested that they might be transport proteins. Such a role could explain the macular dystrophy as a membrane transport defect in the RPE. Indeed, several recent papers reported the appearance of a Cl^- conductance when each of the four human bestrophins or their *Xenopus* homologues were heterologously expressed in HEK-293 cells (Sun et al., 2002; Tsunenari et al., 2003; Qu et al., 2003). Currents induced by the expression of hBest1 described initially by Sun et al. (2002) were sensitive to intracellular $[Ca^{2+}]$.

So why are the results of Qu et al. (2004) so significant if it was already known that bestrophins are Cl^- channels? The fact is that the earlier reports did not actually prove that the bestrophin proteins are a structural part of the pore-containing channel. They were equally compatible with the hypothesis that bestrophins up-regulate a Cl^- current carried by another channel protein. This distinction might seem like hairsplitting, but in the Cl^- channel field such false positive candidates have plagued researchers more than once (for reviews see Clapham, 1998; Jentsch et al., 2002).

Based on the earlier results of Tsunenari et al. (2003), it was relatively accepted that bestrophins are located (at least partially) in the plasma membrane, and that they probably directly interact with (or are themselves) the pore protein. These authors could abolish an inhibitory effect of sulfhydryl reactive MSET by removing specific cysteines. This, together with further biochemical experiments, allowed a tentative assignment of the transmembrane topology of bestrophins.

An additional, and strong, argument for bestrophin being part of the pore-forming protein is provided now by Qu et al. (2004): Currents induced by wild-type mBest2 are blocked by extracellular SCN^- ions with an IC_{50} ~12 mM. The authors identified an amino acid (S79) that practically abolished this block when mutated to cysteine. In addition, the same mutation also altered the relative SCN^-/Cl^- permeability ratio and reduced block by DIDS. These results strongly suggest that the residue S79 directly participates in the ion conduction process and thus that the bestrophin protein is a structural component of the ion conducting pore. Qu et al. (2004) perform further detailed experiments to characterize the channel. For example, they determine

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the Ca^{2+} sensitivity and find an EC_{50} of $\sim$0.2 μM, close to values described for "native" currents.

Collectively, the available data (Sun et al., 2002; Tsunenari et al., 2003; Qu et al., 2003, 2004) establish that bestrophins are able to form Ca^{2+}-dependent Cl^- channels in heterologous systems, but a major question remains: how the "heterologous" currents compare with native Ca^{2+} dependent Cl^- currents. Differences in recording methods, activation protocols, and other parameters make a clear classification difficult. Grossly, at least three different classes of Ca^{2+}-dependent Cl^- channels can be distinguished (even though these need not be mutually exclusive): channels activated directly by micromolar or submicromolar concentrations of Ca^{2+} without the need of additional activators, channels activated/modulated by Ca^{2+}/calmodulin–dependent protein kinase II (CAMKII), and cGMP-dependent channels (Jentsch et al., 2002; Matchkov et al., 2004). Anion selectivity sequences are similar among the endogenous currents and the bestrophin-associated channels do not show any large deviations from the "generic" pattern. Both mBest2 and the two tested *Xenopus* homologues do not need activation by protein kinases or cGMP, and their Ca^{2+} sensitivity is similar to that of the endogenous *Xenopus* oocyte channel, which is well characterized and known to be directly activated by calcium (Qu and Hartzell, 2000, 2001). Thus, these directly by Ca^{2+} activated channels might be considered good candidates as correlates for the bestrophin channels. However, a characteristic property of these "classical" channels does not fit: Ca^{2+}-activated Cl^- currents from *Xenopus* oocytes (and also those from many other preparations) show strong outward rectification and voltage-dependent kinetics at submaximal Ca^{2+} concentrations (Qu and Hartzell, 2000). In contrast, currents carried by mBest2 are linear and time independent. Also, the voltage dependence of DIDS block differs between these two channels. Unfortunately, two important functional properties have not yet been described for bestrophin mediated currents: the single-channel properties and the sensitivity to niflumic acid. These could help to determine more precisely the physiological identity of the bestrophins.

In the meantime, several hypotheses can be formulated. Bestrophins probably form multimeric proteins (Qu et al., 2003), and it might be that the physiologically described Cl^- channels are heteromultimers composed of different bestrophin subunits. It could also be that a subunit, which is structurally unrelated to bestrophins, is missing. Alternatively, it might be that the currents carried by bestrophins do not correspond to any of the Ca^{2+}-activated Cl^- currents described so far in the literature, but rather represent a new type of current that has escaped discovery by electrophysiologists. A pessimistic hypothesis would be that the currents induced by the overexpression of bestrophins were due to an epiphenomenon unrelated to their real physiological function. While this hypothesis has become very unlikely with the new paper of Qu et al. (2004), it cannot be completely dismissed. Further work is necessary to establish the precise function of bestrophins.

Other proteins have been proposed to mediate Ca^{2+}-activated Cl^- currents. Among these are the CLCA proteins (Cunningham et al., 1995; Gaspar et al., 2000), which are cell-adhesion molecules (Elble et al., 1997), and that have been investigated for quite a long time now. However, as discussed in detail by Jentsch et al. (2002), the evidence for a direct-channel function is still relatively weak. More recently, the CLC-3 protein, a member of the CLC-family of Cl^- channels (Jentsch et al., 2002), has been proposed by D.J. Nelson's group to represent the CAMKII activated Cl^- current that is found, for example, in epithelial T84 cells (Huang et al., 2001; Robinson et al., 2004). The properties of the currents reported by this group differ, however, significantly from those described by others (Li et al., 2000, 2002). Thus, this issue still awaits a resolution.

Compared with these other candidates, the evidence in favor of bestrophins being real Ca^{2+}-activated Cl^- channels now should be considered very strong, to the point where they should indeed be considered to be bona fide Cl^- channels. One of the most interesting problems to be solved in the (hopefully not too distant) future will be to understand how precisely a defective Cl^- channel in the RPE leads to macular degeneration.

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REFERENCES

Clapham, D.E. 1998. The list of potential volume-sensitive chloride currents continues to swell (and shrink). *J. Gen. Physiol.* 111:623–624.

Cunningham, S.A., M.S. Awayda, J.K. Bubien, I.I. Ismailov, M.P. Arrate, B.K. Berdiev, D.J. Benos, and C.M. Fuller. 1995. Cloning of an epithelial chloride channel from bovine trachea. *J. Biol. Chem.* 270:31016–31026.

Elble, R.C., J. Widom, A.D. Gruber, M. Abdel-Ghany, R. Levine, A. Goodwin, H.C. Cheng, and B.U. Pauli. 1997. Cloning and characterization of lung-endothelial cell adhesion molecule-1 suggest it is an endothelial chloride channel. *J. Biol. Chem.* 272:27853–27861.

Gaspar, K.J., K.J. Racette, J.R. Gordon, M.E. Loewen, and G.W. Forsyth. 2000. Cloning a chloride conductance mediator from the apical membrane of porcine ileal enterocytes. *Physiol. Genomics.* 3:101–111.

Huang, P., J. Liu, A. Di, N.C. Robinson, M.W. Musch, M.A. Kaetzel, and D.J. Nelson. 2001. Regulation of human CLC-3 channels by multifunctional Ca^{2+}/calmodulin-dependent protein kinase. *J. Biol. Chem.* 276:20093–20100.

Jentsch, T.J., V. Stein, F. Weinreich, and A.A. Zdebik. 2002. Molecular structure and physiological function of chloride channels. *Physiol. Rev.* 82:503–568.

Li, X., K. Shimada, L.A. Showalter, and S.A. Weinman. 2000. Biophysical properties of ClC-3 differentiate it from swelling-activated chloride channels in Chinese hamster ovary-K1 cells. *J. Biol.*

Chem. 275:35994–35998.

Li, X., T. Wang, Z. Zhao, and S.A. Weinman. 2002. The ClC-3 chloride channel promotes acidification of lysosomes in CHO-K1 and Huh-7 cells. *Am. J. Physiol. Cell Physiol.* 282:C1483–C1491.

Marquardt, A., H. Stöhr, L.A. Passmore, F. Kramer, A. Rivera, and B.H. Weber. 1998. Mutations in a novel gene, VMD2, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). *Hum. Mol. Genet.* 7:1517–1525.

Matchkov, V.V., C. Aalkjaer, and H. Nilsson. 2004. A cyclic GMP-dependent calcium-activated chloride current in smooth-muscle cells from rat mesenteric resistance arteries. *J. Gen. Physiol.* 123:121–134.

Petrukhin, K., M.J. Koisti, B. Bakall, W. Li, G. Xie, T. Marknell, O. Sandgren, K. Forsman, G. Holmgren, S. Andreasson, et al. 1998. Identification of the gene responsible for Best macular dystrophy. *Nat. Genet.* 19:241–247.

Qu, Z., R. Fischmeister, and C. Hartzell. 2004. Mouse bestrophin-2 is a bona fide Cl^- channel: identification of a residue important in anion binding and conduction. *J. Gen. Physiol.* 123:327–340.

Qu, Z., and H.C. Hartzell. 2000. Anion permeation in Ca^{2+}-activated Cl^- channels. *J. Gen. Physiol.* 116:825–844.

Qu, Z., and H.C. Hartzell. 2001. Functional geometry of the permeation pathway of Ca^{2+}-activated Cl-channels inferred from analysis of voltage-dependent block. *J. Biol. Chem.* 276:18423–18429.

Qu, Z., R.W. Wei, W. Mann, and H.C. Hartzell. 2003. Two bestrophins cloned from *Xenopus laevis* oocytes express Ca^{2+}-activated Cl^- currents. *J. Biol. Chem.* 278:49563–49572.

Robinson, N.C., P. Huang, M.A. Kaetzel, F.S. Lamb, and D.J. Nelson. 2004. Identification of an N-terminal amino acid of CLC-3 critical in phosphorylation-dependent activation of ICl,CaMKII. *J. Physiol.* 10.1113/jphysiol.2003.058032.

Sun, H., T. Tsunenari, K.W. Yau, and J. Nathans. 2002. The vitelliform macular dystrophy protein defines a new family of chloride channels. *Proc. Natl. Acad. Sci. USA.* 99:4008–4013.

Tsunenari, T., H. Sun, J. Williams, H. Cahill, P. Smallwood, K.W. Yau, and J. Nathans. 2003. Structure-function analysis of the bestrophin family of anion channels. *J. Biol. Chem.* 278:41114–41125.