Perspective

The List of Potential Volume-sensitive Chloride Currents Continues to Swell (and Shrink)

David E. Clapham

From the Howard Hughes Medical Institute, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115

The protein for the swelling-activated chloride conductance is one of the few ion channels left for which we do not have a universally acceptable clone. In this brief note, I hope to describe why chloride channels in general, and $I_{Cl.swell}$ in particular, have been difficult to pin down. Dr. Strange describes why he believes the pICln protein is not $I_{Cl.swell}$. I agree. In 1995 we demonstrated that pICln is unlikely to be a channel itself (Krapivinsky et al., 1994). Voets et al. (1996) also concludes that, although oocyte expression of pICln activates a chloride current, this chloride current is not $I_{Cl.swell}$. pICln is related to chloride channel activation, but how or why is not understood. In an attempt to place the arguments in a broader perspective, Table I lists most of the proposed chloride channel proteins that have been isolated and cloned (see also Jentsch and Gunther, 1997).

Although at least eight proteins have been proposed to comprise chloride channels, only three have been established. When a new protein is proposed to comprise a channel function, it faces more of an uphill battle if it has no homology to known channels. For this reason, I believe it is best to keep an open mind about the phospholemman, p64, and Ca-CC proposed channel types. Of the eight proteins listed, only ClC-2 and ClC-3 are viable candidates for the swelling-activated chloride channel itself, although other proteins cannot yet be excluded from participating in activation of the swelling current.

TABLE I

What Proteins Make Chloride Channels?

Proposed channel protein	Chloride channel?	Initial reference
	Weight of evidence	
GABA, glycine	Yes	Grenninglogh et al., 1987; Schofield et al., 1987
CFTR	Yes	Riordan et al., 1989
CIC class	Yes	Jentsch et al., 1990
CIC-2	Perhaps $I_{Cl.swell}$	Grunder et al., 1992
CIC-3	Perhaps $I_{Cl.swell}$	Duan et al., 1997
P-glycoprotein, or multidrug resistance (MDR) gene product	Not a $C1^-$ channel	Valverde et al., 1992
PICln	Not a Cl^- channel	Paulmichl et al., 1992
p64	Insufficient evidence	Landry et al., 1993
Phospholemman	Insufficient evidence	Moorman et al., 1992
Ca-CC ($I_{Ca.Cl}$?)	Insufficient evidence	Cunningham et al., 1995

Why has there been difficulty in nailing down chloride channels? So far, no chloride channel sequence resembles a known voltage-gated channel, the most well-established group of channel proteins. Thus, at least initially, homology to known channels was not helpful in identifying these channels. Second, if one accepts the results of numerous mutagenesis studies on chloride channels, one must conclude that the chloride pore either involves the whole protein or there is more than one way to make a chloride-conducting pore. From studies on $GABA_A$, glycine, CFTR, and ClC proteins, no chloride-selective consensus domain has emerged. Third, the most common expression cloning system, *Xenopus* oocytes, has numerous background chloride channels, some of which seem to be activated by expression of almost any protein (Tzounopoulos et al., 1995). Finally, the lipid bilayer method is too sensitive to contaminating proteins to use as a reliable assay for identification of novel protein function. In a picogram of 99.9% pure protein, there are >10,000 contaminating protein molecules. Even one of these molecules can be detected if it inserts into the membrane, and this has led to numerous false identifications of various proteins as ion channels. The initial methods that have led to successful identification of chloride channels involved (*a*) ligand binding, purification, and microsequencing (glycine, $GABA_A$), (*b*) genetic approaches (CFTR), and (*c*) a modified method of expression cloning in *Xenopus* oocytes involving hybrid depletion (ClC-0).

Why has there been difficulty in finding the swelling-activated chloride conductance? Most of the difficulties are related to the problems mentioned above. But it is not clear that $I_{Cl.swell}$ is represented by a single channel type, given the range of properties that have been described for these currents (Okada, 1997). Finally, it is likely that $I_{Cl.swell}$ is regulated by other proteins that are involved in cell swelling, such as the cytoskeleton. Swell-

J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/98/05/623/2 $2.00
Volume 111 May 1998 623–624
http://www.jgp.org

ing, like temperature, has fairly broad repercussions in cells. This means that expression of all kinds of proteins may trigger the activation of the swelling current. This activation may be direct—or very indirect.

ICln

We expression-cloned ICln (Paulmichl et al., 1992). Other groups confirmed that its expression leads to activation of a chloride conductance (Abe et al., 1993; Buyse et al., 1997). When we published our expression cloning of pICln, we stated that "the assumption that the protein is a chloride current is the simplest conclusion, but more complex interpretations are feasible" (Paulmichl et al., 1992). After we purified the protein, made antibodies, and studied it for 2 yr, we decided it was unlikely to be the channel itself since it was soluble, mainly cytoplasmic, and abundant (Krapivinsky et al., 1994). We thought it likely the channel was linked somehow indirectly to endogenous oocyte $I_{Cl.swell}$, that it somehow regulated $I_{Cl.swell}$ (Krapivinsky et al., 1994). Gschwentner et al. (1996), however, disagreed and maintain that the protein does comprise the channel itself. Voets et al. (1996) and Buyse et al. (1997) have presented evidence that ICln does not evoke $I_{Cl.swell}$, but does evoke a swelling-insensitive Cl channel with similar sensitivity to nucleotide block. Overall, the weight of the evidence is that pICln is indirectly related to chloride current activation in expression systems, but that it is probably not $I_{Cl.swell}$, nor even a chloride channel itself. Since our finding that pICln is not an integral membrane protein and is associated with several other cytoplasmic proteins (Krapivinsky et al., 1994), my opinion is that pICln's function has yet to be discovered.

CONCLUSION

One way science is done is to formulate a hypothesis, and then subject it to scrutiny. In my opinion, the hypothesis that either P-glycoprotein or pICln comprise chloride channels in themselves has been rejected by experimentation. The current evidence that ClC-2 or ClC-3 are themselves chloride channels is strong. Whether they will turn out to be forms of $I_{Cl.swell}$ as currently defined will require more experiments. Certainly it will be interesting to discover how cell swelling is translated into gating of a presumed integral membrane protein. The swelling-sensing and channel-gating mechanism will likely require several proteins, and pICln may turn out to play a role in one of these steps. But the only comment I can make with certainty is that we have not seen the end of proteins proposed to comprise $I_{Cl.swell}$.

REFERENCES

Abe, T., K. Takeuchi, K. Ishii, and K. Abe. 1993. Molecular cloning and expression of a rat cDNA encoding MDCK-type chloride channel. *Biochim. Biophys. Acta.* 1173:353–356.

Buyse, G., T. Voets, J. Tytgat, C. DeGreef, G. Droogmans, B. Nilius, and J. Eggermont. 1997. Expression of human pICln and ClC-6 in Xenopus oocytes induces an identical endogenous chloride conductance. *J. Biol. Chem.* 272:3615–3621.

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.

Duan, D., C. Winter, S. Cowley, J.R. Hume, and B. Horowitz. 1997. Molecular identification of a volume-regulated chloride channel. *Nature.* 390:417–420.

Grenningloh, G., A. Rienitz, B. Schmitt, C. Methfessel, M. Zensen, K. Beyreuther, E.D. Gundelfinger, and H. Betz. 1987. The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature.* 328:215–220.

Grunder, S., A. Thiemann, M. Pusch, and T.J. Jentsch. 1992. Regions involved in the opening of ClC-2 chloride channel by voltage and cell volume. *Nature.* 360:759–762.

Gschwentner, M., A. Susanna, A. Schmarda, A. Laich, U.O. Nagl, H. Ellemunter, P. Deetjen, J. Frick, and M. Paulmichl. 1996. ICln: a chloride channel paramount for cell volume regulation. *J. Allergy Clin. Immunol.* 98:S98–S101.

Jentsch, T.J., and W. Gunther. 1997. Chloride channels: an emerging molecular picture. *Bioessays.* 19:117–126.

Jentsch, T.J., K. Steinmeyer, and G. Schwartz. 1990. Primary structure of *Torpedo marmorata* chloride channel isolated by expression cloning in Xenopus oocytes. *Nature.* 348:510–514.

Krapivinsky, G., M. Ackerman, E. Gordon, L. Krapivinsky, and D.E. Clapham. 1994. Molecular characterization of I_{Cln} protein: identification as a swelling-induced chloride conductance regulator. *Cell.* 76:439–448.

Landry, D., S. Sullivan, M. Nicolaides, C. Redhead, A. Edelman, M. Field, Q. al-Awqati, and J. Edwards. 1993. Molecular cloning and characterization of p64, a chloride channel protein from kidney microsomes. *J. Biol. Chem.* 268:14948–14955.

Moorman, J.R., C.J. Palmer, J.E. John III, M.E. Durieux, and L.R. Jones. 1992. Phospholemman expression induces a hyperpolarization-activated chloride current in *Xenopus* oocytes. *J. Biol. Chem.* 267:14551–14554.

Okada, Y. 1997. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. *Am. J. Physiol.* 42:C755–C789.

Paulmichl, M., Y. Li, K. Wickman, M. Ackerman, E. Peralta, and D.E. Clapham. 1992. Expression cloning of an epithelial chloride channel. *Nature.* 356:238–241.

Riordan, J.R., J.M. Rommens, B.S. Kerem, A.R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.L. Chou, M.L. Drumm, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science.* 245:1066–1073.

Schofield, P.R., M.G. Darlson, N. Fujita, D.R. Burt, F.A. Stephenson, H. Rodriguez, L.M. Rhee, J. Ramachandran, V. Reale, T.A. Glencourse, et al. 1987. Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor superfamily. *Nature.* 328:223–227.

Tzounopoulos, T., J. Maylie, and J.P. Adelman. 1995. Induction of endogenous channels by high levels of heterologous membrane proteins in Xenopus oocytes. *Biophys. J.* 69:904–908.

Valverde, M.A., M. Diaz, F.V. Sepulveda, D.R. Gill, S.C. Hyde, and C.F. Higgins. 1992. Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature.* 355:830–833.

Voets, T., G. Buyse, J. Tytgat, G. Droogmans, J. Eggermont, and B. Nilius. 1996. The chloride current induced by expression of the protein pICln in Xenopus oocytes differs from the endogenous volume-sensitive chloride current. *J. Physiol.* 495:441–447.