Rat Brain p64H1, Expression of a New Member of the p64 Chloride Channel Protein Family in Endoplasmic Reticulum*

(Received for publication, February 18, 1997, and in revised form, May 12, 1997)

Rory R. Duncan, Paul K. Westwood, Alan Boyd, and Richard H. Ashley‡
From the Department of Biochemistry, University of Edinburgh, Edinburgh EH8 9XD, United Kingdom

Many plasma membrane Cl– channels have been cloned, including the cystic fibrosis transmembrane conductance regulator and several members of the voltage-gated CIC family. In contrast, very little is known about the molecular identity of intracellular Cl– channels. We used a polymerase chain reaction-based approach to identify candidate genes in mammalian brain and cloned the cDNA corresponding to rat brain p64H1. This encoded a microsomal membrane protein of predicted Mr 28,635 homologous to the putative intracellular bovine kidney Cl– channel p64. In situ mRNA hybridization histochemistry showed marked expression in hippocampus and cerebellum, and in vitro expression revealed a large cytoplasmic domain, one membrane-spanning segment, and a small nonglycosylated N-terminal luminal domain. The predicted protein contained consensus phosphorylation sites for protein kinase C and protein kinase A, and protein kinase C-mediated phosphorylation increased the Mr of p64H1 to ~43,000, characteristic of the native protein in Western blots. Recombinant p64H1 was immunolocalized to the endoplasmic reticulum of human embryonic kidney 293 and HT-4 cells, and incorporation of human embryonic kidney 293 endoplasmic reticulum vesicles into planar lipid bilayers gave rise to intermediate conductance, outwardly rectifying anion channels. Although p64H1 is the first intracellular Cl– channel component or regulator to be identified in brain, Northern blotting revealed transcripts in many other rat tissues. This suggests that p64H1 may contribute widely to intracellular Cl– transport.

Ion channel reconstitution followed by single-channel recording and analysis has shown that intracellular organelles contain numerous ion channels. These include channels in the endoplasmic reticulum (ER)1 and sarcoplasmic reticulum (1–8) and ion channels in mitochondria (9–11), secretory vesicles (12, 13), and synaptic vesicles (14). Although a concerted effort to understand the molecular basis of intracellular ion transport has revealed the molecular identities of several intracellular cation channels, intracellular Cl– channels are much less well characterized. Nevertheless, it has been speculated that anion channels may help to regulate organelle volume or conduct counterions to balance intracellular Ca2+ uptake and release. Such channels could therefore have important roles in many cellular processes.

Experimental work by Landry et al. (15) establishes a role for the protein p64 in intracellular Cl– transport. Briefly, Landry et al. (15) identify specific ligands for a bovine kidney microsomal Cl– transporter and purified candidate proteins, including the 64-kDa protein p64, using drug affinity chromatography (16). Reconstitution of partially purified p64 into planar lipid bilayers revealed anion channel activity (16), and incorporation into proteoliposomes conferred a [36Cl] permeability that was abolished by anti-p64 antibodies (17). p64 cDNA was cloned (17) and expressed in Xenopus oocytes, where the recombinant protein was localized to intracellular membranes. Taken as a whole, this work strongly suggested that p64 was a component of an intracellular Cl– channel, and it provided the impetus for the present study.

Genes homologous to bovine p64 are expressed in rat tissues, including brain (18). In addition, p64-expressed sequence tags have been identified in several tissues during the Human Genome Mapping Project. In an attempt to identify p64-related intracellular Cl– channel proteins in mammalian brain, we used a PCR-based approach to clone and functionally express a rat brain homologue of p64, p64H1. This is the first putative intracellular Cl– channel component to be identified in brain, and we suggest that p64- and p64H1-related genes encode a family of proteins associated with intracellular Cl– transport.

EXPERIMENTAL PROCEDURES

RT-PCR and cDNA Library Screening—RT-PCR was carried out on whole rat brain total RNA (19) using two fully degenerate oligonucleotide primers (18) based on the amino acid sequence of bovine kidney p64 (Ref. 17; GenBankTM accession number L16547). We used the random primer method to label a 546-bp EcoRI fragment of BS2, a partial cDNA clone generated by this procedure, with [α-32P]dCTP (2000 Ci/mmol, Amersham). This probe was used to screen an oligo(dT)-primed rat brain poly(A)+ cDNA library (ZAP II cDNA library (Stratagene)) at moderate stringency (6 × SSPE, 5 × Denhardt’s solution, 1% SDS, 100 μg/ml salmon sperm DNA, 65 °C). We isolated 13 clones after washing the filters at moderate stringency (final wash: 2 × SSPE, 65 °C, 30 min), and phagemidal DNA was rescued according to the manufacturer’s instructions. DNA sequencing was carried out using the Sequenase II system (Amersham). This is the first putative intracellular Cl– channel component to be identified in brain, and we suggest that p64- and p64H1-related genes encode a family of proteins associated with intracellular Cl– transport.

Circle (or Concatemer) Rapid Amplification of cDNA Ends-PCR (20)—Poly(A)+ RNA was purified from rat brain total RNA using Poly(A)+ Quick columns (Stratagene). 10 pmol of an antisense oligonucleotide (5′-CGTGGTGACATGAAAAGACATCCT-3′) based on sequence analysis of the longest cDNA clone were used to direct first-strand cDNA synthesis from 1 μg of poly(A)+ RNA by 10 units of Superscript II RNase H–reverse transcriptase (Life Technologies Inc.). The RNA template was destroyed by alkaline hydrolysis, and the cDNA was circularized by incubation with 10 units of T4 RNA ligase (NEB).
This was used as a template in a PCR with the following oligonucleotides: forward primer, 5′-TCAGTTCTGCTCCTGTTAAGCC-3′; reverse primer, 5′-TCAGAGGCTGCTCTGGTCTC-3′; the PCR product (eR1) was ligated to the T/A cloning vector pGEM-T (Promega) and sequenced (Sequenase II, Amersham). eR1 extended an additional 120 bp (5′-TCAGAGGCTGCTCTGGTCTC-3′) and was verified by sequencing the forward primer. eR1 was used to screen a second rat whole brain cDNA library (agt10 5′ STRETCH, CLONTECH) under conditions identical to those previously described. The 1.9-kb insert from the newly isolated clone (p64H1) was PCR-amplified using agt10 insert-flanking primers (forward primer, 5′-AGCAATGTCACCTGGTGCTAAGT-3′; reverse primer, 5′-TATGAG-TGTCTATACGCT-3′) and a polymerase mixture of Taq and Pwo (Exodi, Boehringer Mannheim). The final product was ligated to pTag (R&D Systems).

**Northern Hybridization**—3 µg of tissue-specific poly(A) RNAs purified from total tissue RNAs were electrophoresed in a 1% (w/v) agarose-formaldehyde gel, transferred to Hybond-N (Amersham), and cross-linked in a Stratalinker UV oven (Stratagene). We also used a commercial rat multiple tissue Northern blot (CLONTECH). A 346-bp EcoRI restriction fragment of BS2 labeled with [α-32P]CTP, as described earlier, was hybridized to the membranes and washed under conditions of high (final wash: 2 × SSPE, 0.1% (w/v) SDS, 65 °C for 10 min) or moderate (final wash: 2 × SSPE, 65 °C for 30 min) stringency and exposed to radiographic film for 24 h.

**Western Blotting**—For Western blotting, tissue or cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Immobilon, Millipore). Membranes were incubated with anti-ER Cl− channel polyclonal antiserum (Ab990) to a fusion protein containing a fragment of p64H1 and this was detected with a 1:5000 dilution of rabbit IgG anti-rabbit secondary antibody. Briefly, proteins were transferred onto Hybond-C (Amersham), and excess binding sites were blocked with 1% (w/v) bovine serum albumin in a solution containing 10 mM Tris-HCl (pH 7.4), 140 mM NaCl, and 0.1% (v/v) 3-5% (v/v) TBS. Membranes were exposed to polyclonal antibody (1:2000 dilution in TBS) for 60 min, washed with TBS, and exposed to horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:4000 dilution in TBS, Pierce). Membranes were washed extensively with TBS, and bound antibody complexes were revealed by enhanced chemiluminescence detection.

**Indirect Immunofluorescence**—Transfected HEK-293 and HT-4 cells were grown to confluence on sterile glass coverslips. 48 h after transfection, the cells were washed extensively in ice-cold PBS containing Ca2+ and Mg2+, fixed for 20 min in ice-cold buffered paraformaldehyde, and washed again in PBS. p64H1 was detected using a 1:50 dilution of antiserum (18) in PBS, 0.2% (v/v) fish skin gelatin (Sigma). After extensive washing, the primary antibody was detected using rhodamine-conjugated goat anti-rabbit IgG second antibody (1:100 dilution in PBS, 0.2% (v/v) fish skin gelatin (Sigma)). Nuclear DNA was stained using Hoechst 33258 dye, and ER membranes were visualized using the rhodamine-conjugated goat anti-rabbit IgG and photographed using a Leica DMR fluorescence microscope. Images obtained were analyzed with a Hamamatsu C5810 chilled color CCD camera using standard Nikon filter cubes. Negative controls, using pre-immune serum from the same animal, showed no p64H1-specific staining.

**Planar Bilayer Reconstitution**—Planar lipid bilayers (27) comprising equivalent electrolyte-free films containing a 1:1 mixture of dipalmitoyl lecithin and cholesterol in a 20% (v/v) glycerol solution were formed in 100-mm culture dishes with 1-µl aliquots of calcium phosphate DNA precipitate containing 20 µg of plasmid DNA prepared using standard methods or Tfx-20 (Promega) (28). The transfection was confirmed by Southern blotting after 48–72 h (see below). Negative control transfections were performed in an identical manner using vector alone as a source of DNA. To analyze expressed products in planar lipid bilayers, cells were harvested from transfected or control plates by scraping into ice-cold buffer containing 5 mM Tris-HCl (pH 7.4), 0.32 mM sucrose, 0.1 mM 4:2-aminoethyl/benzene-sulfonyl fluoride, and 10 µg/ml trypsin inhibitor and homogenized on ice using 25 strokes of a Teflon glass homogenizer. The homogenates were fractionated by differential centrifugation to yield microsomal membranes, which were either used immediately or snap-frozen in liquid N2 and stored at −70 °C for up to 6 weeks.
RESULTS

Isolation of p64H1 cDNA—p64 was identified as a Cl$^-$ channel component in bovine kidney (15–17). In this study, we used RT-PCR and cDNA library screening to clone cDNAs encoding p64-related Cl$^-$ channels from rat brain. Sequence analysis revealed that none of the original 13 clones isolated by this approach (see "Experimental Procedures") contained full-length cDNA inserts. Many of the cDNAs contained long 3'-UTR sequences, and computer analysis predicted extensive RNA secondary structure (Genetics Computer Group 8, University of Wisconsin). This probably reduced the abundance of full-length p64H1 cDNAs when the original library was constructed. To overcome these problems, we used circle (or concatemer) rapid amplification of cDNA ends-PCR (20) to extend the partial-length cDNAs obtained by conventional library screening toward the 5'-end of the p64H1 transcript. This technique, briefly described under "Experimental Procedures," produced the probe cRI and, eventually, p64H1. Sequence analysis of two independent PCR clones of p64H1 revealed 100% identity to BS2 and cRI, and the presence of a methionine codon in a consensus region favorable for translation, viz. GGC-CATGG (29, 30). This single potential-initiating methionine codon was followed by a 753-bp open reading frame encoding a protein with a predicted Mr of 28,635. The nucleotide and deduced amino acid sequences of p64H1 and p64H1 are shown in Fig. 1A. The clone also contained 165 bp of 5'-UTR (as shown). The same cDNA was obtained when another cDNA library was screened with the 5'-UTR sequence. There were two in-frame termination codons within the 5'-UTR, and the other reading frames contained multiple termination codons (not shown). Partial p64H1 cDNA clones obtained by screening an oligo(dT)-primed cDNA library contained unusually long 3'-UTR sequences of 3.9 kb.

Predicted Protein Sequence and Secondary Structure—Fig. 1B shows an optimized alignment of the predicted 253 amino acid residues of p64H1 with bovine p64. p64H1 showed 71% identity to the C-terminal portion of p64 (which has an additional 226 N-terminal residues) and appeared to have a single potential membrane-spanning region, which was predicted to adopt an alpha-helical conformation (31). The hydropathy profile of p64H1 closely resembled the C-terminal half of p64 (Fig. 1C). There were no consensus N-glycosylation sites, but p64H1 had multiple consensus sites for phosphorylation by a variety of kinases. These included a single protein kinase A (PKA) site and four protein kinase C (PKC) sites (indicated in Fig. 1A). The putative transmembrane domain was preceded by a relatively short N-terminal domain, but this had no similarity to published cleaved signal peptide sequences, and sequence analysis did not predict a protease cleavage site. Finally, data base searching revealed no similarity to any other protein apart from p64H1.
from bovine p64 and other p64-related proteins.

Distribution of p64H1 mRNA in Brain and Other Tissues—Northern blot analysis at moderate stringency using a 346-bp probe derived from p64H1 cDNA revealed three transcripts of approximately 1.9 kb, 4.8 kb, and 6.5 kb in samples from whole rat brain (Fig. 2A). At higher stringency, the p64H1 transcript hybridized exclusively to the 4.8-kb species (Fig. 2B). The 1.9-kb and 6.5-kb transcripts may represent related members of a gene family or result from alternative mRNA splicing. The 4.8-kb transcript was detected in RNA from several rat tissues, indicating that p64H1 is widely expressed (Fig. 2B). Based on these results, we further localized p64H1 expression in rat brain by in situ mRNA hybridization. Marked expression was observed in the cerebellum, hippocampus, and dentate gyrus, and moderate expression was observed in the cerebral cortex (Fig. 2C). No signal was detected in parallel experiments using a sense probe or in samples treated with RNase (not shown).

In Vitro Expression, Membrane Topology, and Phosphorylation—We expressed p64H1 in vitro to confirm the location of the initiating methionine and to delineate the protein membrane topology. The Mr of p64H1 was unaltered in the presence of canine pancreatic microsomal membranes (Fig. 3A), consistent with the lack of predicted consensus glycosylation sites. Microsomes containing recombinant p64H1 were incubated at high pH to lyse the membrane vesicles and dissociate peripheral membrane proteins. This treatment failed to dissociate the translation product (Fig. 3A, lane 4), consistent with an integral membrane protein (32, 33). Intact microsomes containing recombinant p64H1 were also exposed to proteinase K to determine how much of the protein was accessible to an extravesicular protease. As shown in Fig. 3B, this reduced the apparent Mr of the in vitro translation product by ~27,000, whereas solubilization of the microsomal membranes with 0.1% (v/v) Triton X-100 (not shown) allowed complete digestion. The bands containing unincorporated label are an artifact of the Tris-Tricine gels used to resolve the low Mr (~6,000) peptide. Finally, we demonstrated that PKC phosphorylated p64H1 in vitro. This increased the apparent Mr of the protein by up to ~10,000 (Fig. 3C), with a ladder of phosphorylated proteins consistent with multiple phosphorylation states (four PKC sites could theoretically give rise to $4^{2} = 16$ different phosphoproteins).

Localization of Expressed p64H1 to the ER—To determine the cellular location of p64H1, we transiently transfected HEK-293 cells and the mouse neuronal cell line HT-4 with an expression vector containing p64H1. We detected p64H1 with an apparent Mr of ~33,000 after 48 h using an anti-p64H1 polyclonal antiserum (Ab990). The protein was localized to the P3 membrane fraction, which is known to be enriched in ER but also contains other membranes including Golgi and plasma membranes.
membranes. p64H1 was not detected in the P1 or P2 fractions corresponding to unbroken cells and nuclear membranes (P1) or mitochondria (P2), and it was absent in mock-transfected cells. Its intracellular localization was confirmed by indirect immunofluorescence. As before, p64H1 could be detected 48 h post-transfection, and it appeared to be localized to the ER, with characteristic staining of the outer nuclear membrane and staining of membrane-bound organelles extending toward, but not including, the plasma membrane. It was absent in dummy-transfected cells. In the triple-stained cells in Fig. 4, where the ER of p64H1-transfected HT-4 cells was visualized using DiOC-5(3) (25, 26), p64H1 is clearly localized to ER membranes.

**Single-channel Recording**—P3 microsomal membrane vesicles from p64H1-transfected and mock-transfected HEK-293 cells were incorporated into voltage-clamped planar lipid bilayers in the presence of choline chloride to select for Cl⁻ channels (1, 2, 11). The presence of p64H1 in the transfected samples and its absence in controls was confirmed by Western blotting. Anion channels similar to those illustrated in Fig. 5A were obtained from each of seven independent transfections but not from five mock-transfected control preparations. Like p64 (16), the channels were unaffected by indanyloxyacetic acid 94 (up to 50 μM cis and trans), and they were not blocked by 5-nitro-2-(3-phenylpropylamino)benzoic acid or DIDS (up to 50 μM). The channels showed no obvious voltage-dependence or rectification until perfusion was carried out to give more physiological ionic conditions, viz. 10 mM choline chloride cis (presumed “cytoplasmic,” see “Experimental Procedures”) and 100 mM choline chloride trans (extracytoplasmic, both solutions buffered to a pH of 7.4 with 10 mM Tris-HCl). Under these conditions, the channels showed mild outward rectification (Fig. 5B). The single-channel reversal potential shifted to less negative values in each of four experiments where cis [choline chloride] was increased to 50 mM, but the channels were only poorly selective for anions versus cations. In fusion conditions (450:50 mM choline chloride), P₂Cl⁻/Pcholine was 3.1 ± 0.7, mean ± S.D., n = 16 experiments from seven independent transfections (not corrected for ionic activities). In symmetric 50 mM choline chloride experiments, the channels had a slope conductance of 43 ± 12 picoSiemens, n = 3).

**DISCUSSION**

The newly isolated rat brain cDNA, p64H1, encodes a homologue of the putative bovine kidney Cl⁻ channel p64. Like p64H1, the cDNA encoding bovine p64 includes an unusually long (−5 kb) 3'-UTR (17). Computer predictions of the novel protein membrane topology and in vitro translation exper-
ments showing the pattern of protease protection in the presence of microsomal membranes indicate that p64H1 has a large (M_r ~ 25,000) C-terminal cytoplasmic domain, a single transmembrane domain, and a small intraluminal (M_r ~ 5,000) domain (Fig. 3D). This topology would of course be conserved regardless of an ER, secretory vesicle, or plasma membrane location for p64H1, but Western blotting of the native protein in subcellular fractions from rat brain (18), together with immunoblotting and indirect immunofluorescence studies on transiently transfected HEK-293 and HT-4 cells, indicate that native and recombinant p64H1 are intracellular membrane proteins localized to the ER. The similarities between p64 and p64H1 raise the possibility of a family of homologous genes, possibly with alternative mRNA splicing (see low stringency Northern blots, Fig. 2A). Although p64 was predicted to have up to four transmembrane domains (17), including two within the C-terminal sequence noted to be highly homologous to p64H1, our in vitro translation experiments suggest that this topology is unlikely and p64 may in fact have only one or two transmembrane domains.

With the exception of phospholemman (34), which is also a relatively small protein, no other putative Cl^- channel has a similar topology to p64H1. Apart from polypeptide toxins and ion channels encoded by viruses (35, 36), the smallest proteins currently thought to contribute to ion channel pores are phospholemman and minK (37, 38). These have M_r values of ~8,000 and ~14,000, respectively. The apparent M_r of the muscle plasma membrane protein phospholemman increases markedly (by ~7,000) on phosphorylation by PKC and PKA, whereas the apparent M_r of p64H1 increases by ~10,000 on PKC-mediated phosphorylation to ~43,000, even though the consensus PKC site located between the predicted transmembrane domain and the N terminus (Fig. 1A) may be nonfunctional. This corresponds to the M_r of the native rat brain protein (18), suggesting that p64H1 is phosphorylated in vivo. Western blotting suggested that in our experiments, the recombinant protein (M_r ~ 33 kDa) was not phosphorylated. It may be significant that both PKC and p64H1 are enriched in hippocampus and cerebellum. Phospholemman homo-oligomers formed ion channels following bilayer reconstitution (34), and minK mRNA induced Cl^- currents and K^+ currents in oocytes via interactions involving the N-terminal and C-terminal domains of the expressed protein (39). minK has recently been shown to contribute to the cardiac inward rectifier I_K1, where it associates with the novel K^+ channel subunit K_C10 (40, 41). Interestingly, PKC enhanced minK-associated Cl^- currents and diminished K^+ currents in oocytes by phosphorylating different sites on the protein (35).

Despite the detailed experimental evidence suggesting that p64 is an ion channel component (15–17), we suggest that it is possible that p64H1 may act as a channel regulator or activate endogenous brain ER anion channels. p64H1 may associate with other proteins (18), consistent with this possibility. The difficulty of assigning specific roles to novel proteins has been well illustrated by I_C1n. This widely distributed membrane current has been associated with the expression of a soluble cytoplasmic protein, pICln, which appears to couple the activation of plasma membrane Cl^- channels and cell swelling in, for example, Xenopus oocytes (42). Mutagenesis studies strongly suggested that pICln actually contributes to an ion channel pore (43), although the precise molecular mechanism by which this occurs remains to be elucidated. Meanwhile, it has been shown that expression of either pICln or the unrelated protein CIC-6 can enhance an endogenous Xenopus oocyte current indistinguishable from I_C1n (44). This suggests that in reality I_C1n is an unidentified channel that can be activated by a variety of different proteins. Many proteins, including membrane proteins, are known to act as channel regulators. For example, the integral membrane protein TipE has been shown to enhance the expression of a Drosophila Na^+ channel (33). It is clear that...
detailed information concerning the biophysical and pharmacological properties of identified intracellular Cl⁻ channels would be valuable, but there are very few single-channel data for ER anion channels including p64 (16). Most recordings are from non-neural tissues (3, 5, 7), and many of these channels may be protein translocation pores rather than conventional ion channels. Anion channels reconstituted from rat brain microsomes (2, 45) and cardiac mitoplast membranes (11) are poorly selective for anions versus cations, like p64H1-related channels, but they appear to display different conductances and have different single-channel gating and substrate behavior.

In conclusion, we have described p64H1 as the first brain ER anion channel component to be identified at the molecular level. We have also demonstrated the existence of a new gene family containing p64, p64H1, and possibly other members (as suggested by Northern blotting). p64H1 may contribute directly to an ion channel, as suggested for p64 (16, 17), or its role may be more indirect. For example, it could belong to a new class of membrane proteins coupling ion channels, transporters, and membrane receptors in a cascade of interacting proteins (46). The availability of p64H1 cDNA will enable this and other models to be tested to elucidate the precise role of the protein in neurons and other cells.

Acknowledgments—We thank David Sheppard for helpful discussions, Richard Ribchester for help with image capture and printing, and Ronald McKay for the HT-4 cells.

REFERENCES
1. Smith, J. S., Coronado, R., and Meissner, G. (1985) Nature 316, 446–449
2. Ashley, R. H. (1989) J. Membr. Biol. 111, 179–189
3. Schmid, A., Gogelein, H., Kemmer, T. P., and Schulz, I. (1988) J. Membr. Biol. 104, 275–282
4. Schmid, A., Dehlinger-Kremer, M., Schulz, I., and Gogelein, H. (1990) Nature 346, 374–376
5. Simon, S. M., and Blobel, G. (1991) Cell 65, 371–380
6. Martin, C., and Ashley, R. H. (1993) Cell Calcium 14, 427–438
7. Morier, N., and Sauvé, R. (1994) Biophys. J. 67, 751–765
8. Suhkareva, M., Morrissette, J., and Coronado, R. (1994) Biophys. J. 67, 751–765
9. Schein, S. J., Colombini, M., and Finkelstein, A. (1976) J. Membr. Biol. 30, 99–120
10. Sorgato, M. C., Keller, B. U., and Stuhmer, W. (1987) Nature 320, 498–500
11. Hayman, R. A., Spurway, T. S., and Ashley, R. H. (1993) J. Membr. Biol. 136, 161–190
12. Arispe, N., Pollard, H., and Rojas, E. (1992) J. Membr. Biol. 130, 191–202
13. Ashley, R. H., Brown, D. M., Apps, D. K., and Phillips, J. H. (1994) Eur. J. Biochem. 223, 263–275
14. DeRiemer, S. A., Sakmann, B., and Stadler, H. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5310–5314
15. Landry, D. W., Reitman, M., Cragoe, E. R., and Al-Awqati, Q. (1987) J. Gen. Physiol. 90, 779–798
16. Landry, D. W., Akabas, M. H., Redhead, C., Edelman, A., Cragoe, E. J., and Al-Awqati, Q. (1989) Science 244, 1469–1472
17. Landry, D., Sullivan, S., Nicolaides, M., Redhead, C., Edelman, A., Field, M., Al-Awqati, Q., and Edwards, J. (1995) J. Biol. Chem. 270, 14948–14955
18. Howell, S., Duncan, R. H., and Ashley, R. H. (1996) FEBS Lett. 380, 297–301
19. Chomzinski, P., and Satchi, N. (1987) Anal. Biochem. 162, 156–159
20. Muruyama, I. N., Rakow, T. L., and Muruyama, H. I. (1985) Nucleic Acids Res. 13, 3796–3797
21. Young, W. S., Meezy, E., and Siegel, R. E. (1986) Neurosci. Lett. 70, 198–203
22. Gilmore, R., and Blbef, G. (1985) Cell 21, 497–505
23. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
24. Frederiksen, K., Jat, P. S., Valtez, N., Levy, D., and McKay, R. (1988) Neuron 1, 439–448
25. Sorgato, M. C., Keller, B. U., and Stuhmer, W. (1987) Nature 324, 5310–5314
26. Terasaki, M., Song, J., Wong, J. R., Weiss, M. J., and Chen, L. B. (1984) Cell 38, 101–108
27. Miller, C. D., (ed) (1984) Ion Channel Reconstitution, Plenum Publishing Corp., New York
28. Hodgkin, A. L., and Katz, B. (1949) J. Physiol. 106, 27–77
29. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
30. Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148
31. Root, B., Farinelli, P., and Casadio, R. (1996) Protein Sci. 5, 1704–1718
32. Fujiki, Y., Hubbard, A. L., Fowler, S., and Lazarow, P. B. (1982) J. Cell. Biol. 93, 97–102
33. Feng, G., Deak, P., Chopra, M., and Hall, L. M. (1995) Cell 82, 1001–1011
34. Moorman, J. H., Ackerman, S. J., Kowdley, G. C., Griffin, M. F., Mounsey, J. P., Chen, Z. H., Cala, S. E., Obrian, J. J., Strabo, G., and Jones, L. R. (1995) Nature 377, 737–740
35. Duff, R. C., and Ashley, R. H. (1992) Virology 190, 485–489
36. Pinto, L. H., Holsinger, C. J., and Lamb, R. A. (1992) Cell 70, 517–528
37. Palmer, C. J., Scott, B. T., and Jones, L. R. (1991) J. Biol. Chem. 266, 11126–11130
38. Takumi, T., Okabe, H., and Nakamura, S. (1983) Science 224, 1042–1045
39. Atassi, B., Guillermare, E., Lesage, P., Honore, E., Romey, G., Lazdunski, M., and Barhain, J. (1993) Nature 365, 850–852
40. Barhain, J., Lesage, P., Guillermare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) Nature 384, 78–80
41. Sanguineti, M. C., Curran, M. E., Zou, A., Shn, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80–83
42. Krapivinsky, G. B., Ackerman, M. J., Gordon, E. A., Krapivinsky, L. D., and Clapham, D. E. (1993) Nature 361, 439–448
43. Paulmicl, M., Li, Y., Wickman, K., Ackerman, M., Peralta, E., and Clapham, D. (1992) Nature 356, 238–241
44. Buyse, G., Vos, T., Tygatt, J., De Greef, C., Drougmans, G., Nilius, B., and Eggermont, J. (1997) J. Biol. Chem. 272, 3615–3621
45. Clark, A. G., Murray, D., and Ashley, R. H. (1987) Biophys. J. 73, 168–178
46. Al-Awqati, Q. (1995) Science 269, 805–806
47. Valenzuela, S. M., Martin, D. K., Pur, S. B., Robbins, J. M., Warton, K., Bootcov, M. R., Schofield, P. R., Campbell, T. J., and Breit, S. N. (1997) J. Biol. Chem. 272, 12575–12582
Rat Brain p64H1, Expression of a New Member of the p64 Chloride Channel Protein Family in Endoplasmic Reticulum
Rory R. Duncan, Paul K. Westwood, Alan Boyd and Richard H. Ashley

J. Biol. Chem. 1997, 272:23880-23886.
doi: 10.1074/jbc.272.38.23880

Access the most updated version of this article at http://www.jbc.org/content/272/38/23880

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 10 of which can be accessed free at http://www.jbc.org/content/272/38/23880.full.html#ref-list-1