Interactions between Subunits of the Human Epithelial Sodium Channel*

(Received for publication, July 2, 1997)

Christopher M. Adams‡, Peter M. Snyder§, and Michael J. Welsh¶

From the Howard Hughes Medical Institute and Departments of Internal Medicine and Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242

The human epithelial sodium channel (hENaC) mediates Na\(^+\) transport across the apical membrane of epithelia, and mutations in hENaC result in hypertensive and salt-wasting diseases. In heterologous expression systems, maximal hENaC function requires co-expression of three homologous proteins, the α, β, and γhENaC subunits, suggesting that hENaC subunits interact to form a multimeric channel complex. Using a co-immunoprecipitation assay, we found that hENaC subunits associated tightly to form homo- and heteromeric complexes and that the association between subunits occurred early in channel biosynthesis. Deletion analysis of γhENaC revealed that the N terminus was sufficient but not necessary for co-precipitation of αhENaC, and that both the N terminus and the second transmembrane segment (M2) were required for γ subunit function. The biochemical studies were supported by functional studies. Co-expression of γ subunits lacking M2 with full-length hENaC subunits revealed an inhibitory effect on hENaC channel function that appeared to be mediated by the cytoplasmic N terminus of γ, and was consistent with the assembly of nonfunctional subunits into the channel complex. We conclude that the N terminus of γhENaC is involved in channel assembly.

The human epithelial Na\(^+\) channel (hENaC)\(^1\) mediates Na\(^+\) transport across the apical membrane of epithelia and is essential for normal salt homeostasis. Some mutations in genes encoding hENaC subunits cause a hereditary form of hypertension, Liddle’s syndrome (1–5), whereas other hENaC mutations cause the salt-wasting disease pseudohypoaldosteronism type 1 (6, 7). The hENaC subunits are members of a family of proteins that include the FMRFamide-gated Na\(^+\) channels of the Helix aspersa (8), ion channels from human neurons (BNC1 and BNaC2) (9–12), and the degenerins of Helix pomatia and BNaC2) (9–12), and the degenerins of Caenorhabditis elegans (DEG-1, MEC-4, MEC-10, UNC-105, UNC-8, and DEL-1), which form putative mechanosensory channels (13–19).

Functional studies indicate that epithelial Na\(^+\) channels are composed of at least three homologous proteins, the α, β, and γhENaC subunits (20–24). Amino acid sequence analysis of the subunits and biochemical studies of rat αENaC (25–27) suggest that the subunits possess cytoplasmic N and C termini, two transmembrane domains (M1 and M2), and a large glycosylated, cysteine-rich extracellular domain. Since all three subunits are required for maximal hENaC function, it has been hypothesized that the subunits interact to form a multimeric channel complex. In addition, genetic studies of the degenerins suggest that mechanosensory function and dominant neurodegeneration require three gene products (14–16).

The goal of this study was to answer some fundamental questions about the structure of hENaC. First, do the hENaC subunits interact with each other and with themselves? Second, which parts of an hENaC subunit are necessary for channel function and which are responsible for interactions with other subunits?

EXPERIMENTAL PROCEDURES

DNA Constructs—The cDNAs encoding secreted alkaline phosphatase, and α, β, γ, α\(_{\text{S594X}}\), β\(_{\text{D268X}}\), γ\(_{\text{D102X}}\) hENaC (all in pMT3) are described elsewhere (4, 22, 23, 28). Epitopes were introduced into full-length hENaC subunits, immediately upstream of the stop codon, using the Mutagene Phagemid in vitro mutagenesis kit (Bio-Rad). The FLAG epitope (DYKDDDDK) was inserted into αhENaC (α\(_{\text{FLAG}}\)), βhENaC (β\(_{\text{FLAG}}\)), and γhENaC (γ\(_{\text{FLAG}}\)), and a hemagglutinin (HA) epitope (YPY-DVPDYA) was inserted into βhENaC (β\(_{\text{HA}}\)) and γhENaC (γ\(_{\text{HA}}\)). In α\(_{\text{FLAG}}\), the FLAG epitope replaced the last 8 C-terminal residues of hENaC. In the other tagged subunits, the epitope was inserted immediately after the most C-terminal residue. Epitope-tagged subunits were cloned into pMT3 for expression.

γ\(_{\text{S594X}}\) and γ\(_{\text{D102X}}\) were constructed by single-stranded mutagenesis of γhENaC in pcDNA3 (Invitrogen, San Diego, CA). γ\(_{\text{S594X}}\) and γ\(_{\text{D102X}}\) were amplified by polymerase chain reaction using γhENaC as a template. In γ\(_{\text{S594X}}\), the FLAG epitope was inserted after the last C-terminal residue. γ\(_{\text{FLAG}}\) and γ\(_{\text{D102X}}\) were cloned into pcDNA3 and γ\(_{\text{S594X}}\) into pMT3 for expression.

Antibodies, Immunoprecipitations, and Western Blots—The anti-α monoclonal antibody, 12CA5, was obtained from Boehringer Mannheim, and the anti-FLAG monoclonal antibody, M2, was from Eastman Kodak Co. The anti-α and anti-γ polyclonal antisera were raised against peptides corresponding to the N-terminal 20 amino acids of α and γhENaC, respectively.

COS-7 cells were transfected by electroporation and grown in medium containing 10 μM amiloride. Two to three days post-transfection, cells were lysed at 4 °C in lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.4, 0.4 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 10 μg/ml pepstatin A) with detergent. Metabolic labeling with [\(^{14}C\)]methionine (Amersham Corp.) was performed prior to cell lysis, as described previously (29). To determine the most appropriate method for solubilization of hENaC subunits, we tested lysis buffer with several different detergents. 1% digitonin, 1% Nonidet P-40, and radioimmunoprecipitation buffer (1% Triton X-100, 0.1% SDS, and 1% sodium deoxycholate) each effectively solubilized α\(_{\text{FLAG}}\), whereas 1% CHAPS and 2.5% octyl glucoside were both relatively ineffective (Fig. 1A). 1% Triton X-100, without SDS or deoxycholate, also effectively solubilized α\(_{\text{FLAG}}\). Lysates were cleared by centrifugation at 70,000 rpm at 4 °C for 30 min, then incubated with primary antibody overnight at 4 °C. For immunoprecipitations, we used 5 μg/ml anti-FLAG antibody.
then detected by Western blot as described above. COS-7 cells were transfected with αFLAG, β, and γ. Cells were lysed with various detergents, and αFLAG was immunoprecipitated and detected by Western blot with anti-FLAG antibody. B, glycosylation of epitope-tagged subunits. COS-7 cells were transfected with αFLAG, βFLAG, or γFLAG. Cells were lysed with 1% digitonin, and αFLAG, βFLAG, or γFLAG was immunoprecipitated with anti-FLAG antibody. Immunoprecipitated protein was incubated at 37 °C overnight with or without protein N-glycanase (PNGase), and FLAG-tagged subunits were detected by Western blot with anti-FLAG antibody. C, function of epitope-tagged subunits. Xenopus oocytes were injected with the indicated DNA constructs, and amiloride (100 μM)-
sensitive current (Iamiloride) was measured with a two-electrode voltage-clamp. In this experiment, epitope-tagged subunits were expressed in combinations that were used for co-immunoprecipitation studies. Individual epitope-tagged subunits were also fully functional when expressed with two wild-type subunits in Xenopus oocytes (not shown). Each group represents an average current (± S.E.) from at least four oocytes.

RESULTS

Co-immunoprecipitation of Full-length hENaC Subunits—To test the hypothesis that hENaC subunits form heteromeric complexes, we examined the interaction of hENaC subunits in transfected COS-7 cells. In these experiments, we immunoprecipitated one subunit and detected the other subunit by Western blot; this strategy allowed us to distinguish interactions were not detected. Under identical immunoprecipitation conditions, we could not detect an interaction between αhENaC and the cystic fibrosis transmembrane conduct-
These truncated subunits (\(a_t\)) did not interact nonspecifically with other transmembrane proteins. To test the strength of the heteromeric interactions, we varied the immunoprecipitation conditions; each interaction withstanded all the conditions tested, including a nonionic detergent.

In *Xenopus* oocytes, expression of \(\alpha\)hENaC alone generates current, suggesting that \(\alpha\) may form a homomultimer. Because expression of \(\beta\) and \(\gamma\) alone fails to generate current, it is possible that these subunits do not form homomultimers. To test these possibilities we employed two different strategies. First, we tested the ability of full-length subunits to co-precipitate smaller subunits that lacked the cytoplasmic C terminus. These truncated subunits (\(\alpha_{S594X}\) and \(\beta_{R566X}\)) are functional and are similar to mutant \(\beta\) and \(\gamma\) subunits found in patients with Liddle’s syndrome (1–4). Deletion of the C terminus speeds migration on an SDS-polyacrylamide gel electrophoresis gel, allowing us to distinguish metabolically labeled \(\alpha_{S594X}\) and \(\beta_{R566X}\) from full-length protein. Fig. 4A shows that \(\alpha_{FLAG}\) co-precipitated \(\alpha_{S594X}\) and \(\beta_{FLAG}\) co-precipitated \(\beta_{R566X}\), indicating that both \(\alpha\) and \(\beta\) subunits could form homomultimers. Fig. 4B also shows that \(\alpha_{FLAG}\) co-precipitated \(\beta_{R566X}\), and \(\beta_{FLAG}\) co-precipitated \(\alpha_{S594X}\), consistent with the data shown in Figs. 2 and 3. Our second strategy was to immunoprecipitate one full-length subunit with the anti-HA antibody, and then detect co-precipitating subunits with the anti-FLAG antibody. As shown in Fig. 4B, \(\beta_{FLAG}\) co-precipitated with \(\beta_{HA}\) and \(\gamma_{FLAG}\) co-precipitated with \(\gamma_{HA}\) (Fig. 4A). These results indicated that each of the subunits can associate to form homomultimers. Therefore, the inability of \(\beta\) and \(\gamma\) to generate current when expressed alone is not likely the result of an inability to associate.

### Biochemical Interactions between \(\alpha\)hENaC and Truncated \(\gamma\) Subunits

To define the site(s) of interaction between \(\alpha\)hENaC subunits, we deleted portions of one subunit, \(\gamma\)hENaC, then tested those truncated \(\gamma\) subunits for their ability to associate with full-length \(\alpha\)hENaC. First, we made progressive truncations from the C terminus of \(\gamma\) (Fig. 5A). Deletion of the C terminus, M2, the large extracellular domain, and M1 of \(\gamma\) did not abolish its ability to interact with \(\alpha\) (Fig. 5, B and C). A construct that consisted of only the cytoplasmic N terminus \(\gamma_{L54X}\) was able to precipitate \(\alpha\) (Fig. 5B). Thus, the N terminus of \(\gamma\) was sufficient for an interaction with \(\alpha\). Although constructs that contained M1 \(\gamma_{S594X}\), M1\(\alpha_{S594X}\), or M1\(\alpha_{S594X}\) interacted with glycosylated \(\alpha_{S594X}\), we detected an interaction only between \(\gamma_{L54X}\) and unglycosylated \(\alpha_{S594X}\). To determine if the N terminus was necessary for an association with \(\alpha\), we tested \(\gamma_{L54X}\) with unglycosylated \(\gamma_{L54X}\) and \(\gamma_{L54X}\) with unglycosylated \(\alpha_{S594X}\). As shown in Fig. 5D, \(\gamma_{L54X}\) immunoprecipitated \(\alpha_{S594X}\). This result indicated that the N terminus of \(\gamma\) was not required for association with \(\alpha\) and that other sites of intersubunit association likely exist.

### Function of Truncated \(\gamma\) Subunits in Xenopus Oocytes

Since some pseudohypoaldosteronism type 1-associated mutations are predicted to cause truncation of \(\alpha\)hENaC subunits (6, 7), we tested the ability of truncated \(\gamma\) subunits to contribute to a functional \(Na^{+}\) channel complex. In *Xenopus* oocytes, expression of \(\alpha\)hENaC alone produces a small amiloride-sensitive \(Na^{+}\) current (22). We previously reported that co-expression of \(\gamma\) with \(\alpha\) produced larger currents, and maximal currents were obtained when all three subunits were co-expressed (23). Moreover, in the absence of \(\alpha\)hENaC, \(\beta\) and \(\gamma\) generated no amiloride-sensitive current. Fig. 6 shows that coexpression of \(\gamma\)hENaC with the \(\alpha\) subunit increased current. However, of the truncated \(\gamma\) subunits, only \(\gamma_{K76X}\) augmented a subunit-dependent amiloride-sensitive currents. \(\gamma_{K76X}\), which lacked most of the C terminus and was similar to mutant subunits found in patients with Liddle’s syndrome, increased the current compared with wild-type \(\gamma\). This increase in current was
also co-precipitated expressing all three wild-type subunits. Since these constructs
interact with hENaC (4, 31). Truncated subunits into the channel complex, we expressed
terminated and initiation of assembly required three homologous subunits (33, 34). In contrast, we found that
which the subunits interact to form a heteromeric channel complex. This hypothesis was confirmed by our demonstration that hENaC subunits interact with each other and with themselves. The interactions between hENaC subunits appeared to occur early in biosynthesis, prior to glycosylation. Furthermore, the interactions were relatively strong and were not disrupted by nonionic detergents or high concentrations of salt. In the assembly of some heteromeric channels, such as the nicotinic acetylcholine receptor, interaction between specific subunits requires the presence of a third subunit, suggesting a
that hENaC subunits interact with each other and with themselves. In addition, we detected subunit association with full-length subunits to form nonfunctional channels if nonfunctional channel subunits are co-expressed (30). Fig. 7C shows that oocytes co-expressing αFLAG with γ1102X or γL54X contained much less αFLAG protein than control oocytes. This result was consistent with our hypotheses that truncated γ subunits assemble into an unstable complex with full-length subunits, and that the γN terminus is a site of channel assembly.

**DISCUSSION**

These studies provide both biochemical and functional data showing protein-protein interactions between hENaC subunits. Previous studies indicated that maximal hENaC function required three homologous subunits (α, β, and γ), suggesting that the subunits interact to form a heteromeric channel complex. This hypothesis was confirmed by our demonstration that hENaC subunits interact with each other and with themselves. The interactions between hENaC subunits appeared to occur early in biosynthesis, prior to glycosylation. Furthermore, the interactions were relatively strong and were not disrupted by nonionic detergents or high concentrations of salt. In the assembly of some heteromeric channels, such as the nicotinic acetylcholine receptor, interaction between specific subunits may be a mechanism to generate diversity in channel function and regulation.

To begin to define the subunit domain(s) that participate in intersubunit associations, we performed a deletion analysis of the γ subunit and found that the γN terminus alone could co-precipitate a. Interestingly, the γN terminus interacted only with unglycosylated a and did not co-immunoprecipitate glycosylated a. There are a number of possible explanations. First, the precipitating antibody may not recognize the γN terminus when it is associated with glycosylated a. Second, the γN terminus may interact with full-length a early, then dissociate when a is glycosylated. Third, the N terminus may associate with unglycosylated a and prevent its glycosylation. While our experiments here do not address these issues, the third possibility is consistent with our experiments in Xenopus oocytes, which showed an effect of the γN terminus on a biosynthesis.

Our studies of truncated γ subunits also indicated that there is not a single site that is essential for intersubunit association. This result was consistent with studies of other channels that have shown multiple sites of subunit interactions (35). One interesting structural feature of hENaC subunits is the large number of highly conserved extracellular cysteines, which could potentially participate in intersubunit disulfide bonds. Although these cysteines were not required for intersubunit

---

**Fig. 5. Associations of truncated γ subunits with αhENaC.** A. Diagram showing truncated γ subunits. B–D, interactions of truncated γ subunits with α. COS-7 cells were transfected with the indicated constructs and lysed in 1% Triton X-100 (B and D) or 1% Nonidet P-40 (C). In B and C, γ subunits were immunoprecipitated with anti-Nt antisera, and αFLAG was detected by Western blot using anti-FLAG antibody. In D, γL54X was immunoprecipitated with anti-FLAG antibody, and α was detected by Western blot using anti-Nt antisera. In B and D, immunoprecipitates were washed under high salt conditions. In C, only glycosylated αFLAG was observed, probably due to a lower level of protein expression in this experiment.

probably due to increased cell-surface expression of αγK676X channels, since γK676X lacked a C-terminal sequence thought to mediate endocytosis of hENaC (4, 31). Truncated γ subunits that lacked the N terminus (γA3–53) or M2 (γK576X, γ1102X, and γL54X) were unable to substitute for wild-type γ, indicating a functional requirement for the N terminus and M2. Moreover, co-expression of γA3–53 with γL54X did not reconstitute a functional γ subunit. The functional requirement for the N terminus may reflect its involvement in intersubunit associations, as we observed in the co-immunoprecipitation assay. The requirement for M2 might be explained by its contribution to the channel pore (32) and suggests that pseudohypoparathyroidism type 1-associated mutants that lack M2 are nonfunctional.

**Functional Interactions between Truncated γ Subunits and αhENaC**—We also used a functional assay in Xenopus oocytes to investigate interactions between α and γhENaC. Since hENaC subunits interact to form heteromeric complexes, we hypothesized that the interaction of a nonfunctional, truncated γ subunit with the hENaC channel complex might have an inhibitory effect on channel function. To promote assembly of truncated subunits into the channel complex, we expressed wild-type α, β, and γ subunits with a relative overabundance of truncated γ subunits. To control for nonspecific effects on protein synthesis, some oocytes were injected with a plasmid encoding secreted alkaline phosphatase (28) in place of plasmids encoding truncated γ subunits. Expression secreted alkaline phosphatase does not alter the amount of current generated by coexpression of α, β, and γ. As shown in Fig. 7A, γK576X, γ1102X, and γL54X inhibited amiloride-sensitive current in oocytes co-expressing all three wild-type subunits. Since these constructs also co-precipitated α, their inhibitory effect was consistent with a direct interaction with the αβγ complex, at least in part through the α subunit. To test the possibility that the inhibitory effect required the presence of β or γhENaC, we expressed only wild-type αhENaC with γ1102X or γL54X. In this assay as well, both γ1102X and γL54X inhibited amiloride-sensitive current (Fig. 7B). The functional effect of these constructs was consistent with the results of the co-precipitation assay, which showed that the N terminus of γhENaC was a site of interaction with α.

We hypothesized that if the truncated γ subunits interacted directly with full-length subunits to form nonfunctional channel complexes, these complexes might be degraded by cellular quality control mechanisms, thereby reducing the amiloride-sensitive current. By a similar mechanism, Xenopus oocytes degrade inward rectifier K+ channels if nonfunctional channel subunits are co-expressed (30). Fig. 7C shows that oocytes co-expressing αFLAG with γ1102X or γL54X contained much less αFLAG protein than control oocytes. This result was consistent with our hypotheses that truncated γ subunits assemble into an unstable complex with full-length subunits, and that the γN terminus is a site of channel assembly.
Effects of truncated γ subunits on hENaC. Oocytes were injected with a 1:1:1:4 DNA ratio of α to β to γ to truncated γ or secreted alkaline phosphatase (A), or a 1:4 DNA ratio of α to truncated γ or secreted alkaline phosphatase (B). Amiloride-sensitive currents were measured at a holding potential of −60 mV in A and at −80 mV in B. Average currents were normalized to those of control cells, which expressed secreted alkaline phosphatase instead of a truncated γ or secreted alkaline phosphatase. Oocyte membrane proteins were isolated, then separated by SDS-polyacrylamide gel electrophoresis, and αFLAG was detected by Western blot using anti-FLAG antibody. Longer exposures revealed a difference from control currents (p < 0.004).

Inhibitory effects of truncated γ subunits on hENaC. Expression of truncated γ subunits in Xenopus oocytes identified elements of the γ subunit that are essential for function. γ subunits lacking either the N terminus or M2 were nonfunctional, even though these regions were not required for subunit association. The functional requirement for M2 may be physiologically relevant for two reasons. First, alternatively spliced αhENaC transcripts with stop codons upstream of M2 have been identified in kidney, lung, and taste tissues (36). Like γE518X, γ1102X and γL54X, the α subunits encoded by alternatively spliced messages are nonfunctional. However, our studies suggest that alternatively spliced transcripts, if translated, could associate with full-length subunits, and thus could inhibit hENaC function. Second, our data indicate that pseudohypoaldosteronism-associated mutations that disrupt M2 in hENaC subunits may cause pseudohypoaldosteronism type 1 by abolishing subunit interaction.

In studies of other multimeric ion channels, the interaction of nonfunctional channel subunits with functional ones disrupted channel function and/or cell surface expression (30, 37–39). Therefore, as an additional assay of subunit interactions, we examined the functional effects of truncated γ subunits on wild-type hENaC channels. In oocytes expressing wild-type α, β, and γ hENaC, or α hENaC alone, γ subunits that contained the cytoplasmic N terminus reduced the magnitude of amiloride-sensitive currents. N termini are important in the assembly of other ion channels, including voltage-gated K+ channels and the nicotinic acetylcholine receptor (37, 39–41). In addition, cytoplasmic N termini have been shown to be important regulators of function in channels after they have been assembled (42, 43). Interestingly, proteins closely related to hENaC, the degenerins of C. elegans, also appear to be inhibited by overexpression of their cytoplasmic N termini.2 Thus, the functional effect of the γ N terminus may reflect a general property of this family of ion channels.

Acknowledgments—We thank Pary Weber and Ellen Tarr for excellent assistance. We especially appreciate the discussions and help of Drs. Maggie Price, Lynda Ostedgaard, Fiona McDonald, and Lance Prince. We thank the University of Iowa DNA Core Facility for assistance with sequencing and oligonucleotide synthesis.

2 M. Driscoll, personal communication.
REFERENCES

1. Shimkets, R. A., Warnock, D. G., Bositis, C. M., Nelson-Williams, C., Hansson, J. H., Schambelan, M., Gill, J. R., Jr., Ullick, S., Milora, R. V., Finding, J. W., Canessa, C. M., Rossier, B. C., and Lifton, R. P. (1994) *Cell* 79, 407–414.

2. Hansson, J. H., Nelson-Williams, C., Suzuki, H., Schild, L., Shimkets, R., Lu, Y., Canessa, C., Iwasaki, T., Rossier, B., and Lifton, R. P. (1995) *Nat. Genet.* 11, 76–82.

3. Schild, L., Canessa, C. M., Shimkets, R. A., Gautschi, I., Lifton, R. P., and Welsh, M. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 5699–5703.

4. Snyder, P. M., Price, M. P., McDonald, F. J., Adams, C. M., Volk, K. A., Zeiher, E. G., Stokes, J. B., and Welsh, M. J. (1995) *Cell* 83, 969–978.

5. Buhien, J. K., Ismailov, I. I., Berdiev, B. K., Cornwell, T., Lifton, R. P., Fuller, C. M., Achard, J. M., Benos, D. J., and Warnock, D. G. (1996) *Am. J. Physiol.* 270, C208–C213.

6. Chang, S. S., Grunder, S., Hanukoglu, I., Rösler, A., Mathew, P. M., Hanukoglu, I., Schild, L., Lu, Y., Shimkets, R. A., Nelson-Williams, C., Rossier, B. C., and Lifton, R. P. (1996) *Nat. Genet.* 13, 248–253.

7. Strautnieks, S. S., Thompson, R. J., Gardiner, R. M., and Chung, E. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 11735–11737.

8. Lingueglia, E., Champigny, G., Lazdunski, M., and Barbry, P. (1995) *Nature* 378, 730–733.

9. Price, M. P., Snyder, P. M., and Welsh, M. J. (1996) *J. Biol. Chem.* 271, 7879–7882.

10. Waldmann, R., Champigny, G., Voilley, N., Lauritzen, I., and Lazdunski, M. (1996) *J. Biol. Chem.* 271, 10433–10436.

11. Garcia-Anoveros, J., Derfler, B., Neville-Golden, J., Hyman, B. T., and Corey, D. P. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1459–1464.

12. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., and Lazdunski, M. (1997) *Nature* 386, 173–177.

13. Chaffee, M., and Au, M. (1989) *Nature* 342, 102–1033.

14. Chaffee, M., and Wolinsky, E. (1990) *Nature* 345, 410–416.

15. Driscoll, M., and Chaffee, M. (1991) *Nature* 349, 588–593.

16. Huang, M., and Chaffee, M. (1994) *Nature* 367, 467–470.

17. Hong, K., and Driscoll, M. (1994) *Nature* 367, 470–473.

18. Liu, J., Schrank, B., and Watersston, R. H. (1996) *Science* 273, 361–364.

19. Tavernarakis, N., Shreffler, W., Wang, S., and Driscoll, M. (1997) *Neuron* 18, 107–112.

20. Canessa, C. M., Horisberger, J.-D., and Rossier, B. C. (1993) *Nature* 361, 467–470.

21. Canessa, C. M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.-D., and Rossier, B. C. (1994) *Nature* 367, 463–467.

22. McDonald, F. J., Snyder, P. M., McCray, P. B., Jr., and Welsh, M. J. (1994) *Am. J. Physiol.* 266, L728–L734.

23. McDonald, F. J., Price, M. P., Snyder, P. M., and Welsh, M. J. (1995) *Am. J. Physiol.* 268, C1157–C1163.

24. Benos, D. J., Awaysa, M. S., Ismailov, I. I., and Johnson, J. P. (1995) *J. Membr. Biol.* 143, 1–18.

25. Snyder, P. M., McDonald, F. J., Stokes, J. B., and Welsh, M. J. (1994) *J. Biol. Chem.* 269, 24379–24383.

26. Renard, S., Lingueglia, E., Voilley, N., Lazdunski, M., and Barbry, P. (1994) *J. Biol. Chem.* 269, 12981–12986.

27. Canessa, C. M., Merillat, A. M., and Rossier, B. C. (1994) *Am. J. Physiol.* 267, C1682–C1690.

28. Swick, A. G., Janicot, M., Cheneval-Kastelic, T., McLennathan, J. C., and Lane, M. D. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 1812–1816.

29. Ostedgaard, L. S., Rich, D. P., DeBerg, L. G., and Welsh, M. J. (1997) *Biochemistry* 36, 1287–1294.

30. Tucker, S. J., Bond, C. T., Herson, P., Pessia, M., and Adelman, J. P. (1996) *J. Biol. Chem.* 271, 5866–5870.

31. Firsov, D., Schild, L., Gautschi, I., Merillat, A. M., Schneegger, E., and Rossier, B. C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 15370–15375.

32. Waldmann, R., Champigny, G., and Lazdunski, M. (1995) *J. Biol. Chem.* 270, 11735–11737.

33. Gu, Y., Forayeth, J. R., Verrall, S., Yu, X. M., and Hall, Z. W. (1991) *J. Cell Biol.* 114, 799–807.

34. Green, W. N., and Claudio, T. (1993) *Cell* 74, 57–69.

35. Tu, L., Santarelli, V., Sheng, Z., Skach, W., Pain, D., and Deutsch, C. (1996) *J. Biol. Chem.* 271, 18904–18911.

36. Li, X-J., Xu, R-H., Guggino, W. B., and Snyder, S. H. (1995) *Mol. Pharmacol.* 47, 1133–1140.

37. Li, J., Xu, R-H., and Snyder, S. H. (1995) *Science* 272, 1225–1230.

39. Babila, T., Moscucci, A., Wang, H., Weaver, F. E., and Koren, G. (1994) *Neuron* 12, 615–626.

40. Verrall, S., and Hall, Z. W. (1992) *Cell* 68, 23–31.

41. Shen, N. V., Chen, X., Boyer, M. M., and Pfaffinger, P. J. (1995) *Neuron* 11, 67–76.

42. Shen, N. V., and Pfaffinger, P. J. (1995) *Neuron* 14, 625–633.

43. Hoshi, T., Zagotta, W. N., and Aldrich, R. W. (1990) *Science* 250, 533–538.

44. Zagotta, W. N., Hoshi, T., and Aldrich, R. W. (1990) *Science* 250, 568–571.