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Commentary

Chloride channels and endocytosis: ClC-5 makes a Dent

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Ion channels are ubiquitous pore-forming membrane proteins found in virtually every cell of every living organism. In mammals, a vast array of ion channel types participate in numerous vital physiological processes such as membrane excitability, cell volume regulation, signal transduction, secretion, and absorption. Therefore, it is not surprising that hereditary conditions can occur because of mutations in genes which encode critical components of ion channel molecules (1). Since 1989, more than 25 human disease entities have been identified as "ion channelopathies." Studies of ion channel disease syndromes have contributed greatly to our understanding of specific disease pathogenesis and, in some cases, have revealed new insights into the relationship between ion channel structure and function (2–6).

In this issue of *Proceedings*, Günther, *et al*. study the cellular localization of a kidney chloride channel (ClC-5) implicated in the pathogenesis of X-linked hypercalciuric nephrolithiasis (prototype syndrome, Dent's disease) and infer the probable function of this molecule (7). The ClC chloride channel family, to which ClC-5 belongs, has been recognized only within the past decade (8); yet one-third of all identified mammalian ClC isoforms have been linked to human genetic diseases (Table 1) (9–12). Soon after the first member of this gene family was sequenced from *Torpedo* electric organ (ClC-0) (13), a mammalian homolog expressed exclusively in skeletal muscle (ClC-1) was identified as the gene for myotonia congenita (9, 10, 14), an unusual syndrome associated with muscular stiffness caused by delayed relaxation of muscle following voluntary contractions. The candidacy of ClC-1 was initially based on solid physiological evidence of diminished sarcolemmal chloride conductance in a curious animal model, the myotonic or "fainting" goat (15). Another mammalian ClC isoform expressed in the kidney (ClC-K_B), was added recently to the list of ClC "channelopathies" when mutations were discovered in Bartter's syndrome, a rare disorder associated with renal salt wasting and hypokalemic alkalosis (12). This discovery provides evidence that ClC-K_B is probably the basolateral chloride channel in medullary thick ascending limb cells required for transepithelial transport of Na^+ and Cl^- ions. Dent's disease and a related form of X-linked hypercalciuric nephrolithiasis were genetically mapped to Xp11.22, a region initially devoid of logical candidate genes (16, 17). Subsequently, a microdeletion in a single Dent's disease family was found that deleted a novel ClC sequence (ClC-5), and this finding helped pinpoint the gene (18). Further analysis of ClC-5 has now established that mutations in this gene may cause four related X-linked syndromes of hypercalciuric nephrolithiasis (11).

Syndromes associated with ClC-5 mutations share the following phenotypic features: excessive urinary calcium excretion (hypercalciuria), abnormally high urinary levels of low molecular weight proteins, (low molecular weight proteinuria), and either intrarenal calcification (nephrocalcinosis) or formation of calcium kidney stones (nephrolithiasis). Although Dent's disease was first described in England, the clinical presentation of the three related syndromes, X-linked recessive nephrolithiasis (North America), X-linked hypophosphatemic rickets (Italy), and low molecular weight proteinuria/nephrocalcinosis (Japan) has been related to the geographic location in which they were first described (19). At the present time, there are many uncertainties about the mechanism responsible for hypercalciuria in these syndromes, but the presence of low molecular weight proteinuria in Dent's disease subjects has prompted consideration of a defect in absorptive endocytosis involving the proximal tubule (20). Normally, fluid produced by glomerular ultrafiltration is free of large proteins, such as albumin, but smaller proteins (peptide hormones, enzymes, and secretory and tissue proteins), which may pass the glomerular barrier, are subsequently reabsorbed by epithelial cells lining the proximal tubule. The process of low molecular weight protein reabsorption is mediated by a type of absorptive endocytosis that has been well characterized at both the functional and ultrastructural level (21, 22). Ultimately, proteins absorbed via this pathway are enzymatically degraded in lysosomes. An important functional requirement for absorptive endocytosis and subsequent protein degradation is the maintenance of an acidic intravesicular environment. Acidification is primarily accomplished by the active transport of H^+ by a vacuolar type H^+-ATPase. Also, because active H^+ transport is an electrogenic process, concurrent chloride movement into endosomal compartments is required to maintain electroneutrality (Fig. 1). Intracellular organelles are known to possess Cl^- channels although their molecular identities have been unclear (23).

Table 1. Hereditary syndromes caused by ClC chloride channel mutations

Gene*	Ion Channel	Disease(s)
CLCN1 (7q35)	Muscle ClC-1	Thomsen's disease (myotonia congenita) Recessive generalized myotonia
CLCNKB (1p36)	Kidney ClC-K_B	Bartter's syndrome, type III
CLCN5 (Xp11.22)	Kidney ClC-5	Dent's disease X-linked recessive nephrolithiasis X-linked recessive hypophosphatemic rickets LMWP/nephrocalcinosis

*Human chromosomal location given in parenthesis.
LMWP, low molecular weight proteinuria.

Using ClC-5 specific antibodies, Günther *et al.* (7) now demonstrate localization of ClC-5 to early endosomes in proximal tubular cells of rat kidney. Immunostaining for ClC-5 was virtually absent in the apical membrane microvilli of the proximal tubule where one would expect to find proteins that participate in transepithelial ion movement. Rather, the staining pattern for ClC-5 coincides with that of the vacuolar H^+-ATPase in the subapical cytoplasm. This subcellular location also overlaps the distribution of acutely reabsorbed fluorescently tagged β2-microglobulin, a low molecular weight

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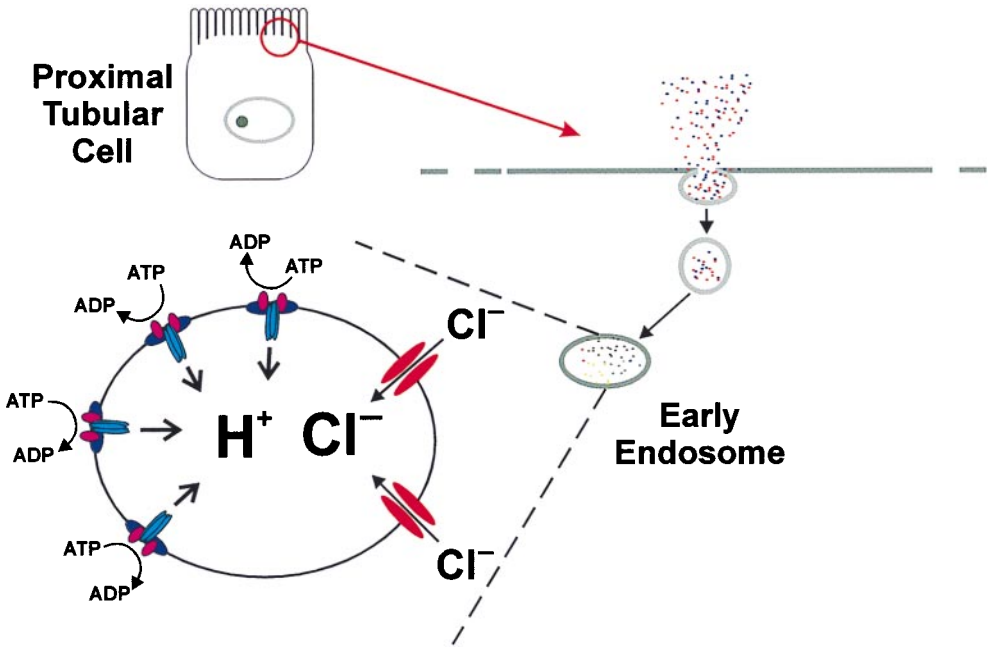

FIG. 1. Illustration of absorptive endocytosis in a proximal renal tubular cell. Expanded view showing contributions of the H^+ATPase and chloride channel to acidification of the endocytic vesicle.

protein normally taken up by proximal tubular cells via endocytosis. Similar findings were observed in ClC-5-transfected COS-7 cells. To further demonstrate the localization of ClC-5 in endosomes, the investigators cotransfected ClC-5 with cDNA encoding a mutant rab5 protein, a small GTPase critical for early endosome fusion (24). Expression of mutant rab5 in COS-7 cells leads to formation of unusually large endocytic vesicles that are associated with intense staining for ClC-5. The accumulation of ClC-5 is isoform-specific as demonstrated by the failure of the homologous ClC-0 to target to the abnormal endosomes. Taken together, the data presented by Günther *et al.* suggest that ClC-5 is a strong candidate for the chloride channel of proximal tubular early endosomes and that its disruption in Dent's disease leads directly to an impairment in the reabsorption of low molecular weight proteins.

The pattern of ClC-5 localization in other nephron segments suggests that it also may participate in transepithelial ion transport processes. In this study, ClC-5 was localized to the apical plasma membrane of medullary and cortical collecting duct intercalated cells. In acid-secreting type A- (α) intercalated cells, ClC-5 colocalizes with the proton pump on the apical membrane. By contrast in bicarbonate-secreting type B- (β) intercalated cells, ClC-5 and vacuolar H^+-ATPase localize to opposite membranes with apical expression of ClC-5 and basolateral expression of the proton pump. It is tempting to speculate that ClC-5 is important for maintaining electroneutrality of apical acid secretion by α-intercalated cells, but its role in β cells is less clear. It would be interesting to examine whether changes in whole animal acid-base balance modulate the polarity of expression of ClC-5 in intercalated cells, similar to the phenomenon demonstrated for the vacuolar H^+-ATPase (25).

Although ClC-5 appears to be an endocytic chloride channel in cells of the proximal tubule, the molecular identity of other endocytic chloride channels in other regions of the nephron and in other tissues that do not express ClC-5 remains unclear. Previously, investigators have identified another intracellular chloride channel, designated p64 (26), and subsequently demonstrated its localization in secretory granules but not in endocytic vesicles of rabbit proximal tubular cells (27). Similarly, the cystic fibrosis transmembrane conductance regulator (CFTR) is present and functional in intracellular compartments (28, 29), and in addition to its clear physiological role in epithelial chloride secretion, CFTR appears to have functional importance in vesicular trafficking (30). However despite early speculation, CFTR is probably not required for the acidification of intracellular compartments (28, 29). Finally, other ClC type chloride channels may turn out to be important for the function of intracellular organelles. Recently, certain ClC-6 splice variants have been localized to the endoplasmic reticulum of transfected cells (31), but studies in native tissues are required before the significance of this finding is known.

In summary, the paper by Günther *et al.* provide evidence that ClC-5 is an important, although highly localized, endocytic chloride channel. This finding contributes to our understanding of absorptive endocytosis in mammalian kidney and helps to explain the low molecular weight proteinuria in Dent's disease. It remains unclear why ClC-5 mutations produce hypercalciuria and other related manifestations of the X-linked disorders listed in Table 1. It also is not completely clear how the unique voltage-dependence of ClC-5 observed in heterologous expression systems (32, 33) relates to its function in vesicular acidification. Lastly, given the availability of numerous cloned ClC subtypes, it would seem feasible to address the question of which functional domains of ClC-5 target this molecule to endosomes. Clearly, we are just beginning to appreciate the importance of ClC type chloride channels in a wide variety of physiological and cellular processes.

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1. George, A. L., Jr. (1995) *Kidney Int.* **48,** 1180–1190.
2. Sheppard, D. N., Rich, D. P., Ostedgaard, L. S., Gregory, R. J., Smith, A. E. & Welsh, M. J. (1993) *Nature (London)* **362,** 160–164.
3. Steinmeyer, K., Lorenz, C., Pusch, M., Koch, M. C. & Jentsch, T. J. (1994) *EMBO J.* **13,** 737–743.
4. Yang, N. & Horn, R. (1995) *Neuron* **15,** 213–218.
5. Staub, O., Dho, S., Henry, P. C., Correa, J., Ishikawa, T., McGlade, J. & Rotin, D. (1996) *EMBO J.* **15,** 2371–2380.
6. Fahlke, Ch., Yu, H. T., Beck, C. L., Rhodes, T. H. & George, A. L., Jr. (1997) *Nature (London)* **390,** 529–532.

7. Günther, W., Lüchow, A., Cluzeaud, F., Vandewalle, A. & Jentsch, T. J. (1998) *Proc. Natl. Acad. Sci. USA*, **95,** 8075–8080.
8. Jentsch, T. J. (1994) *Curr. Top. Membr.* **42,** 35–57.
9. Koch, M. C., Steinmeyer, K., Lorenz, C., Ricker, K., Wolf, F., Otto, M., Zoll, B., Lehmann-Horn, F., Grzeschik, K. H. & Jentsch, T. J. (1992) *Science* **257,** 797–800.
10. George, A. L., Crackower, M. A., Abdalla, J. A., Hudson, A. J. & Ebers, G. C. (1993) *Nat. Genet* **3,** 305–310.
11. Lloyd, S. E., Pearce, S. H. S., Fisher, S. E., Steinmeyer, K., Schwappach, B., Scheinman, S. J., Harding, B., Alessandra, B., Devota, M., Goodyear, P., *et al.* (1996) *Nature (London)* **379,** 445–449.
12. Simon, D. B., Bindra, R. S., Mansfield, T. A., Nilson-Williams, C., Mendonca, E., Stone, R., Schurman, S., Nayir, A., Alpay, H., Bakkaloglu, A., *et al.* (1997) *Nat. Genet* **17,** 171–178.
13. Jentsch, T. J., Steinmeyer, K. & Schwarz, G. (1990) *Nature (London)* **348,** 510–514.
14. Steinmeyer, K., Ortland, C. & Jentsch, T. J. (1991) *Nature (London)* **354,** 301–304.
15. Adrian, R. H. & Bryant, S. H. (1974) *J. Physiol. (London)* **240,** 505–515.
16. Pook, M. A., Wrong, O., Woodling, C., Norden, A. G. W., Feest, T. G. & Thakker, R. V. (1993) *Hum. Mol. Genet.* **2,** 2129–2134.
17. Scheinman, S. J., Pook, M. A., Wooding, C., Pang, J. T., Frymoyer, P. A. & Thakker, R. V. (1993) *J. Clin. Invest.* **91,** 2351–2357.
18. Fisher, S. E., Black, G. C. M., Lloyd, S. E., Hatchwell, E., Wrong, O., Thakker, R. V. & Craig, I. W. (1994) *Hum. Mol. Genet.* **3,** 2053–2059.
19. Scheinman, S. J. (1998) *Kidney Int.* **53,** 3–17.
20. Hebert, S. C. (1996) *Nature (London)* **379,** 398–399.
21. Wall, D. A. & Maack, T. (1985) *Am. J. Physiol.* **248,** C12–C20.
22. Christensen, E. I. & Nielsen, S. (1991) *Semin. Nephrol.* **11,** 414–439.
23. Al-Awqati, Q., Barasch, J. & Landry, D. (1992) *J. Exp. Biol.* **172,** 245–266.
24. Stenmark, H., Parton, R. G., Steele-Mortimer, O., Lutcke, A., Gruenberg, J. & Zerial, M. (1994) *EMBO J.* **13,** 1287–1296.
25. Bastani, B., Purcell, H., Hemkin, P., Trigg, D. & Gluck, S. (1991) *J. Clin. Invest.* **88,** 126–136.
26. Landry, D. W., Sullivan, S., Nicolaides, M., Redhead, C., Edelman, A., Al-Awqati, Q. & Edwards, J. (1993) *J. Biol. Chem.* **268,** 14948–14955.
27. Redhead, C., Sullivan, S. K., Koseki, C., Fujiwara, K. & Edwards, J. C. (1997) *Mol. Biol. Cell* **8,** 691–704.
28. Lukacs, G. L., Chang, X. B., Kartner, N., Rotstein, O. D., Riordan, J. R. & Grinstein, S. (1992) *J. Biol. Chem.* **267,** 14568–14572.
29. Biwersi, J. & Verkman, A. S. (1994) *Am. J. Physiol.* **266,** C149–C156.
30. Biwersi, J., Emans, N. & Verkman, A. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 12484–12489.
31. Buyse, G., Trouet, D., Voets, T., Missiaen, L., Droogmans, G., Nilius, B. & Eggermont, J. (1998) *Biochem. J.* **330,** 1015–1021.
32. Steinmeyer, K., Schwappach, B., Bens, M., Vandewalle, A. & Jentsch, T. J. (1995) *J. Biol. Chem.* **270,** 31172–31177.
33. Sakamoto, H., Kawasaki, M., Uchida, S., Sasaki, S. & Marumo, F. (1996) *J. Biol. Chem.* **271,** 10210–10216.