Hensin: a matrix protein determinant of epithelial polarity.

R J Alpern

Editorial

A fundamental element of acid-base regulation is the ability of the kidney to respond to changes in dietary acid composition by modifying rates of net acid excretion. A key component of this homeostatic response was first demonstrated by McKinney and Burg (1), who examined net H^+/HCO_3^- transport in rabbit cortical collecting ducts removed from the in vivo milieu and perfused in vitro. In vitro perfused cortical collecting ducts from rabbits ingesting an acid diet secreted H^+ into the luminal fluid, whereas cortical collecting ducts from animals ingesting an alkaline diet secreted HCO_3^-. This finding represented an example of long term memory in renal epithelial cells, wherein the in vivo milieu determined cellular behavior in vitro.

The cortical collecting duct is composed of three cell types, a principal cell which does not contribute to H^+/HCO_3^- transport, an α-intercalated cell which secretes H^+ into the lumen, and a β-intercalated cell which secretes HCO_3^- into the lumen. In α-intercalated cells H^+ secretion is mediated by an apical membrane vacuolar H^+ pump and a basolateral membrane Cl^-/HCO_3^- exchanger, whereas in β-intercalated cells HCO_3^- secretion is mediated by similar transporters with reversed polarity, a basolateral membrane vacuolar H^+ pump, and an apical membrane Cl^-/HCO_3^- exchanger. However, in α-intercalated cells the basolateral membrane Cl^-/HCO_3^- exchanger is encoded by the AE1 gene, whereas in β-intercalated cells the apical Cl^-/HCO_3^- exchanger does not label with antibodies against AE1. Roles for an H,K-ATPase in these processes have been proposed but have not been established. Schwartz et al. (2) found that α-intercalated cells demonstrate apical endocytosis whereas β-intercalated cells exhibit basolateral membrane endocytosis; in both cases endo/exocytosis was proposed to mediate trafficking of the vacuolar H^+ pump to and from the plasma membrane.

Using apical endocytosis as a marker for α-intercalated cells, Schwartz et al. (2) found that administration of acid to rabbits caused an increase in the number of α-intercalated cells with a decrease in the number of β-intercalated cells and no change in the total number of intercalated cells. In addition, it was noted that this transition occurred in the absence of mitosis, suggesting that it involved an adaptive change in cell function rather than proliferation and death of specific cell types. Based on these results, it was proposed that this adaptation involved a transition of β- to α-intercalated cells, possibly a reversal of epithelial polarity wherein the apical membrane Cl^-/HCO_3^- exchanger moved to the basolateral membrane and the basolateral membrane vacuolar H^+ pump moved to the apical membrane (2). Subsequent studies using markers similar to those described above, have found that intercalated cells can be divided into more than two subtypes, and that the response to acid may be more complicated than a mere interconversion between α- and β-intercalated cells. Nevertheless, a significant adaptation in intercalated cell function and morphology oc-

curs, and an understanding of the mechanisms responsible is of significant interest.

To address the mechanisms involved, van Adelsberg et al. developed an in vitro model for this adaptation. When primary cultures of β-intercalated cells were plated at low density on permeable supports they expressed HCO_3^- secretion that was dependent on apical Cl^- and inhibited by apical DIDS, an inhibitor of anion exchangers (β-intercalated cell phenotype), whereas when the cells were plated at high density they expressed apical H^+ secretion that was inhibited by basolateral DIDS (α-intercalated cell phenotype) (3). A clonal immortalized cell line was developed from these β-intercalated cells to further address this adaptation. These cells expressed an apical Cl^-/HCO_3^- exchanger as expected, but unexpectedly were shown to express apical AE1 (4). This finding was surprising in that immunohistochemical staining for AE1 has been consistently negative in β-intercalated cells in vivo. It is not presently resolved whether this discrepancy is attributable to a phenotypic alteration in culture or to a technical problem with immunohistochemical staining in vivo. This controversy aside, when these clonal cell lines were plated at low density they exhibited apical AE1 and no apical endocytosis (β-intercalated cell phenotype), while when plated at high density they exhibited basolateral membrane AE1 and apical endocytosis (α-intercalated cell phenotype) (3). This effect of plating density on phenotype was shown to be mediated by a 230-kD matrix protein.

In this issue of *The Journal*, Takito et al. (5) describe the purification and cloning of a partial cDNA for this 230-kD protein, which they have named hensin, the Japanese word for, "change in body." Hensin was secreted by the cultured intercalated cells into the extracellular matrix only when cells were plated at high density. In addition, antibodies against hensin inhibited the development of apical endocytosis in cells seeded at high density. Thus, hensin appears to play a key role in signaling the morphologic plasticity observed in these cells.

Hensin is a member of the scavenger receptor cysteine rich (SRCR) family (6). Proteins in this family are cell surface or secreted polypeptides that contain one or more SRCR domains. These domains are $\sim$ 100 amino acids in length and contain 6 to 8 cysteines, many of which form intradomain disulfide bonds. These domains likely mediate binding to other cell surface or extracellular molecules (6). Hensin is secreted into the extracellular matrix of the cultured intercalated cells, but also localizes to the basolateral membrane of these cells and to the apical and basolateral membranes of renal cells in vivo. Thus, hensin is likely a secreted protein which interacts with a membrane receptor leading to changes in morphology and function. By Northern blot, hensin is expressed in a number of epithelial tissues including small intestine, stomach, liver, kidney, and lung. Thus, hensin may play a key role in the development or regulation of epithelial function.

The specific role of hensin in the kidney is not yet clear. It is localized only to the collecting duct, suggesting some role in the development or regulation of this nephron segment. Although the identification and cloning of hensin was initially inspired by pH-induced adaptations in the collecting duct, it is not presently clear that it plays a role in this process. First,

changes in pH in vitro do not elicit changes in hensin secretion by the β-intercalated cell line (5). However, changes in media pH also do not elicit changes in endocytosis or AE1 localization in these cells. This may be because of a problem inherent in the cultured cells. A second concern relates to the fact that in the intact kidney hensin is expressed not only on α-intercalated cells as predicted, but also on β-intercalated cells and even on principal cells. Thus the mere presence of hensin does not commit cells to express an α-intercalated cell phenotype. Further studies will certainly follow to elucidate the function of hensin. Whether or not hensin mediates the effect of pH on the cortical collecting duct, it seems highly likely that hensin will be an important determinant of some aspect of collecting duct morphology and function.

Robert J. Alpern
Department of Internal Medicine
University of Texas Southwestern Medical Center

References

1. McKinney, T.D., and M.B. Burg. 1977. Bicarbonate transport by rabbit cortical collecting tubules: effect of acid and alkali loads in vivo on transport in vitro. *J. Clin. Invest.* 60:766–768.

2. Schwartz, G.J., J. Barasch, and Q. Al-Awqati. 1985. Plasticity of functional epithelial polarity. *Nature (Lond.).* 318:368–371.

3. van Adelsberg, J., J.C. Edwards, J. Takito, B. Kiss, and Q. Al-Awqati. 1994. An induced extracellular matrix protein reverses the polarity of band 3 in intercalated epithelial cells. *Cell.* 76:1053–1061.

4. van Adelsberg, J.S., J.C. Edwards, and Q. Al-Awqati. 1993. The apical Cl/HCO$_3$ exchanger of β intercalated cells. *J. Biol. Chem.* 268:11283–11289.

5. Takito, J., C. Hikita, and Q. Al-Awqati. 1997. Hensin, a new collecting duct protein involved in the in vitro plasticity of intercalated cell polarity. *J. Clin. Invest.* 98:2324–2331.

6. Resnick, D., A. Pearson, and M. Krieger. 1994. The SRCR superfamily: a family reminiscent of the Ig superfamily. *Trends Biochem. Sci.* 19:5–8.