Differentiation of Intercalated Cells in the Kidney

The intercalated cell of collecting ducts of the kidney is of two forms, the α form secretes acid, whereas the β form secretes HCO₃. Here, we review recent work that shows that the α form is derived from the β form and that the pathway is mediated by an extracellular matrix protein called hensin/DMBT1.

Given that all life started in water, an infinite source of H⁺, and that the primitive oceans had low salinity, it is likely that the first ion transport mechanism to evolve was a proton transport mechanism. The most likely first transport mechanism “invented” by evolution thus may have been proton translocation harnessed to energy transduction such as those in the reactions of cytochrome oxidase. Proton transport in multicellular organisms serves an additional function, that of the control of the pH of the extracellular and intracellular environments. In vertebrates, several organs participate in the defense against untoward acidity or alkalinity of the milieu intérieur; chief among them is the kidney, with participation by the lungs and brain, which control the rate of excretion of the potential acid CO₂. Changes in pH have powerful effects on the structure of proteins, and hence their function and cells had to develop mechanisms of defense against these changes. Often these responses involve profound changes in gene transcription or rapid changes in protein function. Remarkably, the proximate mechanism by which any cell senses the change in extracellular pH remains obscure. Much excitement developed when two orphan G-protein-coupled receptors (OGR1 and GPR4) were found to respond to changes in pH in the physiological range (23). Deletion of at least one of them, GPR4, prevented a normal renal response to an acid load and resulted in a mild distal renal tubular acidosis (dRTA) (36).

To regulate H⁺ balance, the kidney relies primarily in production of new HCO₃ needed to titrate the net acid generated by daily metabolism. Since the kidney suffers from the fact that it is a filtering organ, the majority of its H⁺ secretion mediates re-absorption of filtered HCO₃ before any new HCO₃ can be added to the body fluids. Production of new HCO₃ as well as absorption of filtered HCO₃ proceeds by the same mechanism, H⁺ secretion. In the mammalian kidney, two segments, the proximal tubule and the collecting tubule, perform this process. In the collecting tubule, the intercalated cells are the “professional” H⁺ and HCO₃ transporting cells, and here we will concentrate on recent advances in our understanding of their function.

Two Types of Intercalated Cell

The intercalated cells (IC), also known as mitochondria-rich cells or dark cells, are present throughout the vertebrate kingdom in fish gills, in the skin of amphibians, in the urinary bladder of reptiles and amphibia, and in the collecting tubules of mammalian kidneys. H⁺ and HCO₃ transport is mediated by the vacuolar-type ATPase, a large protein complex composed of at least 14 subunits divided into two sectors: a catalytic sector and a transmembrane ion translocating one. Although the v-ATPase is present in all cells, some of its subunits exist in different forms that are specifically expressed in one or a few cell types. For instance, the B1 subunit is only expressed in intercalated cells (and olfactory epithelium), whereas the B2 subunit is ubiquitously expressed (40, 43). Transepithelial transport requires that the v-ATPase is present in a polarized distribution such that it is present in the apical membrane (for H⁺ secretion) or the basolateral membrane (for HCO₃ secretion into the urine). The excess OH⁻ produced by proton pumping is carboxylated by CO₂ to produce HCO₃, which is then transported across the membrane opposite to that harboring the ATPase. This reaction is catalyzed by carbonic anhydrase II, a high turnover enzyme that is present wherever there is need for HCO₃ or CO₂ movement. HCO₃ transport is mediated by a Cl:HCO₃ exchange. Although these cells are specialized for H⁺ transport, recent studies suggest that they are also part of an immune defense system. The cells express toll-like receptors and synthesize and secrete several antimicrobial peptides (10, 35).

The IC cells exist in two canonical forms: α and β. The α form secretes H⁺ into the urine by an apically located proton translocating ATPase, and the resulting HCO₃ is transported across the basolateral membrane by an alternately spliced form of the red cell band 3 (AE1). The β form secretes HCO₃ into the lumen by an apical Cl:HCO₃ exchanger (pendrin) and a basolateral H⁺-ATPase (see Refs. 4 and 39 for recent reviews). There are a
number of other differences between these two cells; for instance, the β-IC cells have no apical endocytosis, whereas the α form has one of the most vigorous apical endocytosis reported for any cell. In addition, the α-IC responds to a change in cell pH induced by CO₂ (14) or a weak acid (8) by exocytosis of vesicles whose membrane is enriched in H⁺-ATPases, thereby stimulating the rate of transepithelial H⁺ secretion. Exocytosis is mediated by lowering of cell pH, which in turn increases cell calcium, which is the proximate cause of exocytosis (1).

An examination of their morphology also shows that the two cell types differ dramatically (see FIGURE 1; used with permission from Ref. 5). Although both cells are enriched in carbonic anhydrase and in mitochondria, the cell shape is quite different with the α-IC being more columnar with its apical region jutting into the tubular lumen. Underneath the apical surface, their cytoplasm is full of translucent vesicles. That region of the cell in the β-IC form is devoid of vesicles, and the cytoplasm appears more dense and darker in staining by electron microscopy, and the nucleus is more eccentric and its mitochondria more tightly packed than the α-IC phenotype. The apical surface of α-IC has exuberant microvilli and folds that change when the cells are stimulated to secrete acid. By contrast, the apical surface of the β-IC cell is devoid of microvilli. When we developed a clonal intercalated cell line, we also discovered that the cytoskeletal proteins needed to produce microvilli (villin and cytokeratin 18) were absent in the β-IC form but were highly expressed in the α-IC form. These studies led us to the conclusion that these two forms represent different states of differentiation, where the β-IC form is less differentiated than the α-IC phenotype (42).

We found that feeding animals an acid diet resulted in an increase in the number of α-IC cells and a decrease in the number of β cells, while the total number of IC cells remained constant. We concluded that the β cells are the progenitors of the α cells and that this conversion is a direct response to changes in the extracellular pH (32). Based on the distribution of the V-ATPase and the anion exchangers, other forms of IC were also found (non-A, non-B) (44), but we believe that they may be transitional cells.

Hensin/DMBT1 Converts β-IC to α-IC

To identify the molecules involved in this process, we used an immortalized intercalated cell line that reproduced many aspects of this conversion (12). We discovered that when the β-IC were induced to convert to the α-IC phenotype they deposit in their ECM a new extracellular matrix protein (ECM) that is capable by itself of inducing the change in phenotype. We purified this activity using an assay where β-IC cells were seeded on filters coated with the various fractions obtained from the sequential column chromatography purification scheme. The appearance of apical endocytosis represented functional purification, with apical endocytosis being the preeminent characteristic of the α-IC that differentiates it from the β-IC cell. We cloned the

![Figure 1](http://physiologyonline.physiology.org/)

**FIGURE 1.** Intercalated cells in kidney collecting tubule

Figure from Ref. 5.
homogenously purified protein, which we termed hensin (2, 38) (later called by the Mouse Genome Project, DMBT1). Hensin/DMBT1 is a large, multi-domain protein composed of eight SRCR domains, four CUB, domains, and two ZP domains. Although these domains are present in many other proteins, a function is yet to be assigned to any of them. β-IC cells produce this protein but secrete it into the basolateral medium in a soluble form; hence, the major effect of the acid signal is to induce its deposition in the ECM. Unlike the ECM form of hensin, the monomeric soluble hensin is incapable of converting β to α IC. In the polymerized form, hensin is a multimer with a very high molecular weight, and we later found that the polymerized version contains two other proteins, galectin 3 and cypA, a cis trans prolyl isomerase that is the cytoplasmic target of the immunosuppressant cyclosporin.

An antibody to hensin blocked the immortalized clonal β-IC cell line to an α-IC phenotype. Furthermore, the antibody also prevented the conversion of the β-IC to the α-IC form in isolated perfused rabbit tubules. We found that polymerization of hensin, the critical mechanism for the conversion of cell phenotype, was a complex multistep biochemical process. It begins (we think) by an acid-induced activation of inside-out signaling by integrin β1 (41). The next step appears to be the secretion of the two proteins, both of which are necessary for the polymerization. Cyclophilin A by its cis trans prolyl isomerization is able to begin the process of polymerization (25). In vivo, blockade of this enzyme prevents hensin polymerization and causes distal renal tubular acidosis (45) similar to what has been observed in patients treated with cyclosporin (3). A second protein, galectin-3 needs to be secreted to “bundle” the hensin fibers (15); this protein is induced in acidosis and gets secreted into the ECM and is deposited with hensin under the α-IC in vivo (31).

Other investigators also confirmed our finding that acidosis results in the appearance of more α-IC and an equal reduction of the number of β-IC cells especially in the cortex of all species tested. But when discussing their findings, somehow they never seemed to consider the mechanism as due to conversion of β-IC to α-IC but offered no competing hypothesis. The fact that a clonal β-IC cell line converts to an α-IC phenotype and that an antibody to hensin blocks the conversion in isolated perfused tubule seemed compelling enough. However, the most rigorous test would be to block the conversion completely by deletion of hensin. Global deletion of hensin was embryonic lethal (see below). We recently obtained a conditional deletion of hensin in the intercalated cells using a Cre driven by the promoter of the B1 V-ATPase subunit (25). The results of the conditional deletion of hensin in the intercalated cell were dramatic (13) (see FIGURE 2). All IC cells in the cortex were of the pendrin-expressing β form, and there were no α-IC in either cortex or medulla (FIGURE 2A).

In the medulla, the intercalated cells were present, but on ultrastructure they resembled the cortical β-IC. However, unlike the cortical β-IC, they did not express pendrin nor was their cytoplasm as dense, and they had some vesicles underneath the apical membrane. They probably were incapable of transepithelial transport since the vacuolar ATPase was diffusely located in the cell rather than polarized to one or another membrane. These studies provide strong evidence for the hypothesis of conversion of β-IC to α-IC cells. As discussed above, polymerization of hensin is necessary for its action, and to test the idea of whether deposition of hensin in the ECM is indeed necessary for its function we deleted integrin β1 from the intercalated cell. The mice exhibited an identical phenotype to that of the hensin-deleted ones. No α-IC cells were seen in either cortex or medulla.

Physiological studies in these mice showed that they exhibited complete distal renal tubular acidoses (dRTA). These mice had plasma HCO3 concentrations of ~17 meq/l and were acidotic. Their urine pH was higher than that of their littermates by ~0.5 pH units. This represents a new type of dRTA where a major cause of the acidosis is HCO3 wasting due to continued HCO3 secretion by the β-IC cells. This is not expected to occur in most other cases of dRTA (e.g., due to a defect in AE1) since we predict that the acidosis will convert the β-IC from a HCO3-secreting to an α-IC cell that is not functional, i.e., a null cell; hence, in these
syndromes, the only defect in net H+ secretion is that due to inhibition of proton transport by the α-IC cell but without having continued HCO3− secretion. Hence, the presence of functional β-IC cells guarantees continued loss of HCO3− and hence the development of acidosis. The hensin conditional KO studies describe a new form of dRTA where HCO3− secretion is the major culprit. Whether there are human syndromes with this type of dRTA remains to be determined.

**Lineage of Cortical and Medullary Intercalated Cells**

The conditional knockout studies demonstrate that there is a path of differentiation in which the β cell is a progenitor of the α type. However, this simplified scheme seems to apply only to the cortex, suggesting that medullary α-IC cells are derived from a different type of cell. As a preliminary conclusion, we propose that the new cell type that bears many similarities to the β-IC but does not express pendrin is a progenitor of the medullary α intercalated cell.

Intercalated cells are derived from the ureteric bud, an epithelial structure that buds off the Wolffian duct. During embryonic development, it was found that intercalated cells appear early in the presumptive medulla by E15.5 expressing V-ATPase and AE1 (20). Pendrin-expressing intercalated cells appear in the connecting tubule as early as E14 but only after birth in the cortical collecting tubule (34). In medullary collecting tubules, pendrin-positive cells appear during embryonic development but are eliminated after birth by apoptosis (21). The thickness of the medulla actively increases during embryonic life, whereas that of the cortex only begins to expand rapidly after birth in rodents (9). Hence, the environments in which these two segments develop are widely different.

The conversion of ureteric bud cells to principal and intercalated cells is the first evident differentiation step in collecting duct development. The transcription factor Foxi1 was found to be critical in this regard since deletion of this gene resulted in absence of the intercalated cells (6). Consequently, the mice developed dRTA. Interestingly, the collecting tubule of these mice is composed of a single cell type, but these cells express not only the characteristic marker of the principal cell, aquaporin 2, but they also express carbonic anhydrase II, a marker of intercalated cells. However, these cells did not express pendrin or AE1. It is well known that the skin of amphibians and fishes contain intercalated cells, and recent studies in Zebrafish skin showed that foxi1 is critical for the specification of these “ionocytes” (16, 18). Similar studies in *Xenopus* embryo skin demonstrated the critical nature of Foxi1 also (28). Foxi1 begins to be expressed in the collecting tubule early in E15. Another transcription factor, CP2L1 (46) (a grainyhead family member), appears to influence specification of the intercalated cells since its deletion results in absence of intercalated cells in the collecting tubule. In *Xenopus* embryo skin, the transcription factor ubp1, a grainyhead orthologue, is expressed but only in β-IC (28).

Differentiation of the intercalated cell poses an interesting problem in renal development since the collecting tubule is the only nephron segment with two cell types where the IC cells are present in a random distribution among the majority principal cells. Such a pattern of differentiation is often produced using the mechanism of “lateral inhibition” mediated by the Notch signaling pathway. In the various tissues where this “salt and pepper” appearance is maintained, notch signaling is critical for the development of this pattern. These include all distal renal epithelia including those in the Pronephros mesonephros and metanephros from fish to man. In fishes and amphibians, the skin (and gills) also contains this. Notch signaling was found to be critical for the specification of all these cells (22). In mice, deletion of notch from the collecting duct results in an increase in the proportion of intercalated cells in the collecting tubule (19). Expression of active notch intracellular domain in the collecting tubule leads to complete absence of intercalated cells. Thus specification of these cells depends on notch signaling.

A plausible model that incorporates many of these studies is presented in **FIGURE 3**. A likely first differentiation step would be for a ureteric bud cell to be specified into either a principal cell fate (active Notch) or intercalated cell one (inactive notch). In this latter cell, Foxi1 would be active. This is followed by a bifurcation to form two (or perhaps more) lineages, one that would lead to the formation of the β lineage in the cortex and the other that would lead to the similar cell type in the medulla. If it turns out that, like in *Xenopus* skin, grainyhead genes specify β-IC, then that would be the next step in the life cycle of the intercalated cell. Whether that cell (presumably grainyhead+) is an actual HCO3−-secreting β-intercalated cell or a precursor of one is yet to be determined. Since the “medullary” lineage has not been previously detected and was only revealed in the hensin knockout mice, one can only speculate regarding their pathway of differentiation. As a preliminary conclusion, we propose that there is a new cell type that is a medullary pre-α cell. This cell resembles the cortical β-intercalated cell except that it does not express pendrin. These mutant mice should allow further study of this progenitor cell type. Both of these lineages...
ultimately result in formation of α-intercalated cells under the influence of polymerized hensin. Importantly, it appears that hensin/DMBT1 mediates this conversion in both lineages.

**Hensin/DMBT1 Might Identify a General Pathway of Epithelial Differentiation**

Epithelial differentiation begins when stem cells convert to a “proto-epithelial” cell that has all the characteristics of epithelia, e.g., apical and basolateral polarized distribution of proteins and lipids, transepithelial transport, a variety of junctions, and secretion of specific extracellular matrix proteins. Yet all of these embryonic epithelia “look” alike and cannot readily be assigned to a specific organ or tissue. Indeed, the proto-epithelia of even multi-layered epithelia such as the skin begin life as a single layer of flat cells similar to those of the embryonic intestine or kidney. “Terminal” differentiation then sets in with conversion of these proto-epithelia into their mature recognizable forms, e.g., columnar, cuboidal, etc. Differentiation can continue in adult life, e.g., in the intestine, where the crypt cells are less differentiated than the villus cells even though both cell types are capable of transepithelial transport. No comprehensive catalog of the genes that mediate this differentiation is available; yet the cellular changes that are obvious are dominated by changes in the apical compartment. Proto-epithelia have no apical microvilli, whereas many differentiated epithelia have a brush border or exuberant microvilli (see difference between β- and α-IC). Apical functions are also very different; terminally differentiated cells have vigorous apical endocytosis and exocytosis, whereas proto-epithelia have none to speak of. In addition, cell shape is critically different in that proto-epithelia are flat and “squamous,” whereas most terminally differentiated epithelia have their own characteristic shape whether they are columnar or cuboidal or multilayered.

**FIGURE 3. Proposed model for intercalated cell differentiation**
Expression studies showed that hensin is expressed in most epithelial organs in the adult mouse. Since differentiation continues to occur in the intestine and prostate, we examined the staining pattern of hensin in these tissues and found that hensin was present in an ECM extracellular pattern in the villous and surface cells of the ileum and colon, respectively, where it was present in association with galectin 3 (42). In the less differentiated intestinal crypt cells, it was present only in intracellular vesicles. Similar patterns were found in the prostatic epithelium (24). In the embryo, it begins to be expressed in the blastocysts (E3.5) and becomes concentrated in the primitive endoderm and later in the visceral endoderm. We generated a global knockout of hensin and found that it was embryonic lethal at about E4.5. At that time, the first columnar epithelium, the visceral endoderm, develops where it envelops the embryo proper. When we cultured embryonic stem cells on polymerized hensin and compared them to those seeded on different matrices such as fibronectin collagen IV and laminin, ES cells cultured on hensin became columnar in shape and developed apical endocytosis and began to express specific genes of the visceral endoderm (37). These studies demonstrate that hensin mediates differentiation of cells other than the intercalated cells and raises the possibility that it may be a general inducer of epithelial differentiation. Incidentally, two other groups later generated global knockouts of hensin/DMBT1 but found viable and fertile mice (11, 30).

The most likely cause of these disparate results is the obvious one of genetic background since the three groups used different substrains of ES cells from a Sv129, lines well known to harbor extensive “contamination” with other substrains (33, 39). Another example of the differences the use of substrains of 129/Sv ES cell lines caused is that four laboratories obtained a widely divergent pattern of structural and functional anomalies by using different substrains for deletion of the ALS2 gene (7). These studies suggest that genetic background is the basis for these disparate results. Given these background effects, one suspects that it might be possible to observe mutations in hensin in humans that might not cause embryonic lethality.

Terminal Differentiation

The concept of terminal differentiation was developed for cells in culture a few decades ago and remains the most “plastic” of terms, often meaning whatever the author wanted it to mean (for a rigorous and amusing review of the semantics, see Ref. 29). It was originally developed for studies of aging in culture where it was thought that each cell had a fixed number of divisions before it reached a state that is “postmitotic.” Later, this was expanded to the idea that once a cell reaches that phenotype its fate is fixed and that it cannot divide. But for studies in vivo, the term has been maddeningly flexible and is often used by investigators to mean whatever they want it to mean. So, joining in this sport, we used to identify the two different states of the intercalated cells where the β-IC has a simplified morphology and cell shape, whereas the α-IC is a columnar epithelium with a large number of organelles and polarized functions. The reason being that, even for terminally differentiated cells, there are extensive observations during embryonic development showing that one such cell could readily convert to a different phenotype, often another differentiated phenotype and even one from a completely different germinal layer lineage. In mature tissues, it is easy to demonstrate that terminally differentiated epithelia, for instance in the proximal tubule, can readily be converted to mesenchymal cells such as fibroblasts exhibiting terminally differentiated fibroblast proteins. Such a de-differentiation of epithelial cells followed by redifferentiation also occurs during regeneration after renal ischemia in the kidney. In the α-intercalated cells, HCO3 treatment can lead to a beginning of conversion of α- back to β-intercalated cells. But the issue of mitosis was never considered in our papers to be any strict criterion by which one can distinguish the presence or absence of terminal differentiation. Recent studies have documented that, at least in the medullary collecting tubule, fully differentiated α-intercalated cells actively proliferate in response to acidosis, incidentally also demonstrating another difference between cortical and medullary acid-secreting cells (17). So, as Reiner says when we talk about a “terminal whatzit,” one should “silently and prayerfully consider all the logical implications and equip oneself with an ample supply of quotation marks” (29). ■

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References


