Collecting Duct Intercalated Cell Function and Regulation

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Abstract
Intercalated cells are kidney tubule epithelial cells with important roles in the regulation of acid-base homeostasis. However, in recent years the understanding of the function of the intercalated cell has become greatly enhanced and has shaped a new model for how the distal segments of the kidney tubule integrate salt and water reabsorption, potassium homeostasis, and acid-base status. These cells appear in the late distal convoluted tubule or in the connecting segment, depending on the species. They are most abundant in the collecting duct, where they can be detected all the way from the cortex to the initial part of the inner medulla. Intercalated cells are interspersed among the more numerous segment-specific principal cells. There are three types of intercalated cells, each having distinct structures and expressing different ensembles of transport proteins that translate into very different functions in the processing of the urine. This review includes recent findings on how intercalated cells regulate their intracellular milieu and contribute to acid-base regulation and sodium, chloride, and potassium homeostasis, thus highlighting their potential role as targets for the treatment of hypertension. Their novel regulation by paracrine signals in the collecting duct is also discussed. Finally, this article addresses their role as part of the innate immune system of the kidney tubule.


Introduction
Intercalated cells are epithelial cells traditionally associated with the regulation of acid-base homeostasis in distal segments of the kidney tubule (Figure 1) (1). These cells also participate in potassium and ammonia transport and have a role in the innate immune system. Many early studies emphasized that these tubule segments were not part of the classic nephron because they arise from the mesonephric kidney or Wolfian duct, which also gives origin to the male excurrent duct (2). Most collecting duct cells express the epithelial sodium channel (ENaC) and are grouped together as principal cells (3,4). Until recently, our understanding of the collecting duct and the roles of intercalated cells has lagged behind that of other segments. The collecting duct was initially described as not having a specialized function or as having a role only in water reabsorption (5,6).

Intercalated cells are essential in the response to acid-base status of the organism, and they help dispose of acid that is generated by dietary intake and cannot be eliminated via the lungs, the so-called fixed or nonvolatile acid (Figure 2). The kidney contributes to acid-base homeostasis by recovering filtered bicarbonate in the proximal tubule. Distally, intercalated cells generate new bicarbonate, which is consumed by the titration of nonvolatile acid (7). Dysfunction of the proximal tubule, where approximately 90% of the bicarbonate is reabsorbed, leads to proximal renal tubular acidosis (8). The connecting segment and collecting duct rely mostly on their intercalated cells to reabsorb the normally smaller amount of residual bicarbonate. In addition, intercalated cells participate in the excretion of ammonia/ammonium, a topic reviewed in a separate article in this series (9).

The relevance of intercalated cell dysfunction in clinical scenarios is often not as evident as the relevance of principal cell dysfunction, such as in patients who present with diabetes insipidus or the syndrome of inappropriate antidiuretic hormone secretion. In clinical practice, intercalated cell dysfunction is most often associated with metabolic acidosis, although histologic or laboratory confirmation of this dysfunction is seldom performed in the general acute care setting. Moreover, the contribution of intercalated cells in preventing acidemia is often eclipsed by the coordinated compensatory roles of the lung, bone, and more proximal kidney tubule segments. Nonetheless, animals subjected to dietary acid loading have significant increases in the luminal (facing the urine) surface area of intercalated cells, changes that begin within a few hours from the change in diet (reviewed in references 7,10).

Until very recently, intercalated cells were not thought to contribute to extracellular fluid volume regulation, yet now they are firmly established as important contributors to collecting duct NaCl transepithelial transport and the protection of intravascular volume in concert with principal cells (Figure 2) (reviewed by Eladari et al. [4]). An impressive new study has now established that, in vivo, intercalated cells regulate their intracellular volume by a mechanism involving the vacuolar H⁺-ATPase (H⁺-ATPase; also referred to as V-ATPase in the literature). This study is important because it establishes that although most animal cells rely on the Na⁺/K⁺-ATPase (Na,K-ATPase) to energize other
Intercalated Cell Distribution, Nomenclature, Morphology, and Developmental Considerations

The cells lining the proximal tubule, thin limb, and thick ascending limb have a homogeneous ultrastructural appearance. Tubule-specific cells function as a unit within each segment. Distal to the macula densa, however, the epithelia of the distal convoluted tubule, connecting segment, and collecting duct are lined by different types of cells. Most cells in these segments are considered segment-specific principal cells, while the other cells are grouped together and called intercalated cells (5,12). An impediment to the study of these cells has been their pleomorphism and their isolation within the epithelium; they sit surrounded by principal cells. Intercalated cells vary in morphology, localization, and abundance, and these differences are evident not only among species but also among individuals from the same species. In addition, few cell lines replicate their phenotypes in culture. Some of the available immortalized intercalated cell models include the rabbit Clone-C cells, the mouse OMCDis, and the canine MDCKC11 (13–15). The Clone-C cell line is particularly useful because it can switch phenotypes from type B to type A intercalated cells (Figure 2) depending on culture conditions (16,17). Of note, many very relevant studies that have uncovered important regulatory pathways in intercalated cells have been performed in cell lines of inner medullary origin, such as the mLIMCD3 cell line (18,19).

In both normal and pathological conditions, intercalated cells respond to extracellular and intracellular signals that integrate the function of intercalated and principal cells to regulate electrolyte transport processes (31,32) and bicarbonate secretion (33,34). The plasma membrane of each epithelial cell organizes into apical and basolateral domains that develop distinct membrane lipid and transport protein compositions. Thus, within a homogeneous epithelium of one cell type, the apical membranes (facing the tubular lumen) of all cells could function as a unit, and similarly for the basolateral domains of the same cells facing the interstitium. The maintenance of the apical and basolateral domains of the kidney tubular epithelium depends on the presence of functional tight junctions, the differential permeabilities of the two membranes, and the generation of gradients between the lumen and the interstitium. Recent findings have uncovered the paracrine signals that integrate the function of intercalated and principal cells in the collecting duct (35).

Three types of intercalated cells are traditionally recognized, based largely on cell morphology and localization (reviewed in reference 36). These cells fall into the categories of type A (also known as “α”), type B (or “β”), and non-α, non-B intercalated cells (Figure 2) (reviewed elsewhere [10,36]). The initial morphologic classification was upheld after both functional and immunolabeling studies once inhibitors and antibodies for individual transport proteins became available. Currently, intercalated cells are classified with respect to the presence of the chloride-bicarbonate exchanger AE1 (Slc4a1) and to the subcellular

Figure 1. | Intercalated cells in rat collecting duct. The cartoon and confocal micrograph illustrate the intercalated cell distribution along the kidney tubule and within the epithelium. Intercalated cells were detected in the cortical and outer medullary collecting duct (green oval) by immunofluorescence labeling using an antibody against one of the H^+–ATPase subunits (green). Intercalated cells are also located in the connecting segment (red circle). The apical or luminal labeling of the H^+–ATPase indicates that these cells are mostly type A intercalated cells (A-IC). Principal cells were labeled with an antibody against the water channel aquaporin-2 (red). Modified from reference 9, with permission.
Transepithelial transport processes and regulatory mechanisms in type A intercalated cells (A-IC) and type B intercalated cells (B-IC). This cartoon illustrates the major transport proteins expressed in the three main epithelial cell types present in the collecting duct: the principal cell, which expresses the epithelial sodium channel; the acid-secreting type A-IC; and type B-IC, which secretes bicarbonate while reabsorbing NaCl. In the cortical and outer medullary collecting duct, type A-ICs express H⁺-ATPase and the H⁺/K⁺-ATPase at the apical/luminal membrane, while they express the Cl⁻/HCO₃⁻ exchanger AE1 at their basolateral membrane. The bicarbonate sensor soluble adenylyl cyclase (sAC) and protein kinase A (PKA) play important roles in the regulation of the H⁺-ATPase (see Figure 5A). Slc26a11 (A11), an electrogenic Cl⁻ transporter, as well as a Cl⁻/HCO₃⁻ anion exchanger, are also expressed at the apical membrane of the type A-IC. On the other hand, the type B-ICs display an electroneutral NaCl transport/reabsorption pathway at their apical membrane that involves pendrin, a Cl⁻/HCO₃⁻ exchanger, and the Na⁺-driven Cl⁻/HCO₃⁻ exchanger (NDCBE). The proposed basolateral Na⁺ extrusion pathway would involve the cotransporter SLC4A9 (AE4). The mechanism of Cl⁻ exit remains to be elucidated. In type B-ICs, reabsorption of NaCl from the lumen is energized by the basolateral H⁺-ATPase rather than by Na⁺/K⁺-ATPase.
Localization of the multisubunit H\(^{+}\)-ATPase (36,37). This classification has been further modified to reflect whether cells express the transport protein pendrin, a subtype of chloride/bicarbonate exchanger (37). Therefore, another definition for type A intercalated cells combines their function in urinary acidification, their lack of pendrin expression, the presence of H\(^{+}\)-ATPase at their apical membrane, and the expression of AE1 at their basolateral membrane. On the other hand, the intercalated cells involved in bicarbonate secretion express the chloride/bicarbonate exchanger pendrin at their apical membrane. These cells are further classified as type B and non-A, non-B intercalated cells. While type B intercalated cells express the H\(^{+}\)-ATPase at their basolateral pole, the non-A, non-B intercalated cells express both the H\(^{+}\)-ATPase and pendrin at their apical membrane. Moreover, non-A, non-B intercalated cells are located in the connecting segment or connecting tubule (10,38). All three types of intercalated cells express carbonic anhydrase in their cytoplasm (36,39). This metalloenzyme reversibly catalyzes the hydration of CO\(_2\) (40) and thus leads to the formation of bicarbonate. At least 13 isoforms of carbonic anhydrase have been identified in mammals. Carbonic anhydrase II, acting in tandem with H\(^{+}\)-ATPase, facilitates the coordinated proton and bicarbonate secretion from intercalated cells. Table 1 summarizes the current findings on transport or other proteins that can help in the characterization and classification of intercalated cell types.

**Intercalated Cell Development and Plasticity**

Interest in intercalated cell development arose, in part, because of the intriguing presence of the non-A, non-B intercalated cell, which some viewed as an intermediate type. Moreover, other intercalated cells did not fit into any of the three available classifications because of the expression of marker proteins in unexpected subcellular localizations. These other types of intercalated cells were thought to represent a series of intermediate forms that could arise if, for example, a type B intercalated cell were transforming into a type A intercalated cell under the pressure of metabolic acidosis. One such “intermediate” cell might express the H\(^{+}\)-ATPase diffusely in the cytoplasm or at both the apical and basolateral domains (41,42). There is now evidence that type A and type B intercalated cells represent different states of differentiation, as proposed by Al-Awqati and confirmed by others (16). In some of these studies carried out in the rabbit model, adaptation to acidosis was not accompanied by changes in the number of intercalated cells but rather by a change from type B to type A intercalated cells (7,43). The transition process from type B to type A intercalated cell depends on the basolateral deposition of the extraacellular matrix proteins hensin (DMBT1), galectin 3, and other proteins (7,44). Overall, induction of chronic metabolic acidosis increases the proportion of type A intercalated cells while metabolic alkalosis causes an increase of type B intercalated cells (7,45,46). Moreover, mice with a hensin defect in intercalated cells indeed developed metabolic acidosis. In this mouse model, intercalated cells in the cortex had a type B phenotype (44). Interestingly, the hensin-deficient mice had modified the phenotype of medullary epithelial cells. These modified medullary cells had the ultrastructure of the type B intercalated cells usually found in cortex, while they differed from type B cells in that they did not express pendrin (47).

In addition to the above factors, the Notch signaling pathway also controls the development of primitive epithelia, such as the ones in the connecting segment and the collecting duct (20,48). The Notch signaling cascade is essential for a developmental process called lateral inhibition, where
neighboring cells undergo one final step that gives them very different phenotypes. Notch signaling disruption indeed alters collecting duct cellular composition, resulting in more intercalated cells and fewer principal cells; in addition, probably because of the decreased numbers of principal cells, the mice also display a nephrogenic diabetes insipidus phenotype (48). The developmental events would involve ureteric bud cells differentiating into principal cells, with active Notch signaling, while intercalated cells would appear because of inactive Notch signaling (49,50). Intercalated cell development goes one step further to generate cells of type B lineage in the cortex and outer medulla. The expression of grainyhead genes results in the type B intercalated cell phenotype (21,51,52).

In rodent kidney, the appearance of intercalated cells during development has traditionally been tracked by following the expression of several of the intercalated cell–specific H^+-ATPase subunits and other markers. This multisubunit proton pump is ubiquitous, but several of its subunits are specific to distal nephron intercalated cells (47,53). These intercalated cell–specific H^+-ATPase subunits (such as a4 and B1) appear early in the medulla at embryonic day 15.5 (E15.5), after the detection of the expression of Foxi1, a forkhead family transcription factor that is detected in adult intercalated cells (54). Pendrin-positive intercalated cells have been detected in the mouse kidney at E14. There was also a debate on whether intercalated cells developed from principal cells or vice versa, or whether one cell population could contribute to the repopulation of the collecting duct after injury. Some of this controversy has been clarified by the characterization of a mouse strain lacking a transcription factor of the forkhead family, Foxi1 (55). In adult mice, Foxi1 is detected only in intercalated cells, suggesting that this transcriptional regulator is required for intercalated cell development. Indeed, Foxi1 knockout mice lack intercalated cells, and their collecting duct epithelium instead expresses a more primitive cell type with some principal and intercalated cell characteristics. For example, this undifferentiated cell type expresses the water channel aquaporin-2, which is expressed in principal cells, and the cytosolic enzyme carbonic anhydrase II, which is used as a marker of intercalated cells. Foxi1-null mice did not express H^+-ATPase or pendrin in their collecting ducts.

### Type A Intercalated Cells, Their Major Transport Proteins, and Their Hormonal Regulation

Type A intercalated cells are present in the late distal convoluted tubule, the connecting segment, cortical and outer medullary collecting duct, and, in some reports, the early part of the inner medullary collecting duct. They are the more abundant type of intercalated cell in the outer stripe of the outer medulla in most mammalian species (10). Morphologically, they lack a cilium, they have numerous apical microvilli, and mitochondria are abundant near the apical pole (10,28). Their most characterized role is as cells that secrete protons into urine, and they can easily be identified for their lack of pendrin expression.

Type A intercalated cells can secrete H^+ equivalents into urine via the H^+-ATPase or the H^+K^+ATPase (H,K-ATPase) at their apical membrane. The latter pump exchanges one potassium ion for each extruded proton. In addition, these cells express Scl4a1, a splice variant of erythroid band 3, at the basolateral membrane (Figure 1) (42). The secretion of a proton into the tubular lumen, whether it is in exchange for potassium reabsorption or not, results in the generation of intracellular bicarbonate via carbonic anhydrase II, which is reabsorbed into the interstitium in exchange for chloride by AE1. The H^+-ATPase is very abundant at the apical membrane of type A intercalated cells and in subapical vesicles or tubulovesicular structures, and they appear as 10-nm spherical structures or “studs” coating these membranes, also described as rod-shaped particles (56,57).

### Table 1. Relevant proteins expressed in kidney intercalated cells

<table>
<thead>
<tr>
<th>Intercalated Cell Type Protein Expressed</th>
<th>Type A</th>
<th>Type B</th>
<th>Non-A, Non-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase II: catalyzes the reversible hydration of CO2 to bicarbonate and water</td>
<td>Apical</td>
<td>Cytoplasmic</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>H^+-ATPase: pumps H^+ across the (plasma) membrane, and into the extracellular space</td>
<td>Apical^a</td>
<td>Basolateral^a</td>
<td>Apical and diffuse vesicular^a</td>
</tr>
<tr>
<td>AE1 (Slc4a1): anion exchanger 1, exchanges a Cl^- for a bicarbonate</td>
<td>Basolateral^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pendrin (Slc26a4): exchanges a Cl^- for a bicarbonate</td>
<td>Apical^a</td>
<td>Apical^a</td>
<td></td>
</tr>
<tr>
<td>H^+/K^+-ATPase: exchanges an H^+ for a K^+ at the expense of ATP</td>
<td>Apical</td>
<td>Apical? Basolateral</td>
<td></td>
</tr>
<tr>
<td>AE4 (Slc4a4): anion exchanger 4, exchanges a Cl^- for (n) bicarbonate</td>
<td>Apical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RhBG: Rh B glycoprotein, ammonia transporter</td>
<td>Basolateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RhCG: Rh C Glycoprotein, ammonia transporter</td>
<td>Basolateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDCBE (Slc4a8): Na^+-driven Cl^-/bicarbonate exchanger</td>
<td>Apical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11 (Slc26a11): electrolytic Cl^- transporter and Cl^-/HCO3^- anion exchanger</td>
<td>Apical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKCC1: Na^+-K^+-2Cl^- cotransporter</td>
<td>Basolateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK channel or Maxi-K: K^+ secretion</td>
<td>Apical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adapted from reference 36. BK, big potassium.

^aThe differential localization patterns pertaining to the transport proteins identify a combination of key markers that aid in the classification of the different intercalated cell subtypes.
The H⁺-ATPase facilitates the movement of protons across the apical membrane of type A intercalated cells. Other ion movements, such as Cl⁻ and/or bicarbonate extrusion, compensate H⁺ transport in proton-secreting cells (32,58). In the case of H⁺-ATPase, two other main factors affect its function at the plasma membrane: the pH difference across the apical membrane and the transepithelial potential difference (59). For example, this pump mediates H⁺ transport at a rate that is 0 when the luminal pH is <4.5, while the transport rate of the pump is saturated at a pH of 7.0–8.0. In addition, when the lumen potential is 120 mV relative to the interstitium, the H⁺ transport rate is negligible. On the other hand, at an applied potential of −30 mV the H⁺ pumping rate saturates. A hypothesis was then presented that Na⁺ reabsorption through ENaC would create a more lumen negative transtubular potential difference along the distal nephron. This potential difference would favor H⁺-ATPase function in type A intercalated cells. Wagner and colleagues elucidated in vivo that ENaC function in the connecting segment was sufficient to induce this upregulation (60).

Both AE1 and H⁺-ATPase are upregulated in the kidney during metabolic acidosis (45), and the morphology of type A intercalated cells changes (Figure 4) (10). Although the entire collecting duct contributes to urinary acidification, the outer medullary collecting duct is very important, as the entire collecting duct contributes to urinary acidification (46,47). Type A intercalated cells participate in active potassium reabsorption via the H⁺/K⁺-ATPases (2), and they undergo hypertrophy when animals are fed a low-potassium diet. However, the contribution of each α isoform in kidney potassium and acid handling remains to be determined, as the null mice for either the α₁ or α₂ subunits did not have significant changes in their acid-base or potassium excretion (62). In the setting of acidosis, the activity of H⁺/K⁺-ATPase increases in the collecting duct (64,65). These findings indicate that this pump represents a key mechanism of proton secretion by the kidney in metabolic acidosis. In addition to the H⁺/K⁺-ATPase, another important regulator of K⁺ homeostasis, the high-conductance big potassium (BK) or maxi-K channel is also highly expressed at the apical membrane of type A intercalated cells (66), especially in animals fed a high-potassium diet. These channels, which are less abundant in principal cells, are activated by depolarization, stretch/luminal flow, intracellular calcium increases, or hypoosmotic stress (reviewed in reference 67). Moreover, the BK channel is inhibited by the with-no-lysine kinase 4 (WNK4) in a phosphorylation-dependent manner (68,69). In a later study performed in a cell line of intercalated cell characteristics, WNK4 inhibited BK channel activity in part by increasing channel ubiquitination and degradation (70).

Acid-Base Sensing in Intercalated Cells

The activity and subcellular localization of acid-base transport proteins can be quickly altered by changes in extracellular or intracellular pH or bicarbonate changes. These findings have led to an intense search for the pH-sensing mechanism within the intercalated cells. For example, the H⁺-ATPase in type A intercalated cells is acutely activated (within minutes to hours) by kinases such as protein kinase A (PKA), while it is acutely inhibited by the metabolic sensor AMP activated protein kinase (AMPK) (Figure 5) (72–74). This downregulation of the proton pump by AMPK is preliminary evidence that intercalated cell function can be altered when the tubule is under metabolic stress, such as under conditions of ischemia. These two kinases also regulate the H⁺-ATPase in proximal tubule and epididymis (75,76). We have characterized two major phosphorylation sites in the H⁺-ATPase A subunit: Ser-175 is activated (within minutes to hours) by kinases such as cAMP/PKA and it is acutely inhibited by the metabolic sensor AMP activated protein kinase (AMPK) (72,73). In particular, Ser-175 is in part responsible for the activation of H⁺-ATPase in endosomes downstream of G protein-coupled signaling via cAMP/PKA (77).
In addition, PKA activation of the $H^+$-ATPase has also been linked to the acute activation of the bicarbonate-sensor soluble adenylyl cyclase (sAC) in type A intercalated cells (Figure 5A) (74). This cascade links physiologic changes in plasma and/or intracellular bicarbonate to acute intercalated cell phenotypic changes. For example, the PKA activator cAMP directly stimulates proton secretion in primary cultures of mouse intercalated cells (78) while significantly increasing the size of apical microplicae in these cells (78). The role of bicarbonate-activated sAC in intercalated cell function is also supported in cases of increased chronic luminal bicarbonate delivery (over days) to the collecting duct (79). A recent study found that Slc26a11, recognized as an electrogenic $Cl^2$ transport as well as a $Cl^2$/HCO$_3^-$ anion exchanger, facilitates $H^+$-ATPase function in type A intercalated cells (80).

Another important sensor of extracellular pH in the kidney is the G protein–coupled receptor 4 (GPR4). Its function was identified and characterized in cell lines in studies that used the mOMCD1 intercalated cell model (18). GPR4-null mice had decreased kidney net acid secretion and a non-gap metabolic acidosis, suggesting that GPR4 is a pH sensor with a likely important role in promoting acid secretion in kidney collecting duct intercalated cells in vivo. Moreover, the non-receptor tyrosine kinase Pyk2 was studied as a potential sensor of pH changes in the distal tubule. Pyk2 is expressed in kidney, and it becomes autophosphorylated by several stimuli, including decreased pH (81). Studies also performed in mOMCD1 cells revealed that Pyk2 and downstream effectors increased $H^+$-ATPase but not $H^+/K^+$-ATPase in this intercalated cell model.
Type B Intercalated Cells and Their Transport Processes

The classic type B intercalated cells express a chloride-bicarbonate exchanger, pendrin, at their apical membrane and express H⁺-ATPase at their basolateral membrane (Figure 6) (41,82). These cells are thought to be responsible for the secretion of OH⁻ equivalents. Pendrin is encoded by the SLC26A4 PDS gene. This transport protein is a member of the multifunctional sulfate transporter solute carrier 26 family (SLC26). Although it does not transport sulfate (83), it can transport anions such as iodide, Cl⁻, and bicarbonate (84). Pendrin is also expressed in epithelial cells in the inner ear (85) and the thyroid, as well as in other tissues (82,86). This differential tissue expression pattern highlights different transport roles for pendrin. For example, in kidney intercalated cells and in the inner ear, pendrin acts as a chloride-bicarbonate exchanger (85), while in the thyroid gland it mediates iodide transport (86). Therefore, mutations in SLC26A4 involve various organs to differing degrees. Vaughan Pendred described this disorder as the combination of deafness and goiter (87,88). Remarkably, both patients with Pendred syndrome, which is inherited in an autosomal recessive pattern, and pendrin-null mice have normal kidney function under baseline conditions (82,89). However, upon bicarbonate administration, which induces pendrin upregulation in wild-type mice, pendrin-null mice developed a severe metabolic alkalosis. These alkali-loaded animals deficient in pendrin could not secrete bicarbonate, suggesting a critical role for pendrin in the modulation of acid-base status that protects against metabolic alkalosis (90,91).

Initially, the reabsorption of Cl⁻ in the collecting duct was attributed to the paracellular pathway, with some studies indicating that there was transcellular Cl⁻ absorption (92) (reviewed in references 4,37). Indeed, very recent studies have confirmed that the paracellular pathway plays an important role in Cl⁻ reabsorption in the collecting duct (93). On the other hand, the role of type B intercalated cells in Cl⁻ reabsorption became clear when pendrin was identified in these cells (82). Because both sodium and chloride intake can modulate kidney function, pendrin was considered immediately as a potential mediator in the pathogenesis of hypertension (90,94). Several studies have confirmed that pendrin protein expression decreases with maneuvers that increase urinary Cl⁻ excretion, while protocols that caused Cl⁻ depletion, such as furosemide treatment, significantly increased pendrin levels. Later studies showed that metabolic acidosis induced by acetazolamide or ammonium sulfate administration reduced the levels of pendrin irrespective of low Cl⁻ levels in urine (95). Aldosterone and angiotensin II also increase the levels of pendrin (96,97), thus linking this transport protein to the maintenance of adequate extracellular volume and potentially to the development of hypertension. Recently, it has been proposed that pendrin may be activated via a kidney-specific mechanism that does not include circulating aldosterone (98). Furthermore, angiotensin II mediated pendrin upregulation and subcellular localization changes in kidney occur via the angiotensin 1a receptor (99). Pendrin-mediated Cl⁻ uptake leads to vascular volume expansion and pendrin-induced modification of ENaC abundance and function (98). Changes in luminal bicarbonate concentration and/or pH could also contribute to this signaling (100). Thus, pendrin expressed in the kidney might represent a potential target for blood pressure control. This role is illustrated in pendrin-null mice that were resistant to mineralocorticoid-induced hypertension and in turn became hypertensive when fed a low-sodium diet.

Both type B and non-A, non-B intercalated cells (Table 1) express pendrin at their apical membrane and intracellular vesicles (Figure 6) (82,101). Pendrin is more abundant in the model proposed by Chambrey and colleagues (35). These cells participate in electroneutral NaCl absorption, which is energized by H⁺-ATPase. Two cycles of transport via pendrin, when coordinated with one cycle of NDCBE results in net uptake of one Na⁺ and one Cl⁻ and the extrusion of two bicarbonate (HCO₃⁻) ions. The Cl⁻ ions recycle across the apical membrane. A basolateral channel (not shown) mediates Cl⁻ exit. The basolateral anion exchanger 4 (AE4 or Slc4a9), together with the H⁺-ATPase also facilitates Na⁺ and bicarbonate exit.

**Figure 6.** Model of major transport processes and regulatory mechanisms in type B intercalated cells. This cartoon is modified from the model proposed by Chambrey and colleagues (35). These cells participate in electroneutral NaCl absorption, which is energized by H⁺-ATPase. Two cycles of transport via pendrin, when coordinated with one cycle of NDCBE results in net uptake of one Na⁺ and one Cl⁻ and the extrusion of two bicarbonate (HCO₃⁻) ions. The Cl⁻ ions recycle across the apical membrane. A basolateral channel (not shown) mediates Cl⁻ exit. The basolateral anion exchanger 4 (AE4 or Slc4a9), together with the H⁺-ATPase also facilitates Na⁺ and bicarbonate exit.
at the apical plasma membrane than intracellularly in non-A, non-B cells under baseline conditions. This finding suggests that pendrin-mediated anion exchange occurs to a greater extent in non-A, non-B cells than in type B cells (102). This difference in pendrin subcellular distribution also suggests that its function is likely regulated by trafficking between the plasma membrane and cytoplasmic vesicles. Nitric oxide also contributes to intercalated cell transport protein regulation as it reduces pendrin protein abundance in the kidney without modulating pendrin subcellular localization in a CAMP-dependent manner (103). The regulation of pendrin also involves glycosylation-dependent processes (104,105). In addition to the regulation of acid-base status, it appears that pendrin expressed in kidney intercalated cells plays a role in controlling iodide homeostasis as well, especially during high water intake (106).

Type B intercalated cells have recently come to prominence with the discovery that the Na⁺-driven chloride/bicarbonate exchanger (NDCBE) (107) and pendrin colocalize at the apical membrane of these cells (Figure 6). Together these two transport proteins mediate the until recently unexplained thiazide-sensitive electroneutral NaCl reabsorption in the collecting duct (4,11,107). Moreover, pendrin deletion in type B intercalated cells disturbs the function of ENaC in adjacent principal cells (98,100). As in intercalated cells, NaCl absorption is energized by the H⁺-ATPase and not by the Na⁺/K⁺-ATPase (11); Eladari and colleagues hypothesized that Na⁺ transport in type B intercalated cells was mediated by a bicarbonate-dependent transport protein at the basolateral membrane. This transport protein therefore would be energized by H⁺-ATPase. Members of the same research group had previously shown that the anion exchanger 4 (AE4/Slc4a9) was expressed basolaterally in type B intercalated cells (Figure 1) (108).

Acidosis induces important changes in the transport proteins expressed in type B intercalated cells. For example, acidosis downregulates pendrin expression at their apical membrane and in apical recycling endosomes, and moreover, AE4 expression is diminished at their basolateral pole (109).

**Regulation of Intercalated Cell Transport Proteins by Hormones and Other Mediators: Effects of (Pro)renin, Renin, Angiotensin, and Aldosterone**

Steroid hormone receptors, such as the mineralocorticoid receptor (MR), function downstream of the renin-angiotensin-aldosterone system (RAAS). Together with the glucocorticoid receptor these hormones elicit signaling events in the kidney that regulate salt and potassium homeostasis, intravascular volume (110), and cell metabolism (111–113). Aldosterone, via the MR, in turn activates ENaC in principal cells, resulting in an increase in paracellular Cl⁻ transport, H⁺ secretion from intercalated cells, and activation of basolateral Na⁺/K⁺-ATPase (reviewed in references 60,110,114). However, these transcellular transport cascades are mostly relevant to principal cells (11).

During hyperkalemia, aldosterone is also secreted. Its stimulation of ENaC via the MR leads to lumen electronegativity, which promotes K⁺ secretion via the renal outer medullary small-conductance K⁺ channel (ROMK) expressed in principal cells and the flow-mediated, large-conductance, calcium-activated potassium channel or BK channel, which is expressed in intercalated cells (Figure 7) (71,115). Thus, potassium secretion is promoted without retaining sodium. It is paradoxical that activation of the same MR in the intercalated cell would result in increases of both NaCl reabsorption and regulation of acid-base transport proteins (110). The RAAS is activated during metabolic acidosis (116), while RAAS blockade inhibits kidney acid secretion in the setting of acidosi (112,117). The regulatory effects of steroid hormones on individual transport proteins in intercalated cells have been extensively studied. For example, decreased dietary potassium increases type A intercalated cell function apical projections and increases the activity of H⁺/K⁺-ATPase and the BK channel (66). However, under potassium-replete conditions, which are known to increase aldosterone levels, changes in dietary sodium and serum aldosterone did not affect BK expression in intercalated cells (118).

There are several important findings to consider in the regulation of H⁺-ATPase and other transport proteins in intercalated cells by steroid hormones. For example, Wagner and colleagues showed that angiotensin II increases the expression of the H⁺-ATPase (119). Moreover, mineralocorticoids, such as aldosterone, which is released in metabolic acidosis, also increase the expression of pendrin and the H⁺-ATPase (58,97). Recently a novel modulatory pathway that affects the MR in intercalated cells has been uncovered. This pathway, which involves MR phosphorylation, is likely the key to the differential response of these cells when only aldosterone is present (as in hyperkalemia), in contrast to when both aldosterone and angiotensin II are present (as in volume depletion) (see Figure 8) (69,120). The MR can be activated by both aldosterone and cortisol (121–123). While principal cells express the enzyme 11β hydroxysteroid dehydrogenase (11βHSD2), which rapidly converts cortisone to a less active hormone, cortisone, intercalated cells lack this enzyme (69,110,121). Thus, in principal cells the MR is mostly activated by aldosterone and not cortisol, and this activation leads to the coordinated action of ENaC and the Na⁺/K⁺-ATPase. A recent study elegantly showed that a single phosphorylation site, Ser-843, in the MR inactivates it, and this inactive receptor is the major species in intercalated cells (69). When there is volume depletion (Figure 8B), both angiotensin II and WNK4 promote dephosphorylation/activation of the MR. Once active, this receptor binds aldosterone and mediates the increase in H⁺-ATPase and chloride-bicarbonate exchanger activity. This coordinated action increases plasma volume while inhibiting potassium secretion. In the absence of angiotensin II, the receptor remains phosphorylated and thus aldosterone cannot promote H⁺-ATPase activity. In this scenario, aldosterone promotes K⁺ secretion from principal cells. Angiotensin II also increases net transcellular chloride absorption across type B cells via a mechanism that involves both pendrin and activation of the H⁺-ATPase (96). Together these findings show the physiologic relevance of selective mineralocorticoid activation in intercalated cells.

The role of renin on intercalated cell function has come to the forefront because of the identification of the (Pro)renin
receptor [(P)RR] as an accessory protein of H\textsuperscript{+}-ATPase. However, and just like H\textsuperscript{+}-ATPase, (P)RR is ubiquitously expressed. This receptor protein has a large extracellular domain that binds the enzymes renin and pro-renin (124–126). (P)RR was referred to as the ATP6AP2 (ATPase, H\textsuperscript{+} transporting, lysosomal accessory protein 2) (127). (P)RR undergoes proteolytic cleavage to generate soluble (P)RR (128,129). (P)RR is easily detected in type A intercalated cells and not in principal cells (130,131). Binding of renin and prorenin to (P)RR increases the catalytic efficiency of these enzymes in the conversion of angiotensinogen to angiotensin by 4-fold. Prorenin secreted from the principal cells binds to the (P)RR located in the apical membrane of intercalated cells or to the soluble (P)RR, and thus enhances local angiotensin I and angiotensin II formation in the collecting duct (Figure 9) (114,132). (P)RR interaction with the H\textsuperscript{+}-ATPase is required for prorenin-induced activation of Erk1/2 in a cultured cell model of intercalated cells (133). (P)RR has also been shown to play a role in vasopressin-mediated H\textsuperscript{+}-ATPase activity and cAMP accumulation, in a prorenin-independent manner in the intercalated cells. The (P)RR has been implicated in several pathologic conditions, such as diabetic nephropathy, hypertension, albuminuria, and pre-eclampsia. However, (P)RR blockade would likely have adverse effects through its activation of H\textsuperscript{+}-ATPase (130,134–136).

**Figure 7. | Intercalated cells are involved in K\textsuperscript{+} secretion in the collecting duct.** This cartoon illustrates the location of the voltage-dependent renal outer medullary small-conductance K\textsuperscript{+} (ROMK) and the flow-dependent big potassium (BK) channels in the intercalated cells (type A-IC and type B-IC) and principal cells. (A) Under normal dietary conditions, ROMK predominantly secretes K\textsuperscript{+}. However, under the influence of a K\textsuperscript{+}-rich diet, because of the increased activity of the basolateral Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, the transport via the epithelial sodium channel (ENaC)—transport increases, generating a driving force for ROMK to secrete more K\textsuperscript{+} from the principal cells. (B) When high K\textsuperscript{+} secretion is accompanied by increased flow, it stimulates BK channel synthesis and function in the intercalated cells (type A-IC and type B-IC).

**Intercalated Cell Dysfunction and the Pathogenesis of Distal Renal Tubular Acidosis**

Mutations in certain intercalated cell transport proteins, or associated enzymes, can lead to distal renal tubular acidosis (dRTA) type I, a syndrome characterized by a decreased
capability of urinary acidification and variable degrees of hyperchloremic, hypokalemic metabolic acidosis (137). When this condition is severe, the patient cannot excrete the daily acid load, which varies from 50 to 100 mEq/d depending on the patient’s diet. This continued proton accumulation leads to a normal anion gap metabolic acidosis, with values of plasma bicarbonate that can fall below 10 mEq/L. The treatment involves potassium and alkali repletion. When the disease is not so severe, it is called incomplete, type 1 dRTA. These patients still have an inability to acidify the urine (pH 5.5) without a significant drop in plasma bicarbonate. Some forms of dRTA are due to a back-leak of protons resulting from the exposure of the patient to pharmacologic agents that damage the tubular plasma membrane, such as amphotericin (138). However, these patients will often display signs and symptoms associated with principal as well as intercalated cell dysfunction.

Inherited forms of dRTA most often result from the disruption of normal type A intercalated cell physiology. Patients with dRTA tend to have acidaemia because of the lack of acid secretion from type A intercalated cells. This lack of acid secretion, and alkalization of the urine, can present with nephrocalcinosis and/or nephrolithiasis, as well as hypokalemia (139). The inherited forms of dRTA have been described as having autosomal dominant or recessive modes of transmission. Some disease-causing mutations of H+-ATPase a4 and B1 subunits result in early-onset dRTA (140–142). These findings suggest that the differential distribution of isoforms of H+-ATPase in intercalated versus other epithelial cells may be acquired during early infancy in humans. Another important transport protein in intercalated cells is the chloride-bicarbonate exchanger AE1. A large screen of kindreds with dRTA revealed that some mutations in AE1 cause autosomal dominant dRTA, while other defects identified in AE1 appear to result in autosomal recessive dRTA (143–145). In a mouse animal model, that deletion of carbonic anhydrase II reduces the number of intercalated cells in both type A and type B intercalated cells (146).

In humans, dRTA is often accompanied by some degree of salt, water, and potassium losses, and the causes for these findings were not well understood (147). Recently, a mouse model of dRTA, which was deficient in the intercalated cell-specific B1 subunit of H+-ATPase (ATP6V1B1), also presented with urinary losses, leading to hypovolemia, hypokalemia, and polyuria (Figure 10) (35). Although these mice did not present with acidosis unless challenged with an acid load (148), the molecular defect in H+-ATPase led to dysfunction of both ENaC and the combined pendrin/NDCBE (35). Researchers studying this mouse model discovered that the NaCl urinary loss coincided with increased prostaglandin E2 (PGE2) and ATP levels in urine. PGE2 is a known inhibitor of Na+ transport in the collecting duct (149–151).

Figure 8. | Model of regulatory pathways for the mineralocorticoid receptor (MR) in intercalated cells: hyperkalemia and volume depletion. This figure depicts pathways that involve the differential phosphorylation state of the MR in principal versus intercalated cells (type A-IC; type B-IC), that are likely the key to the distinct responses of these cells in two different scenarios. (A) The first scenario involves conditions when only aldosterone is present (as in hyperkalemia). In this case, hyperkalemia leads to aldosterone secretion while no angiotensin II is present. Here, MR is phosphorylated in intercalated but not in principal cells. These conditions lead to aldosterone-mediated Na+ reabsorption via the epithelial sodium channel in principal cells, which drives K+ secretion also in principal cells. (B) In contrast, when both angiotensin II and aldosterone are present (as in intravascular volume depletion), the MR is dephosphorylated downstream of angiotensin II, and the activity of this receptor is thus restored in intercalated cells. In addition, as a result of aldosterone signaling both pendrin and H+-ATPase are upregulated, and in turn there is a decrease drive for K+ secretion. Modified from reference 69.
Indeed, when PGE\(_2\) synthesis was blocked in vivo, the animals displayed restored ENaC levels in the cortex (35). This elegant study proposed a mechanism wherein type B intercalated cells would sense increases in NaCl delivery to the distal nephron, leading to release of ATP from intracellular stores. This ATP release would be followed by an increase in PGE2 from the type B intercalated cells, and then to a decrease in Na\(^+\) absorption by the adjacent principal cells.

In addition, rare genetic defects, such as carbonic anhydrase II deficiency, can lead to intercalated cell as well as proximal tubular dysfunction. This renal tubular acidosis is also referred to as type 3 or mixed renal tubular acidosis (137,152). The patients with this genetic defect also present with osteopetrosis and cerebral calcification.

**Acquired Intercalated Cell Dysfunction**

Amphotericin can cause a generalized dysfunction of the kidney tubule, including intercalated cells, because it damages the plasma membrane and causes an H\(^+\) back-leak of protons due to the exposure (138,153). On the other hand, some conditions, such as Sjögren syndrome and some cases of systemic lupus erythematosus, that can present with dRTA due to more predominant intercalated cell dysfunction. The estimates of patients with Sjögren syndrome and dRTA vary from 30% to 70%; this condition often presents in the context of tubulointerstitial nephritis (154–156). Autoimmune antibodies isolated from patients interact with the intercalated cells, but the precise antigens have not been determined (157). Kidney biopsies of patients with Sjögren syndrome and dRTA reveal a reduced expression of H\(^+\)-ATPase and AE1 (158,159). There is also a decrease in the total number of intercalated cells in these patients. Taken together, Sjögren syndrome is associated with loss of intercalated cell transport activity associated with defects in acid-base balance, although the precise events in the development of these findings are yet to be explored.

Lithium is a proven treatment for bipolar disorder, and approximately 40% of patients receiving this treatment develop urinary concentrating defects and eventually nephrogenic diabetes insipidus, which can contribute to ESRD (reviewed in references 160,161). One of the many renal manifestations of long-term lithium treatment is that patients develop an incomplete form of renal tubular acidosis that is usually not clinically relevant (162). Despite this clinical syndrome, the findings in rats treated with lithium reveal increased numbers of type A intercalated cells that are present even in the inner medulla (163). It has been proposed that lithium decreases the gradient for proton secretion.

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**Figure 9.** Intercalated cells are targets of paracrine angiotensin II signaling. Angiotensin II stimulates secretion of prorenin and renin from principal cells. Prorenin binds to the prorenin receptor at the apical membrane of type A-IC, where in turn it stimulates the synthesis of angiotensin II from angiotensin I via the action of angiotensinogen, which originates in proximal segments of the nephron. Angiotensin II then binds to the angiotensin receptor 1a (AT1aR) in the type A-IC. Moreover, prorenin binds to its receptor in type A-IC to stimulate signaling via p58 or cAMP.
(a voltage-dependent defect) along with a concomitant decrease in \( \text{H}^+ \)-ATPase activity. Therefore, the observed increase in the number of intercalated cells may be a potential defense mechanism against the initial mechanism of lithium-induced acidosis. Moreover, lithium inhibits glycogen synthase kinase 3\( \beta \) (reviewed in reference 164). Inhibition of this kinase during kidney development induces nephron differentiation via the Wnt signaling pathway (165). Wnt signaling, a very relevant cascade needed for normal embryonic development, has been reviewed elsewhere (166). Therefore, another explanation for the increased number of intercalated cells seen with lithium treatment could be the disruption of Wnt signaling downstream of lithium-mediated glycogen synthase kinase 3\( \beta \) inhibition.

Findings similar to those observed with long-term lithium treatment were described after dietary K\(^+\) depletion, with increases in intercalated cells that were reversed upon dietary K\(^+\) repletion (167). These researchers concluded that principal cells and intercalated cells may interconvert in response to changes in dietary K\(^+\) and that autophagy was likely involved in the pathways driving this interconversion.

The antirejection medications cyclosporine and tacrolimus affect intercalated cell function and result in the development of dRTA, numerous changes in proximal and distal nephron epithelial transport proteins, and a decrease in the non–type A intercalated cells (168,169). Cyclosporine also inhibits cytochrome P450 and thus affects hensin polymerization after...
extracellular deposition, a step that when defective reduces terminal differentiation of intercalated cells (170).

Intercalated Cells and the Innate Immune Response

The epithelial cells lining the urinary tract play a major role in maintaining the sterility of urine, which is achieved by mucus production, urinary flow, and bladder emptying, all of which provide immediate defense against microbial invasion. This innate defense cascade is less specific than the adaptive immune response. Among the better-known components of the innate immune response are the Toll-like receptors (TLRs). TLRs play a key role in containing urinary tract infections and pyelonephritis. More than 13 TLRs have been identified in mammals, and kidney tubular epithelial cells express most of them. Among these TLRs, TLR4 has been implicated in the innate immune defense against uropathogenic Escherichia coli, the most frequent pathogen responsible for urinary tract infection (171). TLR4, which recognizes lipopolysaccharides in the outer membrane of gram-negative bacteria, is expressed in mouse collecting duct intercalated cells (172). TLRs induce a dynamic immune response via several signaling pathways, including the production of antimicrobial peptides (AMPs) (Figure 11) (172,173).

AMPs are polypeptides of <100 amino acids that at physiologic concentrations are active against bacteria, enveloped viruses, fungi, and protozoa. AMPs are expressed by neutrophils or epithelial cells constitutively or after induction by exposure of the cells to pathogens (174). Urinary tract and kidney epithelial cells synthesize several AMPs, including defensins, cathelicidin, hepcidin, and ribonuclease 7 (RNAse7). Among these AMPs, defensins and RNAse7 are expressed in the kidney collecting duct (175). Defensins, which have broad-spectrum antimicrobial activity, are classified into two main subfamilies: α- and β-defensins. Of the β-defensins, human β-defensin-1 and β-defensin-2 are expressed in the loop of Henle, distal tubule, and collecting duct. In contrast to human β-defensin-1,
human β-defensin-2 is not constitutively expressed in the kidney, but instead its upregulation has been implicated in the immune response during chronic kidney infection (176). On the other hand, RNase7 is a potent AMP constitutively expressed by the uroepithelium of the bladder, ureter, and intercalated cells. Urinary concentrations of RNase7 are much greater than those of other AMPs, exhibiting a bactericidal activity against both gram-negative and gram-positive bacteria.

Another process that implicates type A intercalated cells in the immune response during urininary tract infection is that these cells secrete the bacteriostatic protein lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL). An important difference between NGAL and other AMPs is that NGAL expression is intensively upregulated by both septic and aseptic causes of AKI (177,178). Barasch and colleagues showed that type A intercalated cells are key elements in the production of this bacteriostatic protein. They used a mouse model expressing an NGAL reporter gene, which showed NGAL expression after kidney ischemia (179,180). Barasch and colleagues showed that type A intercalated cells secrete the bacteriostatic protein lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL). An important difference between NGAL and other AMPs is that these cells secrete the bacteriostatic protein lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL). An important difference between NGAL and other AMPs, exhibiting a bactericidal activity against both gram-negative and gram-positive bacteria.

Summary and Conclusions

When intercalated cells were first identified, their morphology was distinctive and reminiscent of acid-secreting cells in more primitive epithelia. Since the 1960s, the essential role of the intercalated cells in acid-base homeostasis was solidified, first with the characterization of the transport processes in the distal nephron and later with the identification of individual membrane transport proteins, such as H+ -ATPase, AE1, and pendrin, contributing to those processes. The role of intercalated cells in the pathogenesis of distal (type 1) and mixed renal tubule acidosis has been clearly established. In the last few years, the acid-base cellular sensors such as sAC and GPR4, and downstream-activated kinases such as PKA or the metabolic sensor AMPK have been recognized as key regulators of intercalated cell function. Moreover, the role of intercalated cells as regulators of salt reabsorption and intravascular volume preservation was established with the identification of NDCBE and pendrin in type B intercalated cells. Lately, it has come to the forefront that both intercalated cell volume regulation and the functional integrity of the distal nephron depend on H+ -ATPase function in these fascinating cells.

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References


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