Membrane Trafficking of Angiotensin Receptor Type-1 and Mechanochemical Signal Transduction in Proximal Tubule Cells

Robert J. Kolb, Philip G. Woost, Ulrich Hopfer

Abstract—Cellular localization and trafficking of the major angiotensin receptor, AT1, was studied in mouse proximal tubule cell lines because angiotensin II concentrations in the luminal fluid of proximal tubules are greater than the Kd of the receptor and would predict high turnover rates of the receptor. Mouse proximal tubule cells can exist in 2 polarized, differentiated states after confluence: a proteop epithelium and a highly differentiated epithelium. The latter is distinguished by greater polarization of the microtubule cytoskeleton and collection of apical microtubule-dependent membrane proteins in condensed apical recycling endosomes (CARE) in proximity to the primary cilium. AT1, AT2, and the sodium hydrogen exchanger NHE3 are localized to CARE. With fluid movement, AT1 receptors externalize from CARE to the apical plasma membrane and allow luminal angiotensin II to initiate cell signaling. These data suggest that fluid movement controls receptor externalization and, hence, a model in which ciliary deflection results in transduction of a mechanical stimulus into the chemical signaling of the AT1 receptor. (Hypertension. 2004;44:352-359.)

Key Words: receptors, angiotensin ■ epithelium ■ microscopy ■ kidney

Angiotensin II (Ang II) is a key hormone involved in absorption of sodium by proximal tubules.1,2 It acts through the G-protein coupled surface receptors AT1 and AT2 in humans or AT1a, AT1b, and AT2 in rodents. Although much is known about the signaling pathways downstream from the receptors, the physiological mechanisms involved in regulating proximal tubule sodium absorption by Ang II are not fully understood. The proximal tubule contains all elements of a complete renin-angiotensin system (ie, angiotensinogen, renin, angiotensin converting enzyme, and Ang II).3–6 Measured Ang II concentrations in the proximal tubular luminal fluid are 6 to 10 nmol/L,7–9 ie, values that are 100 to 1000× the systemic concentration and above the Kd of Ang II receptors.8 These findings are incompatible with classical concepts of hormonal regulation by modulation of hormone (ligand) concentration. For example, only a limited dynamic range is available for regulation if baseline concentrations of the hormone are already above the Kd of the cognate receptor.

High Ang II concentrations predict high internalization rates for AT1 receptors that are on the surface and therewith high turnover rates. Past experiments have shown G-protein coupled receptor internalization as a way to downregulate from a “baseline” state and that in epithelial cells, apical membrane proteins recycle through an intracellular compartment termed “apical recycling endosomes.”10 Therefore, membrane trafficking of AT1 was investigated in vitro using mouse proximal tubule (mPT) cell lines. Experimental conditions that promote differentiation of polarized cells similar to that of cells in vivo were used so that any findings would be relevant for understanding behavior of cells in vivo. mPT cell lines were derived from microdissected proximal tubule segments of the Immortomouse (Ludwig Institute for Cancer Research, United Kingdom). This transgenic mouse harbors a temperature-sensitive version of the immortalization gene SV40 large T antigen (ts-SV40-l-Tag) under control of interferon-γ response elements. Therefore, the proximal tubule cells from these animals are conditionally immortal and can be expanded at the permissive temperature of 33°C, whereas a switch to the mouse body temperature of 39°C allows a high degree of differentiation under appropriate conditions.11

Materials and Methods

Cell Culture
mPT cell lines were maintained on collagen-coated Millicell-CM culture plate inserts (Millipore Corp) to promote a polarized epithelial phenotype. Two different types of conditions were used: (1) expansion at 33°C until cells formed a confluent and resistive monolayer and (2) differentiation at 39°C. Under expansion conditions, cells were incubated at 33°C with renal tubular epithelial medium2 plus epidermal growth factor, interferon-γ, and 5% FBS applied to both apical and basolateral compartments. For greater differentiation, cells were switched after confluence to 39°C in renal tubular epithelial medium with 1 nmol/L L-3,3′,5-triiodothyronine, no epidermal growth factor or interferon-γ, and 5% FBS only on the
basal side. To assess the effect of fluid movement on cells, culture plates were placed in the incubator on a rotary shaker at ~1 Hz.

**Immunocytochemistry**

Fixation and immunocytochemistry were performed using standard techniques. To preserve the microtubule cytoskeleton, prefixation wash and fixation were carried out at 33°C and room temperature, respectively. Details about antibodies and indirect immunofluorescence are provided in an expanded Methods section available in an online supplement at [http://www.hypertensionaha.org](http://www.hypertensionaha.org). Antibody AT1, N10 (Santa Cruz Biotechnology) was used for Figures 2 to 4 and Figure 6 in this study to detect AT1. Specificity of primary antibodies was established by omission in the immunocytochemical staining protocol and, in the case of peptide-specific antibodies, with blocking peptides in both immunocytochemistry and Western blots (see online supplement).

**Microscopy and Image Analysis**

Image stacks were acquired with a Zeiss 200 M inverted microscope with a DG4 fluorescent light source (Sutter Instrument Co) and a 12-bit CoolSnapHQ camera (Roper Scientific) under control of Metamorph v4.5 (Universal Imaging Corp). Images were deconvolved by Autoquant’s Autodeblur (blind deconvolution) software (AutoQuant Imaging, Inc).

**Statistical Analysis**

Statistical analysis was done by ANOVA and Student paired t test as appropriate.

**Results**

**In Vitro Differentiation States of Polarized Proximal Tubule Cells**

The physiological state of cells in culture and therewith the physiological relevance of experimental results depend strongly on culture conditions. Adherent epithelial cells, such as proximal tubule cells, must be grown on permeable supports and form electrically resistive monolayers to be considered minimally differentiated. However, that is not sufficient because Al-Awjati et al have shown that renal intercalated cells in cell culture can exist in a “protoepithelial” and “terminally differentiated epithelial” state with very different electrolyte transport properties, whereby only the latter state has relevance to the in vivo situation. For proximal tubule cells in culture, cellular changes that occur with differentiation beyond the protoepithelial state have not been described, but are highly relevant in the context of a study on membrane trafficking because the organization of the cytoskeleton is a major determinant of this process.

mPT cells were grown to confluence at 33°C (protoepithelial state) and then switched to 39°C without added growth factors to allow for differentiation (highly differentiated state). This switch resulted in reorganization of a number of cellular parameters and formation of distinct condensed apical recycling endosomes (CARE) described later. The appearance of this distinct cellular compartment is one reason to consider the cells at 39°C as highly differentiated. mPT cells in vivo have been shown to rapidly move certain apical transporters, such as Na+/H+ exchanger type 3 (NHE3), into a distinct subapical compartment from which the transporters can recycle to the surface.

Confluent monolayers of proximal tubule protot epithelia were characterized by tight junctions (specific conductance of 7 mS/cm²), formation of a primary cillum, and active Na+-dependent phosphate as well as succinate absorption from the apical compartment (Philip G. Woost, 2004, unpublished data). Na+-dependent phosphate and succinate transport are typical proximal tubule functions. When cells were switched to differentiation conditions, striking changes were observed in microtubule and apical glycoprotein organization, whereas other parameters, such as Na+-dependent succinate transport, remained unchanged.

In protot epithelial monolayers, microtubules form a meshwork below the apical plasma membrane with dense microtubules running parallel to the lateral plasma membrane and few microtubules extending into the perinuclear region (Figure 1A). In highly differentiated monolayers, microtubules form a ring in the apical cytoplasm with radial extensions. Microtubules were highly diminished along the lateral plasma membrane and not detectable at the basal pole of cells, at least relative to the dense ring and web at the apical pole (Figure 1B). These results with proximal tubule cells are in agreement with previous studies in which filter-grown Madin-Darby canine kidney (MDCK) cells exhibited a polarized distribution of microtubules with major concentration in the supranuclear region of the cell at 48 hours after confluence.

Surface glycoproteins were assessed by binding of wheat germ agglutinin (WGA), a lectin that binds to glycoproteins containing dimers and trimers of N-acetylglucosamine. Prominent, relatively uniform WGA staining of microvilli and the apical portion of lateral borders is illustrated for protot epithelial cells in Figure 1C. With differentiation, WGA staining of the brush border becomes even more pronounced. In addition, a new feature appears, namely the development of a distinct apical plasma membrane area devoid of WGA-stainable glycoproteins. This area is located close to the center of the cell in x-y projections and often bordered by a ring of intense staining (Figure 1D). Notably, this unique region within the apical plasma membrane is located above the microtubule ring that forms in the apical pole under the same differentiation conditions.

The development of a primary cilium is an expression of the polarized nature of cells and is closely associated with the organization of the microtubule cytoskeleton. The primary cilium of proximal tubule cells is nonmotile. Primary cilia are present in protot epithelial cells where they stain with WGA. Figure 1E illustrates a transitional monolayer with formation of an apical plasma membrane region devoid of glycoproteins, but the presence of WGA-stained cilia. In highly differentiated proximal tubule monolayers, cilia are present with an approximate length of 3 to 5 μm (Figure 1F), but they do not stain with WGA. Therefore, absence of WGA-stainable glycoproteins on the ciliary membrane provides one criterion to assess the differentiation state. The described pattern of changes in microtubule structure and organization of surface glycoproteins was seen in at least 5 of 8 different mPT cell lines. Most of the subsequent studies were carried out with cell line mPT 32101.

**Localization of AT₁ Is Microtubule Dependent**

mPT cells stably and abundantly express AT₁ in both protot epithelial and highly differentiated states as judged from Western blots and immunocytochemistry. Antigenic specific-
ity was established by the use of blocking peptides and 3 different antibodies directed against either the N- or C-terminus of AT1 (see the online supplement). The different antibodies gave essentially identical results in immunocytochemical experiments.

In protoepithelia, AT1 is found in vesicles that are dispersed throughout the cell as well as at the apical and basolateral plasma membranes (Figure 2A to 2D). In contrast, in highly differentiated cells, vesicles containing AT1 receptors relocate to the subapical region and give rise to a distinct

![Figure 1](https://example.com/figure1.png)

**Figure 1.** In vitro differentiation states of polarized proximal tubule cells. A, Subapical, supranuclear slice through a protoepithelial mPT monolayer showing microtubules forming a meshwork with heavy staining at cell edges, which represents extensions that run orthogonally to the monolayer and parallel to the lateral plasma membranes. B, Subapical, supranuclear slice through a highly differentiated mPT monolayer showing microtubules forming a ring structure. Amplification of pixel intensities are different in A and B to bring out the major structural features. C, Apical surface of a protoepithelial mPT monolayer labeled with WGA showing a punctate staining on a diffuse background and outline of the cells, corresponding to microvilli, apical plasma membrane, and lateral plasma membrane above the tight junction, respectively. D, Apical surface of a highly differentiated mPT showing the apical plasma membrane that now shows many cells possessing 1 distinct apical plasma membrane region devoid of any WGA-staining glycoproteins. E, Apical surface of an mPT monolayer in transition between protoepithelium and highly differentiated epithelium showing WGA showing heavy staining of the ciliary plasma membrane in some cells that also show plasma membrane regions devoid of WGA-staining. F, Staining of the primary cilium (red) with antiacetylated tubulin antibody and of nuclei with 4',6-diamidino-2-phenylindole (DAPI; blue) from highly differentiated epithelia. All images were acquired at ×100 except F, which was at ×63. 1F is a projection of 2 slices, one 2 μm above the apical membrane for the primary cilium and another at the level of the nucleus. Bars=10 μm.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Localization of AT1, and its microtubule dependence in mPT monolayers. A, Apical WGA staining of a protoepithelial cell monolayer. B, Subapical, supranuclear slice of the same protoepithelial monolayer as in A stained with antibody against AT1. C, Merged apical image slices from the protoepithelium shown in A and B. D, Z slice from the same image stack as shown in Figure C (taken at the red line). Note the predominantly cytoplasmic location of AT1 (green) and the heavy glycosylated brush border stained with WGA (red). E, Subapical, supranuclear slice of a highly differentiated monolayer showing the accumulation of AT1 receptors in CARE. F, Apical WGA surface staining of the same highly differentiated monolayer as shown in E. G, Merged apical image slices from E and F, and counterstained with a nuclear dye (blue; Hoechst 33342). This image shows the relative location of AT1 in CARE and the heavy glycosylated brush border stained with WGA (red). H, Subapical, supranuclear slice of a highly differentiated monolayer showing the accumulation of AT1 receptors in CARE. F, Apical WGA surface staining of the same highly differentiated monolayer as shown in E. G, Merged apical image slices from E and F, and counterstained with a nuclear dye (blue; Hoechst 33342). This image shows the relative location of AT1 in CARE. H, Z slice of the same image stack as shown in G. This image reveals the subapical, supranuclear location of AT1 in CARE, which shows a lobulated doughnut structure with conical sides at high magnification (see supplement Movie I). I, Subapical, supranuclear slice of a highly differentiated monolayer stained with antibody against Gδi (red), and counterstained with a nuclear dye (blue) showing Gδi in CARE. For merged images, the AT1 signal is labeled green and the signal for WGA-Texas-Red is labeled red. Bars=10 μm.
lobular ring structure (Figure 2E to 2H and online supplement Movie I). This structure resembles condensed apical recycling endosomes in MDCK cells\cite{20,21} and is referred to as CARE. A close-up view shows dense staining at the most apical end with extensions and branching that become fainter toward the nucleus. It is likely that AT1 in CARE represents a form of the receptor that is ready to bind ligand because a high proportion of a signal transduction molecule for AT1, namely Go\(\alpha\), is also located in the same structure (Figure 2I).

In x-y projections, the location of the center of CARE coincides with that for the microtubules (Figure 3A to 3C) and the area of the plasma membrane devoid of WGA binding (Figure 2G). However, the width of the 3 structures differs, which, for example, is seen as partial overlap of the AT1 ring with the microtubule ring. Nevertheless, the appearance of the 3 structures in close proximity when cells differentiate indicates that they are related and may operate together in a particular cell function. Recolocalization of AT1 to the subapical, supranuclear region begins within 60 minutes after cells are exposed to differentiation conditions and remains as such for at least 12 hours.

To test the dependence of AT1 collection in the apical ring structure on an intact microtubule cytoskeleton, differentiated monolayers were either exposed to 10 \(\mu\)mol/L nocodazole or to cold temperature (0°C) for 30 minutes. Both treatments result in disassembly of microtubules. Interestingly, both treatments disassembled the ring structure of AT1 and dispersed AT1-containing vesicles throughout the cell, in addition to the expected destruction of the normal distinct structure on an intact microtubule cytoskeleton (Figure 3A to 3F). Colocalization experiments of AT1 with detyrosinated tubulin, which is the specific form of tubulin found in the ciliary axonema, indicate that the ring formed by vesicles containing AT1 is adjacent to the cilium, with minimal overlap (Figure 4A to 4C).

**Coupling of Ciliary Stimulation to Externalization of AT1**

The microtubule dependence of the movement of AT1-containing vesicles to CARE adjacent to the primary cilium in differentiated cells suggests that the cilium may also play a role in trafficking of AT1 through the cell. Recent studies by Praetorius and Spring\cite{22} and Nauli et al\cite{23} in MDCK and collecting duct cells, respectively, indicate that the primary cilium of differentiated cells functions as a flow sensor using Ca\(^{2+}\) influx and elevated cytosolic Ca\(^{2+}\) to signal changes to the rest of the cell. However, in cell culture, there is typically little fluid movement across the apical plasma membrane, and Ca\(^{2+}\) influx via the ciliary route is probably low. As elevated cytosolic Ca\(^{2+}\) constitutes a fusion signal in most cells, the accumulation of AT1 in CARE can be explained by a lack of ciliary Ca\(^{2+}\) influx and, hence, lack of movement of AT1 to the apical membrane by vesicle fusion. In other words, ciliary stimulation may be necessary for externalization of AT1 receptors from intracellular vesicle, at least in highly differentiated cells where microtubules direct apical recycling vesicles to pass through CARE. This concept is summarized in a model in Figure 5.

**Effect of Apical Fluid Movement on AT1 Distribution and Function**

To test this hypothesis, confluent mPT monolayers were placed under differentiation conditions on a rotary shaker to gently move the apical solution across the cells. This fluid movement resulted in externalization of AT1 receptors out of CARE and into the apical surface membrane (Figure 6A and 6B). This effect was most pronounced in the center of the filters on which cells were grown.

To assess the functionality of the newly inserted AT1 receptors, phosphorylated extracellular-regulated kinase (pERK) was measured separately in the nucleus and the rest of the cell after Ang II and mechanical stimulation. Immunofluorescence of image stacks that spanned the entire monolayer was quantified, and the ratio of nuclear to total pERK was calculated. Validity of this method was established by calculating the variance of the ratio from several image stacks of each filter (intrafilter variance). The average SEM of 9 sets was 6%, therewith low enough for meaningful comparison of filters treated differently. Interestingly, the ratio of nuclear to total pERK specifically increased only when stimulation with apical Ang II and fluid movement were both present; this effect was mediated by AT1, as indicated by inhibition by the AT1 blocker candesartan (Figure 6C). The numerical results for the ratio under the interesting condition of fluid movements were: control 0.46±0.03; Ang II 0.62±0.02; and candesartan plus Ang II 0.46±0.05, with n=3 different sets of experiments. Differences between the pairs (Ang II)/control and (Ang II)/(Ang II plus candesartan) are statistically significant (\(P<0.05\)).

**Localization of Other Apical Proteins in Highly Differentiated Proximal Tubule Epithelia**

The studies with AT1 raised the question whether redistribution with differentiation is unique to AT1 or whether other proteins behave similarly. We specifically studied the localization of AT2, another G-protein coupled receptor, and the apical transporter NHE3. mPT cells stably express endogenous AT2 and NHE3 in both proteoepithelial and highly differentiated states, as determined by immunocytochemistry and Western blot analyses. Figure 7A demonstrates for AT2 and Figure 7B for NHE3 that both types of proteins are also collected in the CARE compartment in a manner very similar to AT1 (for NHE3, see also the online supplement Movie II). Membrane trafficking of NHE3 is known to be regulated in vivo, in part by hypertension,\cite{24} and to recycle through CARE.\cite{25}

**Discussion**

The results of this study are important in several respects. At the cell biology level, they extend the concept of specialized CARE to proximal tubule cells and important endogenous receptors and transporters. The importance of CARE for trafficking of integral apical membrane proteins and polarization had previously been shown mainly with a collecting duct cell line\cite{20} (ie, in MDCK cells and with transfected proteins).\cite{21} The present study demonstrates its existence in proximal tubule cells and at physiological abundance of the endogenous AT1 and AT2 receptors and NHE3 transporter.
Further, the importance of microtubule organization for formation of CARE is shown, and its proximity to the primary cilium and the formation of a specialized apical membrane region devoid of WGA-binding proteins is demonstrated for the first time to our knowledge. A specialized apical membrane region, adjacent intracellular accumulation of specific proteins, or both have previously been demonstrated a few times in other epithelial monolayers (eg, SNAP-25 in MDCK cells, NHE3 in opossum kidney cells, and aminopeptidase N in a proximal tubule cell line). However, no function has been established. The coordinated development of the microtubule ring structure, the accumulation of vesicles containing integral apical membrane proteins, and a specialized apical membrane region adjacent to the cilium with differentiation suggest that the architecture of the microtubule cytoskeleton and distribution pattern of the apical glycoproteins are useful criteria to judge the differentiation state of epithelial cells.

At the functional level, the studies establish that fluid flow is important for directing AT1 receptors to the apical surface and allowing them to interact with the hormone Ang II in the adjacent solution to produce cellular signals appropriate for this receptor. The presence of AT1 in the CARE compartment adjacent to the cilium would appear to be important for control of externalization of this receptor by fluid flow and ciliary deflection. Assuming that proximal tubule cilia respond in the same manner as MDCK and collecting duct cells (ie, with localized Ca2+ influx), ample precedence from other cell types suggests that this Ca2+ can promote fusion of nearby vesicle with the plasma membrane (Figure 5). Interestingly, the specialized apical plasma membrane region adjacent to the cilium and devoid of WGA-binding resembles the specialization of the flagellar pocket in trypanosoma, which has been established as a major site for exo- and endocytosis.

The results on AT1 trafficking clearly demonstrate that AT1 externalization of the receptor can be rate-limiting for Ang II signaling and that the dynamics of externalization and endocytosis must be taken into account when considering the situation in vivo. The steady-state situation in vivo is one of fluid flow and constant Ang II secretion into the lumen and, hence, tonic Ang II signaling. Interestingly, expected differences in cell behavior in response to either tonic or acute stimulation can explain differences in the dose-response curve of Ang II on sodium bicarbonate reabsorption with different experimental preparations, particularly between in vivo and in vitro preparations. In vivo, increasing Ang II concentrations monotonically stimulate sodium bicarbonate reabsorption rates up to 2.5-fold above the Ang II-independent ones. In contrast, in the examined in vitro preparations, the concentration–effect curves of Ang II on sodium bicarbonate reabsorption were one- to two-fold above that of the Ang II-independent ones.
preparations, the Ang II dose-response is biphasic with stimulation at very low concentrations and inhibition at higher concentrations, such that at physiological luminal concentrations only minimal stimulation or even inhibition of sodium bicarbonate reabsorption occurs. The biphasic curve results from activation of different cellular signaling pathways by AT1, one that is stimulatory at low Ang II concentrations and another one that is inhibitory at higher concentrations. Interestingly, with tonic stimulation by Ang II and recycling of the angiotensin receptor through the cell, as it occurs in vivo, the concentration of the active Ang II-receptor complex at any time should remain modest even at high Ang II concentrations, thus predicting coupling to only the stimulatory signaling pathway and, hence, a monotonic stimulatory curve. Similarly, the proposed mechanism for Ang II signal activation in Figure 5 allows for shifts in the rate-limiting step depending on preparation and condition, and such a shift can explain the wide dynamic range of Ang II effects on Na+/H+ absorption (6 orders of magnitude from 10^{-12} to 10^{-6} mol/L) in different proximal tubule preparations.

It is unclear at the moment what the dominant functional consequences of basolateral AT1 signaling are. Kreitzer et al convincingly showed that integral “apical” membrane proteins can be inserted into the basolateral plasma membrane of

Figure 5. Model for mechanochemical signal transduction. The top section depicts the effect differentiation on an angiotensin receptor distribution within cells: in protoepithelial cells (left) the bulk of angiotensin receptors is localized in vesicles that are dispersed throughout the cell, whereas with further differentiation (right), vesicles accumulate in a central location adjacent to the primary cilium, as demonstrated in Figure 4. The bottom section depicts the effect of Ca^{2+} influx initiated by ciliary bending on angiotensin receptor distribution: Ca^{2+} influx stimulates vesicle fusion with apical plasma membrane that results in angiotensin receptors moving into the apical plasma membrane (right).

Figure 6. Changes in AT1 distribution by fluid movement across the apical surface membrane and activation of angiotensin signaling. A, Apical slice from a highly differentiated mPT monolayer gently shaken for 12 hours in the incubator. The slice is stained for AT1 (green) and apical and basolateral surface glycoproteins (red; WGA added to apical and basolateral compartments). Note that the bulk of AT1 localizes to the apical plasma membrane. B, Z slice of the image stack used for A. Note that the AT1 receptor localizes predominately to the apical plasma membrane after shaking. C, Ratio of nuclear to total pERK as a function of treatment of differentiated mPT monolayers. pERK was measured through staining with an antibody against dually pERK. Monolayers were maintained in incubator for 12 hours under differentiation conditions either without or with gentle shaking on a rotary shaker (1 Hz). Candesartan was added to the apical compartment at 10 nmol/L for 12 hours when indicated. Ang II was added to the apical compartment at 10 nmol/L for 10 minutes when indicated. n=3 separate filters for each group. Note that the significant increase in the ratio of nuclear to total pERK is dependent on apical fluid movement and presence of Ang II in the apical compartment and is inhibited by the AT1 blocker candesartan. * indicates significant with P<0.05, compared with control without Ang II and to candesartan.
polarized MDCK cells when trafficking is altered by microtubule disrupting procedures, such as exposure to cold or treatment with microtubule disrupting agents. Therefore, AT1 receptors would be expected to reach the basolateral plasma membranes under any condition that would disrupt the normal microtubule architecture, including inflammatory processes. It is interesting to speculate that healthy proximal tubule cells may have evolved microtubule-dependent trafficking of AT1 to direct this receptor away from the basolateral membrane and to concentrate on signal transduction that effects sodium retention.

Perspectives

The results of this study have implications for the mechanism of intrarenal regulation of sodium reabsorption by Ang II. Ciliary control of the movement of angiotensin receptors to the apical surface membrane in proximal tubule cells provides a rationale why the kidney constitutively maintains high luminal Ang II concentrations. Trafficking of apical Ang II receptors through CARE, control of their externalization by ciliary movement, and high luminal Ang II concentrations constitute a mechanochemical signal transduction scheme and thus serve to provide cells with pressure/flow information. Ample ligand (Ang II) in the luminal fluid is necessary so that subsequent ligand binding, downstream signaling, and receptor endocytosis are fast relative to externalization. Furthermore, this complex regulatory scheme allows integration of different types of signals. For example, proximal tubule cells have recently been shown to sense cytosolic Na⁺ concentrations and respond with appropriate changes in the ratio of expression of AT1 and the dopamine receptor D1, which regulate Na⁺ reabsorption in opposite directions. The combination of Na⁺-dependent receptor expression and flow-dependent receptor externalization could provide cells with a mechanism to integrate information on luminal fluid flow and Na⁺ concentration, that is, information about Na⁺ abundance.

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References


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