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Review

Discerning the role of mechanosensors in regulating proximal tubule function

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Raghavan V, Weisz OA. Discerning the role of mechanosensors in regulating proximal tubule function. *Am J Physiol Renal Physiol* 310: F1–F5, 2016. First published October 14, 2015; doi:10.1152/ajprenal.00373.2015.—All cells in the body experience external mechanical forces such as shear stress and stretch. These forces are sensed by specialized structures in the cell known as mechanosensors. Cells lining the proximal tubule (PT) of the kidney are continuously exposed to variations in flow rates of the glomerular ultrafiltrate, which manifest as changes in axial shear stress and radial stretch. Studies suggest that these cells respond acutely to variations in flow by modulating their ion transport and endocytic functions to maintain glomerulotubular balance. Conceptually, changes in the axial shear stress in the PT could be sensed by three known structures, namely, the microvilli, the glycocalyx, and primary cilia. The orthogonal component of the force produced by flow exhibits as radial stretch and can cause expansion of the tubule. Forces of stretch are transduced by integrins, by stretch-activated channels, and by cell-cell contacts. This review summarizes our current understanding of flow sensing in PT epithelia, discusses challenges in dissecting the role of individual flow sensors in the mechanosensitive responses, and identifies potential areas of opportunity for new study.

mechanosensation; shear stress; mechanical stretch; calcium; endocytosis; cilia; microvilli; glycocalyx

THE KIDNEY IS A COMPLEX three-dimensional organ that reclaims water, ions, and small metabolites from the filtered plasma to maintain electrolyte balance. Blood carried by the renal artery enters the capillary loops of Bowman's capsule, and the plasma is filtered through slit diaphragms formed by podocytes to generate an ultrafiltrate that enters the lumen of the proximal tubule (PT). The hydrostatic pressure as the blood enters the parallel arrays of the afferent arteriole is 48 mmHg, whereas the pressure in the efferent arteriole is ~22 mmHg (10). This drop in pressure between the afferent and efferent arterioles builds an intraluminal hydrostatic pressure of ~10–15 mmHg that propels the glomerular ultrafiltrate through the PT. This flow through the PT is pulsatile, with variable oscillations due to the heart rate and to tubuloglomerular feedback mediated by the macula densa.

Epithelial cells lining the PT are responsible for reabsorbing up to 70% of the Na^+, K^+, H^+, NH_4^+, Cl^-, HCO_3^-, P_i, glucose, and water from this ultrafiltrate via transcellular and paracellular transport. Moreover, cells lining this segment express multiligand receptors that mediate the uptake of low-molecular weight proteins that have escaped the filtration barrier to prevent their loss in the urine (8). These cells elaborate a highly differentiated brush border decorated by a glycocalyx, as well as a primary cilium. Although the paracellular spaces are highly permeable to sodium and other ions as well as water, these cells have tight junctions, adherens junctions, and gap junctions. Integrins expressed on the basolateral surfaces of these cells maintain adhesion to the basal lamina and transmit changes in stretch to the cells via intracellular cytoskeletal tethers. Changes in the luminal pressure manifest as changes in axial shear stress that act on the apical surface of the PT epithelia and as radial stretch that may act on both surfaces (Fig. 1).

The role of flow in regulating ion reabsorption across the PT epithelium was initially studied using single-tubule micropuncture and microperfusion of rat PTs in the early 1960s. These studies demonstrated that reabsorption of Na^+, HCO_3^-, K^+, and water were enhanced when perfused at higher flow rates compared with lower flow rates (3, 4, 7, 9, 15). Studies conducted since that time have shown that the apical surface of PT cells sense changes in shear stress through their microvilli and primary cilia, and that integrins and stretch-sensitive channels on the basolateral surface can transduce changes in stretch into physiological responses (12, 27, 36). Our current understanding of the role of these cellular assemblies/structures in PT mechanosensation is summarized below.

PT Mechanosensitive Responses to Shear Stress

The most commonly used methods to study shear stress-dependent effects are microperfusion of individually dissected tubules, tubular micropuncture, and exposure of cultured cells to fluid shear using parallel plate flow chambers. The microvilli, primary cilia, and the glycocalyx on the apical surface of PT cells have all been suggested to sense changes in the flow of glomerular filtrate entering the tubule lumen as described below.

Microvilli. The apical surface of rat PT cells contains ~6,500 microvilli, each ~2.8 μm in length. Each of these structures contains ~25–35 actin filaments arranged in bundles and cross-linked by fimbrin, villin, and espin (31). Beneath the

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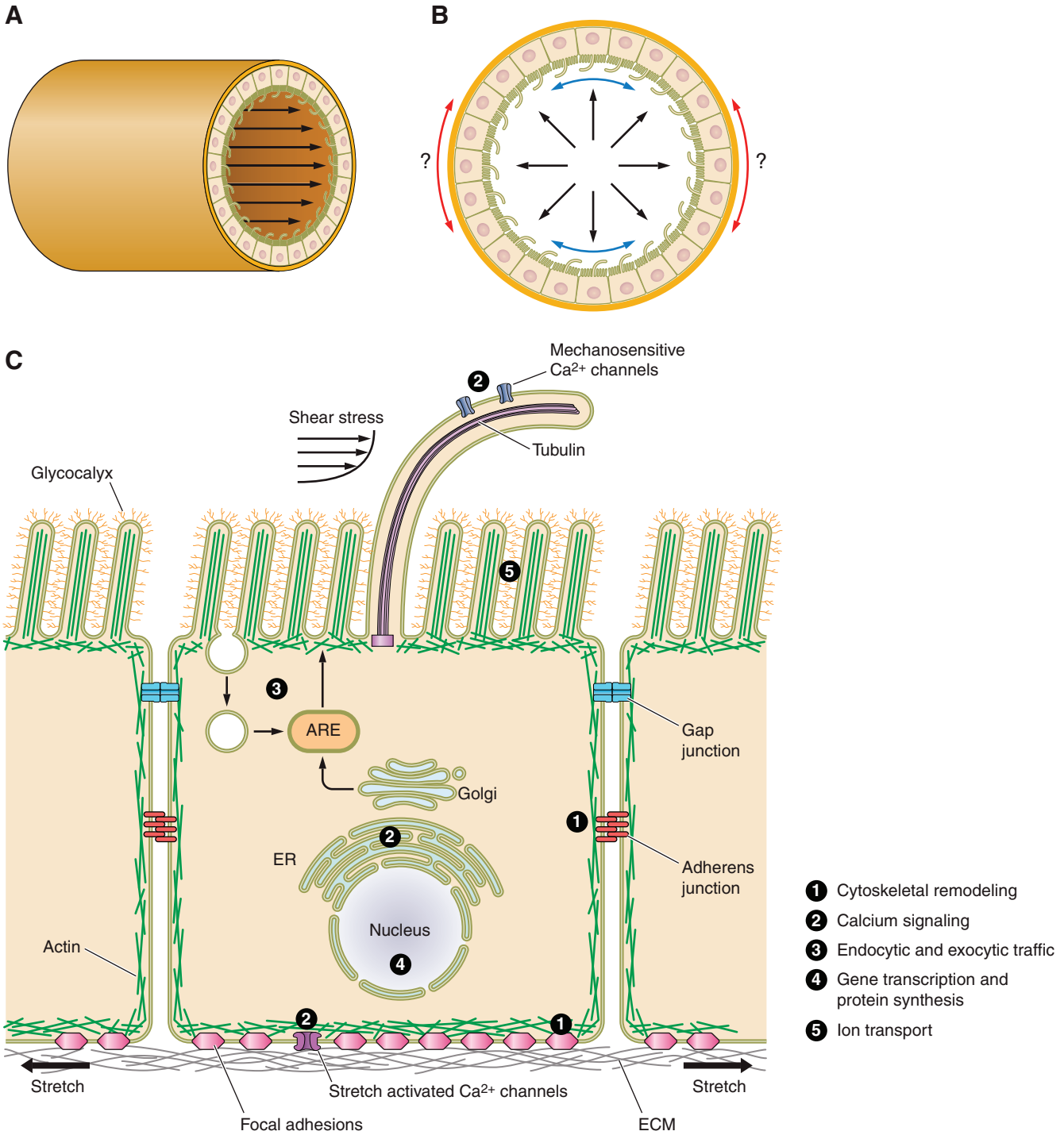

Fig. 1. Mechanosensors of the proximal tubule (PT). *A*: schematic of the manifestation of flow as apical shear stress. *B*: increases in flow also manifest as radial stretch on the walls of the PT to increase the inner diameter of proximal tubules (blue arrows). Whether this stretch is also transduced to the basolateral surface (red arrows) in normal kidney tubules is unclear. *C*: mechanosensory cascades in the PT initiated by shear stress and stretch. Small changes in the bending of microvilli in response to shear stress are amplified by the apical cortical actin network to trigger downstream responses that include cytoskeletal re-organization (i.e., actin restructuring and formation of focal adhesions and tighter adherens junctions), redistribution and activation of ion transporters, and repositioning of apical endosomes. ARE, apical recycling endosomes. The glycocalyx may serve to increase the frictional coefficient of the apical surface and amplify the effects of microvillar bending. Shear stress-dependent bending of the primary cilia causes Ca^{2+} influx through ciliary localized to mechanosensitive Ca^{2+} channels, subsequent Ca^{2+}-stimulated Ca^{2+} release from the endoplasmic reticulum (ER), and increases in apical endocytic capacity. Basolateral and apical stretch cause integrin-mediated cytoskeletal reorganization, Ca^{2+} signaling through stretch-activated calcium channels, and regulation of gene transcription and protein synthesis. See text for further details.

apical surface, these filaments are integrated into a dense actin meshwork that can transduce and amplify even small changes in the bending of microvilli into signaling events that ultimately regulate ion transport, cytoskeletal reorganization, biosynthetic traffic, and changes in apical recycling endosome (ARE) distribution. These changes are believed to be independent of the primary cilium because cells were plated and studied before ciliogenesis (11). For a more detailed review on the role of microvilli in flow sensing, the reader is referred to Ref. 36.

Glycocalyx:. As in most cells, a layer of cross=linked mucins, glycoproteins, and glycolipids coats the apical microvilli of PT cells (37). While the glycocalyx is known to play a critical role in shear stress sensation in the vascular endothelium (reviewed in Ref. 35), its role in mechanosensation in the renal PT has not been rigorously tested. In vascular endothelial cells, the glycocalyx is connected to the cytoskeletal network inside the cell, and when exposed to blood flow, transduces its bending moment to modulate endothelial cell function. The glycocalyx could magnify the physiological effects of flow in the PT by increasing the frictional coefficient at the apical surface. However, treatment of immortalized PT cells with the glycocalyx-digesting enzyme heparinase III did not alter the cellular response to shear stress (11). In this study, only changes in flow-dependent changes in cytoskeleton remodeling were examined, and it remains possible that the glycocalyx mediates other flow-dependent responses.

Primary cilia. Rat PT cells elaborate a single apical primary cilium typically 3–4 μm in length that extends somewhat beyond the microvillar surface (18). Over the past two decades, there has been an increasing appreciation of the function of these complex structures as mechanosensitive flow sensors in many tissue types, including the distal tubule and collecting duct of the kidney. We and others recently demonstrated that PT epithelia respond functionally to changes in fluid shear stress by increasing their endocytic capacity (13) and that this response requires the primary cilium (28, 29). In other tissues, members of the transient receptor potential family of cation channels localized to the cilium are believed to trigger the downstream mechanosensitive responses to ciliary bending (26); however, the role of these channels in the PT has not yet been elucidated. In addition, this cascade is also likely to require membrane proteins that may not be associated with the cilium, including components involved for extracellular ATP release and purinergic receptor signaling. A review of primary cilia as mechanotransducers can be found in (24).

PT Mechanosensitive Responses to Radial Stretch

In addition to increased shear stress, a rise in intraluminal pressure dilates the tubular lumen and potentially triggers a stretch response. In isolated, perfused proximal tubules and in unilateral ureteral obstruction disease models, stretch induced by increased flow is transduced into a change in the outer diameter of the tubule as well as the lumen. It is unclear to what extent these changes in the outer diameter also occur in PTs within the intact normal kidney during normal variations in flow. Stretch is known to modulate Ca^{2+} signaling, cell volume, cytoskeletal reorganization, and gene transcription in kidney tubule cells (1, 5, 16, 21, 23, 33). Integrins, gap junctions, and basolateral stretch-activated calcium channels have been documented to play a role in transducing these changes in stretch (14, 23, 32). In isolated PTs, and in cells cultured on stretchable membranes, mechanical stretch has been suggested to trigger formation of focal adhesions mediated by integrins, and to increase cytosolic levels of Ca^{2+} through stretch-activated calcium channels. More detailed discussions of how stretch regulates PT function can be found in these excellent reviews (27, 36).

Limitations of Current Systems and Alternatives

Notwithstanding the large number of cellular structures and their resident proteins that sense and transduce changes in flow, there are numerous experimental complexities that have limited our ability to generate an integrated model for flow sensing in the PT. One caveat to experiments performed using both parallel plate flow chambers and tubule perfusion is that the cells are generally subjected to a constant rate of flow rather than to the pulsatile flow physiologically experienced by cells in the PT. Moreover, each system has its own unique limitations. The shear forces experienced by individual cells when cultured on flat surfaces may be different compared with what they sense within the cylindrical arrangement of a tubule. The availability of more elastic substrata that can be subjected to defined amounts of stretch, as well as new microfluidic chamber designs for culturing cells, may improve existing limitations (1, 13). Moreover, cultured PT cells used for flow studies frequently lack the exquisite differentiation of apical structures found in vivo, and by necessity must be grown on artificial substrata that may not fully recapitulate the functions of the basal lamina. While these features are preserved in microdissected PTs used for perfusion studies, the extraordinarily efficient reabsorption of water and ions across the tubule means that cells along the tubule are exposed to progressively decreasing intraluminal pressures and flow rates. Additionally, because these isolated tubules are no longer buttressed by the adjacent tubules and vessels within the confines of the kidney cortex, increases in flow may cause the outer diameters of the tubules to stretch beyond their capacity in vivo with consequent changes in signaling. An alternative approach to these models that is being increasingly employed is the use of intravital two-photon microscopy to monitor physiologically relevant changes in rodent kidneys (2, 17, 20).

Another complication in creating a comprehensive model for PT flow sensing is the near impossibility of ablating mechanosensitive structures to test their individual roles without altering global functional parameters. For example, microvilli cannot be easily dismantled without perturbing the global organization of the actin cytoskeleton. Previous studies demonstrating a role for microvilli in flow regulation of ion transport and cytoskeletal remodeling have used the nonselective actin-depolymerizing agent cytochalasin D to collapse microvilli (11). Animal knockouts or gene editing of proteins selectively involved in maintaining microvillar structure may provide a more targeted approach to perturb formation of microvilli (6). However, alteration of microvillar structure by any approach will significantly alter apical membrane morphology with likely consequences to the glycocalyx and ciliary bending, as well as potential changes to lipid composition and microdomains that could affect ion transport and endocytic efficiency.

Similarly, many reports on the role of cilia in mechanosensation, including our own studies, have used ammonium sulfate or chloral hydrate to acutely deciliate cells. Although cells remain impermeable after these treatments and cilia are regenerated within hours and days, these maneuvers likely cause significant cell changes, including changes in Ca^{2+} homeostasis (19, 22). The use of mouse models with defective ciliogenesis, for example, the IFT88 KO which ablates a component of the intra-flagellar transport machinery, may be a good model cell system for evaluating the role of primary cilia in flow sensing (34). Additionally, methods exist to bend individual primary cilia using fine-micropipette manipulation and bead-based optical traps that could be used to selectively modulate responses to ciliary bending at the single cell level (25, 30).

As discussed above, each method of studying the role of mechanical forces has its own limitations, therefore, a thoughtful plan to investigate the role of shear stress and stretch in regulating PT function will combine multiple complementary approaches.

Perspective

The fundamental voids in developing an integrated model for how changes in flow are sensed and recorded by PT cells are the difficulty in identifying the individual roles of the mechanosensors involved and our lack of understanding of the interplay between the several mechanosensory cascades discussed above. In addition to dissecting the individual contributions of microvilli, the glycocalyx, and cilia in sensing apical shear stress, the role of radial stretch in the overall cellular response remains unclear. A few of the many unanswered questions that remain to be addressed include the following: *1*) How do microvilli and cilia coordinate membrane traffic and cytoskeletal reorganization in PT epithelia in response to flow? *2*) Is there a role for the glycocalyx in PT flow sensing? *3*) Is stretch of the PT outer diameter relevant in the context of normal kidney function? *4*) How do shear stress and stretch synergistically alter PT cytoskeletal reorganization? and *5*) How do shear stress and stretch coordinate other facets of PT function? Answering these questions would broaden our understanding of normal PT function and has important implications for identifying new molecular targets for diseases caused by PT dysfunction. We anticipate that creative science and new advances in technology will help us iterate toward more physiologically relevant models to propel our understanding of mechanosensation in PT epithelia.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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