J Physiol 599.14 (2021) pp 3437–3446

The Journal of Physiology

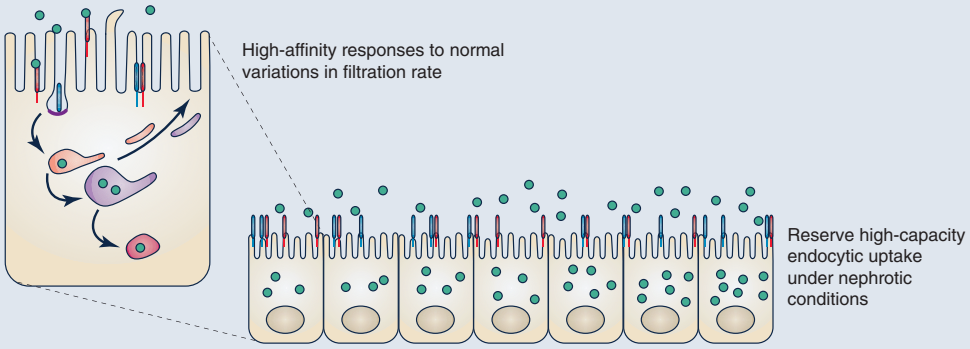
TOPICAL REVIEW

Endocytic adaptation to functional demand by the kidney proximal tubule

Ora A. Weisz

Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261, USA

Edited by: Ian Forsythe & Helle Praetorius



Abstract The kidney proximal tubule (PT) efficiently recovers the low level of albumin and other proteins that normally escape the glomerular filtration barrier. Two large receptors, megalin and cubilin/amnionless (CUBAM), bind to and efficiently retrieve these predominantly low molecular-weight proteins via clathrin-mediated endocytosis. Studies in cell culture models suggest that PT cells may sense changes in shear stress to modulate recovery of filtered proteins in response to normal variations in filtration rate. Impairments in PT endocytic function lead to the excretion of filtered proteins into the urine (tubular proteinuria). Remarkably, when the glomerular filtration barrier is breached, the PT is able to recover excess albumin with a capacity that is orders of magnitude higher than normal. What mediates this excess capacity for albumin uptake under nephrotic conditions, and why doesn't it compensate to prevent tubular proteinuria? Here we propose an integrated new working model to describe the PT recovery of filtered proteins under normal and nephrotic states. We hypothesize that uptake via the fluid phase provides excess capacity to recover high concentrations of filtered proteins under nephrotic conditions. Further, concentration of tubular fluid along the tubule axis will enhance the efficiency of uptake in more distal regions of the PT. By contrast to cells where fluid phase and receptor-mediated uptake are independent pathways, expression of megalin is required to maintain apical endocytic pathway integrity and is essential for both uptake mechanisms. This model accounts for both the

Ora Weisz is a Professor of Medicine, Cell Biology, and Clinical and Translational Science at the University of Pittsburgh School of Medicine. Her research programme seeks to understand how genetic disorders and other diseases affect the development and function of the kidney proximal tubule.

DOI: 10.1113/JP281599

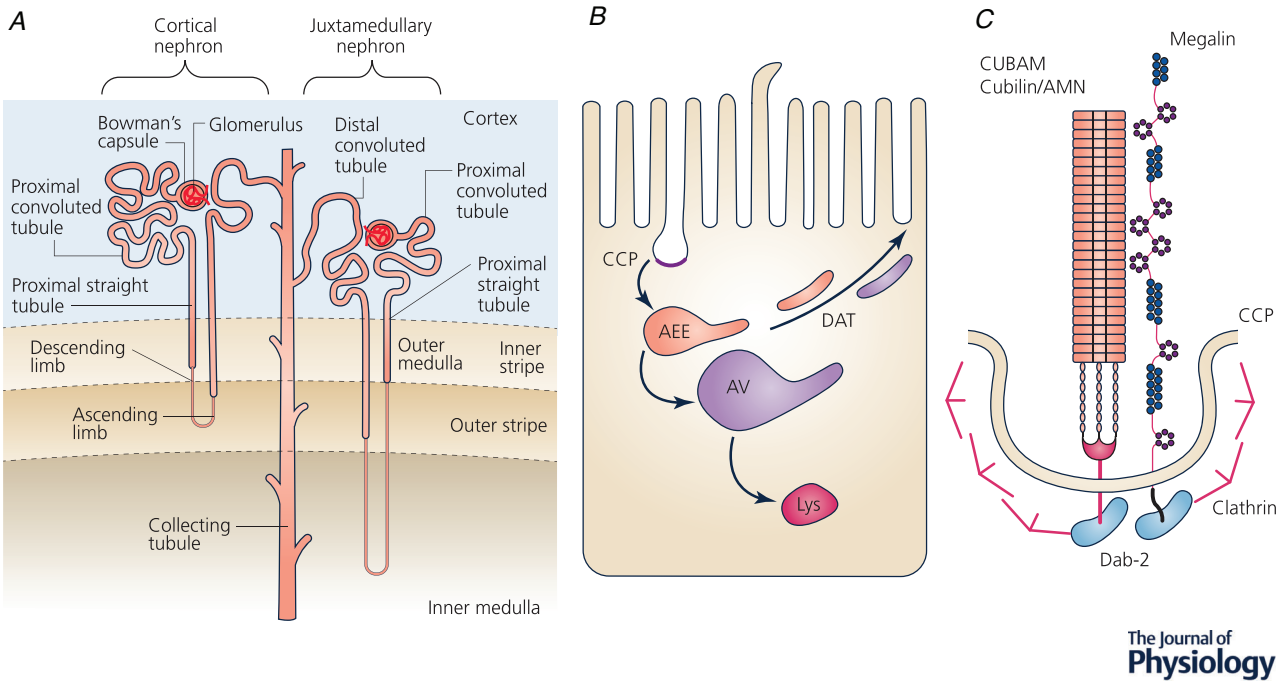
high-affinity and the high-capacity responses to filtration load in physiological and pathological states.

(Received 3 March 2021; accepted after revision 6 May 2021; first published online 25 May 2021)
Corresponding author O. A. Weisz: Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA. Email: weisz@pitt.edu

Abstract figure legend Multiligand receptors on the kidney proximal tubule (PT) efficiently recover the low level of albumin and other proteins that normally escape the glomerular filtration barrier. However, the PT has the capacity to internalize much higher amounts of proteins, as revealed when the glomerular filtration barrier is breached. Data from cell culture and mouse studies suggest a unifying model to explain how the PT handles normal and nephrotic levels of filtered proteins.

Structure and function of the PT

The vertebrate kidney comprises individual nephrons which function co-ordinately to maintain the diverse roles of this organ in the control of body fluid composition, activation of vitamin D, hormone synthesis and excretion of toxins. The kidney proximal tubule (PT), which comprises the first segment of the nephron, plays an outsized role in all of these functions. This segment is responsible for reabsorbing approximately two-thirds of the filtered water and sodium and generates driving forces for selective reabsorption of other ions in downstream nephron segments. The PT is structurally divided into a proximal convoluted tubule (PCT) that emanates from



The Journal of Physiology

Figure 1. PT organization and endocytic uptake of filtered ligands
A, the proximal tubule consists of convoluted (PCT) and straight (PST) regions, and can be further subdivided into S1, S2 and S3 segments. The S3 segment extends into the outer stripe of the medulla and may have different lengths in cortical *vs*. juxtamedullary nephrons. *B*, ligands that normally escape the filtration barrier are internalized by receptor mediated endocytosis in clathrin-coated pits (CCPs) that invaginate from the base of apical microvilli on PT cells. Ligands dissociate from their receptors as acidified apical early endosomes (AEEs) mature into apical vacuoles (AVs) and are delivered to lysosomes (Lys) for degradation, whereas receptors are recycled to the apical surface in dense apical tubules (DAT). *C*, megalin and cubilin receptors mediate the internalization of filtered ligands by PT cells. Megalin contains a single transmembrane domain whereas cubilin trimers traffic as a CUBAM complex together with the membrane spanning protein amnionless (AMN). Megalin and AMN cytoplasmic tails contain motifs that engage the Dab2 endocytic adaptor protein, which facilitates receptor recruitment to clathrin-coated pits. Figure created with BioRender.com.

the glomerulus and a proximal straight tubule (PST) that extends across the outer stripe of the outer medulla. The overall lengths and geometries of the PT may vary between individual PTs, with tubules emanating from deeper, more juxtamedullary glomeruli having shorter or more tortuous PSTs (Zhai *et al*. 2003) (Fig. 1*A*). The PT has further been subclassified into three segments (S1, S2 and S3) that are distinguished by distinct cell morphologies, mRNA and protein expression (Christensen *et al*. 2012*b*; Lee *et al*. 2015; Limbutara *et al*. 2020). The S1 segment and S3 segments are confined to the PCT and PST, respectively, whereas the S2 segment overlaps the boundary between the PCT and PST (Christensen *et al*. 2012*b*).

Plasma proteins are generally excluded from the tubule lumen by the glomerular filtration barrier, a tripartite structure comprising endothelial cells, a basement membrane and podocytes (Haraldsson *et al*. 2008). While this assemblage presents a formidable physical obstacle, a small fraction of proteins escapes the barrier and is delivered in the ultrafiltrate to the tubule lumen. Smaller proteins generally have higher sieving coefficients and are preferentially filtered; there may also be some charge selectivity (Haraldsson *et al*. 2008). Among these proteins are carriers for vitamins A, D, and E, as well as albumin. A major role of the PT, in addition to regulating fluid and ion homeostasis, is to recover these and other proteins from the ultrafiltrate to prevent their loss in the urine.

Retrieval of filtered proteins is mediated by two large multiligand receptors, megalin and cubilin, that are abundantly expressed at the apical surface of PT cells, facing the tubule lumen. These receptors bind to and internalize >50 different plasma proteins (Christensen *et al*. 2012*a*; Eshbach & Weisz, 2017). Megalin is a ∼600 kDa single-pass transmembrane protein related to the low-density lipoprotein receptor. Cubilin (∼460 kDa) lacks a membrane domain and associates with an auxiliary membrane protein called amnionless (AMN; ∼50 kDa) to reach the apical membrane as a CUBAM complex (Strope *et al*. 2004; Coudroy *et al*. 2005; Christensen *et al*. 2013). Uptake of megalin and CUBAM receptors occurs via clathrin-coated pits that form at the base of PT microvilli (Rodman *et al*. 1984; Gekle *et al*. 1997) (Fig. 1*B*). The cytoplasmic domains of megalin and AMN contain endocytic motifs that bind to the clathrin adaptor disabled-2 (Dab2) and mediate endocytosis of the receptors into apical endocytic compartments (Fig. 1*C*). The acidified milieu of these compartments triggers dissociation of internalized ligands from megalin and CUBAM; the receptors are recycled to the apical membrane via membrane-rich structures termed dense apical tubules (DATs) whereas soluble released cargoes are retained in fluid-rich endocytic structures that mature and deliver their contents to lysosomes for degradation. While a small fraction of cargo may escape this route and be delivered intact across the PT cell monolayer for direct release into the bloodstream, there is little evidence that this transcytotic route represents a major pathway for recovery of filtered proteins (Park, 1988; Christensen *et al*. 2007).

Impairment in PT function that prevents efficient endocytic retrieval of normally filtered proteins results in their excretion into the urine. Clinically, this phenomenon is variously referred to as low molecular weight proteinuria, or tubular proteinuria. Tubular proteinuria is characterized by the preferential excretion of low molecular weight proteins (<50 kDa) such as β_2-microglobulin (MW ∼11 kDa) and retinol binding protein (MW ∼20 kDa) that have relatively high sieving coefficients and thus more easily pass through the glomerular filtration barrier than larger proteins (Norden *et al*. 2001). By contrast, dysfunction of the glomerular filtration barrier that overwhelms PT uptake capability results in a very different urinary excretion profile that includes higher molecular mass proteins. The proteinuric urinary profile is typically dominated by albumin (MW ∼66.5 kDa), which is by far the most abundant protein in plasma (∼40 mg/ml). Albumin binds with higher affinity to cubilin than to megalin, suggesting that cubilin mediates the direct binding and internalization of this and structurally related ligands (Birn *et al*. 2000; Ren *et al*. 2020). However, loss of either megalin or cubilin expression results in tubular proteinuria, suggesting a requirement for both receptors for PT retrieval of normally filtered proteins (Leheste *et al*. 1999; Nykjaer *et al*. 2001). The two proteins colocalize in PT cells and can be isolated as a complex (Moestrup *et al*. 1998; Ahuja *et al*. 2008), however, megalin and CUBAM are differentially expressed in some tissues and can traffic independently (Dietrich *et al*. 2008; Jensen *et al*. 2014). In the PT, megalin appears to play a major regulatory role in the maintaining a robust apical endocytic pathway, because knockout of the receptor in the PT results in a dramatic reduction in the number of apical endocytic compartments (Christensen & Willnow, 1999; Leheste *et al*. 1999). Low levels of megalin (and cubilin) expression have also been documented in podocytes, and patients and mice lacking megalin develop glomerular proteinuria (Charlton *et al*. 2020). Whether megalin expression is directly or indirectly required to maintain podocyte health remains unclear. As a consequence, dissecting the specific roles of megalin *vs*. CUBAM in the binding and recovery of filtered ligands *in vivo* has proven somewhat challenging.

High-affinity *vs*. high-capacity PT endocytic uptake

In general, the affinities of native receptor-ligand interactions are tuned to match physiological concentrations.

In the PT, the concentration of albumin entering the tubule lumen in rats has been estimated by micropuncture to be ∼23 μg/ml (Tojo & Endou, 1992). This is remarkably close to the single saturable site measured for albumin binding to the apical membrane of the proximal tubule opossum kidney (OK) cell culture model [∼20–50 μg/ml half-maximal binding affinity; (Schwegler *et al.* 1991; Gekle *et al.* 1996; Ren *et al.* 2020)].

The capability of the PT to retrieve much higher amounts of plasma proteins than those normally filtered is revealed when the glomerular filtration barrier is breached. This is perhaps best illustrated by recent experiments in mice quantifying the additive effects on urinary albumin excretion when megalin and cubilin were knocked out in PTs in addition to disrupting the glomerular filtration barrier by knockout of podocin (Nielsen *et al.* 2013; Wagner *et al.* 2016; Weyer *et al.* 2018). Knockout (KO) of megalin and cubilin together increased baseline (0.05 mg/mg albumin/creatinine) urinary albumin levels by ∼20-fold (to 1.1 mg/mg albumin/creatinine), consistent with the known requirement for these receptors in the recovery of normally filtered amounts of albumin. By contrast, disruption of the glomerular filtration barrier by knockout of podocin resulted in a ∼3000-fold increase over baseline in urinary albumin excretion (to 157 mg/mg albumin/creatinine), with no change in megalin or cubilin mRNA levels. Remarkably, this was increased by an additional 40% (225 mg/mg albumin/creatinine), in triple KO mice lacking renal megalin, cubilin and podocin (Weyer *et al.* 2018). Together, these data suggest that megalin/cubilin reabsorption capacity when the glomerular barrier is breached is ∼60-fold greater than that required to recover normally filtered levels of proteins.

The presence of a saturable binding site for normal ligand uptake is seemingly at odds with the demonstration of a large reserve capacity for albumin uptake observed under nephrotic conditions and raises two interrelated questions. First, what accounts for the excess capacity for albumin uptake? Second, why doesn't this large reserve capacity compensate to prevent tubular proteinuria? Several recent studies from various laboratories have shed new light on these questions. Based on our current understanding of endocytic uptake in the PT derived from studies in cultured cells and in rodents, we propose a new model for protein recovery whereby uptake of normal *vs.* nephrotic levels of albumin occurs via differential contributions of receptor-mediated and fluid phase uptake along the PT axis.

Endocytic adaptation to variations in GFR

Normal glomerular filtration rate (GFR) in humans varies widely, reflecting the dynamic response of the kidney to normal physiological conditions including diet, sex, body mass index, age and pregnancy (Cachat *et al.* 2015). While it is difficult to define hyperfiltration in absolute terms, there is considerable clinical and experimental evidence that individuals routinely experience periods of relative hyperfiltration, such as after ingesting a protein-rich meal (King & Levey, 1993). The PT is remarkably able to rapidly adapt to wide variations in GFR in order to maintain stable fractional reabsorption of water and ions. This redistribution is essential to enable increased Na^+ reabsorption required to maintain tubuloglomerular balance. In perfused tubules, flow-induced changes in torque on brush border microvilli correlated nearly identically with changes in Na^+/H^+ exchange (Duan et al. 2006). These mechanosensitive changes in PT ion transport *in vivo* are thought to be mediated by drag forces that greatly amplify the effect of small changes in microvillar bending in response to shear stress (Guo *et al.* 2000).

During hyperfiltration, more filtered protein also enters the tubule lumen per unit time, although its concentration in the tubular fluid is unchanged. How does the PT efficiently recover filtered proteins across a broad range of flow rates? Recent studies using OK cells in culture suggest that these cells rapidly adapt their endocytic capacity to changes in fluid flow. Initial studies performed using cells grown on microfluidic chambers quantified a ∼2-3-fold increase in albumin uptake capacity when cells were acutely subjected to FSS (0.1–1 dyne/cm^2) for 3 h (Ferrell *et al.* 2012; Raghavan *et al.* 2014). Uptake occurred via a clathrin- and dynamin-dependent pathway, and rapidly returned to baseline levels when the FSS was discontinued (Raghavan *et al.* 2014). Under these culture conditions, flow-dependent modulation of endocytic capacity required FSS-induced Ca^{2+} release and intact primary cilia. Subsequent studies demonstrated that growing OK cells under continuous shear stress and with increased O_2 availability dramatically improved their baseline endocytic capacity by roughly 5-fold compared with cells maintained under static conditions (Long *et al.* 2017). In addition to increased expression of megalin and cubilin, cells cultured in this manner acquire morphological, transport, metabolic and transcriptional adaptations characteristic of PTs *in vivo*, including dense microvilli, greater sodium transport capacity, and oxidative metabolism (Long *et al.* 2017; Ren *et al.* 2019; Park *et al.* 2020). Similar to the observations using cells cultured under static conditions and exposed acutely to flow, endocytic capacity in this more differentiated cell model is rapidly and reversibly regulated by changes in FSS (Long *et al.* 2017). However, whether microvilli and/or primary cilia are required for the mechanosensitive responses has not been established.

In principle, an increase in the number of clathrin-coated pits that form and/or an increase in the size of individual pits could account for the enhanced

endocytic capacity in response to FSS (Fig. 2). There is precedence in the literature for actin-dependent modulation of coated pit size to accommodate the internalization of larger cargoes in non-polarized cells (Cureton *et al.* 2009). Consistent with the possibility of dynamic regulation of endocytic vesicle size, ultrastructural studies of rodent kidneys reveal large and irregular clathrin-coated invaginations at the base of PT apical microvilli where endocytic uptake occurs (Rodman *et al.* 1984; Birn *et al.* 1993; Hatae *et al.* 1997). Mechanosensitive changes in actin dynamics or in the rate of coated pit maturation and fission could cause these clathrin-coated structures to expand in volume. Future studies quantifying coated pit and vesicle size in PT cells using super-resolution microscopy may provide a means to help unravel the mechanism that drives FSS-modulated uptake in cultured cells. However, extending these findings to determine whether variations in GFR alter endocytic capacity *in vivo* is a technical challenge that is unlikely to be resolved soon. The glomerular filtration barrier precludes the possibility of delivering a known concentration of filtered ligand into a given tubule at a specified flow rate. Additionally, there may be variability in single nephron GFR and consequent differences between ligand uptake in individual PTs. These variables make it nearly impossible to reliably determine whether endocytic capacity in cells within a given tubule responds acutely to changes in flow.

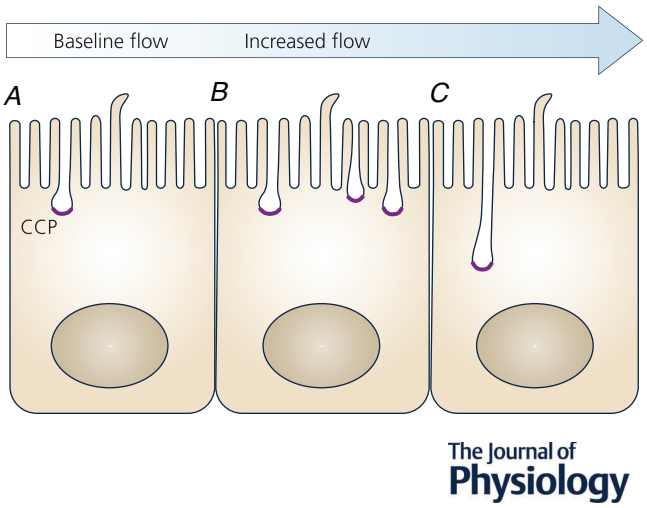


Figure 2. Possible mechanisms for modulation of apical endocytic capacity by flow
A, endocytosis of megalin and cubilin normally proceeds via clathrin-coated pits (CCP) that bud from the base of PT microvilli. We speculate that under conditions of increased flow, there could be an increase in the number or maturation of pits that form (*B*) and/or an increase in the average size of clathrin-coated structures (*C*). Bending of the microvilli and/or the primary cilium at the centre of the apical plasma membrane may mediate flow-dependent changes in endocytic uptake. Figure created with BioRender.com.

Endocytic recovery of supra-normal concentrations of filtered albumin

Rapid modulation of endocytic capacity may enable PT cells to accommodate normal variations in the flow of filtered ligands; however, it is unlikely to account for the extraordinary ability of this nephron segment to recover very high concentrations of albumin under nephrotic conditions. As described below, data from several studies are consistent with the idea that, in addition to its receptor-mediated recovery, uptake via the fluid-phase may contribute significantly to the PT recovery of albumin under nephrotic conditions. In this model, the progressive concentration of albumin as water and salt are reabsorbed along the tubule axis further drives the contribution of fluid-phase uptake *vs*. receptor-mediated uptake in more distal segments of the PT. The overall profile of albumin recovery thus depends on the number of available receptors and kinetics of receptor-mediated endocytosis in S1, S2 and S3 segments, the fluid-phase volume that accompanies receptor-mediated uptake, and the tubular concentration of albumin along the tubule axis.

The ability of the PT to recover concentrations of albumin far higher than those normally filtered was first demonstrated in a heroic study by Park and Maack using isolated perfused rabbit PCTs (Park & Maack, 1984). In this study, albumin reabsorption rates were measured in 58 individual tubules each perfused with a given concentration of radioiodinated albumin (between 1 μg/ml and 10 mg/ml). The dose-dependent uptake profile revealed two sites for uptake – a low-capacity, high-affinity site of K_m ~31 μg/ml, and a high-capacity, low-affinity site with a K_m of ~1200 μg/ml (Park & Maack, 1984). Although fluid-phase uptake is not thought to contribute significantly to normal ligand uptake in the PT (Christensen & Maunsbach, 1979), the authors speculated that high-capacity uptake occurred via fluid-phase endocytosis. More recent studies in polarized OK cells have enabled a more granular dissection of concentration-dependent albumin uptake. Mathematical deconvolution of albumin uptake curves distinguished three independent components: a high-affinity component with a K_m of ~50 μg/ml, a lower-affinity component with a K_m of ~300 μg/ml, and a non-saturable component that probably represents fluid-phase uptake. Knockdown of cubilin preferentially reduced the high-affinity component whereas depletion of megalin obliterated the low-affinity component (Ren *et al.* 2020). These observations suggest that megalin expression is preferentially required for uptake at higher concentrations of albumin. Moreover, the progressive contribution of the non-saturable component to the recovery of increasing albumin concentrations suggests that uptake in the fluid phase may contribute significantly

to albumin uptake when its concentration rises above saturation levels.

Uptake via the fluid phase in post-S1 segments of the PT was also recently documented by Hall and colleagues, who observed that fluorescent proteins injected into the circulation of mice and filtered into the tubule lumen were comprehensively recovered by cells of the S1 segment (Schuh *et al*. 2018; Polesel & Hall, 2019). By contrast, uptake of fluid-phase markers occurred well beyond this segment (Schuh *et al*. 2018). Fluid-phase uptake can occur via a variety of clathrin-dependent and -independent pathways (Mettlen *et al*. 2018; Sandvig *et al*. 2018). While the specific mechanism that drives internalization in these cells is unknown, it is unlikely to be dependent on caveolae, which can mediate clathrin-independent basolateral uptake in some polarized epithelial cells but which are not found in PT cells *in vivo* (Scheiffele *et al*. 1998; Zhuang *et al*. 2011). Indeed, there is no evidence for any clathrin-independent uptake pathway in the PT, so presumably the robust internalization of dextran and ligand (above saturating conditions) occurs via vesicles whose formation is dependent on megalin and clathrin expression.

The idea that the primary contribution of receptor-mediated *vs*. fluid phase uptake shifts between the early and late PT is also supported by recent studies in normal and proteinuric rats (Christensen *et al*. 2021), which found that uptake of filtered ligands normally occurs in S1 and S2, whereas the S3 segment functions as a reserve capacity to internalize proteins when earlier segments are overloaded. Although megalin is expressed at comparable levels in S1 and S3 segments, expression of Dab2 declines precipitously in S3 (Lee *et al*. 2015; Limbutara *et al*. 2020; Christensen *et al*. 2021). The relative paucity of this critical protein required for clathrin-mediated uptake of megalin is consistent with the idea that while both mechanisms operate simultaneously, fluid-phase rather than receptor-mediated uptake has a greater capacity to recover supranormal levels of filtered albumin in the S3 segment. The discrepant role assigned to the S2 segment in normal uptake in the two studies described above remains to be resolved, and might reflect species differences in the function of these segments between mice and rats, differences in the ligands examined, or differences in experimental design (Schuh *et al*. 2018; Christensen *et al*. 2021).

Perhaps surprisingly, expression of megalin appears to be required for this reserve pathway to operate in the distal PT. Knockdown of megalin in OK cells reduces the capacity of the non-saturable component of albumin uptake (Ren *et al*. 2020). Moreover, uptake of nephrotic concentrations of albumin in mice is dependent on megalin but not cubilin expression (Ren *et al*. 2020). Presumably megalin expression is required in this segment, as in earlier segments of the PT, to maintain the integrity of the apical endocytic pathway in addition to its function as a ligand receptor.

An integrative model for albumin handling by the PT

A new model that integrates results from studies in cell culture and animals is emerging to explain the remarkable ability of PT cells to recover filtered albumin and other ligands over the broad range of concentrations encountered in normal and pathological conditions (Fig. 3). The model is described below using albumin as an example ligand, but is generally applicable to other normally filtered ligands. While the model is meant to provide a testable framework to describe albumin handling along the PT axis, it does not account for potential changes in receptor expression or other cell functions resulting from proteinuric damage that might affect endocytosis.

Under normal conditions, essentially all the albumin that enters the ultrafiltrate binds to cubilin and is internalized in the S1 segment of the PT (Fig. 3*A*). Changes in tubular flow may alter endocytic capacity to maintain efficient uptake, or the S1 (and possibly S2) segments may harbour sufficient baseline capacity to comprehensively clear filtered proteins over normal variations in GFR. Loss of cubilin expression or mutants that impair albumin binding prevent efficient uptake of filtered albumin and result in tubular proteinuria (Birn *et al*. 2000; Weyer *et al*. 2011; Ahluwalia *et al*. 2019; Bedin *et al*. 2020). Loss of megalin function that affects endocytic pathway integrity will also lead to urinary protein excretion (Weyer *et al*. 2011; Storm *et al*. 2013) (Fig. 3*A*). Additionally, defects in receptor trafficking, shortened PT length, or impaired response to flow would also be predicted to cause tubular proteinuria and may contribute to the pathogenesis of genetic diseases characterized by tubular proteinuria (De Matteis *et al*. 2017; Gliozzi *et al*. 2020; Shipman & Weisz, 2020; Edwards *et al*. 2021).

When the glomerular barrier is breached, albumin levels rapidly overwhelm the capacity for retrieval by cubilin, and may also saturate lower-affinity binding to megalin receptors. If this occurs, fluid phase internalization can contribute significantly to limit urinary albumin excretion, beginning in the S1 segment. Because fluid phase uptake is dependent on megalin expression, loss of this receptor is predicted to have a considerably greater effect on urinary albumin levels compared with loss of cubilin (Fig. 3*B*). Because 70% of water entering the tubule lumen is reabsorbed by transcellular and paracellular pathways along the PT, tubular albumin will become increasingly concentrated, preferentially driving fluid-phase recovery of albumin in more distal PT segments. Taking this further, one could imagine that

differences in PST segment lengths between subcortical *vs*. juxtamedullary nephrons may affect the sensitivity of individual nephrons to nephrotic tubular damage. While the pathway(s) that mediates endocytosis in the PST is unknown, the hypertonic interstitial milieu of the outer medulla could promote a shift to fluid phase uptake in the PST. In this regard, it is notable that hypertonic medium has been previously shown to impair clathrin-mediated but not fluid-phase uptake in cells (Cupers *et al*. 1994).

Defining the relative contributions of receptor-mediated to fluid-phase uptake in each nephron segment *in vivo* will be challenging. Studies of fixed nephrotic tissue confirm that high levels of filtered proteins accumulate along the tubule axis but qualitatively reveal only their steady state levels (Christensen *et al*. 2021). A critical unknown variable is the profile of ion transport and consequent fluid recovery along the tubule axis under nephrotic conditions, which could be affected by indirect disease responses or perhaps even by the presence of excess 'colloid' in the tubule lumen (Schafer, 1990). We also lack quantitative data about the relative efficiency of receptor-mediated uptake in PT nephron segments that would allow estimation of the concomitant volume available for fluid-phase retrieval.

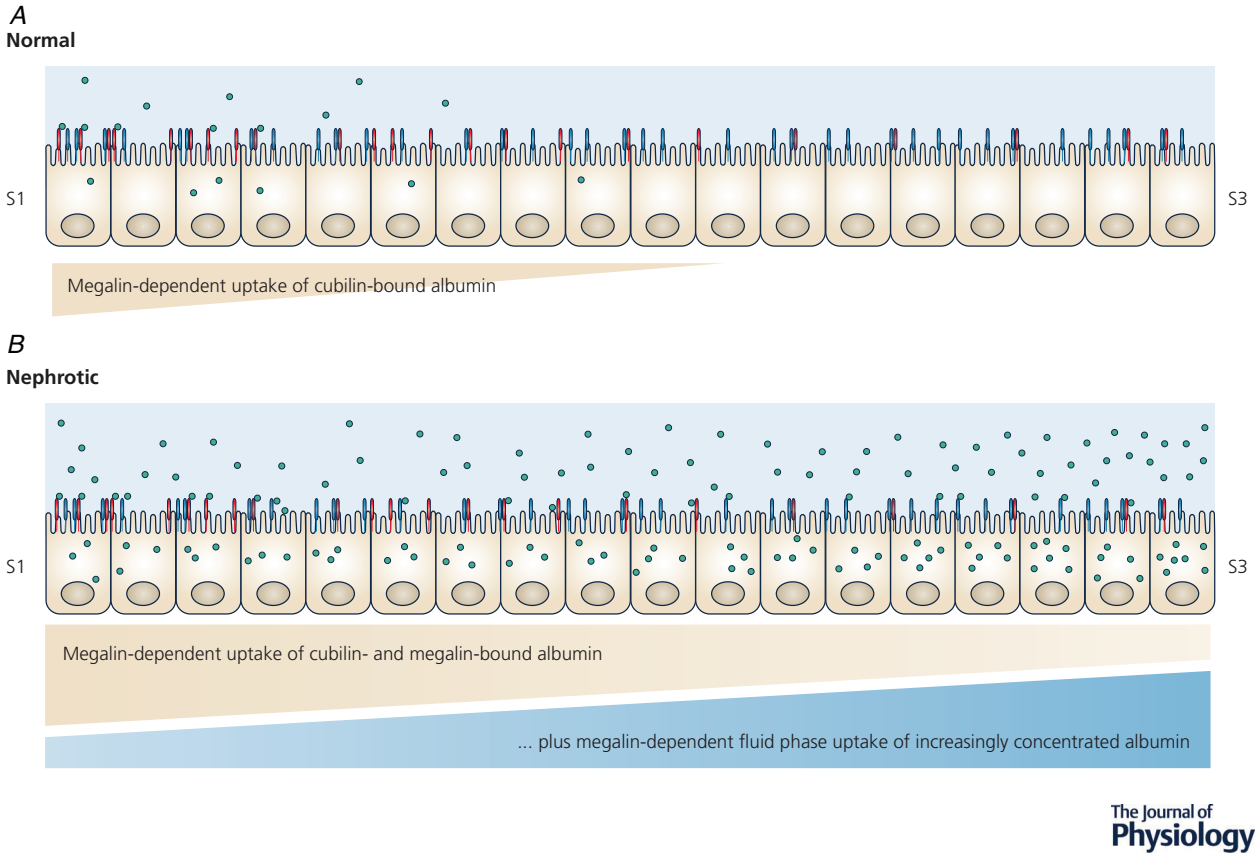


Figure 3. Models for PT uptake of normal and nephrotic levels of albumin
A, under normal conditions, filtered albumin is retrieved largely in the S1 segment and is mediated by direct binding to cubilin receptors. Tubular proteinuria results from loss of either megalin or cubilin function: loss of cubilin prevents high-affinity albumin uptake, whereas loss of megalin ablates the low-affinity uptake site and also compromises the integrity of the apical endocytic pathway. Modulation of endocytic capacity may enable PT cells to accommodate normal variations in GFR as shown in Fig. 2. *B*, when high concentrations of albumin enter the tubule lumen, as occurs under nephrotic conditions, uptake of cubilin- and megalin-bound albumin along the tubule axis becomes saturated. Under these conditions, non-saturable fluid-phase uptake provides a reservoir for retrieval of excess albumin. Uptake via this pathway occurs via the same endocytic compartments that mediate the internalization of receptor-bound albumin. Fluid phase uptake becomes more efficient in distal regions of the PT because the concentration of ligand increases as tubular fluid is progressively removed by ion transport mechanisms along the PT axis. Although albumin uptake in the figure is depicted to increase preferentially towards the distal end of the PT to illustrate this point, the actual profile of albumin recovery in S1, S2 and S3 segments will reflect the balance between the availability of binding sites and kinetics of receptor-mediated endocytosis, the endocytic volume that accompanies receptor-mediated uptake, and the tubular concentration of albumin along the tubule axis. Figure created with BioRender.com. [Correction made on 15 July 2021, after first online publication: Figure 3 has been updated to re-instate the albumin "dots" in the cells, which were previously missing.]

Our working model to describe PT albumin uptake posits that acute cellular adaptations to flow in the S1 (and possibly S2) segment modulate receptor-mediated albumin uptake in response to normal variations in GFR, while the S3 segment of the PT also kicks in to help retrieve overflow albumin in disease states. Many facets of this model remain to be tested, and indeed, some aspects run counter to our current understanding of nephron function. In general, the bulk of filtered ions and solutes that enter the tubule lumen are recovered in the early segments of the nephron, whereas more distal segments make finely tuned adjustments to the ultrafiltrate to preserve fluid homeostasis. This paradigm is present even within the PT, where the S1 segment expresses the high-capacity, low-affinity glucose transporter SGLT2, which normally retrieves ∼90% of filtered glucose, while the remainder is captured by the high-affinity, low-capacity SGLT1 expressed in later PT segments (Shepard & Pluznick, 2017). By contrast, PT endocytic uptake appears to follow the opposite trend, with the S1 segment being responsible for recovery of low levels of filtered proteins and more distal PT regions harbouring additional reserve capacity to recover excess protein. If our somewhat counterintuitive model for axial and mechanistic segregation of normal *vs.* nephrotic protein recovery does indeed hold true, the attractive possibility exists that therapeutic approaches may be found to disengage nephrotic uptake from the recovery of normally filtered proteins.

References

Ahluwalia TS, Schulz C-A, Waage J, Skaaby T, Sandholm N, van Zuydam N, Charmet R, Bork-Jensen J, Almgren P, Thuesen BH, Bedin M, Brandslund I, Christensen CK, Linneberg A, Ahlqvist E, Groop P-H, Hadjadj S, Tregouet D-A, Jørgensen ME, Grarup N, Pedersen O, Simons M, Groop L, Orho-Melander M, McCarthy MI, Melander O, Rossing P, Kilpeläinen TO & Hansen T (2019). A novel rare CUBN variant and three additional genes identified in Europeans with and without diabetes: results from an exome-wide association study of albuminuria. *Diabetologia* **62**, 292–305.

Ahuja R, Yammani R, Bauer JA, Kalra S, Seetharam S & Seetharam B (2008). Interactions of cubilin with megalin and the product of the amnionless gene (AMN): effect on its stability. *Biochem J* **410**, 301–308.

Bedin M, Boyer O, Servais A, Li Y, Villoing-Gaudé L, Tête M-J, Cambier A, Hogan J, Baudouin V, Krid S, Bensman A, Lammens F, Louillet F, Ranchin B, Vigneau C, Bouteau I, Isnard-Bagnis C, Mache CJ, Schäfer T, Pape L, Gödel M, Huber TB, Benz M, Klaus G, Hansen M, Latta K, Gribouval O, Morinière V, Tournant C, Grohmann M, Kuhn E, Wagner T, Bole-Feysot C, Jabot-Hanin F, Nitschké P, Ahluwalia TS, Köttgen A, Andersen CBF, Bergmann C, Antignac C & Simons M (2020). Human C-terminal CUBN variants associate with chronic proteinuria and normal renal function. *J Clin Invest* **130**, 335–344.

Birn H, Christensen EI & Nielsen S (1993). Kinetics of endocytosis in renal proximal tubule studied with ruthenium red as membrane marker. *Am J Physiol Renal Physiol* **264**, F239–F250.

Birn H, Fyfe JC, Jacobsen C, Mounier F, Verroust PJ, Orskov H, Willnow TE, Moestrup SK & Christensen EI (2000). Cubilin is an albumin binding protein important for renal tubular albumin reabsorption. *J Clin Invest* **105**, 1353–1361.

Cachat F, Combescure C, Cauderay M, Girardin E & Chehade H (2015). A systematic review of glomerular hyperfiltration assessment and definition in the medical literature. *Clin J Am Soc Nephrol* **10**, 382–389.

Charlton JR, Tan W, Daouk G, Teot L, Rosen S, Bennett KM, Cwiek A, Nam S, Emma F, Jouret F, Oliveira JP, Tranebjærg L, Frykholm C, Mane S, Hildebrandt F, Srivastava T, Storm T, Christensen EI & Nielsen R (2020). Beyond the tubule: pathological variants of LRP2, encoding the megalin receptor, result in glomerular loss and early progressive chronic kidney disease. *Am J Physiol Renal Physiol* **319**, F988–F999.

Christensen EI, Birn H, Rippe B & Maunsbach AB (2007). Controversies in nephrology: renal albumin handling, facts, and artifacts! *Kidney Int* **72**, 1192–1194.

Christensen EI, Birn H, Storm T, Weyer K & Nielsen R (2012*a*). Endocytic receptors in the renal proximal tubule. *Physiology (Bethesda)* **27**, 223–236.

Christensen EI, Kristoffersen IB, Grann B, Thomsen JS, Andreasen A & Nielsen R (2021). A well-developed endolysosomal system reflects protein reabsorption in segment 1 and 2 of rat proximal tubules. *Kidney Int* **99**, 841–853.

Christensen EI & Maunsbach AB (1979). Effects of dextran on lysosomal ultrastructure and protein digestion in renal proximal tubule. *Kidney Int* **16**, 301–311.

Christensen EI, Nielsen R & Birn H (2013). From bowel to kidneys: the role of cubilin in physiology and disease. *Nephrol Dial Transplant* **28**, 274–281.

Christensen EI, Wagner CA & Kaissling B (2012*b*). Uriniferous tubule: structural and functional organization. *Compr Physiol* **2**, 805–861.

Christensen EI & Willnow TE (1999). Essential role of megalin in renal proximal tubule for vitamin homeostasis. *J Am Soc Nephrol* **10**, 2224–2236.

Coudroy G, Gburek J, Kozyraki R, Madsen M, Trugnan G, Moestrup SK, Verroust PJ & Maurice M (2005). Contribution of cubilin and amnionless to processing and membrane targeting of cubilin-amnionless complex. *J Am Soc Nephrol* **16**, 2330–2337.

Cupers P, Veithen A, Kiss A, Baudhuin P & Courtoy PJ (1994). Clathrin polymerization is not required for bulk-phase endocytosis in rat fetal fibroblasts. *J Cell Biol* **127**, 725–735.

Cureton DK, Massol RH, Saffarian S, Kirchhausen TL & Whelan SPJ (2009). Vesicular stomatitis virus enters cells through vesicles incompletely coated with clathrin that depend upon actin for internalization. *PLoS Pathog* **5**, e1000394.

Dietrich MO, Spuch C, Antequera D, Rodal I, de Yébenes JG, Molina JA, Bermejo F & Carro E (2008). Megalin mediates the transport of leptin across the blood-CSF barrier. *Neurobiol Aging* **29**, 902–912.

Du Z, Yan Q, Duan Y, Weinbaum S, Weinstein AM & Wang T (2006). Axial flow modulates proximal tubule NHE3 and H-ATPase activities by changing microvillus bending moments. *Am J Physiol Renal Physiol* **290**, F289–F296.

Edwards A, Christensen EI, Unwin RJ & Norden AGW (2021). Predicting the protein composition of human urine in normal and pathological states: Quantitative description based on Dent1 disease (CLCN5 mutation). *J Physiol* **599**, 323–341.

Eshbach ML & Weisz OA (2017). Receptor-mediated endocytosis in the proximal tubule. *Annu Rev Physiol* **79**, 425–448.

Ferrell N, Ricci KB, Groszek J, Marmerstein JT & Fissell WH (2012). Albumin handling by renal tubular epithelial cells in a microfluidic bioreactor. *Biotechnol Bioeng* **109**, 797–803.

Gekle M, Mildenberger S, Freudinger R, Schwerdt G & Silbernagl S (1997). Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases. *Am J Physiol Renal Physiol* **272**, F668–F677.

Gekle M, Mildenberger S, Freudinger R & Silbernagl S (1996). Functional characterization of albumin binding to the apical membrane of OK cells. *Am J Physiol Renal Physiol* **271**, F286–F291.

Gliozzi ML, Espiritu EB, Shipman KE, Rbaibi Y, Long KR, Roy N, Duncan AW, Lazzara MJ, Hukriede NA, Baty CJ & Weisz OA (2020). Effects of proximal tubule shortening on protein excretion in a lowe syndrome model. *J Am Soc Nephrol* **31**, 67–83.

Guo P, Weinstein AM & Weinbaum S (2000). A hydrodynamic mechanosensory hypothesis for brush border microvilli. *Am J Physiol Renal Physiol* **279**, F698–F712.

Haraldsson B, Nyström J & Deen WM (2008). Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev* **88**, 451–487.

Hatae T, Ichimura T, Ishida T & Sakurai T (1997). Apical tubular network in the rat kidney proximal tubule cells studied by thick-section and scanning electron microscopy. *Cell Tissue Res* **288**, 317–325.

Jensen LL, Andersen RK, Hager H & Madsen M (2014). Lack of megalin expression in adult human terminal ileum suggests megalin-independent cubilin/amnionless activity during vitamin B_{12} absorption. *Physiol Rep* **2**, e12086.

King AJ & Levey AS (1993). Dietary protein and renal function. *J Am Soc Nephrol* **3**, 1723–1737.

Lee JW, Chou C-L & Knepper MA (2015). Deep Sequencing in microdissected renal tubules identifies nephron segment-specific transcriptomes. *J Am Soc Nephrol* **26**, 2669–2677.

Leheste JR, Rolinski B, Vorum H, Hilpert J, Nykjaer A, Jacobsen C, Aucouturier P, Moskaug JO, Otto A, Christensen EI & Willnow TE (1999). Megalin knockout mice as an animal model of low molecular weight proteinuria. *Am J Pathol* **155**, 1361–1370.

Limbutara K, Chou C-L & Knepper MA (2020). Quantitative proteomics of all 14 renal tubule segments in rat. *J Am Soc Nephrol* **31**, 1255–1266.

Long KR, Shipman KE, Rbaibi Y, Menshikova EV, Ritov VB, Eshbach ML, Jiang Y, Jackson EK, Baty CJ & Weisz OA (2017). Proximal tubule apical endocytosis is modulated by fluid shear stress via an mTOR-dependent pathway. *Mol Biol Cell* **28**, 2508–2517.

De Matteis MA, Staiano L, Emma F & Devuyst O (2017). The 5-phosphatase OCRL in Lowe syndrome and Dent disease 2. *Nat Rev Nephrol* **13**, 455–470.

Mettlen M, Chen P-H, Srinivasan S, Danuser G & Schmid SL (2018). Regulation of Clathrin-Mediated Endocytosis. *Annu Rev Biochem* **87**, 871–896.

Moestrup SK, Kozyraki R, Kristiansen M, Kaysen JH, Rasmussen HH, Brault D, Pontillon F, Goda FO, Christensen EI, Hammond TG & Verroust PJ (1998). The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. *J Biol Chem* **273**, 5235–5242.

Nielsen R, Mollet G, Esquivel EL, Weyer K, Nielsen PK, Antignac C & Christensen EI (2013). Increased lysosomal proteolysis counteracts protein accumulation in the proximal tubule during focal segmental glomerulosclerosis. *Kidney Int* **84**, 902–910.

Norden AG, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FW, Thakker RV, Unwin RJ & Wrong O (2001). Glomerular protein sieving and implications for renal failure in Fanconi syndrome. *Kidney Int* **60**, 1885–1892.

Nykjaer A, Fyfe JC, Kozyraki R, Leheste JR, Jacobsen C, Nielsen MS, Verroust PJ, Aminoff M, de la Chapelle A, Moestrup SK, Ray R, Gliemann J, Willnow TE & Christensen EI (2001). Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D(3). *Proc Natl Acad Sci U S A* **98**, 13895–13900.

Park CH (1988). Time course and vectorial nature of albumin metabolism in isolated perfused rabbit PCT. *Am J Physiol Renal Physiol* **255**, F520–F528.

Park CH & Maack T (1984). Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of the rabbit. *J Clin Invest* **73**, 767–777.

Park HJ, Fan Z, Bai Y, Ren Q, Rbaibi Y, Long KR, Gliozzi ML, Rittenhouse N, Locker JD, Poholek AC & Weisz OA (2020). Transcriptional programs driving shear stress-induced differentiation of kidney proximal tubule cells in culture. *Front Physiol* **11**, 587358.

Polesel M & Hall AM (2019). Axial differences in endocytosis along the kidney proximal tubule. *Am J Physiol Renal Physiol* **317**, F1526–F1530.

Raghavan V, Rbaibi Y, Pastor-Soler NM, Carattino MD & Weisz OA (2014). Shear stress-dependent regulation of apical endocytosis in renal proximal tubule cells mediated by primary cilia. *Proc Natl Acad Sci U S A* **111**, 8506–8511.

Ren Q, Gliozzi ML, Rittenhouse NL, Edmunds LR, Rbaibi Y, Locker JD, Poholek AC, Jurczak MJ, Baty CJ & Weisz OA (2019). Shear stress and oxygen availability drive differential changes in OK proximal tubule cell metabolism and endocytosis. *Traffic* **20**, 448–459.

Ren Q, Weyer K, Rbaibi Y, Long KR, Tan RJ, Nielsen R, Christensen EI, Baty CJ, Kashlan OB & Weisz OA (2020). Distinct functions of megalin and cubilin receptors in recovery of normal and nephrotic levels of filtered albumin. *Am J Physiol Renal Physiol* **318**, F1284–F1294.

Rodman JS, Kerjaschki D, Merisko E & Farquhar MG (1984). Presence of an extensive clathrin coat on the apical plasmalemma of the rat kidney proximal tubule cell. *J Cell Biol* **98**, 1630–1636.

Sandvig K, Kavaliauskiene S & Skotland T (2018). Clathrin-independent endocytosis: an increasing degree of complexity. *Histochem Cell Biol* **150**, 107–118.

Scheiffele P, Verkade P, Fra AM, Virta H, Simons K & Ikonen E (1998). Caveolin-1 and -2 in the exocytic pathway of MDCK cells. *J Cell Biol* **140**, 795–806.

Schafer JA (1990). Transepithelial osmolality differences, hydraulic conductivities, and volume absorption in the proximal tubule. *Annu Rev Physiol* **52**, 709–726.

Schuh CD, Polesel M, Platonova E, Haenni D, Gassama A, Tokonami N, Ghazi S, Bugarski M, Devuyst O, Ziegler U & Hall AM (2018). Combined structural and functional imaging of the kidney reveals major axial differences in proximal tubule endocytosis. *J Am Soc Nephrol* **29**, 2696–2712.

Schwegler JS, Heppelmann B, Mildenberger S & Silbernagl S (1991). Receptor-mediated endocytosis of albumin in cultured opossum kidney cells: a model for proximal tubular protein reabsorption. *Pflugers Arch* **418**, 383–392.

Shepard BD & Pluznick JL (2017). Saving the sweetness: renal glucose handling in health and disease. *Am J Physiol Renal Physiol* **313**, F55–F61.

Shipman KE & Weisz OA (2020). Making a dent in dent disease. *Function* **1**, zqaa017.

Storm T, Tranebjærg L, Frykholm C, Birn H, Verroust PJ, Nevéus T, Sundelin B, Hertz JM, Holmström G, Ericson K, Christensen EI & Nielsen R (2013). Renal phenotypic investigations of megalin-deficient patients: novel insights into tubular proteinuria and albumin filtration. *Nephrol Dial Transplant* **28**, 585–591.

Strope S, Rivi R, Metzger T, Manova K & Lacy E (2004). Mouse amnionless, which is required for primitive streak assembly, mediates cell-surface localization and endocytic function of cubilin on visceral endoderm and kidney proximal tubules. *Development* **131**, 4787–4795.

Tojo A & Endou H (1992). Intrarenal handling of proteins in rats using fractional micropuncture technique. *Am J Physiol Renal Physiol* **263**, F601–F606.

Wagner MC, Campos-Bilderback SB, Chowdhury M, Flores B, Lai X, Myslinski J, Pandit S, Sandoval RM, Wean SE, Wei Y, Satlin LM, Wiggins RC, Witzmann FA & Molitoris BA (2016). Proximal tubules have the capacity to regulate uptake of albumin. *J Am Soc Nephrol* **27**, 482–494.

Weyer K, Andersen PK, Schmidt K, Mollet G, Antignac C, Birn H, Nielsen R & Christensen EI (2018). Abolishment of proximal tubule albumin endocytosis does not affect plasma albumin during nephrotic syndrome in mice. *Kidney Int* **93**, 335–342.

Weyer K, Storm T, Shan J, Vainio S, Kozyraki R, Verroust PJ, Christensen EI & Nielsen R (2011). Mouse model of proximal tubule endocytic dysfunction. *Nephrol Dial Transplant* **26**, 3446–3451.

Zhai XY, Birn H, Jensen KB, Thomsen JS, Andreasen A & Christensen EI (2003). Digital three-dimensional reconstruction and ultrastructure of the mouse proximal tubule. *J Am Soc Nephrol* **14**, 611–619.

Zhuang Z, Marshansky V, Breton S & Brown D (2011). Is caveolin involved in normal proximal tubule function? Presence in model PT systems but absence in situ. *Am J Physiol Renal Physiol* **300**, F199–F206.

Additional information

Competing interests

No competing interests declared.

Author contribution

Sole author.

Funding

HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK): Ora Weisz, R01 DK118726; HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK): Ora Weisz, R01 DK125049.

Acknowledgements

I am indebted to the members of the Weisz laboratory for their dedication to rigorous science and for maintaining esprit de corps despite the challenges brought on by the pandemic. I am grateful to Drs. Catherine Baty and Aurelie Edwards, and to the anonymous reviewers for their careful reading and thoughtful comments for improving the manuscript. Our studies on endocytosis in the PT are supported by NIH R01 DK118726 and DK125049.

Keywords

albumin, cubilin, endocytosis, glomerular filtration rate, megalin, proteinuria