Renal Epithelial Traffic Jams and One-Way Streets

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The kidney contains the body’s second largest number of different cell types, modulating the great diversity of different functions that allow complex homeostasis to occur. A feature that unites all these cell types in the postglomerular nephron is that they are highly polarized; one surface being in contact with the urine and the other with interstitial fluid. As such, there is a strict requirement for vectorial transport of ions, proteins, and other molecules across both apical and basolateral plasma membranes. The cell surface resident proteins by which this transport occurs are tremendously varied, and their repertoires vary at different points along the nephron. Moreover, highly specialized machinery is required to bring these transport proteins to their appropriate site of action. This is a dynamic process, with regulated recycling between the cell surface and intracellular vesicular structures. The purpose of this review is to highlight various renal diseases where the pathogenetic mechanism involves disruption of one or other of these processes.

Basic Trafficking

Resident proteins are synthesized and assembled in the rough endoplasmic reticulum (ER), where there is an in-built quality control and degradation mechanism that is designed to ensure that only properly folded and assembled proteins pass onwards through the Golgi apparatus before being directed to their final destination (1). Quality control is achieved by the association of ER chaperone molecules with unfolded or misfolded polypeptide chains. If these proteins fail to assume their correct structure, they are retained in the ER and targeted for ubiquitination and degradation by the 26S proteasome. While disease-causing mutations may result in complete failure of transcription or translation of the encoded protein, many others will result in structurally abnormal proteins. Although a full discussion of ER function is beyond the scope of this review, it is clear that in many diseases the ER is a common site for retention of these mutant proteins, and loss-of-function is often manifested by intracellular accumulation of abnormal protein that should have been delivered to the cell surface (2). Some published examples of renal diseases where this has been documented include the following: the familial juvenile hyperuricemic nephropathy/medullary cystic disease constellation (caused by mutations in the uromodulin gene UMOD (3,4)), where uromodulin is seen within loop of Henle epithelial cells rather than at the apical surface (5,6); the various forms of Bartter syndrome, including the recently described type 4, where the problem is that Barttin (an essential co-factor for CLC-Kb’s basolateral chloride transport function in the loop) becomes intracellularly retained when mutated and retards CLC-Kb with it, presumably because of failure of correct assembly of the two subunits (7); Gitelman syndrome, which often involves retention of the mutant thiazide-sensitive NaCl co-transporter in the distal convoluted tubule (8,9); and X-linked or autosomal recessive nephrogenic diabetes insipidus, where missense vasopressin receptor or aquaporin-2 (AQP2) mutants respectively are retained within collecting duct principal cells (10,11).

Normal membrane proteins progress from the ER to the Golgi (Figure 1), where they may be modified (for example by the addition of carbohydrate moieties), and on to the trans-Golgi network (TGN) by means of a complex process that allows for both anterograde and retrograde trafficking. Thereafter, different regulated processes direct proteins from the TGN to the apical, basolateral, or endosomal compartments, depending on their functions. Many, but by no means all of the molecular players in this orchestrated system have been elucidated, and some human diseases have provided natural knock-out models, contributing to this knowledge. An example that includes a renal phenotype is Lowe syndrome, a rare X-linked disorder characterized by severe mental retardation, congenital cataracts, and renal Fanconi syndrome. The defective protein is OCRL, which appears to have two functions. First, it is a phosphatase that resides in the TGN, removing the 5' phosphate group from another TGN resident, phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) (12), which is known to play a role in the regulation of vesicular trafficking (13). Phosphorylation and dephosphorylation of PI molecules signals either the recruitment or the activation of proteins essential for vesicular transport. Second, Ocr11 associates with Rab GTPases (14), and crosstalk between PI metabolites and guanosine triphosphatases is an important feature of these regulatory mechanisms. Thus, the association of proximal tubular dysfunction with absence of an important modifier of PI-4,5-P2 reveals its importance in coordinated intracellular trafficking in the nephron.

The final residency of membrane proteins is governed by sorting motifs contained within their sequences, which interact

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specifically with the cellular sorting machinery. These complexes are incorporated into distinct vesicular structures whose surface components further direct sorting by virtue of interactions between attachment proteins known as SNARES (15). The mechanisms and machinery that control this trafficking are currently under extensive investigation, and it is becoming clear that there are multiple pathways for vectorial transport. Moreover, these are dynamic processes, with turnover of cargo between compartments in both forward and reverse directions (Figure 1) becoming increasingly recognized (16).

Several different basolateral plasma membrane targeting motifs have been described, generally residing in the cytosolic C-terminal domains of transmembrane proteins. These include the YXXØ motif (where X is any amino acid, and Ø is one possessing a hydrophobic side chain), the NPXY motif, and the di-leucine motif. The first of these is the best characterized. YXXØ-containing proteins associate with one of the adaptor protein (AP) complexes, which are heterotetrameric structures. AP-1 is found in the TGN and endosomes (17). In polarized epithelial cells, the AP-1β type and AP-4 have been implicated in sorting from the TGN to the basolateral cell surface (18–20), while AP-2 functions in the opposite direction to internalize proteins from the surface (21). In contrast, AP-3 appears to bind cargo destined for the lysosome (22). Lysosomes are a major site of intracellular degradation, and classical lysosomal targeting motifs also involve tyrosine or leucine residues (reviewed in reference 16).

The mechanism of apical membrane targeting is at present less well understood, particularly because there is emerging evidence for crosstalk and transient movement between apical and basolateral compartments (23). Like basolateral sorting, apical sorting information is thought to be localized in the cytosolic domain of transmembrane proteins; in the case of proteins anchored to the membrane via a GPI anchor, sorting information may be provided by lipid moieties.

A number of renal diseases are now recognized to be associated with aberrant trafficking. This may be because of failure of normal recycling from the membrane, or mis-targeting away from the correct cell surface compartment, or misdirection into the wrong intracellular organelle. Examples of each of these are discussed below.

Liddle Syndrome

In 1963, Liddle described a three-generation kindred with autosomal dominant inheritance of early-onset hypertension and hypokalemia (24). The clinical picture in Liddle syndrome is consistent with hyperaldosteronism, but aldosterone levels are suppressed—often, as in the original report, to undetectable levels. The abnormalities in Liddle syndrome can be ameliorated by a low-salt diet plus antagonists of the epithelial sodium channel of the collecting duct, amiloride or triamterene, but they are not improved by mineralocorticoid receptor antagonists. This suggested excessive sodium reabsorption in the kidney as the primary defect rather than activity of an unknown mineralocorticoid. The index patient received a cadaveric renal transplant in 1989, after which her disorder resolved, with normalization of the aldosterone and renin responses to salt restriction. This confirmed a primary renal tubular origin for the salt retention and hypertension (25).

Using a positional cloning approach, Shimkets et al. (26) found that Liddle syndrome is associated with mutation in the β-subunit of the epithelial sodium channel (ENaC) gene, after which similar alterations in the γ-subunit were identified in other kindreds (27). By and large, these mutations are premature stop codons, resulting in truncations of the cytoplasmic C-terminal tail of the relevant subunit. Consistent with the dominant inheritance pattern, these are associated with a gain-of-function in ENaC.

In the kidney, functional ENaC channels are expressed at the apical surface of collecting duct principal cells and are composed of at least three subunits: α, β, and γ (28). The stoichiometry may be 2α:1β:1γ (29) or 1α:1β:1γ (30,31). ENaC activity is mainly regulated by variation in the number of channels present at the cell surface. When necessary, ENaC are removed from the apical surface by ubiquitination and internalization, followed by proteasome-mediated degradation (Figure 2A). Ubiquitination can occur at the N-terminal lysine residues of α- and γ-subunits (32), and the E3 ligases responsible are members of the Nedd4 family (33). Under normal circumstances, ENaC activity is regulated by aldosterone and by a variety of hormones, including insulin, ANP, and ADH (29,34), acting via a range of intracellular kinases. Both Nedd4 and the C-terminal cytoplasmic tail of ENaC have been shown to be phosphorylated (35). Nedd4 is phosphorylated via the action of SGK-1 (serum and glucocorticoid kinase 1), the
function of which is upregulated by aldosterone (36,37). After Nedd4 is phosphorylated, it loses the ability to interact with ENaC, leading to the observed increase in activity (Figure 2B).

The first clue as to the mechanism of disease in Liddle syndrome came from the finding of a de novo missense mutation in a highly conserved β-subunit proline residue within the C-terminus of the γ-subunit, identifying this as the critical region of the molecule (38). Snyder (39) reported that this conserved proline-rich motif, present in the C-terminus of all three subunits, reproduced the affect of Liddle truncations when mutated and expressed in Fischer rat thyroid epithelia. The significance of these findings was clarified with the discovery that it is this proline-rich region, since termed the PY motif, that mediates removal of ENaC from the cell surface via direct interaction with Nedd4 (Figure 2C). Nedd4 is an E3 ligase that was demonstrated to bind ENaC by association of its WW domains with the XPPXY motif present in each ENaC subunit (40–42). Recently, it has been demonstrated that both Nedd4–2, a splice variant of Nedd4, and WWP2, a related family member, can regulate ENaC (43,44). Other ubiquitin machinery proteins such as TSG101 are also potentially implicated (45). It is concluded that ENaC lacking the PY motif are inefficiently removed from the cell surface, leading to increased channel concentration and thus increased sodium reabsorption (46). Although these findings were not initially devoid of controversy, evidence for the alternative hypothesis, that mutations affect open probability (the activity level of individual channels) of ENaC, has not been substantiated.

In vitro evidence of internalization failure has also been substantiated by animal studies. Mice transgenic for the R566X β-subunit mutation that was present in the original kindred show hypervolemia, hypertension, and metabolic alkalosis (47). In mutant compared with wild-type mice, open probability of ENaC was similar; transepithelial potential differences were higher; urinary sodium excretion was lower on recovery from sodium depletion; and ENaC were retained at the apical membrane (48,49).

Nephrogenic Diabetes Insipidus

Nephrogenic diabetes insipidus (NDI) is a condition that results from failure of the kidney to concentrate urine in response to circulating antidiuretic hormone (vasopressin, AVP), which is released from the hypothalamus in response to hypernatremia or hypovolemia. Most cases of NDI are acquired; for example, secondary to hypokalemia, hypercalcemia, or lithium toxicity. Of the primary forms of NDI, three patterns of inheritance are described: X-linked recessive, autosomal recessive, and autosomal dominant. Clinical presentation of these different forms is similar, usually occurring within the first 2.5 yr of life and often in the neonatal period. Polyuria is the primary clinical sequela, with secondary polydipsia, but common presenting symptoms include anorexia, vomiting, constipation, fever, and failure to thrive. Growth retardation occurs commonly, and mental retardation has been described (50). If the diagnosis is made promptly, all of these complications can be prevented by adequate water intake (51).

The basic paradigm of AVP-mediated reabsorption of water in the collecting system involves the circulating hormone binding to a receptor (V2R) on the basolateral side of the principal cell, which leads indirectly to the shuttling of water channels...
(AQP2) from intracellular vesicles to the apical surface of this polarized cell (Figure 3A) (52,53). Water is thereby able to enter the otherwise impermeable cell from the collecting duct lumen, down an osmotic gradient, and then passes through other water channels (AQP3 and AQP4) in the basolateral membrane, to be reclaimed into the interstitium.

The detail of some of these steps has not yet been fully elucidated, but V2R is known to be a G-protein coupled receptor, which on binding its agonist results in activation of adenylate cyclase, production of cyclic AMP (cAMP) with consequent activation of protein kinase A (PKA), and phosphorylation of a serine residue (S256) in the C-terminus of AQP2 (54). This is followed by translocation of the AQP2-containing vesicles to the apical surface, with incorporation of AQP2 into the plasma membrane in tetrameric form. V2R itself, having bound its ligand, is phosphorylated and internalized by a clathrin-mediated mechanism, resulting in downregulation.

Recent data have added to the complexity of this model, with evidence that cell surface expression of AQP2 may be regulated by pathways other than the cAMP cascade. Elevated cGMP, stimulated by hormones such as ANP, may also lead to recruitment of AQP2 to the cell surface, as may depolymerization of the actin cytoskeleton (55). In addition, it has been observed that constitutive recycling of AQP2 occurs between vesicles and the cell surface, independent of changes in the levels of cAMP (56). This alone does not result in accumulation of AQP2 at the surface because of a dynamic equilibrium between vesicle fusion with the plasma membrane, and endocytosis. The latter is of potential interest therapeutically, because it has been demonstrated in cell culture that blocking this endocytosis results in cell surface accumulation of AQP2, independent of the function of V2R, thus bypassing any defect in the receptor or in the subsequent signaling cascade (57).

Using cell culture systems, studies of the molecular pathogenesis of the three inherited forms of the disease have revealed a diversity of points at which intracellular processing and trafficking of proteins may be disrupted, some of which may emerge as potential therapeutic targets.

X-linked recessive NDI is the most common form, accounting for approximately 90% of cases. The disease is caused by loss of function mutations in the AVPR2 gene on the long arm of the X-chromosome, which encodes the seven-transmembrane domain V2R protein (58–60). Over 160 mutations have been described in all regions of the gene. In some of these mutations, the resulting protein is so severely truncated it could not be functional. As noted above, it is likely that such proteins would be retained by the ER and then degraded.

However, while this is the fate of over 70% of V2R mutants, this form of the disease merits further discussion because there is evidence that in some cases cell surface expression of functional V2R can be rescued pharmacologically. Morello et al. (61) investigated two different cell permeable V2R antagonists using nonpolarized cell systems and observed rescue in 8 of 15 mutants. Tan et al. (62), using polarized cells, observed cell surface rescue in 2 of 3 mutants. This was achieved not only with a permeable V2R antagonist, but also by reducing the temperature of the cell culture. It was suggested that both of these interventions enabled stabilization or completion of protein folding, allowing subsequent escape from the ER or Golgi.

Autosomal recessive NDI accounts for approximately 10% of inherited cases. It is caused by mutations in the AQP2 gene (63), almost all of which appear to be retained in the ER, suggesting that in each case, incomplete or misfolding of the mutant protein occurs. In some of these cases it has been demonstrated that the mutant protein was monomeric, rather than the normal homotetrameric configuration (64).

In contrast, the rare dominantly inherited NDI, also caused by mutations in AQP2, appears to result from mistargeting of functional protein to the wrong cellular compartment (Figure 3B). Six mutations have been studied to date, using a variety of cell systems. In each case, mutant protein is able to pass
through the ER and to assemble with wildtype protein, encoded by the normal allele, into functional heterotetramers. A dominant negative mechanism has been proposed whereby the mutant components of the heterotetramer influence its targeting (65,66). There have been some differences in the observed final destination of the heterotetramers among these six mutations. For example, the missense mutant AQP2-E258K was reported to target to the Golgi complex of Xenopus oocytes, whereas the deletion mutant AQP2-727delG was reported to accumulate in late endosomes, lysosomes, and the basolateral plasma membrane in MDCK cells (66,67). Clearly, different mutations might cause disease by different mechanisms, but it is also likely that trafficking behavior of proteins varies between different in vitro cell systems, and caution is required when attempting to infer in vivo pathophysiology. In particular, the commonly used oocyte system is nonpolarized, whereas in vivo the collecting duct principal cell is highly polarized. Indeed, three other AQP2 mutants have behaved differently in oocytes and polarized MDCK cells, the mutant tetramer appearing at the (wrong) cell surface in the polarized cell line in each case, but being retained intracellularly in the nonpolarized line (68).

When mutant AQP2 is delivered erroneously to the basolateral rather than to the apical surface, thus disrupting the function of the cell, possible mechanisms include loss of an apical targeting signal in the protein, or the gain or activation of a basolateral signal that overrides any inherent apical signal. Recently, Kamsteeg et al. (69) described a case of dominantly inherited NDI resulting from a frame-shift mutation that caused an extension of the C-terminus of AQP2 and the introduction of two basolateral targeting motifs, one tyrosine-based and one di-leucine. They proposed that these new motifs present on the mutant components of the heterotetramer dominated any apical motifs present in the mutant or wild-type subunits. This mechanism is likely to be particularly rare, but it does provide an interesting contrast to the mechanism described below for a form of dominant distal renal tubular acidosis, where loss of a basolateral sorting motif results in aberrant targeting.

AQP2 has been dissected further to try to delineate targeting motifs involved in its shuttling between intracellular vesicles and the apical cell surface. Van Balkom et al. (70) identified a region in the C-terminal tail that was required for apical localization, but the cytosolic N-terminal domain was also required to achieve localization in the intracellular vesicles with subsequent movement to the apical surface when the action of AVP was simulated. Not surprisingly then, the trafficking of AQP2 appears to be complex, involving more than one motif.

Autosomal Dominant Distal Renal Tubular Acidosis

Distal renal tubular acidosis (dRTA) is a disease of defective urinary acidification that is caused by dysfunction of α-intercalated cells (α-IC) in the collecting system. dRTA is characterized by hypokalemic metabolic acidosis, metabolic bone disease, and nephrocalcinosis and/or nephrolithiasis. As with NDI, most cases of dRTA are acquired; for example in association with autoimmune disease such as Sjögren syndrome (71). Of the inherited forms, autosomal dominant dRTA (ddRTA) generally presents later, occasionally in adulthood, and with milder phenotype, than does the recessive form (72). Treatment for both is with alkali replacement, which corrects most of the biochemical abnormalities.

In the α-IC, protons are secreted actively across the apical surface of the cell by the H⁺-ATPase into the collecting duct lumen. This process is coupled to the reclamation of bicarbonate ions across the basolateral plasma membrane via the chloride-bicarbonate exchanger, AE1 (Figure 4A) (73). Mutations in SLC4A1, encoding AE1, are to date the only genetic cause of ddRTA (74–78).

With respect to mechanism of disease, it had been realized for several years that ddRTA is unlikely to result from simple haploinsufficiency. First, the evidence for this was that numerous heterozygous AE1 mutations have been described that cause the autosomal dominant erythrocyte diseases hereditary spherocytosis and ovalocytosis (the red cell being the other site of expression of AE1), but these are not associated with a urine acidification defect (79). Second, all of the dRTA-associated AE1 mutations thus far studied have demonstrated near-normal anion exchange function when expressed in Xenopus oocytes (74,75,80). The majority of these are missense mutations of R589 (R589H, R589S, and R589C) as well as S613F, and a complex mutation resulting in an 11–amino acid truncation at the C-terminus, R901X.

The hypothesis of AE1 mistargeting as a mechanism of disease therefore arose. Possible explanations included the protein being retained intracellularly or reaching the surface but losing its specific basolateral distribution such that the electrochemical balance of the cell might be disturbed (Figure 4B). Given that 50% of functional AE1 appears to be sufficient for normal urinary acidification, intracellular retention of the mutant AE1 would also have to be associated with retention of a significant proportion of the wildtype AE1 encoded by the normal allele. Indeed expression of AE1 mutants in (nonpolarized) HEK293 cell line has suggested that they are retained intracellularly and that they exert a dominant negative influence by preventing co-expressed wildtype AE1 from reaching the cell surface (81,82).

Similarly, Toye et al. (80) suggested intracellular retention of the R901X mutant, this time in MDCK cells. However, the conditions used did not lead to polarization of the cells, which may have had an effect on trafficking behavior. We subsequently demonstrated that in adequately polarized MDCK cells, the R901X mutant protein appears at both the basolateral and the apical surfaces, with a proportion being retained intracellularly. We went on to confirm these findings in another mammalian renal epithelial cell line, IMCD (83).

The R901X mutant had offered more of a clue to a possible targeting defect, as the missing 11–amino acid tail contains the sequence YDEV, which could represent a basolateral targeting motif of the YXXØ type. We demonstrated that the 26–amino acid C-terminal cytosolic domain of AE1 containing this putative motif, when transplanted onto an apical reporter protein in place of its own C-terminus, caused basolateral redistribu-
tion of a proportion of the protein. In addition, we found that the tyrosine component of the motif, Y904, was essential for basolateral targeting of wildtype AE1. When changed to alanine, a nonpolarized distribution was observed.

As for missense mutations involving R589, there are as yet no published data from adequately polarized renal epithelial cell lines, so it is not clear whether mutation of this residue disrupts a different basolateral targeting motif or whether it causes a conformational change leading to intracellular retention of the protein.

Cystinosis

Nephropathic cystinosis is characterized by poor growth, renal tubular Fanconi syndrome, glomerular failure, and involvement of other tissues and organ systems (reviewed in reference 84). In untreated children, poor growth is generally evident by 9 mo of age. Signs of renal tubular Fanconi syndrome, including polyuria, polydipsia, dehydration, and acidosis, appear as early as 6 mo of age and are irreversible if tubular damage has already supervened. In untreated patients, glomerular function gradually deteriorates, resulting in renal failure at approximately 10 years of age. Intermediate cystinosis occurs in all patients, usually between 15 and 25 yr of age. Adolescent nephropathic cystinosis manifests itself first at age 10 to 12 yr with proteinuria due to glomerular damage rather than with the manifestations of tubular damage that occur first in infantile cystinosis. There is no excess aminoaciduria and stature is normal. Photophobia, late development of pigmentary retinopathy, and chronic headaches are features. Non-nephropathic cystinosis is characterized by photophobia only.

The underlying metabolic defect in all forms of cystinosis is defective transport of cystine, released from the hydrolytic cleavage of peptides, across the lysosomal membrane and into the cytosol (85,86). The lysosomal build-up of cystine can be reduced by frequent oral administration of cysteamine. This may slow or stop the progression of glomerular damage, may attenuate the Fanconi syndrome, and can delay or prevent the need for renal transplantation. Growth hormone may also be required.

All forms of cystinosis have been shown to be allelic (87) and are due to mutations in CTNS (84,88). The CTNS transcript encodes a 367–amino acid protein named cystinosin, which is a lysosomal membrane cystine transporter (88–90). Cystinosin appears to function as a proton/cystine symporter (91), protons being pumped into the lysosome via vacuolar ATPases (Figure 5A). It is highly specific for cystine. Cystinosin is predicted to be a seven-transmembrane domain protein, with seven potential N-glycosylation sites in the N-terminal region and a classic tyrosine-based lysosomal targeting signal (GYDQL) in the C-terminal tail (88). Mutation analysis revealed that the correct sorting of cystinosin to the lysosomal membrane requires not only the GYDQL motif but also a second signal, because missense alterations in this motif caused only partial redirection to the plasma membrane (89). Site-directed mutagenesis then uncovered a novel conformational motif, the core of which is YFPQA, situated in the fifth loop of the protein (89). When both motifs were altered, the protein completely relocated to the plasma membrane (Figure 5B). This second motif is the first example of a non-C-terminal lysosomal sorting signal.

Thus the trafficking abnormalities that cause cystinosis may be either because cystinosin is absent or nonfunctional (for example, where there are truncation mutations) and therefore the cargo cannot move appropriately, or because this transporter cannot itself traffic normally, as would be predicted from reported missense mutations in the fifth loop or C-terminal truncations (92,93), both of which affect the targeting signals.

Dent Disease

Dent disease is an X-linked recessive renal tubular disorder characterized by low–molecular weight proteinuria, hypercalciuria, nephrolithiasis, nephrocalcinosis, and progressive im-
pairment of renal function. Other features may include rickets, hypophosphatemia with renal phosphate wasting, aminoaciduria, glycosuria, and uricosuria. Consequently the disease may be classified as a form of Fanconi syndrome.

Dent disease is caused by mutations in CLCN5, which encodes CLC-5, a member of a family of mammalian voltage-gated chloride channels (94). Since the identification of this as the responsible gene, three similar conditions have been found to be allelic and are often referred to under the collective term of Dent disease: X-linked recessive nephrolithiasis (95); X-linked recessive hypophosphatemic rickets (96); and idiopathic low-molecular weight proteinuria of Japanese children (97).

CLC-5 is a 746-amino acid protein with multiple membrane-spanning domains and intracellular N- and C-terminal domains (98). In the mammalian nephron, it is expressed in the proximal tubule, in the thick ascending limb, and in IC (99). Electrophysiologic and immunofluorescence studies of transfected cells have demonstrated that CLC-5 is located predominantly in subapical vesicles, with some reported cell surface expression. It colocalizes with the vacuolar H⁺-ATPase in the PT and IC, suggesting that it functions to permit proton pump-mediated acidification of endosomes by providing the anion influx required to maintain electroneutrality (100). Endosomes recycle between the surface and subapical compartments; in fact, a putative PY internalization signal, similar to that seen in ENaC, has been identified in CLC-5’s C-terminal domain (101). Over 60 mutations have now been described in CLCN5, located throughout the gene. Many of these are predicted to result in loss of function or absence of CLC-5 (102). A resulting reduction in endosome acidification may be associated with disruption of their cycling and endocytic function (Figure 6) (103,104).

It is not known precisely how absence or reduced activity of CLC-5 in the endosome and impaired endosomal acidification result in impaired endocytosis. Normally endocytosed LMWP have been demonstrated to colocalize with CLC-5 (100). Christensen et al. (105) suggested that reduced apical surface expression of the multiligand receptors, megalin and cubulin, involved in LMWP endocytosis could in part be responsible.

They demonstrated that, in a CLC-5 knockout mouse, there was a redistribution of these receptors from the apical surface of proximal tubule cells to endosomes and suggested that this arises from retardation of receptor recycling. They proposed that this, in addition to reduced apical internalization of these receptors and slower progression of endosomes to lysosomes, could account for reduced LMWP endocytosis.

This model provides a possible explanation for the proteinuria associated with Dent disease, but how CLC-5 mutation might cause the other clinical features of Dent disease is less clear. Using a CLC-5 knockout mouse, Günther et al. (106) have suggested that hyperphosphaturia and hypercalciumuria are both indirect consequences of impaired endocytosis of PTH.

Defects in the intracellular trafficking of CLC-5 itself may be involved in the mechanism of disease in some cases. Carr et al. (107) conducted renal epithelial cell expression studies of 3 CLC-5 truncated mutants to investigate the possible significance of the second of two CBS domain motifs in the C-terminal domain. These motifs, approximately 50 residues in length and named after their description in cystathione β-synthase, are of unknown function, but they are present in all members of the CLC family. The intracellular distribution of these three mutant constructs was mainly perinuclear. The authors concluded that this CBS domain is required for correct intracellular trafficking of CLC-5.

CLCN5 mutations might therefore result in disruption of trafficking of CLC-5 itself, or by loss of function they might disrupt endosome cycling, which in turn influences the cell surface expression of receptors that mediate endocytosis. The details of these complex interactions remains to be elucidated (Figure 6).

**Hyperoxaluria**

Type I primary hyperoxaluria is a rare autosomal recessive disorder characterized by high urinary oxalate excretion, progressive bilateral calcium oxalate stone formation, and nephrocalcinosis. Extrarenal deposits of oxalate occur in later stages, including in the heart, vessels, bones, and retina. Death from renal failure occurs in childhood or early adult life. Therapy
with pyridoxine, phosphates, magnesium, and citrate have proven helpful (108), but combined liver and kidney transplants are required to effect a cure because the metabolic abnormality originates in the liver (109).

The disease is caused by functional defects of the liver enzyme alanine-glyoxylate aminotransferase (AGT). This results in failure of catabolism of glyoxalate to glycine, leading to overuse of the alternate pathway whereby oxalate is generated. The kidney represents the only excretory pathway for oxalate, which cannot be further metabolized. AGT normally resides in peroxisomes. In some patients, mutations in the AGT gene on chromosome 2 do not lead to loss of the protein, but instead promote mistargeting to the mitochondrion (110,111). AGT mistargeting results from the combination of a common Pro11Leu polymorphism, which generates a cryptic but functionally weak mitochondrial targeting sequence (MTS) and a rare Gly170Arg mutation. The combination of these increases the efficiency of this MTS by slowing the rate at which AGT dimerizes. This type of mistargeting represents a highly unusual mechanism of disease.

**Conclusions**

Over the past few years, an increasing number of human diseases have become attributable to defects in the normal dynamic trafficking processes in all cells, and investigating the molecular bases for these will continue to be a fertile field for new insights into cell biology. Indeed, such mechanistic defects are not just the preserve of rare disorders. For example, there is evidence

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**Table 1. Trafficking disorders with a primary renal effect**

<table>
<thead>
<tr>
<th>Disease</th>
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<th>Protein</th>
<th>Normal Function</th>
<th>Consequence of Mutation</th>
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<td>PKD1,2</td>
<td>Polycystin 1, 2</td>
<td>Ion channel/? cell-cell adhesion</td>
<td>Intracellular retention/?mistargeting</td>
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<td>NKCC2</td>
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<td>Co-factor for CLC-Kb</td>
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<td></td>
<td>Intracellular retention</td>
</tr>
<tr>
<td>Liddle syndrome</td>
<td>SCNN1B, SCNN1G</td>
<td>ENaC β or γ subunit</td>
<td>Electrogenic sodium transporter</td>
<td>Retention at plasma membrane</td>
</tr>
<tr>
<td>Lowe syndrome</td>
<td>OCRL</td>
<td>OCRL</td>
<td>Phosphatidylinositol 4,5-bisphosphate 5-phosphatase</td>
<td>Abnormal trans-Golgi network function</td>
</tr>
<tr>
<td>Nephrogenic diabetes insipidus</td>
<td>AQP2</td>
<td>Water channel</td>
<td></td>
<td>Inappropriate basolateral targeting or intracellular retention</td>
</tr>
<tr>
<td>Dominant distal RTA</td>
<td>AVPR2, SLC4A1</td>
<td>AE1</td>
<td>Vasopressin receptor Cl/HCO₃ exchanger</td>
<td>Non-polarized plasma membrane targeting</td>
</tr>
</tbody>
</table>

* Other than loss of transcription/translation/function.
that a problem in autosomal dominant polycystic kidney disease might be aberrant targeting of mutant polycystins, thereby disrupting normal polarity and cell-cell adhesion (112). However, these data are still to some extent preliminary and are the subject of intensive study. The next decade will undoubtedly increase our understanding of the complex ways in which the kidney carries out its many interrelated functions.

Appendix

Mendelian Inheritance in Man (MIM) Numbers (http://ncbi.nlm.nih.gov/Omim)

- Autosomal dominant distal renal tubular acidosis: 179800
- Autosomal dominant polycystic kidney disease: 173910, 601313
- Bartter syndrome types 1–4: 241200, 601678, 602522, 607364
- Cystinosis: 219750, 219800, 219900
- Dent disease: 300009
- Familial juvenile hyperuricemic nephropathy/medullary cystic disease: 603860, 162000
- Gitelman syndrome: 263800
- Hyperoxaluria type I: 259900
- Liddle syndrome: 177200
- Lowe syndrome: 309000
- Lowe syndrome: 125800, 222000, 304800
- Nephrogenic diabetes insipidus: 125800, 222000, 304800
- Nephrogenic diabetes insipidus: 125800, 222000, 304800
- Nephrogenic diabetes insipidus: 125800, 222000, 304800
- Nephrogenic diabetes insipidus: 125800, 222000, 304800
- Nephrogenic diabetes insipidus: 125800, 222000, 304800

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References


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