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Role of mTOR Signaling for Tubular Function and Disease

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The mechanistic target of rapamycin (mTOR) forms two distinct intracellular multiprotein complexes that control a multitude of intracellular processes linked to metabolism, proliferation, actin cytoskeleton, and survival. Recent studies have identified the importance of these complexes for transport regulation of ions and nutrients along the entire nephron. First reports could link altered activity of these complexes to certain disease entities, i.e. diabetic nephropathy, acute kidney injury or hyperkalemia.

ENaC; endocytosis; mTOR; proximal tubule; ROMK

Introduction

The serine/threonine protein kinase mechanistic (formerly known as "mammalian") target of rapamycin (mTOR) is a conserved member of the phosphoinositide 3-kinase (PI3K)-related kinase family and is the key component of two distinct multiprotein complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (1). Besides the catalytic subunit mTOR, both complexes share mammalian lethal with Sec13 protein 8 (mLST8), a protein that potentially stabilizes mTOR, and DEP domain-containing mTOR-interacting protein (Deptor), a negative regulator of the mTOR kinase (2, 3). mTORC1 is distinguished from mTORC2 by the presence of the regulatory-associated protein of mTOR (Raptor, also known as RPTOR), a protein decisive for mTORC1 localization and substrate recruitment, and the proline-rich AKT substrate of 40 kDa (PRAS40, also known as AKT1S1), an insulin-responsive mTORC1 inhibitory protein (2, 4–6). mTORC2 contains mSIN1, Protor, and the rapamycin-insensitive companion of mTOR (Rictor), a protein that may have analogous function to Raptor (7–9). These two complexes perform distinct functions in the cell: mTORC1 primarily controls cell growth and metabolism, while mTORC2 participates in the control of cell survival and proliferation (10) (FIGURE 1).

The mTORC1 protein kinase responds to a variety of intra- and extracellular nutrient signals. To facilitate growth, mTOR coordinates the synthesis and recycling of essential biomolecules, such as lipids, proteins, and nucleotides. While mTOR is tightly controlled under physiological conditions, the loss of negative regulation can lead to unrestrained cell growth found in cancers or benign tumors (11, 12). On the other hand, a downregulation of mTORC1 activation is associated with longevity (13, 14). Such a broad role in cell growth necessitates a specialized regulation of mTOR kinase activity.

mTORC1 activity can be inhibited by the immunosuppressant rapamycin while mTORC2 is insensitive to rapamycin. mTORC1 orchestrates the signals of a wide variety of nutrient factors, including growth factors, amino acids, cellular energy content, and cellular stress (15). This is accomplished by phosphorylation of different downstream substrates, which until now have not been entirely defined and may in addition vary in a cell-specific manner. Important effectors are ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4B (EIF4B) that regulate the translation of genes important for cell growth (15). mTORC2 is activated by insulin and growth factors via PI3K-dependent (phosphoinositide-3 kinase) signaling and phosphorylates several AGC kinases such as Akt (protein kinase B) or SGK1 (serum-and-glucocorticoid-dependent kinase 1) at their hydrophobic motif (16). As a consequence, mTORC2 is involved in maintaining the cytoskeleton, promoting cell survival and as most recently discovered regulating ion transport. Despite the different functions of mTORC1 and mTORC2, they are interrelated with each other. mTORC1 activity can be indirectly stimulated by mTORC2/Akt that in turn phosphorylates mTORC1 and inactivates TSC1/2 (tuberosis sclerosis complex) and Ras homolog enriched in brain (Rheb), both of which are upstream negative effectors of mTORC1 (1).

In the kidney, mTORC1 and mTORC2 are ubiquitously expressed in the glomerular endothelium, in podocytes and in the tubular epithelium (17). Significant progress on the understanding of the renal actions of mTOR complexes have been made using genetically modified mice lacking specifically mTORC1 and/or mTORC2 in podocytes or in the tubular epithelium (18–20). This review highlights results obtained from recent studies investigating the tubular function of mTOR complexes. These indicate that mTOR complexes regulate a wide array of different tubular transport processes that are of

outmost physiological relevance such as albumin retrieval or K^+ secretion. Moreover, mTOR function seems to be essential for maintaining tubular integrity.

mTORC1 Orchestrates Proximal Tubular Transport

The proximal tubule is the most metabolically active part of the kidney. It is a central and nonredundant structure for bulk nutrient reabsorption out of 180 liters of primary urine every day, from which it reclaims principal nutrients, small proteins, electrolytes, and trace elements to preserve normal cell and tissue homeostasis (21, 22). The sophisticated endocytotic machinery of the proximal tubule enables proper reabsorption of all filtered low-molecular-weight (LMW) proteins. Cells lining the proximal tubule house an extraordinary and rapidly adaptive transport system by maintaining highly structured apical and basolateral membrane domains. The apical brush border with luminal microvilli as well as apical and basolateral membrane infoldings tremendously augments the reabsorptive capacity (23). A high number and density of mitochondria are needed to encounter the energy demand of all transport processes. Genetic diseases, disrupted energy metabolism, or toxic injury can simultaneously affect several transport systems, leading to a syndrome of substrate loss, called Fanconi syndrome (21, 24, 25). In addition to aminoaciduria, glucosuria, as well as phosphaturia, Fanconi syndrome is characterized by low-molecular-weight proteinuria (24). This is caused by dysfunction of receptor-mediated endocytosis, which is dependent on two scavenger receptors, MEGALIN and CUBILIN, followed by clathrin-mediated internalization of

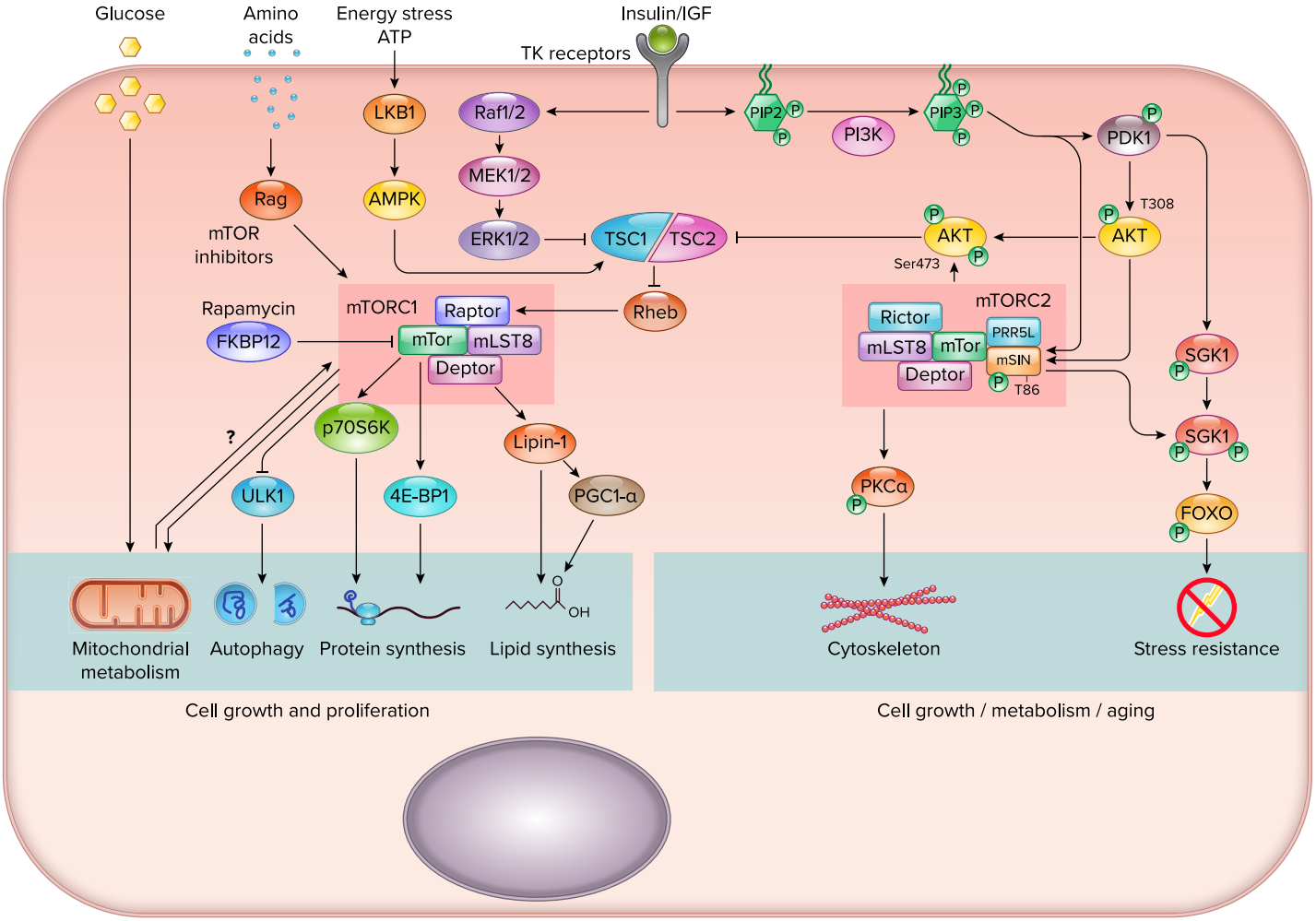

FIGURE 1. **Complex-specific mTOR signaling: mTOR kinase associates into 2 multiprotein complexes with RAPTOR being decisive for designating mTORC1 and RICTOR to form mTORC2**

Activation of the mTOR kinase via nutrients, growth factors or cell stress leads to phosphorylation of complex specific downstream targets involved in cell growth, proliferation, metabolism and aging. AKT, protein kinase B; AMPK, AMP-activated protein kinase; Deptor, DEP domain-containing mTOR-interacting protein; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein; ERK1/2, extracellular signal-regulated kinase 1/2; FKBP12, FK506-binding protein of 12 kDa; FOXO, forkhead box O1; IGF, insulin growth factor; LKB1, serine threonine kinase 11; MEK1/2, mitogen-activated protein kinase mLST8, mammalian lethal with Sec 13 protein 8; mSIN1, mammalian stress-activated protein kinase-interacting protein; mTOR, mammalian target of rapamycin complex; p70S6K, p 70 ribosomal S6 kinase; PI3K, phosphoinositide-3 kinase; PGC1-α, PPARγ coactivator 1-α; PKCα, protein kinase Cα; PRR5L, proline rich protein 5 like; Rag, Ras-related GTP-binding protein; Raptor, regulatory-associated protein of TOR; Rheb, Ras homologue enriched in brain; Rictor, rapamycin-insensitive companion of mTOR; SGK-1, serum- and glucocorticoid-induced protein kinase 1; TK, tyrosine kinase; TSC, tuberous sclerosis complex; TSC1, hamartin; TSC2, tuberin; ULK1, unc-51-like kinase 1. Taken from Ref. 20 and modified according to Ref. 57.

the ligand-receptor complex (23). Fusion of these clathrin-coated vesicles with early endosomes delivers the complex to the endosomal-lysosomal compartment, leading to recycling or degradation of retrieved proteins (23). The prominent endocytotic activity of the proximal tubule (PT) is important to retrieve vital plasma proteins and maintain body homeostasis (26).

Experiments on proximal tubular cell lines and model organisms and observations in patients treated with mTORC1 inhibitors such as rapamycin indicated that mTOR is involved in transport and metabolic processes in the proximal tubule. Initially, in patients treated with mTORC1 inhibitors after renal transplantation, tubular albuminuria and LMW proteinuria, two well-established features of Fanconi syndrome, were recognized (27). Subsequent studies using rapamycin-treated mice (28) and RNAi-mediated knockdown in *Drosophila melanogaster* (29) confirmed these clinical observations. To gain further pathophysiological insights and to circumvent compensatory mechanisms, mice with inducible deletion of mTOR complexes in the renal tubule were generated using the Pax8 promotor (30). In this study, mTORC1 function was disabled by inducible inactivation of *Raptor* (*Raptor*$^{fl/fl}$*Pax8rtTA*TetOCre* mice subsequently termed *Rap*$^{\Delta iTubule}$), mTORC2 function by inducible inactivation of *Rictor* (mice subsequently termed *Ric*$^{\Delta iTubule}$), or both (mice subsequently termed *Rap/Ric*$^{\Delta iTubule}$). Deletion of *Raptor* or both subunits but not *Rictor* alone led to reduced tubular internalization of LMW proteins, pointing to a decisive role of mTORC1 but not mTORC2 in regulation of tubular protein retrieval. Interestingly short-term follow-up in *Rap*$^{\Delta iTubule}$ or *Rap/Ric*$^{\Delta iTubule}$ mice did not show a direct regulation of the two main scavenger receptors MEGALIN and CUBILIN by mTORC1, which is in contrast to long-term rapamycin treatment in mice (28). It is known that long-term administration of rapamycin in high doses additionally inhibits mTORC2 and exerts cytotoxic effects (31). In agreement with this previous observation, long-term (3 mo) follow-up studies in *Rap*$^{\Delta iTubule}$ and *Rap/Ric*$^{\Delta iTubule}$ mice showed a reduced MEGALIN expression in the latter while it remained stable in *Rap*$^{\Delta iTubule}$. In line with this finding, a shortening of the S1/S2 segment was observed in *Rap/Ric*$^{\Delta iTubule}$ mice underlining the importance of mTORC signaling for general cellular programs, such as cell growth, proliferation, and metabolism (1) (FIGURE 2).

If the scavenger receptors are not influenced directly, how does mTORC1 regulate proximal tubular endocytosis? The clue to this question comes from the observation that increased amounts of both endocytosed albumin as well as LMW proteins, such as angiotensinogen, retinol binding protein, vitamin D binding protein, and β2-macroglobulin were found in proximal tubular cells of *Rap*$^{\Delta iTubule}$ mice. These proteins are all physiologically endocytosed by PT cells and further processed for degradation or transcytosis (32). For both processes intracellular trafficking is a prerequisite, which disturbance leads to intracellular accumulation. A reduced expression or phosphorylation level of proteins, i.e., BCL-xL, GBF-1, SNX8, RAB10, and DOCK8, which are all directly or indirectly involved in these processes, could be identified, explaining the reduced endocytosis rate, altered apical membrane differentiation, and impaired intracellular trafficking (33–40). Taken together these data revealed a novel theme of mTORC1 function: regulating a series of proteins being specifically involved in endocytosis and vesicle transport.

Aside endocytotic retrieval of proteins, bulk retrieval of nutrients is an equally important task of the proximal tubule. Indeed, increased glucosuria and aminoaciduria could be seen in mice devoid of tubular mTORC1 (30). The aforementioned urinary losses raised the question whether mTORC1 influences transport by regulation of transporter expression or phosphorylation status, respectively. SGLT2 (SLC5A2) is the main sodium-coupled apical glucose transporter in the PT S1 and S2 segments, with an exclusive localization to the brush-border membrane (41). Inhibition of SGLT2 most recently has become a new antidiabetic strategy with far reaching protective effects on heart, kidney and overall survival of patients (42). Transporter activity is decisively regulated by phosphorylation (43). Phosphoproteomics of *Rap*$^{\Delta iTubule}$ mice pointed to a reduced phosphorylation of SGLT2 at S623 in comparison with control animals (30). This residue is a highly conserved phosphosite between species, including human SGLT2 (S624) (43). Augmented phosphorylation at this site was shown to enhance glucose transport by increasing membrane insertion of SGLT2 (43). Loss of mTORC1-induced phosphorylation at S623 could, hence, contribute to glucosuria seen in *Rap*$^{\Delta iTubule}$ and *Rap/Ric*$^{\Delta iTubule}$ animals. Of interest, the activation loop of SGLT2 and mTORC1 seems to be reciprocal. In a recent report, increased glucose transport in several murine type 1 and type 2 models of diabetic kidney disease showed increased mTORC1 activity in proximal tubular cells (44). Application of the SGLT2 inhibitor dapagliflozin normalized mTORC1 activity and ameliorated the deleterious effects of diabetic nephropathy. At least part of the fibrogenetic effects were mediated by mTORC1 dependent increased urinary aminoacid retrieval (44).

In line with these observations *Rap*$^{\Delta iTubule}$ and *Rap/Ric*$^{\Delta iTubule}$ mice displayed a highly increased excretion of neutral, basic, and acidic amino acids compared with control mice (30). Proteomic and phosphoproteomic analyses of *Rap*$^{\Delta iTubule}$ animals identified reduced expression or phosphorylation states of many amino acid transporters known to be highly expressed in the PT (45). Among these, SLC6A19 (B0AT1), SLC7A7 (y + LAT1), and SLC3A2 (4F2hc) had reduced expression levels, whereas diminished phosphorylation was found in SLC7A9 (b0,+ AT) at position S15 and S18 (30, 45).

Both pharmacological inhibition as well as genetic ablation of mTORC1 led to urinary phosphate losses

(28, 30, 46). These occurred, despite unchanged expression of the major proximal tubular phosphate transporters (NaPi-IIa, NaPi-IIc, and Pit-2), in all reports published to date (28, 30, 46). The influence of mTORC1 on so far unidentified interaction partners might be responsible for this enigmatic finding and warrants further experimental follow-up. In summary, mTORC1 deletion leads to a generalized PT dysfunction, mimicking the clinical diagnosis of Fanconi Syndrome.

mTORC1 Is Required for Energy Metabolism of the Proximal Tubule and Thick Ascending Limb of the Loop of Henle

To study the role of mTORC1 in more distal nephron segments, raptor was constitutively deleted using the *kidney-specific cadherin* promoter (*Raptor*$^{fl/fl}$**Ksp Cre;* mice subsequently termed *Rap*$^{\Delta TAL/CD}$) (47, 48). This led to absent mTORC1 activity in the thick ascending limb (TAL) of the Henle loop as evidenced by absent phosphorylation of the mTORC1 downstream target S6K1. After weaning, *Rap*$^{\Delta TAL/CD}$ mice were found to be polyuric and had a reduced urinary osmolality. Water deprivation excluded osmotic diuresis and confirmed a defect in the urine concentrating ability. The response to furosemide was blunted in *Rap*$^{\Delta TAL/CD}$ mice and paralleled by reduced expression of the NKCC2 (sodium potassium cotransporter 2; *Slc12a1*) suggesting, a TAL defect with impaired countercurrent multiplication. In addition to slight abnormalities in light microscopy, ultrastructural analyses by electron microscopy revealed a severe mitochondrial defect in the

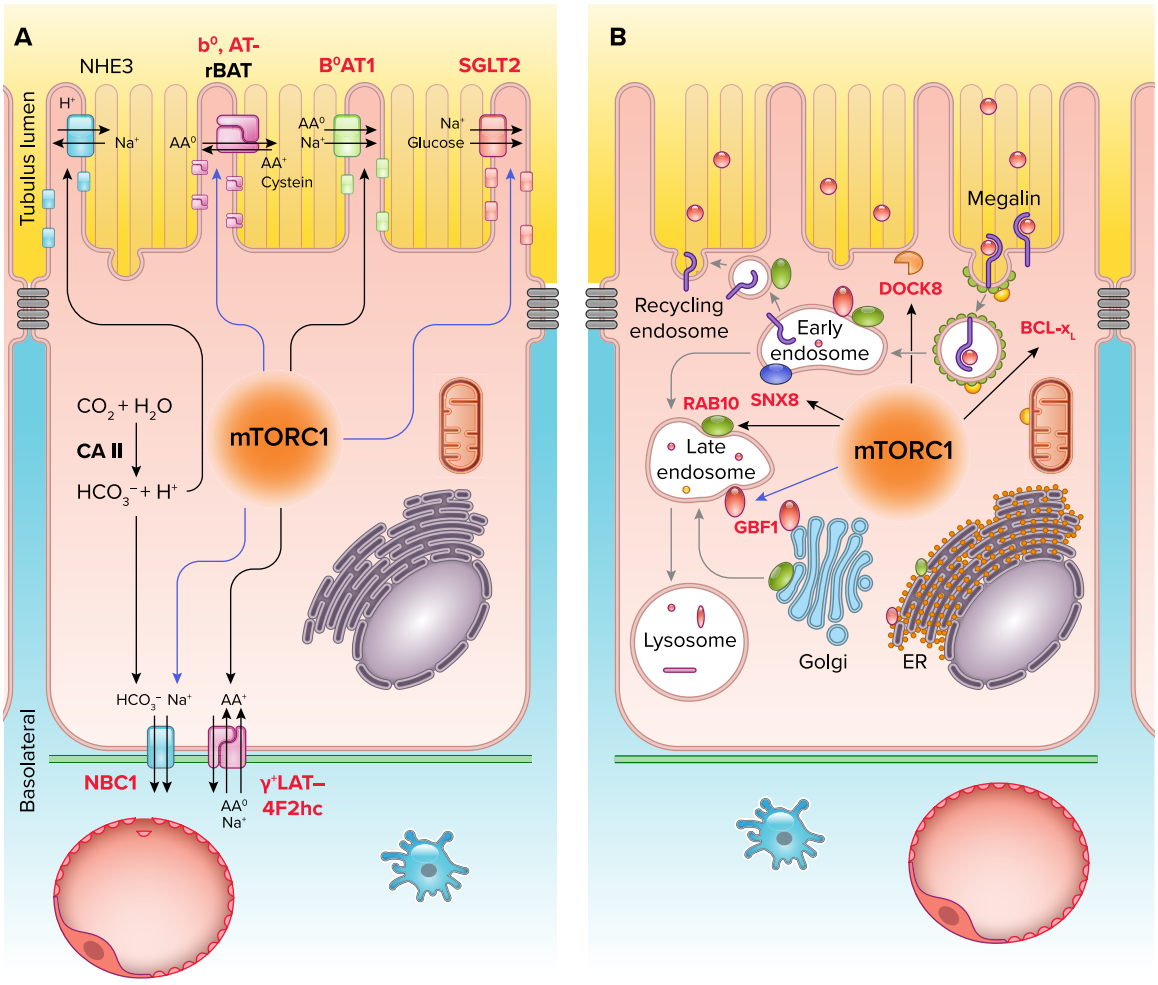

FIGURE 2. **Role of mTORC1 in the proximal tubule: putative mTORC1-dependent regulation of proximal tubular transport and endocytosis**
A: mTORC1 seems to regulate apical and basolateral proximal tubule transport proteins. mTORC1 could directly phosphorylate SGLT2 and NBC1 to increase reabsorption of glucose and sodium and excretion of sodium and bicarbonate. mTORC1 might positively influence the expression level of the amino acid transporter B0AT1 and y + LAT-4F2hc and the phosphorylation status of b0, + AT1-rBAT to reabsorb filtered amino acids and secrete them into the bloodstream. *B*: mTORC1 does regulate PT endocytosis, membrane biogenesis, vesicle trafficking, and cell polarity. mTORC1 likely controls apical membrane biogenesis and cell polarity by affecting expression level of DOCK8, apical clathrin-coated pit and vesicle formation, and endocytosis by affecting the expression levels of BCL-xL, SNX8, and RAB10 and phosphorylation level of GBF-1. Also, it controls proper endoplasmic reticulum (ER) function and Golgi integrity again by influencing the expressions and phosphorylation levels of RAB10 and GBF-1, respectively. Figure taken from Ref. 30. mTORC1, mammalian target of rapamycin complex 1.

tubular epithelial cells of the TAL and the collecting duct (CD). Expression of the peroxisome proliferator-activated receptor coactivator-α, a key regulator of mitochondrial biogenesis and downstream target of mTORC1, was reduced in $Rap^{\Delta TAL/CD}$ mice. Additional deletion of Rictor ($Rap/Ric^{\Delta TAL/CD}$) aggravated the phenotype and led to growth retardation and early mortality. Histologic analysis demonstrated a severe defect in TAL and interstitial fibrosis (47).

It should be remembered that tubular cells of both the proximal convoluted tubule (PCT) and the TAL have a high-energy demand and therefore have a high density of mitochondria, explaining the vulnerability of these segments for disturbances of energy metabolism and acute kidney injury (49). To further study the role of mTOR in this setting mice with inducible deletion of raptor in the entire tubulus ($Raptor^{fl/fl}$*Pax8rtTA*TetOCre mice subsequently termed $Rap^{\Delta iTubule}$) were used, circumventing the consequences of a constitutive loss of mTORC1 (47). When subjected to ischemia-reperfusion, acute tubular necrosis was markedly pronounced in these mice and was evidenced by cell flattening or distal tubular cast formation, involving both the PCT and TAL. Excretion of urinary neutrophil gelatinase-associated lipocalin was increased and paralleled by a higher rate of apoptotic cells in $Rap^{\Delta iTubule}$ mice. This study indicates that mTORC1 is an essential regulator of the energy metabolism of tubular epithelial cells and suggests that pharmacological inhibition by rapamycin might interfere with tubular energy metabolism, eventually facilitating acute kidney injury (AKI) or delaying the recovery from it. This was demonstrated in rapamycin-treated rats after ischemia-reperfusion where recovery of tubular function was considerably delayed (50, 51). In a recent meta-analysis involving 4,039 patients from 9 randomized controlled trials, 2,313 patients treated with the mTORC1 inhibitors everolimus and temsirolimus for renal cell carcinoma or breast cancer were compared with 1704 patients without the use of these drugs (52). AKI defined by increase of plasma creatinine concentration of at least 0.3 mg/dl or higher occurred in 15.7% of the patients receiving mTOR inhibitors compared with 11.7% patients without. The relative risk for patients receiving mTOR inhibitors was significantly higher (1.55, P = 0.010, 95% confidence interval: 1.113 to 2.162). However, there was significant evidence of heterogeneity in the studies. When analyzing the incidence of severe AKI defined by at least twofold of plasma creatinine concentration, the relative risk was not significantly increased (1.29, P = 0.118, 95% confidence interval: 0.94 to 1.77). This meta-analysis suggests that mTORC1 inhibition might be associated with a small but significant risk of AKI although the exact mechanism might be multifactorial and vary from patient to patient.

mTORC1/2 Regulate Sodium and Potassium Homeostasis in the Distal Tubule

Recent work has demonstrated that mTOR signalling is critically involved in the regulation of sodium and potassium homeostasis by the distal nephron (53–56) (FIGURE 3). In this tubular segment, principal cells express the electrogenic epithelial sodium channel (ENaC; encoded by Scnn1a/b/d/g) and the family of renal outer medullary potassium channels (ROMK1-3; encoded by KCNJ1) under the control of aldosterone, thereby matching sodium reabsorption to potassium secretion (58). Loss of ENaC activity in adult mice with an inducible deletion of the γ-subunit of ENaC leads to rapid and lethal hyperkalemia within a few days (59). In the distal nephron, the serum-and-glucocorticoid kinase 1 (SGK1) is a serine threonine kinase of the AGC (protein kinase A/protein kinase G/protein kinase C) family that is transcriptionally upregulated by aldosterone and participates in the regulation of ENaC and ROMK activity (58). SGK1 increases the membrane abundance of ENaC and ROMK by promoting channel insertion and reducing channel retrieval. This is achieved by phosphorylation of both channel subunits (60, 61) and negative regulators such as the ubiquitin ligase Nedd4-2 or WNK4 (lysine-deficient protein kinase 4) (62, 63). SGK1 itself has to be activated by a sequential two-step phosphorylation, first taking place at Ser422 in the hydrophobic motif (HM) near the COOH terminus, followed by phosphorylation at Thr256 in the catalytic domain (64). Whereas the latter has long been known to be mediated by the phosphoinositide-dependent protein kinase PDK1 (65), mTORC2 has only been recently identified as the HM kinase (16). Noteworthy, phosphorylation of SGK1 at Ser422 cannot be mediated by mTORC1 and is not inhibited by rapamycin, indicating substrate specificity of mTORC1 and mTORC2. In renal epithelial cells, mTORC2 was found to phosphorylate SGK1 via interaction with mSIN1, thereby stimulating ENaC currents (66, 67).

The relevance of mTOR on sodium and potassium homeostasis was investigated using pharmacological inhibition of mTOR activity (53, 55). Gleason et al. (53) used catalytic-site inhibitors of the central mTOR kinase subunit (PP242 and AZD8055) which in contrast to rapamycin equally inhibit mTOR activity in both mTORC1 and mTORC2. The authors found that acute administration of PP242 and AZD8055 but not rapamycin induced natriuresis without kaliuresis and reduced the expression of phosphorylated SGK1. The effects of PP242 and AZD8055 were blunted by concomitant administration of the ENaC blocker amiloride but not by the NCC blocker HCTZ, suggesting that the action of PP242 and AZD8055 were mediated by inhibition of ENaC-mediated sodium transport. In another study, treatment of spontaneously hypertensive rats

with PP242 prevented the development of hypertension on a high-salt diet (55). After a single dose, PP242 induced significant natriuresis and inhibition of phosphorylation of NDRG-1, a target of activated SGK1. However, both studies are limited by the lack of complex selectivity of the pharmacological approach to inhibit mTORC2 selectively. It is conceivable, that the observed natriuretic effect of PP242 might well be related to inhibition of sodium reabsorption in the proximal tubule and loop of Henle's loop, likely involving mTORC1.

To study the role of mTORC2 in the distal nephron, mice were generated with genetic deletion of *Rictor* using the *Ksp* promoter (mice subsequently termed $Ric^{\Delta TAL/CD}$), an essential component of mTORC2 (54). $Ric^{\Delta TAL/CD}$ mice were found to be viable yet had increased plasma aldosterone at rest. While adapting adequately to a low-Na^+ diet, they developed fatal hyperkalemia on a high-K^+ diet despite a massive increase in plasma aldosterone. In addition, they were intolerant to ENaC inhibition by triamterene over 4 days. These results recapitulated what had been previously observed in SGK1 deficient mice (70, 71). Immunofluorescence revealed absent phosphorylation of SGK1 in kidneys from $Ric^{\Delta TAL/CD}$ mice and absence of ROMK at the apical membrane. Patch-clamp experiments on split-open tubular segments from the transition zone of the late connecting tubule and early cortical CD demonstrated that Ba^{2+}-sensitive apical K^+ currents were barely detectable in the majority of $Ric^{\Delta TAL/CD}$ mice whereas ENaC activity was largely preserved. This study underscored that in addition to

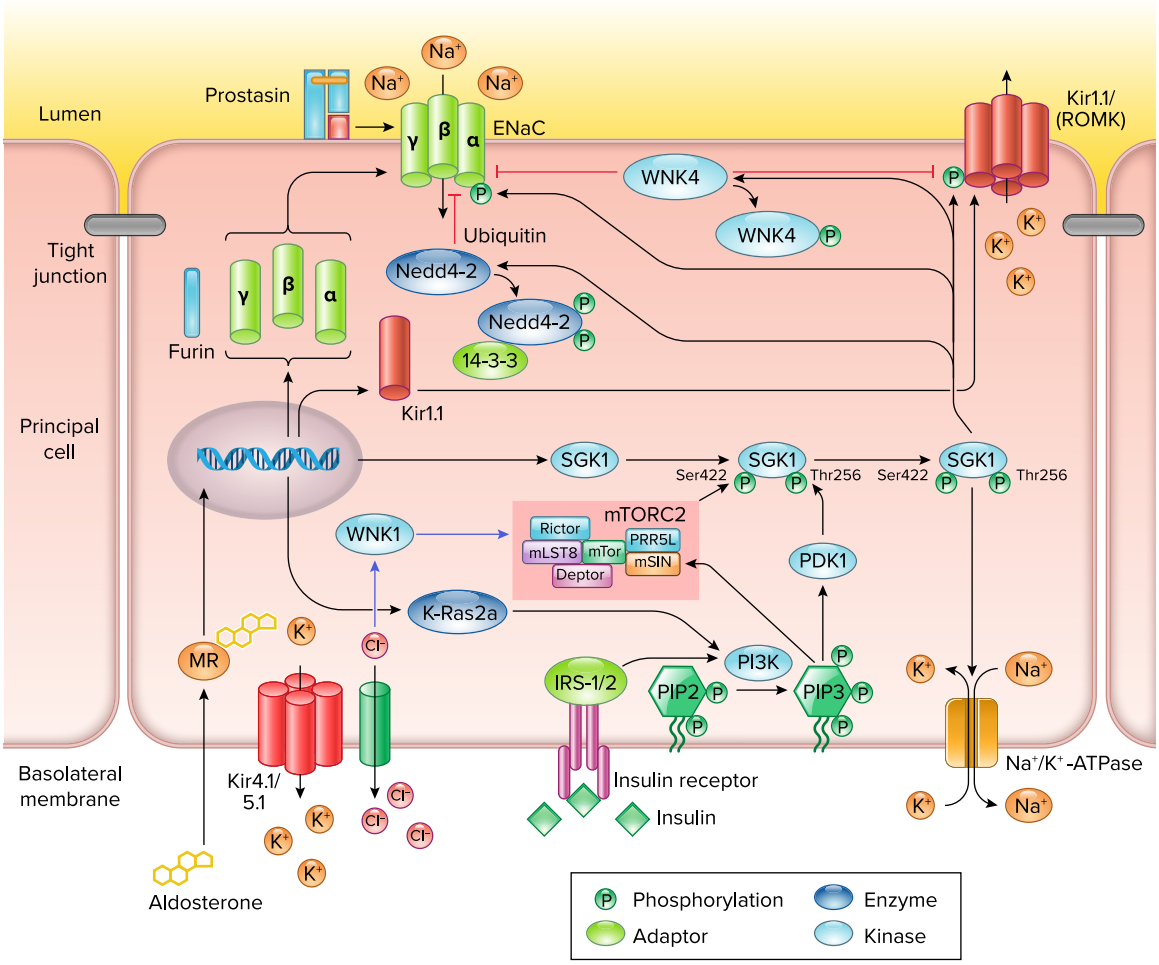

FIGURE 3. **Transport regulation in the distal tubule by mTORC2: mTORC2 is activated by PI3K signaling that is stimulated by both aldosterone and insulin**
In addition, mTORC2 seems to be directly stimulated by WNK1 upon change in basolateral K^+ and Cl^- concentrations. mTORC2 phosphorylates SGK1 at ^{422}Ser, which is required for binding of PDK1 to mediate a 2nd phosphorylation that activates SGK1. SGK1 may phosphorylate and activate ENaC and ROMK directly. In addition, SGK1 phosphorylates and inactivates the ubiquitin ligase Nedd4-2 and WNK4 to reduce membrane retrieval of both ENaC and ROMK. There are no data whether the mTORC2/SGK1 axis stimulate ENaC by proteolytic activation, e.g. via prostasin (68). Taken from Ref. 69 and modified according to Refs. 56, 58. PI3K, phosphoinositide-3 kinase; mTORC2, mammalian target of rapamycin complex 2; WNK, sensitive with-no-lysine kinase 1 and 4; ENaC, epithelial sodium channel; ROMK, renal outer medullary potassium channel; SGK1, SGK-1, serum- and glucocorticoid-induced protein kinase 1; IRS1/2, insulin receptor substrate; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, and phosphatidylinositol (3,4,5)-trisphosphate.

stimulating ENaC mTORC2 is essential for maintaining K^+ homeostasis by regulation of ROMK (54).

In that study, protein expression of Rictor was upregulated in kidneys from triamterene-treated mice, which develop compensatory hyperaldosteronism, raising the possibility that Rictor might be an aldosterone-induced gene. However, this finding could not be reproduced in kidneys from healthy mice treated with aldosterone (72). This suggests the presence of an aldosterone-independent mechanism of mTORC2 regulation, which was substantiated recently (56). In that study, an acute oral K^+ load induced rapid SGK1 phosphorylation and ENaC activation within 1 h in wild-type mice that could not be inhibited by blockade of the mineralocorticoid receptor (MR). In renal epithelial cells, high basolateral K^+ concentration led to phosphorylation of SGK1 and ENaC activation, both of which required mTORC2 activity. This effect was found to be mediated by basolateral Kir4.1 channels (encoded by *Kcnj10*) and the authors proposed a concept that in principal cells mTORC2 activity might be directly and independently from MR signaling stimulated by a depolarization of the basolateral membrane in response to a high extracellular K^+ concentration. This would be analogous to the effect of hyperkalemia on the early distal tubule, which is sensed by basolateral heterotetrameric Kir4.1/5.1 (encoded by *Kcnj10/Kcnj16*) channels, thereby leading to downregulation of NCC expression (73–75). In contrast to NCC, the concept suggests upregulation of ENaC to enhance apical K^+ secretion by ROMK (56). The mechanism linking altered basolateral membrane potential to the effects on NCC and ENaC expressed at the luminal side is thought to involve alteration of intracellular Cl^- concentrations and activity of the Cl^--sensitive with-no-lysine kinase 1 and 4 (WNK) (76). In renal epithelial cells, K^+-stimulated phosphorylation of SGK1 but not that of Akt was undetectable in the absence of WNK1 or kinase-dead WNK1 (56). The authors concluded that WNK1 might act through a noncatalytic mechanism to modulate SGK1 phosphorylation by mTORC2. At present, more studies are needed to decipher the regulation and intracellular signaling cascade of mTORC2. If not transcriptionally upregulated by aldosterone, is RICTOR and subsequently mTORC2 expression regulated by RICTOR's protein half-life e.g., by phosphorylation? Given that SGK1 deficiency does not alter ROMK membrane expression (70, 77), how does *Rictor* deficiency lead to a loss of ROMK membrane abundance and might this relate to dysregulation of trafficking or apical membrane targeting of ROMK? Does mTORC2 activation induce proteolytic ENaC activation and if so which proteases are involved in this cleavage?

Conclusions

There is a broad expression of both mTOR complexes along the nephron, yet regulation of transport processes involving these complexes in each tubular segment is complex and cell-type specific. In the proximal tubule, regulation of nutrient reabsorption and endocytosis by mTORC1 is paramount. Future work will have to delineate which other disease entities could benefit from altered mTORC1 signaling. Site-specific modulation of mTORC1 activity seems an achievable goal given the diverse available transport properties in this part of the nephron. A remaining challenge will be titration of mTORC1's effects on energy metabolism considering prevalent metabolic disease, i.e., diabetes and obesity: partial inhibition will likely be beneficial, complete blockage might become deleterious in the long-run. Regulation of endocytosis needs to be explored in further detail. Especially the known link of mTORC1 to lysosomes and autophagy need to be further explored in the context of the proximal tubule. Although phosphaturia has been consistently reported in both pharmacological as well as genetic studies, it remains elusive on a molecular level.

mTORC2 is vital in the regulation of K secretion. Studies using mTOR kinase inhibitors need to be cautiously interpreted, as all of these drugs also effect mTORC1. Currently, there are no selective pharmacological mTORC2 inhibitors or activators available. Given the reported independence of mTORC2 regulation from the MR activators of mTORC2 could help to prevent hyperkalemia associated with inhibition of the renin-angiotensin-aldosterone system. ■

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