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mTOR signaling in autophagy regulation in the kidney

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Abstract

Cells possess adaptive biosynthetic systems to maintain cellular energy levels for survival under adverse environmental conditions. Autophagy is an evolutionarily conserved cellular catabolic process that breaks down and recycles cytosolic material including macromolecules and organelles through lysosomal degradation. This catabolic process, represented by macroautophagy, is induced by a variety of cellular stresses such as nutrient starvation, which causes a shortage of cellular energy for cells to maintain cellular homeostasis and essential biological activities. In contrast, upon nutrient availability, cells stimulate anabolic processes. The mechanistic/mammalian target rapamycin (mTOR), a serine/threonine protein kinase, is a key player in stimulating cellular anabolism in response to nutrients and growth factors, and plays a crucial role in suppressing autophagy activity. Growing evidence has suggested that autophagy activity is required for the maintenance and physiological functions of renal cells including proximal tubular cells and podocytes. In this section, we will discuss recent progresses in the regulation of autophagy by the mTOR signaling.

Keywords

Autophagy; rapamycin; mTOR; mTORC1; AMPK; renal proximal tubular cell; podocyte

Mechanistic/mammalian target of rapamycin (mTOR)

mTOR protein kinase stimulates many cellular anabolic processes and plays a key role in inhibiting the initiation of autophagy [1]. mTOR is a phosphatidylinositide 3-kinase (PI3K)-related protein kinase conserved from yeast to mammal [2]. mTOR forms at least two distinct functional complexes termed mTOR complex1 (mTORC1) and mTORC2 [3-7]. mTORC1 exists as a multi-protein complex containing mTOR, RAPTOR (Regulatory-associated protein of mTOR), PRAS40 (Proline-rich AKT substrate 40 kDa), MLST8 (Mammalian Lethal with SEC13 protein 8), and DEPTOR (DEP domain-containing protein 6) [8-11], while mTORC2 consists of mTOR, RICTOR (Rapamycin-insensitive companion of mTOR), SIN1

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(Stress-activated map kinase-interacting protein1/MAPKAP1), PRR5 (Proline-rich protein 5/Protor-1), MLST8, and DEPTOR [4,5,12-16]. The configuration of each mTORC is also conserved across species [3]. Importantly, mTORC1 activity is sensitive to rapamcin, whereas mTORC2 activity is resistant.

mTOR possesses multiple domains including HEAT (Huntington, elongation factor-3, a subunit of protein phoshpatase-2A, TOR1) repeats, a FAT (FRAP, ATM, and TRRAP) domain, an FATC domain, a kinase domain, and an FRB (FKBP12-rapamycin-binding) domain [2]. Rapamycin, a macrolide antibiotic originally purified from *Streptomyces hygroscopicus*, is an allosteric inhibitor of mTORC1 [17]. It interacts with the intracellular receptor FKBP12 to form a drugprotein complex. This formation is necessary to block mTORC1 phosphorylation of substrates such as S6 kinase (S6K). Recent structural studies provide insight into the differential sensitivity of mTORC1 and mTORC2 to rapamycin [18,19]. The FRB domain of mTOR, which interacts with rapamycin, resides in close proximity to the active site of mTOR kinase. In mTORC1, the binding of rapamycin-FKBP12 complex to the FRB domain sterically hinders the kinase cleft of mTOR, thereby blocking the accessibility of substrates to the active site of mTOR kinase [19]. In mTORC2, it is conceivable that rapamycin binding to FKBP12 prevents its interaction with the FRB domain of mTOR, likely due to steric hindrance of the FRB domain with a specific component of mTORC2 such as RICTOR or SIN1. However, at high, micromolar concentrations, rapamycin is also able to inhibit mTORC2 activity, when it binds to the FRB in the absence of FKBP12 [20]. It has also been demonstrated that rapamycin treatment reduces the integrity of mTORC1 where the rapamycin-FKBP12 complex destabilizes the interaction between mTOR and RAPTOR. Prolonged treatment of rapamycin however also decreases the integrity and activity of mTORC2 possibly by preventing RICTOR interaction with mTOR during de novo mTORC2 formation [21]. These observations indicate that mTOR is unable to keep or form a multiprotein complex once the rapapmycin-FKBP12 complex binds to the FRB domain [19].

Specific components that interact with mTOR kinase determine substrate specificity for mTORC1 and mTORC2. For example, RAPTOR, an essential scaffolding component of mTORC1 recruits mTORC1 substrates including S6Ks, eIF4E-binding proteins, and ATG1 [2,22,23], while RICTOR or SIN1 may recruit the mTORC2 substrates Akt, PKC, and SGK1 for phosphorylation [4,24-28]. Through substrate phosphorylation, mTORC1 stimulates a wide array of cellular anabolic processes including protein and lipid synthesis, and mitochondria biogenesis, whereas it inhibits catabolic processes such as autophagy (see later section). In contrast, the biological roles of mTORC2 are relatively unknown. However, by activating SGK1 and Akt, and stabilizing conventional PKCs, mTORC2 is likely to play major roles in the regulation of cell survival and cytoskeletal reorganization that are the known functions of these AGC kinases [29,30].

Regulation of mTOR signaling: Growth factor-mediated mTORC1 activation

mTORC1 activity is regulated by multiple extra- and intracellular cues including growth factors, oxidative stress, and nutrients such as glucose and amino acids (Figure 1). Among these cellular cues, both growth factor and amino acid inputs are indispensable for the full

activation of mTORC1. The most proximal molecule that elicits a key role in activating mTORC1 activity is the small GTPase Rheb (Ras homolog enriched in brain). Rheb is a Ras-related GTPase originally identified as a gene rapidly induced in brain neurons by synaptic activity [31]. Genetic studies in both *Drosophila* and mice have shown that Rheb functions as an essential activator of mTORC1 [32,33]. Biochemical studies have demonstrated that active Rheb directly associates with mTOR and potently stimulates its kinase activity in vitro [8,34,35]. Loss of Rheb function eliminates mTORC1 activity and blunts any effects of stimuli including growth factors and amino acid. However, the molecular mechanism by which active Rheb stimulates mTORC1 kinase activity in vitro remains unclear. Since active Rheb specifically stimulates mTORC1 but not mTORC2, the direct target of Rheb is likely to be a specific component of mTORC1. Active Rheb may change the conformation of mTORC1 and open the kinase cleft of mTOR to increase the accessibility of substrates to the active site of mTOR kinase [19]. Consistent with this idea, amino acid stimulation, oxidative stress, or active Rheb overexpression weakens the association between mTOR and Raptor [6,36].

The activity of Rheb, and mTORC1, is inhibited by the tuberous sclerosis complex gene products, TSC1 (hamartin) and TSC2 (tuberin), which form a GTPase activating protein (GAP) complex [37-42]. Mutations in *TSC1* or *TSC2* are associated with the disease tuberous sclerosis complex (TSC), characterized by the formation of hamartomas in multiple organs. TSC1 stabilizes TSC2, which possesses a GAP domain in its carboxyl terminus [43,44]. Multiple growth-related kinases such as AKT, ERK (Extracellular signal-regulated kinase), and RSK (p90 ribosomal protein S6 kinase) phosphorylate and inhibit TSC2 function, thereby activating the Rheb-mTORC1 pathway [45-48]. Consistently, in TSC1 or TSC2 deficient cells, mTORC1 is constitutively activated and no longer sensitive to the inhibitory effects of growth factor deprivation. Accordingly, autophagy activity is largely diminished in TSC null cells [49]. Although both biochemical and genetic studies demonstrate that Rheb is a critical activator of mTORC1 in response to growth factor and amino acid stimulation, amino acid starvation still inhibits mTORC1 activity even in TSC null cells [50], where Rheb is constitutively active, suggesting that the mechanism underlying amino acid-induced mTORC1 is parallel but dominant to Rheb-mediated mTORC1 activation.

Regulation of mTOR signaling: Amino acid-mediated mTORC1 activation

How amino acids, especially leucine, play critical roles in activating mTORC1 remains a longstanding question in the mTOR field. A series of recent studies have elucidated the molecular mechanisms by which amino acids enhance the mTORC1 pathway in coordination with growth factor signaling. Two independent studies identified that Rag (Ras-related GTP-binding protein), another Ras-related GTPase, mediates amino acid-induced mTORC1 activation [51,52] (Figure1). The mammalian Rag subfamily of GTPase consists of Rag A, B, C, and D [53]. RagA and RagB are homologous to yeast Gtr1p, while RagC and RagD are homologous to yeast Gtr2p [54]. The mammalian RagA or RagB forms a heterodimer with RagC or RagD. This formation can also be seen in the yeast Gtr1p/Gtr2p complex. A unique feature of this conserved hetrodimeric Rag complex is that RagA or B (RagA/B) is the GTP form whereas RagC/D is the GDP form in the active complex. The Rag heterodimer is expressed on the lysosomal membrane, and upon amino acid stimulation,

GTP-bound RagA/B interacts with mTORC1 through Raptor [51]. Indeed, immunofluorescence studies revealed that mTORC1 translocates to the LAMP2/Rab7-positive endosome (late endosome/lysosome) in response to amino acid stimulation [51] (Figure 1). Importantly, ectopic expression of GDP-bound RagA/B prevents translocation of mTORC1 to the lysosomal membrane and its activity, whereas GTP-bound active RagA/B renders mTORC1 resistant to amino acid deprivation [51,52]. Accordingly, mTORC1 constitutively localizes to the lysosomal membrane in cells expressing GTP-bound RagA/B even under amino acid starvation conditions. These data indicate that Rags play a critical role in recruiting mTORC1 to the lysosome where mTORC1 can be activated by Rheb [51]. This spatial regulation of mTORC1 by Rag and Rheb explains how the signals from amino acids and growth factors are integrated to fully activate the mTORC1 pathway (Figure 1).

Unlike other small GTPases, the Rag family of GTPases lack lipid modification motifs such as those for farnesylation or myristoylation, even though they localize on the lysosomal membrane. Using proteomics approaches, Sabatini and colleagues identified a Rag heterodimer-associated complex termed as “Ragulator” that consists of at least five distinct proteins including MP1 (MAPK scaffolding protein 1), p14, p18 (MAKSP1), ROBLD3 (Roadblock domain-containing protein 3), and c11orf59. Three (MP1/p14/p18) of these five proteins were known to be associated with the lysosomal membrane to regulate endosome/lysosome organization [55]. Disruption of Ragulator inhibits amino acid-induced mTORC1 activation and causes mislocalization of Rags, indicating that Ragulator plays an important role for lysosomal localization of Rags. Further analysis demonstrated that the Ragulator possesses guanidine exchange factor (GEF)-like activity for both RagA and RagB [56]. These results indicate that Ragulator plays key roles in not only localization but also activation of Rags, thereby stimulating mTORC1 activity on the lysosomal membrane (Figure 1). Furthermore, the activity of vATPases required for lysosomal acidification plays an important role in activating Ragulator to stimulate Rag GTPases in response to amino acid availability [57]. Recent studies also revealed that two protein complexes, termed “GATOR1 (GAP activity toward Rags) and GATOR2”, regulate Rag activity in response to cellular amino acid availability [58]. GATOR1 consists of at least three proteins including NPRL2 (Nitrogen permease regulator 2-like protein), NPRL3, and DEPDC5 (DEP domain-containing protein 5), of which NPRL2 and NPRL3 have been demonstrated to inhibit mTORC1 activity in response to amino acid starvation in yeast [59]. In addition, a recent study by Sabatini and colleagues identified GATOR1 as specifically possessing GAP activity for RagA and RagB [58]. Intriguingly, GATOR2, which consists of 5 distinct WD40 repeat-containing proteins, associates with and inhibits GATOR1 to suppress RagA/B activity. However, the precise molecular mechanisms by which the GATOR complexes sense amino acids and which component of GATOR1 has GAP activity remain unclear. Overall, these series of studies have clarified the pathway and signals from amino acid sufficiency to mTORC1 activation. Unexpectedly, these studies also revealed that the activity of lysosomes plays critical roles for mTORC1 activation, which are also paradoxically important for cellular autophagy.

Mechanism of mTORC1-dependent autophagy inhibition

Although the activity of lysosomes is essential for both mTORC1 activation and autophagy, mTORC1 has been long recognized as an essential negative regulator for autophagy induction. Autophagy is an evolutionarily conserved process that recycles macromolecules and organelles through lysosome-mediated degradation to generate the source of cellular energy during nutritional stress [60]. Upon activation of autophagy, unnecessary cellular components are encapsulated in a double-membrane vesicle structure (autophagosomes), which targeted to lysosomes (autolysosome). Fusion of the outer autophagosomal membrane with the lysosome releases the cargo-containing inner membrane to the lumen of the lysosome for further breakdown and recycling, thereby providing a nutrient source to maintain vital cellular activities [61].

TORC1 in *S. cerevisiae* (budding yeast) negatively regulates autophagy. Rapamycin treatment is sufficient to induce autophagy even in the presence of nutrients, providing key evidence that TORC1 elicits an essential negative role in suppressing autophagy. Previous genetic and biochemical studies demonstrated that TORC1 suppressed the function of ATG1, an autophagy-initiating kinase [62,63]. The budding yeast *atg1* mutant is defective in autophagy induction even under nutrient starvation or rapamycin treatment conditions, indicating that ATG1 acts downstream of TORC1 to induce autophagy. ATG1 forms a complex with other autophagy proteins such as ATG13 and ATG17. The integrity of the ATG1-ATG13-ATG17 complex is important for ATG1 kinase activity, and rapamycin treatment or nutrient starvation enhances the integrity of this complex [62]. It has been postulated that TORC1 enhances the phosphorylation of ATG13 on multiple residues to weaken the integrity of the ATG1 complex and repress autophagy induction [63,64]. Interestingly, the molecular mechanism underlying TORC1-mediated autophagy inhibition through the post-translational modifications of the ATG1 complex seems to be conserved albeit more complicated in higher eukaryotes [65]. The mammalian ATG1 orthologs, Unc-51-like kinase 1 (ULK1) and ULK2, also play important roles in autophagy induction in mammalian cells [66,67]. ULK1 is phosphorylated and activated by 5′-AMP-activated protein kinase (AMPK), an essential energy sensor, in response to metabolic stress [23,68]. In contrast, ULK1 is phosphorylated and inactivated by mTORC1 in response to nutrient availability (Figure 1). ULK1 is stably bound to AMPK, and this interaction is suppressed by the mTORC1-dependent ULK1 phosphorylation, indicating that mTORC1 disrupts the process of ULK1 activation by AMPK under nutrient-rich conditions [23]. Consistently, the interaction between ULK1 and AMPK, and the phosphorylation of ULK1 by AMPK are enhanced by rapamycin treatment. These results indicate that mTORC1 phosphorylation of ULK1 maintains ULK1 in an inactive state. Although ATG13 can also be subjected to mTORC1-dependent phosphorylation in mammalian cells as it is in yeast, it remains unclear the physiological roles of ATG13 phosphorylation in the ULK1 complex because the interaction between ULK1 and ATG13 is maintained even under nutrient-rich conditions [69]. In addition to the above mechanisms, recent studies also revealed that mTORC1 directly phosphorylates AMBRA1 (activating molecule in Beclin-1-regulated autophagy) [70], a component of the VPS34-Beclin1 (ATG6) complex [71], which recruits downstream effectors to the site where nucleation of autophagosomes occurs (Figure 1). AMPBRA1 induces autophagosome

nucleation by promoting Beclin1 interaction with the lipid kinase VPS34 [72]. AMBRA1 plays a key role in stabilizing ULK1 and activating ULK1 kinase activity by facilitating ULK1 dimerization [70]. Interestingly, mTORC1 directly phosphorylates AMBRA1 and inhibits its function in activating ULK1 under nutrient-rich conditions. Taken together, mTORC1 phosphorylates multiple autophagy proteins leading to the blockade of ULK1 functions and inhibiting the induction phase of autophagy.

Role of the mTORC1-autophagy pathway in kidney cells

A series of studies demonstrated that induction of autophagy plays an important role in protecting renal tubular cells (especially proximal tubular cells) from many stresses including ischemia [73-75]. Renal tubular cells show the highest level of mTORC1 activity in renal tissues as determined by rapamycin-sensitive S6 phosphorylation, suggesting that basal autophagy activity is presumably low under normal physiological conditions. A recent study also demonstrated that autophagy in renal tubular cells is stimulated by proteinuria, and the autophagy induction in tubular cells plays an important role in protecting cells from proteinuria-induced apoptosis [76]. Intriguingly, excess calorie uptake, such as in high fat diets, enhances mTORC1 activity and suppresses autophagy induction in the renal tubular cells, resulting in higher susceptibility of tubular injury to proteinuria. The role of autophagy has also been studied in glomerular podocytes [77-79]. Glomerular podocytes display higher autophagy activity compared to other glomerular cells [79]. Lack of autophagy in podocytes causes slowly progressive podocyte loss and glomerulosclerosis in aged mice, indicating that basal autophagy activity plays an important role in maintaining healthy podocytes in older mice. Intriguingly, podocytes also exhibit higher mTORC1 activity compared to other glomerular cells [80-82]. These observations suggest that podocytes create a unique environment where both mTORC1 and autophagy mutually and exclusively function in a single cell. The mechanism by which autophagy activity is maintained in podocytes may be due to their specific cellular shape and the structure of their organelles, which are coupled to provide fundamental podocyte functions. Recent studies demonstrate that podocytes possess large Golgi apparatuses and develop lysosomes at the trans-side of the Golgi, where a large amount of cellular mTORC1 is sequestered on the lysosomal surface in perinuclear regions [83]. Furthermore, podocytes have long foot processes that provide a large surface area for filtration. This unique structure and organellar position may provide a gradient of mTORC1 expression within a podocyte, and allow the cells to activate both autophagy and mTORC1 in different areas. Such a system may have beneficial roles in generating sufficient secretory proteins with a constant energy supply derived from autophagy. Consistently, phosphorylated S6, a substrate of mTORC1 localized to active polysomes, is predominantly expressed in the perinuclear region where active mTORC1 stimulates translation in podocytes [80,82]. It will be important to explore the physiological functions of this coordinated spatial regulation of mTORC1 and autophagy in podocytes and address the questions of whether disruption of this system causes podocyte and glomerular dysfunction.

In summary, a series of studies have proposed that autophagy plays important roles in keeping renal cells healthy by protecting them from metabolic stress. Given that mTORC1 is a potent suppressor for autophagy, any inappropriate mTORC1 activation should elicit deleterious effects on renal cell function. Thus, future studies clarifying the signals and

mechanisms underlying dys-regulation of mTORC1 activity in renal cells promises to shed further light into the interplay between mTORC1 activity and autophagy in renal cell function.

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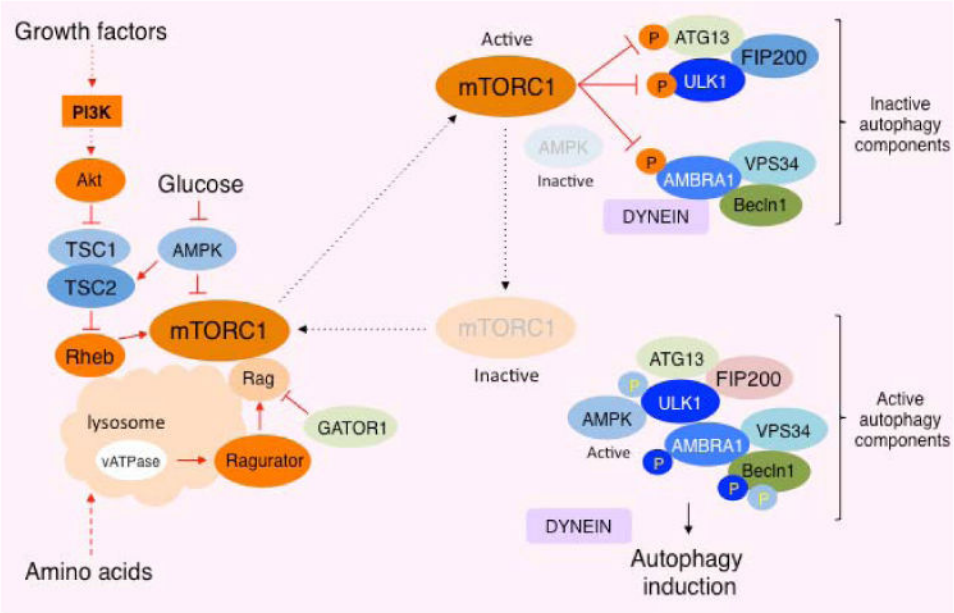

Figure 1. Signal transduction in the regulation mTORC1 activation and autophagy inhibition

Two small GTPases, Rheb and Rags cooperatively stimulate mTORC1 on the lysosomal membrane in response to growth factor and amino acid, respectively. Active mTORC1 phosphorylates multiple components in the ULK1 (ATG1) complex and inhibits its function, whereas AMPK and ULK1 phosphorylation of the components in the complex stimulates its function to induce autophagy.