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# mTOR and lysosome regulation

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## Abstract

Lysosomes are key cellular organelles that play a crucial role in catabolism by degrading extracellular and intracellular material. It is, therefore, very intriguing that mTORC1 (mechanistic target of rapamycin complex 1), a major promoter of anabolic processes, localizes in its active form to the surface of lysosomes. In recent years, many exciting observations have revealed a tightly regulated crosstalk between mTORC1 activity and lysosomal function. These findings highlight the complex regulatory network that modulates energy metabolism in cells.

## Introduction

Target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that regulates cell growth and division in response to energy levels, growth signals, and nutrients [1]. Control of mTOR activity is critical for the cell since its dysregulation leads to cancer, metabolic disease, and diabetes [2]. In cells, mTOR exists as two structurally distinct complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), each one with specificity for different sets of effectors. mTORC1 couples energy and nutrient abundance to cell growth and proliferation by balancing anabolic (protein synthesis and nutrient storage) and catabolic (autophagy and utilization of energy stores) processes. Although different cellular locations for mTORC1 and mTORC2 have been reported (these studies were recently summarized in an excellent review by Betz and Hall [3]), there is a consensus that the localization of mTORC1 to lysosomes is critical for its ability to sense and respond to variations in the levels of amino acids.

When amino acid levels are high, mTORC1 is recruited to the lysosomal surface, where it is activated by the guanosine-5'-triphosphate (GTP)-loaded form of the small GTPase Rheb (Ras homolog enriched in brain) [4,5]. The amino acid-dependent translocation of mTOR to the lysosome requires active Rag GTPases and a Ragulator, a pentameric protein complex that anchors the Rag GTPases to lysosomes [6-8]. The Rag proteins function as heterodimers in which the active complex consists of GTP-bound RagA or B complexed with guanosine diphosphate (GDP)-bound RagC or D. It has been proposed that amino acids trigger the GTP loading of RagA/B proteins, thus promoting the binding to raptor and assembly of an activated mTORC1 complex. However, it is important to mention that this model was recently challenged by a study suggesting that the activation of mTORC1 is not dependent on the Rag GTP charging [9]. Therefore, further studies will be required to solve this apparent discrepancy and fully characterize the mechanism of mTORC1 translocation to lysosomes upon amino acid stimulation. Meanwhile, the activity of Rheb is regulated by a complex consisting of tuberous sclerosis complex 1 (TSC1), TSC2, and TBC1 domain family member 7 (TBC1D7) [10,11]. This complex also localizes to lysosomes and functions as a GTPase-activating protein (GAP) that inhibits the activity of Rheb [12,13]. In the presence of growth factors or insulin, TSC releases its inhibitory activity on Rheb, thus allowing the activation of mTORC1. Therefore, different stimuli must cooperate to ensure proper mTORC1 activation.

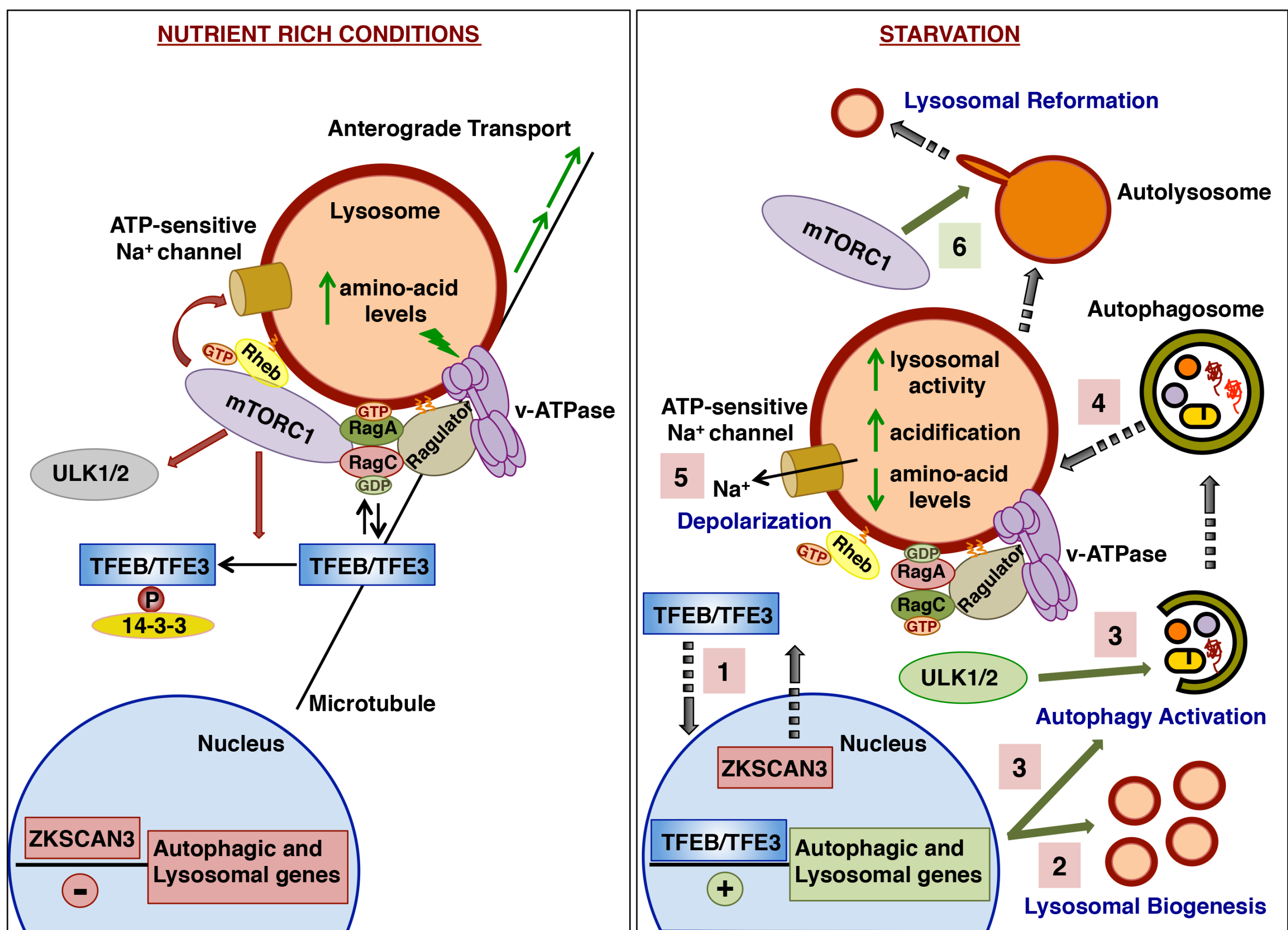
It is important to keep in mind that lysosomes do not simply serve as platforms for the proper assembly of the

mTORC1 regulatory pathway. In the last few years, it has become apparent that the activities of mTORC1 and lysosomes are intimately interconnected. The level of amino acids inside the lumen of lysosomes directly modulates mTORC1 activity through the vacuolar $H^+$-adenosine triphosphatase (v-ATPase) [14]. In addition, recent evidence suggests that mTORC1 may play a crucial role in lysosomal function by regulating biogenesis, distribution, and activity of lysosomes (Figure 1).

## Lysosomal biogenesis

It was long presumed that the expression of lysosomal genes was constitutive. However, recent evidence revealed that cells constantly monitor lysosomal function and possess the ability to increase the number and activity of lysosomes in response to their energetic or degradative needs. The transcription factor EB (TFEB), a member of the basic helix-loop-helix leucine zipper family of transcription factors, promotes expression of many lysosomal proteins and is considered a master regulator of lysosomal biogenesis. Upon activation, TFEB binds directly to a 10-base pair motif (GTCACGTGAC), known as Coordinated Lysosomal Expression and Regulation (CLEAR) element, enriched in the promoter regions of numerous lysosomal genes, thus promoting their transcription [15]. Interestingly, the activation of TFEB is regulated by

**Figure 1. mTORC1 regulates lysosomal function in normal and starvation conditions**




Under nutrient-rich conditions (left panel), active mTORC1 promotes the retention of TFEB and TFE3 in the cytosol as well as inhibition of the ULK1/2 complex and the ATP-sensitive $Na^+$ channel. In addition, lysosomes move toward the periphery of the cell. In contrast, under starvation conditions (right panel), the inactivation of mTORC1 leads to rapid translocation of TFEB and TFE3 to the nucleus (1), induction of lysosomal biogenesis (2), autophagy activation (3), and changes in lysosomal membrane potential (5). Inactivation of mTORC1 might also be required to facilitate the fusion between autophagomes and lysosomes (4). After prolonged periods of starvation, reactivation of mTORC1 is critical to induce autophagic lysosomal reformation (6). Abbreviations: mTORC1, mechanistic target of rapamycin (serine/threonine kinase) complex 1; RHEB, Ras homolog enriched in brain; TFE3, transcription factor binding to IGHM enhancer 3; TFEB, transcription factor EB; ULK, uncoordinated 51-like kinase; v-ATPase, vacuolar $H^+$-adenosine triphosphatase; ZKSCAN3, zinc finger with KRAB and SCAN domains 3.

mTORC1 [16-18]. In fed cells, active mTORC1 phosphorylates TFEB in several serine residues. mTORC1-dependent phosphorylation of TFEB in serine 211 (S211) is particularly important, as this residue mediates the interaction between TFEB and the cytosolic chaperone 14-3-3 and causes retention of TFEB in the cytosol [16,17]. Following starvation and consequent inactivation of mTORC1, the TFEB/14-3-3 complex dissociates, allowing rapid transport of TFEB to the nucleus and TFEB-regulated expression of lysosomal genes.

Active TFEB also upregulates the expression of critical regulators of the autophagic process, including several proteins implicated in the formation of autophagosomes as well as proteins required for the fusion between autophagosomes and lysosomes [19]. Therefore, TFEB contributes to synchronize the two major cellular degradative systems: autophagy and lysosomes. The ability of mTORC1 to block autophagy initiation was well established but was thought to occur at the protein level. mTORC1 directly phosphorylates and inhibits key components of the uncoordinated 51-like kinase 1/2 (ULK1/ULK2) complex, required for autophagy initiation [20,21]. However, the above-mentioned findings indicate that, by promoting the sequestration of TFEB in the cytosol, mTORC1 also modulates autophagy at the transcriptional level. Finally, TFEB induces expression of critical regulators of lipid metabolism that facilitate the use of energy stores during prolonged periods of starvation and this is a role well conserved during evolution [22-24].

Phosphorylation of TFEB by mTORC1 requires the recruitment of TFEB to lysosomes, the compartment where active mTORC1 resides. This recruitment is mediated by the direct interaction between TFEB and active Rag GTPases [25]. Rags also bring folliculin (FLCN) and TSC to lysosomes [13,26-28]. FLCN functions as a RagC/D GAP and is thought to be critical for mTORC1 reactivation when cells change from starvation to nutrient-rich conditions [27]. Therefore, Rags play an important role as docking sites for recruiting mTORC1, mTORC1 effectors (TFEB), and mTORC1 regulators (FLCN and TSC) to the lysosomal surface in a nutrient-dependent manner. Interestingly, TFEB (as well as TFE3; see below) induces the expression of FLCN [26], suggesting the presence of a regulatory loop in which TFEB may contribute to mTORC1 reactivation and therefore its own inhibition.

TFEB is not the only transcription factor implicated in lysosomal biogenesis. TFEB belongs to the MiTF/TFE family of transcription factors that includes MITF, TFE3, and transcription factor EC (TFEC). TFE3 was previously implicated in the development of osteoclasts, activation of the immune system, and control of the allergic response. However, recent evidence showed that TFE3 also induces autophagy and lysosomal biogenesis in starved cells by binding to the CLEAR elements present in the promoter regions of autophagic and lysosomal genes [26]. At the same time, the transcription factor ZKSCAN3 functions as a negative regulator of lysosomal biogenesis and autophagy in fed cells [29]. Interestingly, the nuclear localization of both, TFE3 and ZKSCAN3, is directly regulated by mTORC1 [26,29]. Overall, the emerging picture reveals a close collaboration between mTORC1 and several master regulators of autophagy and lysosomal biogenesis to facilitate an efficient response to the varying energetic demands of the cell.

## Lysosomal activity

To maintain an efficient autophagic flux during starvation conditions, cells must couple formation and degradation of autophagosomes. For this, synthesis of autophagosomes ideally should be linked to increased autophagosome-lysosome fusion and increased lysosomal degradative activity. Recent evidence suggests that mTORC1 serves as a negative regulator of lysosomal function. Zhou and colleagues compared lysosomal function between fed and starved cells by measuring lysosomal acidification, cathepsin activity, and rate of proteolysis [30]. They found that the activity of lysosomes was significantly augmented in starved cells, a factor that is probably critical to ensure efficient degradation of the autophagic content. Increased lysosomal activity under starvation conditions required the inactivation of mTORC1, translocation of TFEB to the nucleus, and fusion of autophagosomes with lysosomes. Early investigations revealed that the inhibition of protein synthesis by cycloheximide (CHX) resulted in diminished lysosomal function [31]. Since CHX causes a dramatic increase in the amount of intracellular amino acids, it is possible that the observed effect was due to CHX-mediated activation of mTORC1. The requirement of autophagosome-lysosome fusion for efficient lysosomal degradation is very intriguing and suggests that autophagosomes might contribute important regulators of lysosomal activity. This idea is supported by the finding that TFEB-mediated lysosomal exocytosis is significantly reduced in autophagy-defective cells [32].

The current model suggests that mTORC1 regulates lysosomal function by directly preventing autophagy and TFEB activation. Under starvation, inactivation of mTORC1 releases the inhibition of the ULK1/ULK2 complex, thus promoting formation of autophagosomes. In addition, TFEB is free to translocate to the nucleus and upregulate the expression of critical regulators that further enhance autophagic flux. TFEB also activates expression of genes that directly increase lysosomal activity, including several subunits of the v-ATPase, lysosomal hydrolases,

and receptors required for the delivery of those hydrolases to lysosomes [33]. In addition, the inactivation of mTORC1 seems to be required to allow the fusion between autophagosomes and lysosomes. Choi and colleagues (2012) recently identified a synthetic compound, MHY1485, which inhibits lysosomal fusion during starvation-induced autophagy by directly binding and activating mTORC1 [34].

mTORC1 might also regulate lysosomal function by directly modulating the activity of key lysosomal proteins. The v-ATPase is a multisubunit proton pump composed of a V1 complex that catalyzes ATP hydrolysis and a transmembrane VO complex that rotated upon ATP hydrolysis. The best-characterized role of v-ATPase is to pump protons inside the lumen of the lysosome, thus promoting lysosomal acidification. However, recent evidence suggests a novel function of v-ATPase in nutrient sensing [14]. When the level of amino acids in the lumen of lysosomes is low, the v-ATPase interacts with the Ragulator and prevents the activation of Rag GTPases. In contrast, when amino acids are abundant, the v-ATPase undergoes conformational changes that release the guanine nucleotide exchange factor (GEF) activity of Ragulator, leading to the activation of Rag heterodimers and the recruitment of mTORC1 to lysosomes. Although it is still undetermined whether the v-ATPase is a direct sensor of amino acid levels or additional proteins are implicated in this process, it is clear that v-ATPase plays a critical role linking amino acid levels to mTORC1 activation. Open questions that remain unanswered include how the structural rearrangements undergone by the v-ATPase in different nutrient conditions affect lysosomal acidification and whether mTORC1 directly modulates v-ATPase activity as part of a regulatory feedback loop. In this regard, it was recently shown that mTORC1 is involved in controlling v-ATPase assembly in dendritic cells [35].

Another interesting possibility was recently suggested by Cang and colleagues [36]. Under nutrient- and ATP-rich conditions, mTORC1 binds and inhibits the activity of an endolysosomal ATP-sensitive $Na^+$ channel formed by the two-pore channels TPC1 and TPC2. When the levels of ATP are reduced, something that occurs under starvation and other stress conditions like hypoxia, ischemia, or hyperosmotic stress, mTORC1 redistributes to the cytosol, allowing opening of the channel and the release of $Na^+$ and other ions from the lumen of the lysosome to the cytosol. The depolarization of the lysosomal membrane may potentially affect many different lysosomal parameters, including the luminal pH, fusion of lysosomes with autophagosomes, or transport of nutrients.

## Lysosomal positioning

In the presence of amino acids and growth factor, lysosomes tend to localize closer to the plasma membrane. It has been suggested that lysosomal positioning may have an important impact on mTOR activity by regulating the proximity of the kinase to upstream signals. In contrast, the retrograde transport of lysosomes to the perinuclear area under starvation conditions is thought to be critical to facilitate fusion with autophagosomes. The Rubinsztein group recently used different approaches to alter the distribution of lysosomes within cells [37]. As expected, the localization of lysosomes in the periphery correlated with increased mTOR activity, whereas the inhibition of lysosomal scattering resulted in diminished mTOR activity and, consequently, increased number of autophagosomes. The Rubinsztein group observed that, under starvation conditions, certain proteins implicated in the anterograde movement of lysosomes, such as kinesin KIF2 (kinesin heavy chain member 2) and ARL8B (ADP-ribosylation factor-like 8B), dissociate from lysosomal membranes, thus facilitating the movement of lysosomes towards the center of the cell. The mechanism by which nutrients regulate lysosomal distribution is currently unknown, but, as mentioned in the previous section, it is possible that nutrient-dependent changes in lysosomal membrane potential or mTORC1 activity regulate the association of lysosomes with microtubules or specific motors.

## Lysosomal reformation

The catabolic activity of autophagy not only is critical to ensure cell survival when nutrients are limited but also regulates autophagy termination and lysosomal homeostasis. The Lenardo group found that, after long periods of starvation, mTORC1 is reactivated [38]. This is probably due to the degradation of the autophagosome content and consequent increase in the level of amino acids inside lysosomes. In agreement with this idea, the inhibition of lysosomal degradation was sufficient to suppress mTORC1 reactivation. Additional factors may contribute to regulate mTORC1 reactivation. For example, the depletion of spinster, a late endosomal/lysosomal sugar transporter, prevented mTORC1 reactivation and caused the accumulation of enlarged autolysosomes [39]. The role of spinster in lysosomal homeostasis was further supported by a recent study showing that this protein regulates fertility and fat content of lipid droplets in *Caenorhabditis elegans* by modulating lysosomal function and morphology [40]. Importantly, mTORC1 reactivation is required for the formation of nascent lysosomes from autophagosomes, a process known as autophagic lysosomal reformation (ALR). In this process, long and stable tubules emanate from autolysosomes and eventually pinch off to form

nascent lysosomes. Over time, these proto-lysosomes become acidic and acquire degradative capacity, thus becoming mature lysosomes. The mechanism that orchestrates ALR is very complex and requires the synthesis and accumulation of specific phosphatidylinositols in certain regions of the reformation tubules as well as recruitment of clathrin and clathrin adaptors AP2 and AP4 [41,42]. Although it is still unclear how mTORC1 reactivation promotes ALR, these findings reveal an important role of mTORC1 in the recycling of critical lysosomal components for which synthesis and transport are energetically demanding. Reactivation of mTORC1 may also halt or at least slow down autophagy, thus preventing autophagic cell death [43,44]. The participation of mTORC1 in ALR may seem contradictory with its inhibitory role in lysosomal biogenesis and function. However, these observations probably reflect a broader role of mTORC1 in coordinating autophagy termination. It is also worth mentioning that ALR was observed when autophagy was induced by serum and glutamine starvation. Further studies will have to determine whether ALR also occurs under other forms of starvation (for example, glucose or amino acid starvation).

The function of mTORC1 in lysosomal reformation is conserved during evolution. In yeast, TORC1 has been shown to mediate vacuolar fission (but not fusion) [45]. Under nutrient restriction, TORC1 inactivation could alter the fusion-fission equilibrium, resulting in an increase in the size and a reduction in the number of vacuolar (lysosomal) structures, as it has been observed in both yeast and mammalian cells.

mTORC1 activity is also critical for fission of phagosomes and entotic vesicles [46]. Internalization of pathogens or live cells by phagocytosis or entosis, respectively, results in the formation of large macroendocytic vacuoles that fuse with lysosomes. Upon degradation, nutrients and vacuolar components are recycled back to the lysosomal network by the mTORC1-regulated fission of the phagosome/entotic vesicle. Although this recycling closely resembles ALR, it is important to point out that there are important differences between the two processes. The two most remarkable are that the vesicles produced by phagosome and entotic vacuole fission contain luminal components and that inhibitors of mTORC1 do not completely block tubulation (although the vesicle shrinkage is significantly delayed). Overall, the aforementioned studies indicate an essential role of mTORC1 in lysosome reformation.

## Concluding remarks

mTORC1 stimulates important anabolic processes, such as protein synthesis and the accumulation of energy stores, whereas lysosomes are critical mediators of catabolism. Therefore, it is not surprising that the activities of mTORC1 and lysosomes are deeply interconnected. In the last few years, we have begun discerning the molecular mechanism that governs this connection. The emerging picture points to lysosomes as key regulators of nutrient signaling and energy homeostasis. However, many exciting questions still await clarification, including how the whole cell adapts to starvation conditions, the identification of novel regulators of the lysosomal/mTORC1 pathway, and the interplay between nutrient sensing and disease.

## Abbreviations

ALR, autophagic lysosomal reformation; CLEAR, Coordinated Lysosomal Expression and Regulation; CHX, cycloheximide; FLCN, folliculin; GAP, GTPase-activating protein; MITF, microphthalmia-associated transcription factor; mTORC1, mechanistic target of rapamycin (serine/threonine kinase) complex 1; RHEB, Ras homolog enriched in brain; TFE3, transcription factor binding to IGHM enhancer 3; TFEB, transcription factor EB; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2; ULK1, uncoordinated 51-like kinase 1; v-ATPase, vacuolar $H^+$-adenosine triphosphatase; ZKSCAN3, zinc finger with KRAB and SCAN domains 3.

## Disclosures

The author declares that she has no disclosures.

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