

The lysosome as a command-and-control center for cellular metabolism

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Lysosomes are membrane-bound organelles found in every eukaryotic cell. They are widely known as terminal catabolic stations that rid cells of waste products and scavenge metabolic building blocks that sustain essential biosynthetic reactions during starvation. In recent years, this classical view has been dramatically expanded by the discovery of new roles of the lysosome in nutrient sensing, transcriptional regulation, and metabolic homeostasis. These discoveries have elevated the lysosome to a decision-making center involved in the control of cellular growth and survival. Here we review these recently discovered properties of the lysosome, with a focus on how lysosomal signaling pathways respond to external and internal cues and how they ultimately enable metabolic homeostasis and cellular adaptation.

Introduction

“Lysosome” is a term originally coined by Christian de Duve in 1955 (de Duve, 2005) to describe a newly discovered organelle that housed a pool of soluble hydrolases capable of degrading proteins, nucleic acids, carbohydrates, lipids, and cellular debris. Because of these easily detectable activities, the lysosome quickly earned its reputation as the cell’s “trash can” or “recycle bin.” There are multiple routes via which lysosomes receive their substrates. In general, extracellular material destined for degradation is delivered to the lysosome via endocytosis (Luzio et al., 2009), whereas intracellular waste is disposed of by the lysosome via a self-catabolic process known as autophagy (Rabinowitz and White, 2010; Singh and Cuervo, 2011). These catabolic events occur in the highly acidic lumen (pH of ~4.5–5.0) of the lysosome, which is segregated by a single lipid bilayer from the cytoplasm. To maintain the steady acidic environment required for its internal hydrolytic activities, the lysosome constantly pumps in protons (H⁺ ions) across its limiting membrane by means of the vacuolar H⁺-ATPase (v-ATPase). This proton gradient also provides the driving force for the proton-coupled

transport of metabolites, ions, and soluble substrates into and out of the lysosomal lumen (Forgac, 2007) and is necessary for proper targeting of newly synthesized lysosomal enzymes from the Golgi to the lysosome. Dissipation of the transmembrane proton gradient results in inefficient cargo sorting, altered membrane traffic, impaired degradation of cellular waste, and eventually metabolic derangement (Saftig and Klumperman, 2009). In addition to its established role in cellular clearance, the lysosome engages in various biological processes including secretion, plasma membrane repair, immune response, cholesterol transport, and metal ion homeostasis, along with recently discovered roles in nutrient sensing and gene regulation (Fig. 1).

Multiple lines of evidence have highlighted a close link between lysosomal activities and metabolic regulation at the systemic level. For example, regulation of lysosomal biogenesis and function appears critical for the execution of lipid catabolic programs in the liver (Settembre et al., 2013b). Moreover, inactivating mutations in genes encoding for lysosomal hydrolases and transporters results in a spectrum of metabolic diseases known as lysosomal storage disorders (Futerman and van Meer, 2004; Platt et al., 2012; Parenti et al., 2015). Timely activation of autophagy in neonatal tissues is also necessary for the survival of organisms, as genetic manipulation of several genes involved in autophagy and lysosomal signaling leads to embryonic lethality in mice (Kuma et al., 2004; Komatsu et al., 2005; Efeyan et al., 2013). Yet, we still lack a complete knowledge of the structural and functional organization of the lysosome and the mechanisms that enable its communication with other cellular compartments. Moreover, we are only beginning to appreciate how lysosomal composition and function evolve dynamically both within a cell and across different organs and tissues, as organisms transition through different metabolic states. In this review, we summarize recent advances in our current understanding of the molecular mechanisms of lysosomal adaptation, and we discuss how the lysosome may be a key mediator of physiological responses to changing metabolic conditions.

The lysosome as a metabolic signaling center

To cope with ever-changing external conditions, cells have evolved sophisticated signaling pathways that sense available nutrient and energy inputs and couple them with specific metabolic outputs. Many of these pathways, such as insulin–phosphoinositide 3-kinase (PI3K), are organized in a

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Abbreviations used: CLEAR, coordinated lysosomal expression and regulation; CREB, cAMP-responsive element binding protein; EGFR, epidermal growth factor receptor; FOXO, forkhead box O; FXR, farnesoid X receptor; GAP, GTPase-activating protein; HLH, helix-loop-helix; MiT, microphthalmia; mTORC1, mechanistic target of rapamycin complex 1; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; RTK, receptor tyrosine kinase; TFE, transcription factor E; TFEB, transcription factor EB; TIR, Toll-IL-1 receptor; TLR, Toll-like receptor; TSC, tuberous sclerosis complex; v, vacuolar.

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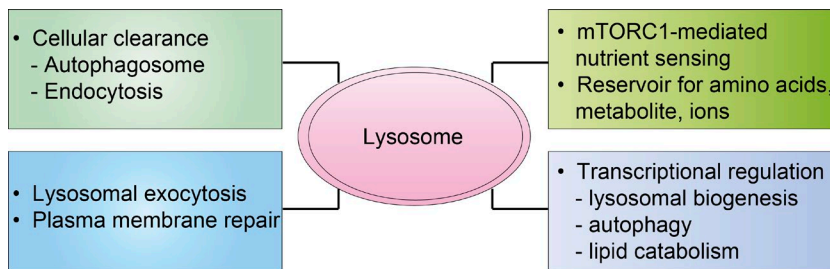


Figure 1. Expanding roles of the lysosome in key cellular processes. Lysosomes play pivotal roles in cellular clearance by engaging with either autophagosomes or late endosomes to facilitate the degradation and recycling of internal and external substrates. Upon plasma membrane injury, lysosomes can repair the damaged site by fusing locally with the plasma membrane. Specialized cell types such as cytotoxic T cells and natural killer cells are capable of secreting cytolytic proteins from lysosomes to destroy infected or tumorigenic cells. Furthermore, lysosomes act as a storage site where amino acids, phosphate, ions, and intermediate metabolites can be selectively transported and retained. Emerging evidence indicates that the lysosome functions as a signaling hub for nutrient-sensing pathways converging on the mTORC1 kinase and can elicit a transcriptional response to meet cellular demands for nutrients and energy.

“top-down” manner, as they involve the engagement of a growth factor ligand to its receptor on the cell surface, followed by signal propagation inside the cell (Taniguchi et al., 2006). Growth factor–derived signals trigger changes in the rate of biochemical reactions occurring in the cytoplasm and inside specialized compartments such as mitochondria, peroxisomes, and lysosomes, ultimately steering the cell toward an anabolic or catabolic path (Ward and Thompson, 2012). In contrast to pathways originating at the cell surface, little is known as to whether intracellular organelles are capable of initiating signaling events on their own, particularly in response to changing metabolic conditions, and to communicate their internal status to each other.

Because it represents the endpoint of multiple catabolic pathways, the lysosome also serves as a nutrient reservoir that buffers variations in nutrient availability and can actively modify the composition and abundance of the cytoplasmic metabolite pool. The key role of the lysosome in maintaining metabolic homeostasis emerged early on from studies in yeast, a model organism that offers two key advantages, namely the ability to easily isolate intact, functional vacuoles (the equivalent structure of the mammalian lysosome), coupled with powerful genetic approaches. It was found that the yeast vacuolar membrane hosts an array of nutrient transporters and permeases that allow bidirectional transport of solutes (Ohsumi and Anraku, 1981; Li and Kane, 2009). Metabolite transport across the vacuolar membrane is highly regulated and leads to the buildup of major stores of cationic amino acids, polyphosphates, ions, and other building blocks that can be subsequently released on demand. Because of the high conservation of lysosomal enzymes and permeases between yeast and mammals, it is likely that the mammalian lysosome has a similar ability for selective retention and release of metabolic building blocks. Through these processes, the lysosome not only can affect the rate of metabolic reactions occurring elsewhere in the cell, but also can communicate the overall metabolic state of the cell to nutrient-sensing modules. One such module is an ancient protein kinase known as the mechanistic target of rapamycin complex 1 (mTORC1), which has recently been shown to be functionally and physically associated with the lysosome from yeast to humans (Sancak et al., 2008, 2010; Sturgill et al., 2008; Binda et al., 2009; Zoncu et al., 2011a). Elucidating the connection between mTORC1 and the lysosome has brought about a paradigm shift in the way we understand lysosome biology.

Functional organization of the mTORC1 pathway at the lysosome

The mTORC1 pathway was identified because its core component, the large (230-kD) serine/threonine kinase mTOR, is the target of the growth-inhibiting macrolide rapamycin (Heitman et al., 1991; Brown et al., 1994; Sabatini et al., 1994). A vast body of research has shown that the main role of mTORC1 is to integrate environmental and intracellular cues, such as growth factors, nutrient availability, energy status, and stresses, to actively drive cell growth and proliferation (Laplanche and Sabatini, 2012; Dibble and Manning, 2013). Under favorable growth conditions, mTORC1 and its downstream effectors promote anabolic programs including mRNA translation, ribosome biogenesis, and lipid synthesis. Conversely, under stressful conditions, mTORC1 activities are largely inhibited to give way to catabolic programs such as autophagy, which allow mobilization of nutrient and energy stores.

mTORC1 is a multi-subunit protein kinase complex that, in addition to the core kinase mTOR, includes the large adaptor subunit RAPTOR (KOG1 in yeast), which is thought to mediate substrate binding (Hara et al., 2002; Kim et al., 2002) and subcellular localization of the complex (Sancak et al., 2008, 2010); two components, PRAS40 (Sancak et al., 2007) and DEPTOR (Peterson et al., 2009), that inhibit intrinsic mTOR kinase activity, and G protein β subunit–like mLST8 (Kim et al., 2003), whose function remains obscure (Guertin et al., 2006). Multiple metabolic inputs including amino acids, glucose, and growth factors control mTORC1 via distinct mechanisms (Jewell and Guan, 2013; Shimobayashi and Hall, 2014; Efeyan et al., 2015), albeit to varying degrees, as none of them alone can fully stimulate mTORC1 on its own. In nutrient-starved mammalian cells, mTORC1 is diffuse throughout the cytoplasm. Readdition of nutrients, particularly amino acids, causes the rapid translocation of mTORC1 to the surface of the lysosome. At the lysosome, the kinase activity of mTORC1 is turned on in a growth factor–dependent manner (Sancak et al., 2008, 2010; Zoncu et al., 2011a). Thus, the prevailing model is that of coincidence detection: for mTORC1 to become fully activated, both local nutrients (particularly amino acids) and long-range nutritional signals carried by insulin must be present (Sancak et al., 2008, 2010; Zoncu et al., 2011a).

These discoveries established a key role for the lysosome in nutrient sensing, as no other organelle is able to support mTORC1 recruitment and activation. The lysosome membrane harbors specialized molecular machinery that recruits and activates mTORC1 in response to amino acids (Sancak et al., 2010), and several components of this machinery are conserved

all the way to yeast (Chantranupong et al., 2015). However, in yeast, mTORC1 remains stably associated with the vacuolar membrane even when amino acids are low (Binda et al., 2009; Péli-Gulli et al., 2015). This important difference may reflect the more complex cellular organization of higher eukaryotes. For instance, budding yeast lacks a canonical insulin-PI3K pathway, thus negating the need for a coincidence detection mechanism (Efeyan et al., 2012).

mTORC1 activation by the PI3K-RHEB axis

The small GTPase Ras homologue enriched in brain (RHEB) contains a C-terminal farnesylation motif that mediates its association with the endomembrane system, including, but not limited to, the lysosome, where it serves as a potent activator of mTORC1 kinase activity (Inoki et al., 2003b; Saucedo et al., 2003; Stocker et al., 2003; Zhang et al., 2003). Rheb is indispensable for mTORC1 activation by virtually all stimuli. However, exactly how Rheb turns on mTORC1 is yet to be resolved.

Because of its intrinsically slow GTP hydrolysis activity, Rheb is preferentially in its GTP-bound form at all times and therefore has to be kept under stringent regulation by its inhibitor, the trimeric tuberous sclerosis complex (TSC, composed of TSC1, TSC2, and TBC1D7 subunits; Inoki et al., 2003b; Zhang et al., 2003; Dibble et al., 2012). Specifically, the TSC2 component displays GTPase-activating protein (GAP) activity that converts Rheb into its GDP-bound state and therefore negatively regulates mTORC1 (Inoki et al., 2003b; Zhang et al., 2003). Upon stimulation by growth factors such as insulin, the serine/threonine kinase AKT is activated in a PI3K-dependent manner and phosphorylates TSC2 (Dan et al., 2002; Inoki et al., 2002). AKT-dependent TSC2 phosphorylation induces dissociation of TSC from the lysosome, where TSC was shown to reside in growth factor-deprived conditions, and thus blocks its inhibitory effects toward Rheb (Demetriades et al., 2014, 2016; Menon et al., 2014). In addition to growth factors, the activity of TSC is regulated by low energy (Inoki et al., 2003a), hypoxia (Brugarolas et al., 2004; Reiling and Hafen, 2004), and genotoxic stress (Budanov and Karin, 2008), which collectively restrict mTORC1-mediated cell growth. Thus, TSC is one, but not the only, integration node for multiple signals that ultimately affect the kinase output of mTORC1.

mTORC1 recruitment by Rag GTPases

To be activated, mTORC1 needs to translocate to the lysosome membrane where Rheb resides. It turns out that amino acids directly regulate the lysosomal recruitment of mTORC1 by modulating the guanine nucleotide state of the Rag GTPases (Kim et al., 2008; Sancak et al., 2008; Binda et al., 2009). The Rags assemble as obligate heterodimers composed of RagA or RagB (which are similar to each other and homologous to Gtr1 in *Saccharomyces cerevisiae*) associated with RagC or RagD (homologous to yeast Gtr2) and are tethered to the lysosomal membrane by the pentameric Ragulator complex (known as the Ego complex in yeast), composed of the LAMTOR1-5 proteins (Binda et al., 2009; Nada et al., 2009; Sancak et al., 2010; Bar-Peled et al., 2012; Powis et al., 2015). Under amino acid sufficiency, the Rag GTPase complex becomes active by adopting a nucleotide state in which Rag-A/B is GTP-loaded and Rag-C/D is GDP-loaded and facilitates the lysosomal attachment of mTORC1 by directly interacting with Raptor (Sancak et al., 2010; Bar-Peled et al., 2012). As such, the cycling of Rag heterodimers between their active and inactive states in an amino

acid-sensitive fashion is tightly regulated by their corresponding GAPs and guanine nucleotide exchange factors (GEFs), as well as posttranslational modifications (Table 1). Specifically, Ragulator acts as a GEF that promotes the loading of Rag-A/B with GTP and thus activates mTORC1 (Binda et al., 2009; Bar-Peled et al., 2012). Two GAP complexes stimulate GTP hydrolysis. Under low amino acids, GATOR1 (SEACIT in yeast) promotes GTP hydrolysis by Rag-A/B, thus switching off the pathway (Dokudovskaya and Rout, 2011; Bar-Peled et al., 2013; Panchaud et al., 2013). In contrast, in the presence of amino acids, Folliculin/FNIP (yeast Lst4/Lst7) causes Rag-C/D to become GDP loaded, enabling the Rags to bind to mTORC1 and recruit it to the lysosome (Tsun et al., 2013; Péli-Gulli et al., 2015). The placement of the Rag GTPases downstream of amino acids in the mTORC1 pathway has provided important clues toward the long-sought-after questions of how and where amino acids are sensed in the cell.

Amino acid sensing inside the lysosome

It was initially proposed that plasma membrane amino acid transporters be potential candidates for amino acid sensors because of their roles in controlling the influx of amino acids into the cell (Christie et al., 2002; Beugnet et al., 2003). However, treatment with cycloheximide, a protein synthesis blocker that increases the concentration of free amino acids in the cytoplasm, is sufficient to restore mTORC1 signaling in cells that have been deprived of extracellular amino acids. This evidence strongly suggests that amino acid sensing should originate intracellularly (Price et al., 1989; Christie et al., 2002; Beugnet et al., 2003; Sancak et al., 2008). The presence of the molecular machinery for amino acid-regulated mTORC1 activation at the lysosome membrane also implies that amino acids may be sensed somewhere in close proximity to the lysosome. Similar to the vacuole in yeast, the lysosome appears to accumulate significant amounts of amino acids within its lumen (Harms et al., 1981; Zoncu et al., 2011b). Using a cell-free assay, it was shown that binding of mTORC1 to the Rag GTPases is stimulated by entry of amino acids into the lysosomal lumen. Conversely, both in vitro and in cells, preventing lysosomal amino acid accumulation blocked mTORC1 binding to the lysosomal surface (Zoncu et al., 2011b; Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015a). These results are compatible with a model for amino acid sensing by mTORC1 in which accumulation of amino acids in the lysosomal lumen is relayed to the Rag GTPases at the lysosomal surface in an inside-out manner.

An RNAi screening in *Drosophila melanogaster* S2 cells revealed that the v-ATPase is a component of the lysosomal amino acid sensing machinery along with the Rag GTPases and Ragulator (Zoncu et al., 2011b). The v-ATPase forms a supercomplex with Ragulator and the Rag GTPases, and its catalytic activity is essential for mTORC1 recruitment in response to amino acids (Zoncu et al., 2011b; Bar-Peled et al., 2012; Dechant et al., 2014; Jewell et al., 2015). Although the precise mechanism of action of the v-ATPase in amino acid sensing remains to be elucidated, an attractive possibility is that amino acids may regulate the assembly and/or activity of the complex (Stransky and Forgac, 2015).

Among the 20 amino acids, leucine and arginine are key activators of mTORC1 (Hara et al., 1998; Wang et al., 1998) upstream of the Rag GTPases (Sancak et al., 2008). Of note, arginine, an amino acid crucial for mammalian embryogenesis and early development, is highly concentrated in rat liver lysosomes

and yeast vacuoles (Wiemken and Dürr, 1974; Boller et al., 1975; Dürr et al., 1979; Harms et al., 1981; Kitamoto et al., 1988). SLC38A9, a putative sodium-coupled amino acid transporter in the lysosome membrane, recently has been proposed as a sensor that signals arginine sufficiency to mTORC1 (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015a). Biochemical analysis demonstrated that SLC38A9 acts upstream of the Rag GTPases and Ragulator and in parallel with the v-ATPase. In amino acid transport assays using reconstituted liposomes, SLC38A9 transports arginine, but not leucine, into the lysosome, albeit with relatively low affinity compared with other amino acid transporters. Overexpression of the N-terminal cytoplasmic domain of SLC38A9 is sufficient to render the mTORC1 signaling resistant to amino acid depletion (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015a), suggesting that this domain acts downstream of the amino acid transport function. Thus, SLC38A9 may function as a “transceptor” that, by transporting arginine across the lysosomal membrane, relays an activating signal toward mTORC1. How arginine binding mechanistically regulates SLC38A9 remains to be determined. Also, the functional relationship between SLC38A9 and the v-ATPase is unclear. Interestingly, deleting SLC38A9 reduces mTORC1 substrate phosphorylation but not its localization to the lysosome (Jung et al., 2015; Rebsamen et al., 2015), whereas v-ATPase inhibition affects both (Zoncu et al., 2011b).

Other lysosomal amino acid transporters implicated in mTORC1 activation include a histidine transporter, SLC15A4 (Kobayashi et al., 2014), as well as proton-assisted amino acid transporter 1 (PAT1)/SLC36A1 (Ögmundsdóttir et al., 2012), which has transport specificity toward small neutral amino acids. Whether and how SLC15A4 and PAT1 function upstream of the Rag GTPases remains to be determined.

Glutamine, the most abundant free amino acid in the human body, provides a carbon and nitrogen source for cell growth. On one hand, several studies indicated that glutamine and glutamine-derived metabolites appear to function upstream of the Rag GTPase orthologues, Gtr1 and 2 (Binda et al., 2009;

Durán et al., 2012; Péli-Gulli et al., 2015). On the other hand, it was shown that glutamine can stimulate lysosomal translocation and activation of mTORC1 via a Rag GTPase-independent mechanism, as revealed in recent studies using yeast (Stracka et al., 2014) and Rag-A/B deleted cells (Jewell et al., 2015). Interestingly, stimulation of mTORC1 by glutamine does not require Ragulator, but still relies on the lysosome and the activity of the v-ATPase. Moreover, ADP-ribosylation factor 1 (ARF1), a Golgi-localized small GTPase, is required in an undefined pathway that links glutamine to mTORC1 localization at the lysosome (Jewell et al., 2015). Further investigations are necessary to determine the location of the mTORC1-activating glutamine pool and to establish the glutamine sensor upstream of Arf1.

Amino acid sensing in the cytoplasm

Recent evidence indicates that cytosolic free amino acids also play a major role in mTORC1 activation. Sestrin-2, a member of the Sestrin family of stress-responsive proteins (Budanov and Karin, 2008), has been shown to be a specific sensor for leucine in mammalian cells (Saxton et al., 2016; Wolfson et al., 2016). Under leucine deprivation, Sestrin-2 inhibits mTORC1 signaling by sequestering GATOR2, which represses the GAP activity of GATOR1 toward Rag-A/B. The crystal structure of Sestrin-2 revealed that Sestrin-2 contains a leucine-binding pocket localized to its C-terminal domain (Kim et al., 2015; Saxton et al., 2016). Binding of leucine disrupts the Sestrin2–GATOR2 interaction and thus allows GATOR2 to promote mTORC1 activation via inhibition of GATOR1 activity. Mutations of Sestrin-2 that abolish binding to GATOR2 lead to constitutive activation of mTORC1 in the absence of leucine, whereas those that diminish binding to leucine suppress mTORC1 activation regardless of leucine availability.

In contrast, a newly characterized vertebrate-specific protein named CASTOR1 (previously named GATSL3) functions as a cytoplasmic arginine sensor for mTORC1 by binding to physiological concentrations of arginine through the conserved ACT domains (Chantranupong et al., 2016). The CAS

Table 1. Regulators of the RAG GTPases involved in mediating amino acid signaling to mTORC1

| Yeast orthologues | Mammalian orthologues | Component | Function | mTORC1 activation | References |
|--|-----------------------|---|--|-------------------|--|
| EGO complex (Ego1-Ego2-Ego3); VAM6 | Ragulator | LAMTOR1 (p18), LAMTOR2 (p14), LAMTOR3 (MP1), LAMTOR4 (C7orf59), LAMTOR5 (HBXIP) | GEF for Rag-A/B; tethers the Rags to the lysosome | Up | Binda et al., 2009; Nada et al., 2009; Sancak et al., 2010; Bar-Peled et al., 2012; Powis et al., 2015 |
| SEACIT (Sea1, Npr2, Npr3) | GATOR1 | DEPDC5, Npr12, Npr13 | GAP for Rag-A/B | Down | Dokudovskaya and Rout, 2011; Bar-Peled et al., 2013; Panchaud et al., 2013 |
| SEACAT (Sea4, Seh1, Sea2, Sea3, Sec13) | GATOR2 | Mios, Seh1L, WDR24, WDR59, Sec13 | Inhibits GATOR1 | Up | Dokudovskaya et al., 2011; Bar-Peled et al., 2013; Panchaud et al., 2013 |
| Lst4/Lst7 | FOLLICULIN | FNIP1/2-FLCN | GAP for Rag-C/D | Up | Petit et al., 2013; Tsun et al., 2013; Péli-Gulli et al., 2015 |
| | SH3BP4 | SH3BP4 | Inhibits Rag-A/B through direct binding | Down | Kim et al., 2012 |
| | RNF152 | RNF152 | Ubiquitinates (K63) RagA, promotes interaction between RagA and GATOR1 | Down | Deng et al., 2015 |
| | SKP2 | SKP2 | Ubiquitinates (K63) RagA, promotes interaction between RagA and GATOR1 | Down | Jin et al., 2015 |

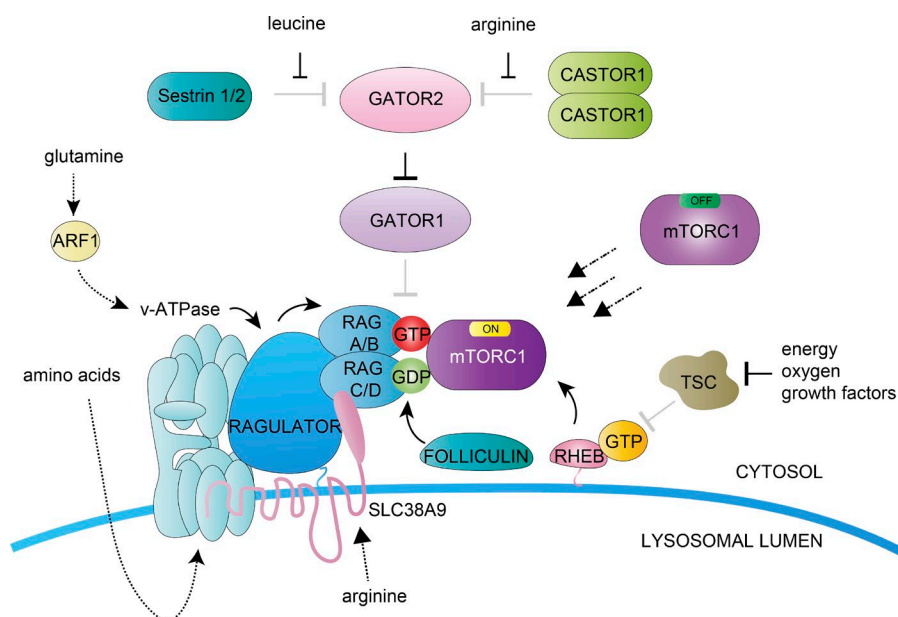


Figure 2. The lysosome as a signaling hub for nutrient sensing. Under growth-promoting conditions, signals from amino acids, energy, oxygen, and growth factors are integrated upstream of the Rag and Rheb GTPases to facilitate the recruitment and activation of mTORC1. Loss of any of these inputs leads to shutdown of mTORC1 signaling by blocking its lysosomal recruitment, kinase activation, or both.

TOR1-dependent mechanism of arginine sensing is highly analogous to leucine sensing by Sestrin-2. Under arginine deprivation, homodimers of CASTOR1, or heterodimers of CASTOR1 and CASTOR2, bind tightly to GATOR2. Refeeding of arginine liberates GATOR2 from this inhibitory interaction, thereby promoting mTORC1 activation. Additional work elucidating the structure and function of GATOR2 is required to clarify whether the inhibitory actions of Sestrin-2 and CASTOR1 toward GATOR2 operate through similar or distinct mechanisms. The presence of both cytoplasmic and lysosomal amino acid sensing systems raises intriguing questions about their relative importance and the mechanisms that coordinate their activities upstream of mTORC1.

Additional proteins have been proposed to play a role in relaying an amino acid signal to mTORC1, including the Ste20 family kinase MAP4K3 (Findlay et al., 2007; Bryk et al., 2010; Yan et al., 2010), leucyl-tRNA synthetase (Bonfils et al., 2012; Han et al., 2012), the scaffold protein SH3BP4 (Kim et al., 2012), and the autophagic adaptor p62/SQSTM1 (Duran et al., 2011). Further work is needed to determine the specific role of each protein and how their regulatory inputs are coordinated upstream of mTORC1.

In summary, the aforementioned discoveries are illuminating the pivotal roles of the lysosome in regulating the switch between catabolic and anabolic metabolism and foster a unifying model of nutrient sensing by which all the signals from intracellular nutrients and exogenous growth factors are integrated at the lysosomal surface (Fig. 2). This model likely undergoes variations between species or even among different organs and tissues of multicellular organisms. Interestingly, *S. cerevisiae* lacks Sestrin and CASTOR homologues, suggesting that yeast mTORC1 may preferentially sense amino acids in the vacuole (the main cellular repository for these metabolites) or that it may be more concerned with nitrogen abundance than with the levels of any specific amino acid (Bahn et al., 2007).

Also, any proteins or small molecules able to interact with and regulate the mTORC1-activating supercomplex may provide additional regulatory mechanisms. Thus, it is conceivable that additional nutrient inputs upstream of mTORC1 wait to be discovered.

Transcriptional regulation of lysosomal function

For a long time, the lysosome was thought of as a metabolic “dead end,” a static compartment that, unlike mitochondria or peroxisomes, is not subjected to feedback regulation by the nutrient state of the cell. This view has been radically altered by the recent discovery of a vast and coordinated transcriptional program controlled by the transcription factor EB (TFEB), along with other members of the microphthalmia-transcription factor E (Mit/TFE) subfamily (Rehli et al., 1999; Sardiello et al., 2009; Settembre et al., 2011).

These basic helix-loop-helix (HLH) transcription factors up-regulate the expression of genes encoding for lysosomal and autophagic proteins by preferentially binding to a 10-bp GTC ACGTGAC motif found within their promoters and termed coordinated lysosomal expression and regulation (CLEAR) element (Sardiello et al., 2009; Palmieri et al., 2011; Settembre et al., 2011). The nutrient status of the cell, along with other environmental cues, tightly controls the expression of the CLEAR network through the cytoplasmic-nuclear shuttling of TFEB. Under nutrient-rich conditions, TFEB is recruited to the lysosomes via its physical interaction with the Rag GTPases. At the lysosomal surface, mTORC1 phosphorylates TFEB on two critical residues, Ser142 and Ser211. Phosphorylated TFEB is then retained in the cytoplasm via binding to 14-3-3 proteins (Martina et al., 2012; Rocznik-Ferguson et al., 2012; Settembre et al., 2012; Martina and Puertollano, 2013). Upon nutrient withdrawal or lysosomal stress, TFEB undergoes dephosphorylation and rapidly translocates to the nucleus to activate the transcription of CLEAR genes, including lysosomal hydrolases, pumps, and permeases, along with autophagic regulatory proteins. Thus, the net effect of TFEB activation is an increase in autophagic flux matched by an expansion of the lysosomal compartment, thereby boosting the ability of the cell to adapt to nutrient-poor and stressful conditions.

Calcineurin, a calcium-dependent phosphatase, is responsible for TFEB dephosphorylation on Ser142 and Ser211 and thus promotes TFEB entry into the nucleus (Medina et al., 2015). Interestingly, it is thought that the lysosomal calcium pool controls calcineurin activation and TFEB dephosphorylation.

Non-mTORC1-related signaling functions of the lysosome

In addition to regulating mTORC1, the lysosome plays an important role in other major signaling pathways by mediating either the breakdown of activated receptors for signal termination or the proteolytic activation of signaling ligands. Two notable examples are discussed here:

(1) In canonical receptor tyrosine kinase (RTK) pathways, binding of the epidermal growth factor (EGF) to its receptor EGFR/ErbB1 at the cell surface triggers receptor dimerization and cross-phosphorylation of the EGFR intracellular kinase domains; phosphorylated domains then recruit and activate effectors such as the small GTPase Ras and the lipid kinase PI3K. Activation of these effectors is essential for the propagation of the EGF-initiated signal to a set of protein kinases such as the MAPK/ERK kinases, which convert the initial ligand–receptor binding into a mitogenic response (Avraham and Yarden, 2011; Tomas et al., 2014). The degradation of activated EGFR in the lysosome is a key step for termination of this highly mitogenic signal. Phosphorylated EGFR triggers its own ubiquitination by the E3 ligase Cbl; endocytic adaptors containing ubiquitin-interacting motif, such as epsin and Eps15, recognize ubiquitinated EGFR and promote its internalization into endocytic vesicles (Tomas et al., 2014). Internalized EGFR is then trafficked via Rab5-positive early endosomes to Rab7-positive late endosomes and progressively removed from the endosomal-limiting membrane via ESCRT-mediated budding of intraluminal vesicles. Through further rounds of fusion, these EGFR-loaded late endosomes then convert into mature lysosomes, wherein cathepsin proteases and lipases degrade the EGFR-loaded intraluminal vesicles (Tomas et al., 2014). The key role of this lysosome-based degradative pathway in signal down-regulation is highlighted by the presence of inactivating mutations of Cbl in various malignancies (Makishima et al., 2009; Tan et al., 2010), resulting in constitutive EGFR signaling at the plasma membrane. Moreover, this mode of regulation is shared by other RTKs, including platelet-derived growth factor receptor and insulin-like growth factor receptor. Thus, in the context of RTK signaling, the lysosomal lumen functions primarily as an endpoint for signal down-regulation, thereby playing an important role in limiting the mitogenic effect of EGFR.

(2) The lysosome is also an important signaling station for innate immunity. This is an ancient pathogen-defense system in which specialized pattern recognition receptors (PRRs) recognize and bind to molecular signatures known as pathogen-associated molecular patterns shared by several classes of pathogens. A prominent class of PRRs is the Toll-like receptor (TLR) family (O'Neill et al., 2013). TLRs consist of leucine-rich repeat motifs in an antigen-binding ectodomain, a single pass transmembrane portion, and an intracellular Toll-IL-1 receptor (TIR) domain. Upon binding of the ectodomain to microbial ligands such as viral or bacterial proteins and nucleic acids, the ectodomain undergoes a conformational change that leads to the recruitment of specific adaptor proteins to the TIR. This binding event initiates a signaling cascade that mounts several anti-pathogen responses, including secretion of inflammatory cytokines and anti-microbial peptides, as well as activation of dendritic cells. Of the 13 known TLRs, TLR 3, 7, 8, and 9 localize to the endolysosomal compartment, with their ectodomain protruding into the lumen and the TIR facing the cytoplasm. These TLRs specialize in recognizing nucleic acids, which are released from invading pathogens that were taken up in intracellular compartments. Localization of TLRs to endocytic compartments is thought to prevent them from recognizing “self” nucleic acids and thus mounting an autoimmune response. Moreover, full activation of these TLRs requires proteolytic processing of their ectodomain by proteases such as asparagine endopeptidases and cathepsins in the acidic lumen of the lysosome (Lee and Barton, 2014). After ligand binding and activation, TLR3 and TLR9 recruit adaptor proteins, such as TRIF and MyD88, respectively, to their TIR, triggering parallel signaling cascades that culminate with the activation of transcription factors and the release of inflammatory cytokines and interferons. Thus, in the context of innate immunity, the lysosomal lumen provides an ideal environment in which TLRs become fully activated and where they bind to their respective ligands, whereas the cytoplasmic face provides a platform for the recruitment of secondary effectors that propagate the pathogen-initiated signal all the way to the nucleus.

Starvation triggers release of calcium from the lysosomal lumen via MCOLN1/TRPML1, a multispans lysosomal ion transporter, and inhibition of MCOLN1 by genetic inactivation and

pharmacological approaches impaired TFEB nuclear translocation and autophagy induction (Medina et al., 2015; Wang et al., 2015b). MITF and TFE3, which are also members of the MiT/TFE family, can form homodimers or heterodimers with TFEB and are regulated by similar mechanisms (Roczniak-Ferguson et al., 2012; Martina and Puertollano, 2013). How starvation increases MCOLN1-mediated lysosomal Ca^{2+} release remains elusive. A recent study (Wang et al., 2015b) has suggested that nutrient-induced changes in the levels of phosphatidylinositol-(3,5)-bisphosphate, a known activator of TRPML1, may be involved in this process.

Phosphoproteomic studies revealed that TFEB possesses multiple sites of phosphorylation (Dephoure et al., 2008; Peña-Llopis et al., 2011; Settembre et al., 2011; Ferron et al., 2013), suggesting that mTORC1-independent signaling pathways could also modulate the nuclear translocation of TFEB. Consistently, the ERK2 kinase phosphorylates TFEB at serine 142 in response to growth factor stimulation and restricts its nuclear localization (Settembre et al., 2011). MITF is also subjected to phosphorylation by c-kit and WNT signaling on the sites that are conserved in TFEB and TFE3 (Wu et al., 2000; Ploper et al., 2015). All these findings indicate that the phosphorylation-dependent regulation of TFEB represents a universal mechanism of lysosomal adaptation to combat cellular stresses.

TFEB regulation and function are evolutionarily conserved from nematodes to humans, and at the organism level TFEB-driven transcriptional responses mediate important physiological processes such as lipid catabolism, longevity, and organismal survival. Experimental evidence indicates that TFEB expression is up-regulated in mice after food deprivation or energy expenditure (Settembre et al., 2013b; Medina et al., 2015). Overexpression of TFEB in mouse liver attenuated diet-induced obesity by promoting lipid catabolism. In contrast, lipid degradation pathways were impaired in hepatocytes from liver-specific TFEB knockout mice (Settembre et al., 2013b). These liver-specific functions of TFEB result from its ability to activate a transcriptional program for lipid catabolism through direct up-regulation of peroxisome proliferator-activated receptor α (PPAR α) and peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC1 α), which are key regulators of lipid breakdown in response to starvation. Thus, TFEB-mediated programs allow the organism to derive energy from the stored lipids, linking lysosomal function to the maintenance of cellular energy balance (Rabinowitz and White, 2010; Singh and Cuervo, 2011).

A TFEB-mediated adaptive response could also contribute to the extended lifespan seen in the nematode *Caenorhabditis elegans*, in which the TFEB homologue, known as HLH-30, acts similarly to its human counterpart to promote lipid mobilization and autophagy in fasting worms (Kaeberlein et al., 2006; Lapierre et al., 2013; O'Rourke and Ruvkun, 2013), whereas loss of HLH-30 diminishes the starvation-induced lifespan extension (Settembre et al., 2013b). Further investigation is required to determine whether modulating the expression and activity of TFEB would impact the lifespan of higher organisms. Of note, the capability of TFEB to promote cellular clearance could also be exploited to develop novel therapeutics for diseases associated with lysosomal and autophagic dysfunction such as lysosomal storage diseases (Settembre et al., 2013a; Spanpanato et al., 2013; Lim et al., 2015) and common neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's diseases. It was observed that TFEB activation by

overexpression or pharmacological stimulation can attenuate protein aggregation in cellular and mouse models of neurodegenerative disease, likely through increased autophagic clearance of protein aggregates (Polito et al., 2014; Xiao et al., 2014, 2015; Chauhan et al., 2015).

At the organismal level, elimination of the TFEB gene in mouse is embryonically lethal because of defects in placental vascularization (Steingrímsson et al., 1998). Prosurvival effects of autophagy elicited by TFEB may also induce metabolic reprogramming that favors cancer growth, for example, by deliberately accumulating nutrients such as amino acids in lysosomes. One notable example is human pancreatic ductal adenocarcinoma, which displays increased number of lysosomes and enlarged autophagosomes as a result of constitutive nuclear localization and activation of MiT/TFE transcription factors, which are decoupled from mTORC1 regulation (Perera et al., 2015). Moreover, gene fusions involving TFE3 or TFEB have been identified in patients with sporadic renal cell carcinoma (Komai et al., 2009; Mosquera et al., 2011; Zhong et al., 2012). A feature common to all TFE3 and TFEB fusion proteins is the retention of the wild-type protein C-terminus that is required for DNA binding, dimerization, and nuclear localization. How the translocated TFE3 and TFEB genes contribute to renal carcinogenesis remains poorly understood. A detailed investigation of the transcriptional programs that become constitutively activated will shed light on this important question (Kauffman et al., 2014; Magers et al., 2015).

The MiT/TFE proteins are prominent members of a rapidly expanding group of transcription factors involved in autophagy-lysosome gene regulation. Emerging evidence has also suggested a key role for forkhead box O (FOXO) transcription factor family in the regulation of autophagy (Webb and Brunet, 2014). Insulin and growth factor signaling negatively regulate FOXO transcriptional activity through AKT/SGK1-dependent phosphorylation, leading to FOXO exclusion from the nucleus and inhibition of its transcriptional activity (Biggs et al., 1999; Brunet et al., 1999, 2001; Kops et al., 1999). During starvation, when insulin and growth factors are absent, FOXO translocates into the nucleus and activates the expression of genes involved in stress response, metabolism, and cellular quality control (Calnan and Brunet, 2008). It was shown that FOXO3 is required for fasting-induced autophagy in muscles (Mammucari et al., 2007; Zhao et al., 2007). Overexpression of FOXO3 is sufficient to induce autophagosome, as revealed by increased foci of LC3-GFP in C2C12-derived myotubes and primary mouse myofibers, whereas knockdown of FOXO3 leads to decreased autophagosome formation (Mammucari et al., 2007; Zhao et al., 2007). Chromatin immunoprecipitation analysis in mouse muscle cells demonstrated that FOXO3 directly binds to the promoters of key autophagy genes including *LC3b*, *Gabap11*, *Atg12l*, *Bnip3*, and *Bnip3l* (Mammucari et al., 2007; Zhao et al., 2007). Therefore, FOXO3 may synergize with TFEB to maintain muscle functionality during fasting.

Just as turning on autophagy and lysosomal biogenesis in response to nutrient scarcity is critical for cellular survival, turning off these processes is equally important, as it allows cells to readjust their metabolic requirements when nutrients are replete. ZKSCAN3, a zinc finger transcription factor containing KRAB and SCAN domains previously identified as a “driver” of cell proliferation (Ma et al., 2007; Yang et al., 2008), has been proposed as a master transcriptional repressor of autophagy (Chauhan et al., 2013). ZKSCAN3 directly represses the

expression of a repertoire of genes involved in sequential steps of autophagic process ranging from lysosome biogenesis to trafficking and autophagosome-lysosome fusion. Similar to TFEB, ZKSCAN3 activity is regulated by nuclear-cytoplasmic shuttling but in the opposite way. Nutrient deprivation or mTORC1 inhibition triggers cytoplasmic localization of ZKSCAN3 and silences its activity, whereas nutrient-rich conditions promote nuclear translocation of ZKSCAN3, leading to suppression of the autophagic response (Chauhan et al., 2013). Hence, by switching on and off multiple components of the autophagy-lysosome system through reciprocal regulation of TFEB and ZKSCAN3, the lysosome exerts its adaptation to meet metabolic demands according to nutrient levels.

Farnesoid X receptor (FXR), which is a bile acid-activated nuclear receptor involved in regulation of bile acid, lipid, and glucose homeostasis, has also been shown to negatively regulate autophagy in the liver through multiple mechanisms (Lee et al., 2014; Seok et al., 2014). In the fed state, FXR competes with PPAR α for a common binding site in the promoter regions of key autophagy genes, resulting in their repression (Lee et al., 2014). Interestingly, mTORC1 also attenuates PPAR α activity by promoting nuclear translocation of nuclear receptor corepressor 1, which is a transcriptional repressor of PPAR α (Sengupta et al., 2010). Furthermore, in fed mice, FXR blocks the transcriptional activity of cAMP-responsive element binding protein (CREB) by disrupting the functional interaction between CREB and its coactivator CRTC2. Because CREB promotes the expression of TFEB and other autophagic regulators, decreased assembly of CREB/CRTC2 complex suppresses catabolism (Seok et al., 2014). Conversely, under fasting conditions, FXR becomes inactive, thus leading to de-repression of the transcriptional activity of TFEB, PPAR α , and the CREB–CRTC2 complex. Together, these studies establish a mechanistic link between nutrient-sensing transcription factors/nuclear receptors and the regulation of autophagy and lysosomal function and delineate an integrated regulatory network for metabolic adaptation of increasing complexity.

Concluding remarks

The discovery of the lysosome-centric signaling networks for nutrient sensing and metabolic adaptation described herein has projected this organelle into the pilot’s seat of cellular physiology. Clearly, more studies are required to achieve a comprehensive understanding of how the lysosome’s many parts interact under various physiological and pathological conditions. Increasing evidence also suggests that this organelle may constantly communicate with other cellular structures to carry out specific metabolic programs. For instance, a contact site between the yeast mitochondria and vacuole named vCLA MP (vacuole and mitochondria patch) provides an alternative route to phospholipid transfer to the conventional route via mitochondria–endoplasmic reticulum contacts, and thus participates in mitochondria biogenesis (Elbaz-Alon et al., 2014; Hönscher et al., 2014).

From a cell biological standpoint, studying lysosomal organization and plasticity will answer longstanding questions regarding the functional diversity of lysosomes in different tissues and organs, which is mediated by tissue- and cell-type specific gene expression but is also likely influenced by local metabolic conditions and age. Deciphering the molecular basis that determines the differences in lysosomal composition and function will help us understand how the lysosome acquires

specialized functions to carry out specific metabolic tasks. A good example is provided by lysosome-related organelles known as melanosomes, which specialize in the synthesis and storage of melanin pigment (Raposo and Marks, 2002, 2007). The molecular composition of melanosomes changes through sequential and well-defined stages of maturation (Raposo and Marks, 2002, 2007). Moreover, melanosomes function differently according to cell type. In retinal pigment epithelial cells, melanosomes help detoxify phagocytosed photoreceptor outer membranes, whereas in epidermal melanocytes, melanosomes contribute to generation of the pigmentation of skin and hair by supplying melanins to neighboring keratinocytes (Dell'Angelica, 2003). Importantly, loss of ability to synthesize pigments and disorganization of melanosomal structures are associated with development of malignant melanoma. Hence, functional characterization of the molecular components of melanosomes throughout different stages of maturation and across cell types will not only provide insights into how they deviate from conventional lysosomes, but also help unravel the pathogenesis of melanoma.

A further emerging aspect is the heterogeneity of lysosomes within a cell. Lysosomes appear to have different abilities to internally acidify and, potentially, to generate metabolic signals (Korolchuk et al., 2011; Johnson et al., 2016). These intrinsic differences may stem from the positioning of lysosomes within cells, which is controlled by specialized protein complexes at the lysosomal surface and by the activity of lysosomal ion channels (Pu et al., 2015; Li et al., 2016).

With rapid advances in genomic editing and proteomic technologies, comparative analyses of lysosomal composition and function will allow us to better appreciate the important contributions of this organelle to many aspects of cellular metabolism, organismal physiology, and disease.

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