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The Lysosome at the intersection of cellular growth and destruction.

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Abstract

The lysosome is an essential catabolic organelle that consumes cellular biomass to regenerate basic building blocks that can fuel anabolic reactions. This simple view has evolved more recently to integrate novel functions of the lysosome as a key signaling center, which can steer the metabolic trajectory of cells in response to changes in nutrients, growth factors and stress. Master protein kinases and transcription factors mediate the growth-promoting and catabolic activities of the lysosome, and undergo a complex interplay that enables cellular adaptation to ever-changing metabolic conditions. Understanding how this coordination occurs will shed light on the fundamental logic of how the lysosome functions to control growth in the context of development, tissue homeostasis and cancer.

Anabolism and Catabolism

At the cellular level, growth is the process of biomass accumulation that precedes cell division and ensures continued rounds of proliferation. During development, organs and organisms grow in size primarily via the addition of new cells through the stereotypic pattern of growth-then-division (Boulan et al., 2015). Adult organs can grow in size as their constituent cells accumulate mass, such as myofibrils in muscle and lipid droplets in adipose tissue, according to the availability of nutrients and metabolic building blocks. Inside each cell, growth relies on a careful interplay of transport systems that import basic building blocks into the cell, sensors that detect the availability of these building blocks, signaling pathways that direct their transformation into more complex molecules by metabolic enzymes and assembly factors (Ben-Sahra and Manning, 2017).

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Declaration of Interest

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In addition to processes that build biomass, cells also harbor machinery that consumes it through active destruction. Biomass consumption is key for fine-tuning the rate of growth, to enable nutrient recycling during scarcity, and for elimination of toxic or damaged cellular components. In both unicellular and multicellular organisms, programs for biomass production and consumption must be carefully coordinated, usually in a reciprocal manner, so as to ensure that the cell does not engage in futile, energy-wasting programs or neglect essential repair functions. This regulation is carried out by growth factors, hormones, stressors and countless other stimuli that constantly feed information about the internal state of the cell and that of distant cell populations in the body, but how these diverse signals are integrated has been a long-standing mystery (Ben-Sahra and Manning, 2017; Liu and Sabatini, 2020).

Recent work has brought forward the idea that anabolic and catabolic programs converge at specific cellular locations within the cell, where their optimal integration is facilitated by spatial proximity, shared molecular components and direct access to upstream nutrient, growth factor and energy stimuli.

Overview of the lysosome.

Discovered in the 1950s by Christian DeDuve, the lysosome is a membrane-bound organelle that plays a well-established role in cellular catabolism (De Duve et al., 1955; Novikoff et al., 1956; Perera and Zoncu, 2016). Its highly acid internal lumen harbors hydrolases that break down complex macromolecules such as glycoprotein, glycolipids and nucleotides, along with large protein complexes and even entire organelles, while its limiting membrane is spanned by permeases that export the products of its digestive activity to the cytoplasm (Figure 1). The ‘power plant’ of the lysosome is the vacuolar ATPase (v-ATPase), an ATP-driven proton pump that acidifies the lysosome interior (Abbas et al., 2020). In turn, the proton gradient established by ATP hydrolysis supports the catalytic activity of its luminal hydrolases, which have a pH optimum between 4.5 and 5.5. Moreover, the pH gradient is used by many transmembrane permeases to export digestion products to the cytoplasm including amino acids, lipids and nucleotides [reviewed in (Perera and Zoncu, 2016)] and, at least in fungi, to import and accumulate metabolites within the vacuole (the fungal equivalent to the lysosome) for long-term storage [reviewed in (Li and Kane, 2009)].

The lysosome is connected to other vesicle populations of the endomembrane system via canonical vesicular trafficking pathways. In general, lysosomes can be thought of as dynamic organelles, which are continuously generated from the encounter of Golgi-derived vesicles carrying resident hydrolases, transporters and pumps, with plasma membrane-derived vesicles loaded with cargo from the cell periphery (endosomes, phagosomes) or interior (autophagosomes) (Luzio et al., 2007). Several pathways recognize newly synthesized lysosomal hydrolases and membrane proteins and deliver them to lysosomes. The main delivery system centers on the mannose-6-phosphate (M6P) receptor (M6PR), which recognizes M6P tags that selectively decorate many important lysosomal hydrolases including proteases, lipases and ribonucleases, and diverts them away from the secretory pathway to the lysosomal lumen (Sleat et al., 2013). Lysosomal membrane proteins, including permeases that transport amino acids, sugars, lipids and ions across the limiting

membrane, are trafficked from the trans-golgi network (TGN) to lysosomes either directly or via the plasma membrane. Sorting signals such as dileucine or tyrosine-based motifs mediate recognition of these client proteins by adaptor protein 1 (AP-1) or Golgi-localized, gamma adaptin ear-containing, ARF-binding (GGA) proteins, which in turn mediate loading of the client protein into clathrin-coated vesicles (Saftig and Klumperman, 2009). The v-ATPase, a multi-subunit complex that comprises 11 core subunits in mammalians plus several accessory components, relies on dedicated chaperones for its delivery from the site of assembly on the ER and Golgi membranes to the lysosome (Cotter et al., 2015; Abbas et al., 2020).

In addition to vesicular pathways it has recently emerged that, both in mammalian and yeast cells, lysosomes engage in non-vesicular communication through the establishment of physical contacts with organelles such as the endoplasmic reticulum, mitochondria and lipid droplets (Elbaz-Alon et al., 2014; Hönscher et al., 2014; Schroeder et al., 2015; Kumar et al., 2018; Wong et al., 2018). This mode of communication relies on specialized tethering proteins, which maintain the respective membranes within 10–30nm from each other while at the same time enabling the exchange of lipids and other metabolites between the two membranes. Similar to contacts established by different organelle pairs, such as ER with mitochondria, membrane contacts between lysosomes and other compartments play key functions such as facilitating metabolite export, fine-tuning the lipid composition of the lysosomal limiting membrane, as well as help maintaining the correct morphology, size and localization of lysosomes (Rowland et al., 2014; Dong et al., 2016; Murley et al., 2017; Thelen and Zoncu, 2017; Lim et al., 2019).

The lysosome as a negative regulator of growth

The main biomass-consuming process mediated by the lysosome is known as autophagy. In response to starvation and other stressors, double-membraned structures known as autophagosomes grows out of a population of precursor vesicles and become decorated with ubiquitin-like proteins known as MAP1LC3s (commonly referred to as LC3A-C) and GABARAPs, which function as docking sites for the capture of diverse cargo (i.e. proteins, protein complexes, organelles, intracellular pathogens) by specialized adaptor proteins (Tsukada and Ohsumi, 1993; Hurley and Schulman, 2014; Mizushima, 2019). The double-membrane autophagosome is eventually completed, trapping its cargo inside and delivering it to the lysosome via fusion with the lysosomal limiting membrane through pairing of complementary SNAREs on the two organelles (Itakura et al., 2012; Guo et al., 2014; Diao et al., 2015). Fusion enables the hydrolases in the lysosomal lumen to attack and degrade the cargo within the autophagosome, along with the bound adaptor proteins. Eventually, metabolites produced via autophagic digestions such as amino acids, sugars, lipids and nucleotides are released from the lysosomal lumen, often with the help of the proton gradient, whereas some molecules may be retained within the lysosome for later use (Perera and Zoncu, 2016). Finally, a membrane sorting process separates lysosomal components from the hybrid organelle to regenerate new, functional lysosomes (Yu et al., 2010; Shen and Mizushima, 2014).

The molecular steps of autophagy have been presented in many comprehensive reviews. Here we summarize regulatory mechanisms that couple autophagy initiation and termination to cellular metabolic state.

In mammalian cells, the molecular cascade that leads to membrane conjugation of LC3A-C and GABARAPs is under the control of a protein complex composed of the Uncoordinated-like kinases (ULK) 1 or 2 (homologue to yeast *ATG1* kinase), together with cofactors ATG13, ATG101 and FIP200 (*ATG17* in yeast). ULK1/2 phosphorylates the other components, leading to stabilization of the entire complex, as well as downstream effectors that kick-start autophagosome formation. These include the Vps34, Atg14 and Beclin components of the phosphatidylinositol 3-kinase (PI3P) complex, which generates phosphatidylinositol 3-phosphate (PI3P), a key phospholipid that makes the site of LC3A-C lipidation and subsequent autophagosome assembly (Kihara et al., 2001; Russell et al., 2013; Egan et al., 2015; Park et al., 2016). Under conditions in which autophagy suppression is desirable, such as high nutrients, ULK1/2 and ATG13 are phosphorylated and inhibited by the master growth regulatory kinase, mechanistic Target of Rapamycin Complex 1 (mTORC1), which also reduces ULK1 protein stability, resulting in suppression of autophagy initiation. Conversely, under nutrient or energy limitation, AMP-activated protein kinase (AMPK) phosphorylates the ULK1/2 complex (Egan et al., 2011; Kim et al., 2011; Puente et al., 2016) as well as other components of the autophagy machinery such as the VPS34 complex and Beclin1 (Kim et al., 2013; Zhang et al., 2016) leading to their activations and initiation of autophagy.

AMPK is a key energy sensor that is activated by high ADP/ATP and AMP/ATP ratios (Herzig and Shaw, 2018). The main function of AMPK is to promote catabolic pathways while shutting down ATP-consuming processes required for cell growth. One way in which AMPK inhibits cell growth is by suppressing mTORC1 signaling (Inoki et al., 2003b; Gwinn et al., 2008) and protein synthesis downstream of mTORC1 (Leprivier et al., 2013).

It is thought that distinct AMPK complexes, containing unique catalytic and regulatory subunit isoforms, exist in different cellular locations. One such complex has been proposed to localize at the lysosomal membrane, where it is phosphorylated and activated by its upstream regulator, liver kinase B1 (LKB1) (Zhang et al., 2013, 2014).

Autophagy is also regulated, both positively and negatively, at the transcriptional level. The MiT/TFE family basic helix loop-helix (bHLH) transcription factors, TFEB, TFE3, TFEC and MiTF, promote the transcriptional activation of autophagic components (e.g. LC3B, autophagic adaptors, autophagosome biogenesis) as well as lysosomal resident proteins (e.g. luminal hydrolases, permeases, v-ATPase subunits) to coordinately increase the degradative capacity of the cell (Sardiello et al., 2009; Settembre et al., 2011; Palmieri et al., 2011). As such, MiT/TFE factors play key roles in adaptation to stress including starvation, energy depletion and various forms of cellular damage, and are suppressed by pro-growth signals. Conversely the Farnesoid X-receptor (FXR) is activated by bile acids, which are elevated in high nutrient states, and suppresses the expression of several autophagic genes including TFEB, thus switching the metabolic balance toward net growth (Seok et al., 2014).

In addition to consuming biomass during autophagy, lysosomes play an important growth-suppressing role through downregulation of mitogenic signals. For example, under high concentrations of the epidermal growth factor (EGF) ligand, the EGF receptor is rapidly removed from the plasma membrane via a clathrin-independent endocytic route, sequestered within the lumen of early- and late endosomes through the action of the endosomal sorting complex required for trafficking (ESCRT), a multi-subunit membrane-remodeling machinery that performs membrane bending and scission away from the cytoplasm, and destroyed upon fusion of late endosomes with lysosomes, thus terminating its pro-anabolic and mitogenic signals (Sigismund et al., 2008; Eden et al., 2012).

The ESCRT machinery also mediates a degradative process known as microautophagy, which has been largely described in yeast, where invaginations of the vacuolar limiting membrane lead to capture of cytoplasmic proteins and their subsequent breakdown in the lumen (Oku and Sakai, 2018).

The lysosome as a positive regulator of growth

As the endpoint of several vesicular pathways that mediate import and degradation of substrates from the extracellular space, including clathrin-mediated endocytosis (CME), fluid-phase endocytosis and phagocytosis, the lysosome can function as a source of building blocks for biomass. For example, uptake of low-density lipoprotein (LDL) via clathrin-mediated endocytosis leads to their lysosomal breakdown and liberation of sterols, fatty acids and phospholipids, which are exported to the ER for membrane biogenesis or stored in lipid droplets [reviewed in (Goldstein and Brown, 2015)].

The lysosome also plays an important function in supplying iron to the cell. Transferrin (Tfn), an iron-binding secreted glycoprotein, becomes loaded with ferric (Fe^{3+}) iron in the plasma and is recognized by the transferrin receptor (TfnR) on the plasma membrane, followed by internalization of the Tfn-TfnR complex via CME. In mildly acidic early endosomes, the TfnR dissociates from Tfn and is recycled to the plasma membrane, whereas iron-loaded Tfn is delivered to the lysosome (Hanover et al., 1984; Cheng et al., 2004). In the acidic environment of the lysosomal lumen, transferrin releases its bound iron, which is then reduced to ferrous (Fe^{2+}) iron and transferred to the cytoplasm and mitochondria through poorly understood mechanisms involving the Dmt1 transporter. The lysosome-supplied iron is essential for synthesis of iron-sulfur (Fe-S) cluster-containing enzymes, which mediate oxidation-reduction reactions that enable mitochondrial respiration. Dissipating lysosomal luminal pH with v-ATPase inhibitors prevents iron dissociation from Tfn and severely impairs cell proliferation (Yambire et al., 2019; Weber et al., 2020).

Recently, a key growth-promoting role of the lysosome emerged from its identification as the cellular platform for the activation of the master regulator, mechanistic Target of Rapamycin Complex 1 (mTORC1) kinase (Figure 2).

mTORC1 is one of the two protein complexes built around the highly conserved mTOR kinase, which is conserved from yeast to humans (Heitman et al., 1991; Sabatini et al., 1994). In eukaryotes from yeast to humans, actively growing cells rely on mTORC1 to

increase the utilization of basic building blocks, primarily glucose and amino acids, which in turn fuel the production of proteins, nucleotides and lipids. At the same time, mTORC1 actively suppresses catabolic processes such as autophagy [reviewed in (Lawrence and Zoncu, 2019; Liu and Sabatini, 2020)]. mTORC1 performs these functions by phosphorylating numerous substrates including protein kinases, translation regulators and transcription factors (Kang et al., 2013). While some of these phosphorylation events activate positive regulators of growth, others inhibit negative regulators of growth as well as positive regulators of catabolism. However, a key requirement is that the protein kinase function of mTORC1 is only turned on under favorable conditions for growth. It is therefore essential for mTORC1 to properly and accurately sense its surrounding environment.

Regulation of mTORC1 signaling pathway

A key finding in the field has been the identification of the lysosomal limiting membrane as the cellular site where two main inputs to mTORC1, nutrients and growth factors, converge to trigger mTORC1 kinase activation (Figure 2). Extensive biochemical studies have shown that mTORC1 is recruited and activated on the surface of the lysosomes by the coordinated action of two small G-proteins: the heterodimeric Rag GTPases and the Rheb GTPase (Sancak et al., 2008; Kim et al., 2008; Sancak et al., 2010; Menon et al., 2014). This basic layout is found across eukaryotes; in budding and fission yeast, TORC1 also binds to and becomes activated on the limiting membrane of the vacuole (the equivalent of the lysosome in these organisms), and many of the molecular players involved in vacuolar TORC1 recruitment are structurally and functionally equivalent to their mammalian counterparts [reviewed in (Nicastro et al., 2017)]. However, important differences between yeast and metazoans have been reported, including the possibility that yeast TORC1 may engage different substrates on separate vesicle populations (Hatakeyama et al., 2019) and the observation that, under nutrient starvation, yeast TORC1 does not disperse from the vacuole but instead polymerizes into large helical assemblies that help maintain it in an inactive state (Prouteau et al., 2017).

The Rag GTPases are obligate heterodimers responsible for mTORC1 recruitment to the lysosome: RagA and RagB (homologous to yeast Gtr1) are highly similar with each other, and each of them can bind either RagC or RagD (homologous to yeast Gtr2), allowing a total of 4 different nucleotide combinations. In response to high nutrients, especially amino acids, glucose and cholesterol, the Rag GTPases become active by adopting a nucleotide state where RagA/B is GTP-loaded, and Rag C/D is GDP-loaded. This conformation allows the Rag GTPases to bind to mTORC1 and anchor it to the lysosomal membrane, a step that allows its subsequent interaction with GTP-bound Rheb (Anandapadamanaban et al., 2019; Rogala et al., 2019). In turn, Rheb triggers a conformational change that makes the kinase site competent for catalysis (Yang et al., 2017). Thus, the emerging model is that of a ‘coincidence detector’, whereby nutrients place mTORC1 in the correct cellular location, the lysosome, via the Rag GTPases, whereas growth factors turn on mTORC1 kinase activity via Rheb. If either input is absent, mTORC1 activation cannot occur. This safety mechanism thus prevents the cell from engaging in anabolic programs when conditions are not appropriate, either due to shortage of local building blocks or absence of mitogenic long-range signals [reviewed in (Lawrence and Zoncu, 2019; Liu and Sabatini, 2020)].

Given the prominent role of Rag and Rheb GTPases in mTORC1 signaling, proteins that modulate the nucleotide state of both Rags and Rheb are key to mTORC1 regulation. The evolutionarily conserved GATOR1 complex, composed of the three-subunits Nprl2, Nprl3 and Depdc5, is a GTPase Activating Protein (GAPs) for RagA/B, hence a negative regulator of mTORC1 activation. In low nutrients, GATOR1 promotes the accumulation of GDP-bound RagA/B on the surface of the lysosomes, thereby blocking the recruitment of mTORC1. In high nutrients, a second, 5-subunit complex known as GATOR2 inhibits the GAP activity of GATOR1, thus allowing RagA/B to become GTP-loaded and promoting lysosomal mTORC1 recruitment (Bar-Peled et al., 2013; Panchaud et al., 2013; Shen et al., 2018).

Both GATOR1 and GATOR2 interact with primary nutrient sensors, i.e. proteins that bind a specific metabolite ligand (Figure 2). Sestrin1 and 2 are cytoplasmic leucine-binding proteins that regulate GATOR2 (Wolfson et al., 2016). The dimeric CASTOR1/2 complex binds to another key amino acid for mTORC1 activation, arginine, and is thought to regulate GATOR2 in a similar manner (Chantranupong et al., 2016; Saxton et al., 2016). The Samtor protein directly modulates the GAP activity of GATOR1 toward RagA in response to its ligand S-adenosylmethionine (SAM), a methyl donor derived from methionine, another key amino acid for mTORC1 activation (Gu et al., 2017).

The machinery described here enables mTORC1 to integrate information about several growth promoting nutrients. Cytoplasmic pools of leucine, arginine and methionine signal to the Rag GTPases via their dedicated sensors, Sestrin1/2, CASTOR1/2 and SAMTOR, respectively (Wolfson et al., 2016; Chantranupong et al., 2016; Gu et al., 2017). Amino acid abundance within the lysosomal lumen, particularly of arginine, is detected by SLC38A9 via its interaction with Ragulator and Rag GTPases (Rebsamen et al., 2015; Wang et al., 2015). Other luminal amino acids may also contribute to mTORC1 activation via dedicated transporters and the v-ATPase also participate in the process (Zoncu et al., 2011; Chung et al., 2019). These lysosomal proteins do not appear to control Rag GTPase nucleotide state via GATOR1. Instead, they may function as guanine nucleotide exchange factors (GEFs) for RagA or RagC (Bar-Peled et al., 2012; Shen and Sabatini, 2018) as well as modulate the ability of either Rag component to spontaneously self-load with GTP (Lawrence et al., 2019). In yeast the Vam6/Vps39 protein, a subunit of the homotypic fusion and vacuole protein sorting (HOPS/class C-Vps) endosomal tethering complex, has been proposed to function as a GEF for the RagA orthologue, Gtr1 (Binda et al., 2009).

Amino acid abundance may also regulate mTORC1 via aminoacyl-tRNA synthetases, which load each transfer RNA (tRNA) with its cognate amino acid. In particular, leucyl-tRNA synthetase (LRS) has been proposed to promote mTORC1 activation by leucine by modulating the nucleotide state of RagD and the RagA/B ortholog, Gtr1, in mammalian and yeast cells, respectively (Han et al., 2012; Bonfils et al., 2012).

Whereas GATOR1 is a RagA-specific GAP, a complex of Folliculin (FLCN) and FLCN-interacting protein (FNIP)1/2 promotes GTP hydrolysis on RagC (Tsun et al., 2013; Petit et al., 2013; Lawrence et al., 2019; Shen et al., 2019). Unlike GATOR1, which inhibits the ability of the Rag heterodimer to interact with mTORC1 under starvation, FLCN:FNIP helps

convert the Rag GTPases to the active $RagA^{GTP}$-$RagC^{GDP}$ configuration under high nutrients. No dedicated amino acid sensors are known to converge on FLCN:FNIP. Rather, its GAP activity appears to be controlled by the nucleotide state of RagA (Meng and Ferguson, 2018; Lawrence et al., 2019).

Glucose also activates mTORC1 through the Rag GTPases, although the sensing mechanism remains to be elucidated (Efeyan et al., 2013). Cholesterol, an important building block for cellular membranes, promotes mTORC1 recruitment to the lysosomes upstream of the Rag GTPases, which senses the cholesterol pool of the lysosomal limiting membrane (Castellano et al., 2017). mTORC1 regulation by cholesterol requires SLC38A9 in a manner that is separable from its arginine sensing function. The lysosomal cholesterol transporter NPC1 negatively regulates cholesterol-mTORC1 signaling by promoting export of cholesterol to acceptor compartments, such as ER, Golgi and plasma membrane (Lim et al., 2019).

Dedicated mechanisms control the nucleotide state of Rheb, which enables mTORC1 kinase activation when growth factors, mitogens and cytokines are plentiful (Yang et al., 2017). A subpopulation of Rheb is known to reside on the surface of the lysosome, where it can interact with mTORC1 to allosterically trigger its kinase activation (Demetriades et al., 2014; Menon et al., 2014). The tuberous sclerosis complex (TSC), which functions as GTPase-activating protein (GAP) toward Rheb, catalyzes the conversion from the active GTP-bound Rheb to inactive GDP-bound (Inoki et al., 2003a; Tee et al., 2003). Growth promoting hormones such as insulin signals through the PI3K-Akt pathway to inhibit the Rheb GAP function of TSC and therefore promote mTORC1 kinase activation. In particular, Akt (as well as ERK and RSK functioning downstream of Ras signaling) phosphorylates TSC at multiple sites and causes dissociation from the lysosomal membrane, where TSC resides in the absence of growth factors (Demetriades et al., 2014; Menon et al., 2014).

TSC is an integration hub for stress signals that antagonize growth including hypoxia, energy depletion, DNA damage and purine depletion (Brugarolas et al., 2004; Inoki et al., 2006; Budanov and Karin, 2008; Hoxhaj et al., 2017). TSC may also provide an additional regulatory input by amino acids, as its localization to the lysosome was proposed to be promoted not only by growth factor withdrawal but also amino acid withdrawal. In contrast, lysosomal Rheb localization appears to be independent of amino acids and growth factors (Demetriades et al., 2014; Menon et al., 2014). Whether Rheb activation requires a dedicated GEF is debatable based on the observation that Rheb spontaneously loads itself with GTP *in vitro* (Rehmann et al., 2008).

Pro-growth actions of lysosomal mTORC1 signaling

In conditions that support cell growth, namely abundant nutrients and growth factors as well as absence of stress, mTORC1 promotes protein synthesis by mainly phosphorylating two key effectors: p70S6 Kinase 1 (S6K1) and eIF4E Binding protein 1 (4E-BP1). Phosphorylation by PDK1 and mTORC1 on two distinct Thr residues of S6K1 results in its activation. In turn, active S6K1 phosphorylates numerous substrates including S6, eIF4B and PDCD4 to promote overall mRNA translation (Ma and Blenis, 2009). On the other hand, by phosphorylating 4E-BP1, mTORC1 triggers its dissociation from eIF4E leading to increase

5’-cap dependent mRNA translation (Dowling et al., 2010; Thoreen et al., 2012; Hsieh et al., 2012).

De novo synthesis of fatty acids, triglycerides and cholesterol, an obligate requisite for cell growth is tightly controlled by a family of transcription factor known as sterol regulatory element-binding proteins (SREBPs). In high nutrients and growth factors, mTORC1 promotes expression, proteolytic processing and nuclear translocation of SREBP1 and SREBP2, which in turn transcriptionally activate enzymatic cascades for the synthesis of fatty acids and sterols, respectively (Porstmann et al., 2008; Düvel et al., 2010; Li et al., 2010; Milkereit et al., 2015). SREBP activation also increases flux towards the pentose phosphate pathway, leading to the production of NAPDH and carbon-rich precursors for the synthesis of lipids and nucleotides (Düvel et al., 2010).

Additionally, mTORC1 directly promotes *de novo* purine and pyrimidine synthesis pathways as well as serine/glycine biosynthesis to support DNA replication and RNA synthesis in growing cells (Ben-Sahra et al., 2013, 2016).

Finally, mTORC1 is a potent regulator of glycolysis. Increased HIF1α expressions by mTORC1 allows the shift from oxidative phosphorylation to glycolysis as HIF1is known to upregulate the expressions of glucose transporters and most enzymes involved in glycolysis (Düvel et al., 2010; He et al., 2018). In turn, glycolysis supports anabolic metabolism by facilitating the use of glycolytic intermediates towards pentose-phosphate and serine/glycine synthesis pathways, both of which provide essential precursors for the synthesis of proteins, nucleotides and lipids [reviewed in (Lunt and Vander Heiden, 2011)].

While triggering anabolic programs, mTORC1 actively suppresses programs required for adaptation to low nutrients, including generation of ketone bodies in the liver (Sengupta et al., 2010) and autophagy through phosphorylation of several autophagosome-initiation factors (Hosokawa et al., 2009; Kim et al., 2011; Puente et al., 2016). mTORC1 also represses catabolism more broadly through regulation of the MiT/TFE transcription factors, the master regulators of autophagy, lysosomal biogenesis and lipid catabolism (Martina et al., 2012; Settembre et al., 2012; Roczniak-Ferguson et al., 2012; Settembre et al., 2013). mTORC1 suppresses TFEB by phosphorylating it on conserved serine residues causing its cytoplasmic retention, whereas recent evidence suggests that mTORC1 can also induce export of TFEB from the nucleus (Napolitano et al., 2018). When nutrient levels drop and mTORC1 becomes inactive, TFEB is dephosphorylated and accumulates in the nucleus, where it activates its target genes.

Given the broad control of metabolic program towards promoting biosynthetic processes, dysregulation of mTORC1 signaling has been implicated in numerous pathologies such as cancer, metabolic and neurological disorders.

Emerging themes in lysosomal growth signaling

Emerging evidence has placed the lysosome at the center of growth regulation via its ability to connect and coordinate anabolic and catabolic programs at the cellular and organismal level. mTORC1 is the most prominent example of lysosomal-based signaling directly

connected to growth. Despite remarkable progress in the identification of many components of the mTORC1 pathway, the full list of mTORC1-regulating nutrients, and how these chemically diverse species are integrated to fine-tune mTORC1 activity needs further investigation.

Moreover, given the number and variety of integral and peripheral protein on the lysosomal membrane, more may be found to connect lysosomal function to cellular metabolism and convey nutrient signals to the mTORC1 scaffolding machinery. For example, a novel ion transporter localized to the lysosomal membrane of the fly larval gut, named 'Hold on don't Rush' (Hodor), was recently shown to function as a sensor for dietary zinc (Redhai et al., 2020). Hodor may represent the first example of 'micronutrient' sensing, whereby metal ions with important pro-growth functions such as zinc, iron and copper control cell- and organism-wide metabolic programs through regulation of the lysosomal mTORC1 machinery.

The increasing power of organelle-specific profiling via proteomic, lipidomic and metabolomic approaches will be useful in uncovering novel nutrient sensors as well as answering critical questions such as how sensing mechanisms varies within different tissues and organs, and how their dysregulations contribute to disease progression (Rhee et al., 2013; Sleat et al., 2013; Abu-Remaileh et al., 2017; Wyant et al., 2018; Lim et al., 2019).

Another developing area involves the ability of lysosomes to interacts with other cellular compartments, both physically and functionally, to regulate metabolism and homeostasis. For instance, in the nematode *C. elegans* the lysosomal lipase LIPL4 was shown to generate a lipid ligand that triggers nuclear translocation of the lipid chaperone LBP8. In the nucleus, ligand-bound LBP8 activates two nuclear receptors that promote mitochondrial lipid oxidation and longevity (Folick et al., 2015).

Lysosomes also engage in two-way communication with other organelles through the formation of physical connections known as membrane contact sites (MCSs). Specialized contacts with the ER regulate actin polymerization and dynamics at the lysosomal membrane, which in turn helps regulate fission and motility of these organelles (Dong et al., 2016; Hoyer et al., 2018). ER-lysosome MCSs also mediate bi-directional transfer of cholesterol and phospholipids, and possibly other metabolites, between the lysosome and the ER, in organisms ranging from yeast to mammals (Murley et al., 2015; Dong et al., 2016; Wilhelm et al., 2017; Zhao and Ridgway, 2017).

The lipid transfer activity of ER-lysosome contacts directly impacts growth signaling. For instance the sterol carrier Oxysterol-binding protein (OSBP), in team with its ER anchors VAPA and VAPB, was shown to transport in the ER-to-lysosome direction a pool of cholesterol that is essential for mTORC1 activation and to plays an important role in the regulation of mTORC1-dependent anabolic programs and autophagy (Lim et al., 2019). Additionally, similar to ER-mitochondria contacts, ER-lysosome MCSs have recently been proposed to facilitate calcium transfer between these two organelles and possibly contribute to replenishing of lysosomal calcium stores (Atakpa et al., 2018). In turn lysosomal calcium, exported to the cytoplasm via the mucolipin 1 (MCOLN1) channel, promotes

dephosphorylation and nuclear translocation of TFEB via the calcium-dependent phosphatase calcineurin, ultimately leading to activation of the CLEAR genome (Medina et al., 2015; Martina et al., 2016).

In addition to ER-lysosome MCSs, increasing evidence supports the existence of membrane contacts between lysosomes and mitochondria of yeast and mammalian cells (Elbaz-Alon et al., 2014; González Montoro et al., 2018; Wong et al., 2018, 2019). Although the functional significance of these structures is only beginning to be elucidated, they could have profound roles in growth regulation by enabling high efficiency transfer of lysosome-derived metabolites to the mitochondria for utilization in growth processes.

Although our understanding on inter-organelle communications has deepened, it is still unclear how dysregulated MCSs are connected to diseases, especially to those related to aberrant growth and metabolism. Recently developed tools to visualize and manipulate MCSs will surely provide new insights on their roles in metabolic signaling and growth regulation, and point the way to novel therapeutic strategies.

Lysosomal signaling in growth processes and growth-related diseases

The ability to precisely control and terminate growth is fundamental to correct development and adult homeostasis of multicellular organisms. Thus, in metazoans a carefully balanced combination of growth, mitogenic and mechanical signals ensures the correct morphogenesis and maintenance of multiple organs, and alterations in this balance leads to both developmental defects and disease in the adult.

As the cellular site for mTORC1 activation, the lysosome is actively involved in cellular and organismal growth. Because loss of mTOR in mice causes embryonic lethality (Gangloff et al., 2004; Murakami et al., 2004; Guertin et al., 2006), our understanding of the role of TOR signaling during development mainly comes from studies in lower organisms. In both fruit flies and nematodes, genetic manipulations of the IGF/PI3K/AKT/mTOR pathway leads to striking alterations in size at the cellular, organ and organismal levels. Inactive TOR mutants reduce cell size, hinders proliferation, and delays or arrests development, while its hyper-activation in specific cell population leads to autonomous increases in cell and organ size (Oldham et al., 2000; Zhang et al., 2000; Long et al., 2002).

In humans, somatic activating mutations in the PI3K/AKT/mTOR pathway have been shown to cause a spectrum of overgrowth syndromes including PIK3CA-Related Overgrowth Spectrum, Proteus syndrome, and brain overgrowth conditions (Keppler-Noreuil et al., 2016; Venot et al., 2018). The requirement for proper mTORC1 regulation is particularly evident in the brain, as both hypo- and hyper-activation of mTORC1 signaling have been associated with abnormal neuronal development, as well as neurological disorders (Lawrence and Zoncu, 2019; Blair and Bateup, 2020; Baldassari et al., 2019).

Cardiac hypertrophy is another disease condition associated with dysregulated mTORC1. Defects in heart growth control is often associated with aging. Indeed, cardiac hypertrophy is commonly observed in older animals, consistent with increased mTORC1 activity during aging (Sciarretta et al., 2014). Studies have shown that genetic or pharmacological inhibition

of mTORC1 is cardioprotective, as it reduces pathological hypertrophy and prevents heart failure (Shioi et al., 2003; Völkers et al., 2013).

Aberrant activation of mTORC1 signaling is also commonly observed in cancer. Mutations in mTOR kinase itself have been reported but mutations have been predominantly observed upstream of mTORC1, especially in pro-growth oncogenic pathways such as PI3K/Akt and RAS-ERK pathways. These genetic alterations acquired by cancer cells often leads to growth-factor independent mTORC1 activation. On the other hand, mutations in components of the nutrient sensing machinery upstream of mTORC1, such as GATOR1 in glioblastoma (Bar-Peled et al., 2013), RagC in follicular lymphoma (Okosun et al., 2016) and FLCN in the Birt-Hogg-Dube hereditary syndrome (Nickerson et al., 2002), may provide cancer cells with powerful pro-growth signals and the ability to bypass critical metabolic checkpoints.

Lysosomal function as an end point of autophagy and macropinocytosis is often hijacked in aggressive cancers, providing survival advantage in harsh microenvironments. These tumors became reliant on lysosome-dependent recycling pathways to generate building blocks necessary for growth. Pancreatic ductal adenocarcinoma (PDA) and lung adenocarcinoma, highly aggressive tumor driven by oncongenic KRas mutations (i.e. Gly12 to Val, Asp or Cys), exhibit high basal lysosomal activity and extreme dependency on autophagy both within the tumor cells and in the surrounding microenvironment (Mancias et al., 2014; Guo et al., 2016; Sousa et al., 2016; Yang et al., 2018).

The constitutive and growth-promoting activation of the autophagy-lysosome system in PDA was shown to stem from increased expression and constitutive nuclear localization of the MiT/TFE transcription factors, leading to a dramatically increased number and activity of lysosomes in PDA cells (Perera et al., 2015). The concomitant upregulation of oncogenic KRas-dependent (pro-anabolic) ERK signaling and MiT/TFE-dependent autophagy-lysosomal catabolism confers a unique metabolic profile to PDA, but also makes it highly susceptible to combined inhibition of these two pathways, as suggested by recent reports in mouse models and human patients (Bryant et al., 2019; Kinsey et al., 2019).

The coexistence of anabolism (ERK or mTORC1 activation) and lysosomal catabolism seem to be an essential adaptation in some non-KRas-driven tumors as well. For example, a sub-population of renal cell carcinomas and soft-tissue sarcomas are driven by chromosomal rearrangement and amplification of TFEB or TFE3 (Sidhar et al., 1996; Weterman et al., 1996). Given the recently reported ability of the MiT/TFE factors to promote mTORC1 activation in a feedback manner via transcriptional upregulation of RagD (Di Malta et al., 2017), it is likely that their translocation and constitutive activation may be instrumental to the establishment of this unique metabolic state.

Concluding remarks

Anabolism and catabolism must be carefully coordinated in order to avoid energy-wasting cycles of simultaneous biomolecule synthesis and degradation, and to allow rapid metabolic responses to sudden variations in nutrient levels, growth signals and stressors. The lysosome plays a key role in coordinating anabolic and catabolic programs. Long known as the end

point of cellular degradation, lysosomes have emerged as sophisticated signaling centers, and the start point of cellular metabolism and growth control. Increasing evidence also suggests that lysosomes constantly communicate with other cellular compartments to carry out specific metabolic programs. Major focus of lysosome research moving forward will be to understand its functions in the context of whole cells and tissues, and implications in organismal homeostasis. With recent advances in mass spectrometry-based and imaging approaches to study organelle physiology, future discoveries will provide better understanding of the lysosome growth control, and point the way to new therapeutic approaches in pathologies associated with lysosomal dysfunctions.

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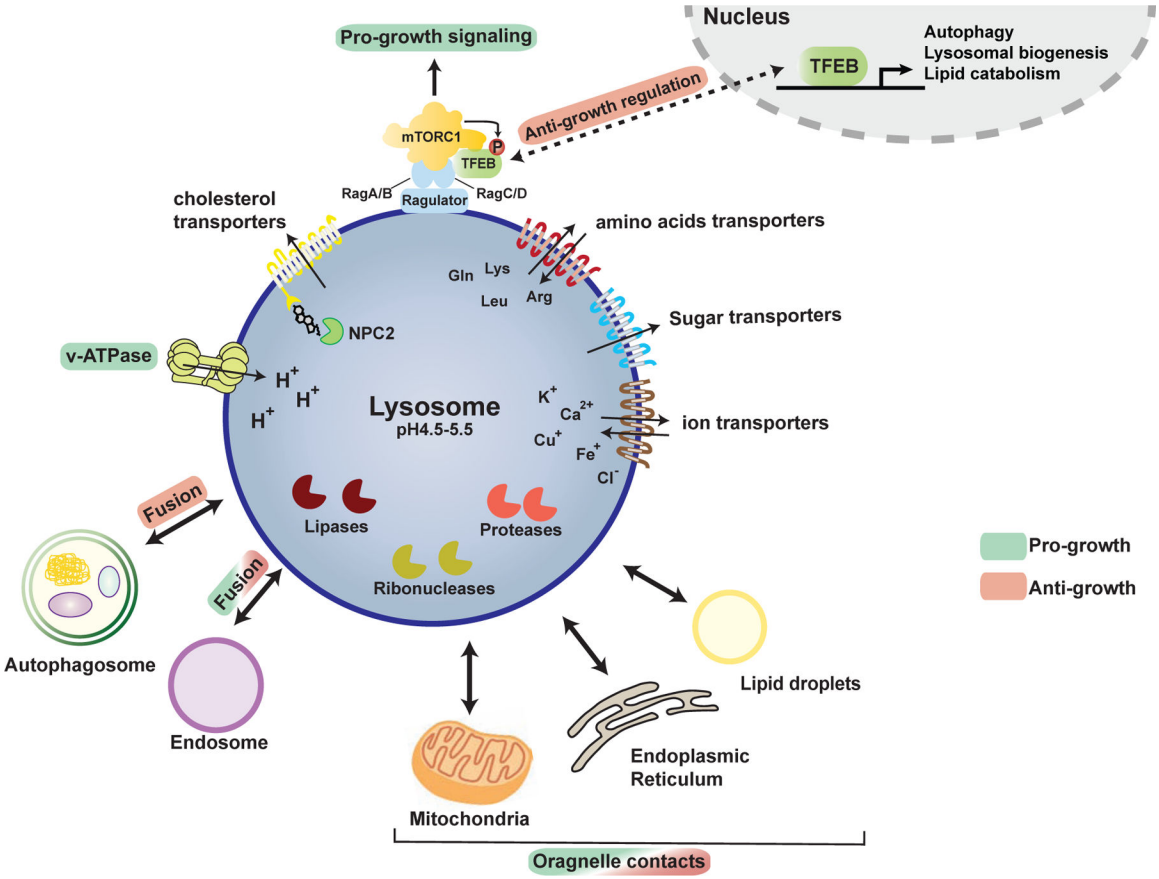

Figure1: Pro- and anti-growth properties of the lysosome

The lysosome is characterized by its low pH (ranging between 4.5 and 5.5), which is mainly established by the vacuolar H^+ ATPases (v-ATPases)-dependent pumping of protons from the cytosol into the lysosomal lumen, aided by the import of anions and antiport of cations which neutralize imbalance in ionic charges and allow further proton transport. The internal acidic environment is critical for the activity of luminal proteases, lipases and ribonucleases that degrade incoming macromolecular substrates. Lysosomal membrane proteins include permeases that transport amino acids, sugars, lipids and ions across the limiting membrane. The limiting membrane functions as a platform for the assembly of a macromolecular complex centered on the mTORC1 protein kinase, which translates nutrient and growth factor abundance into metabolic instructions via downstream effectors such as the TFEB transcription factor.

Lysosomes are the end point for multiple trafficking routes, including endocytic and scavenging pathways. Endosome-lysosome fusion can either promote the breakdown and utilization of extracellular nutrients (pro-growth), or the degradation and silencing of signaling receptors (anti-growth). The lysosome also fuses with autophagosome as part of the autophagy process for the degradation of intracellular constituents. Building blocks generated by lysosomal degradation are either stored inside the lysosomes or transported to the cytoplasm. Lysosomes also engage in direct interactions with other organelles such as mitochondria, endoplasmic reticulum and lipid droplets via specialized structures known as membrane contact sites.

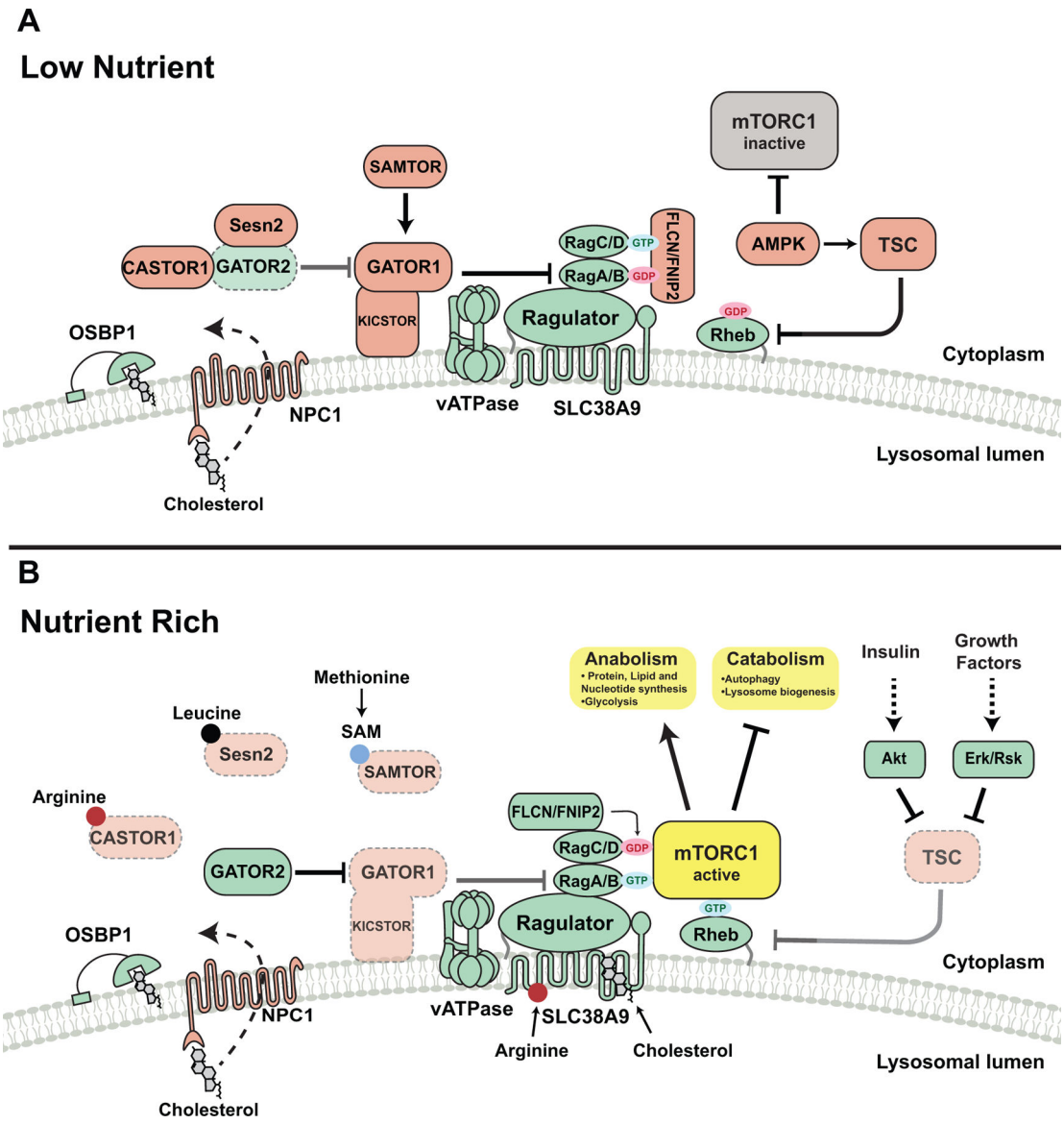

Figure2: Regulation of mTORC1 signaling pathways

The growth-promoting role of the lysosome emerged from its identification as the cellular platform for the activation of the master growth regulator, mTORC1 kinase. A coincidence detection mechanism ensures that the kinase activity of mTORC1 is turned on at the lysosomal limiting membrane only when both nutrients and growth factors are plentiful. Nutrient signals converge on the Rag GTPases, whereas growth factor signals converge on Rheb GTPase.

(A) Under low nutrient conditions the Rag GTPases, which are anchored to the lysosomal membrane by the Ragulator/Lamtor complex, are in the inactive state, in which RagA/B is GDP loaded and RagC/D is GTP loaded. Inactive Rag GTPases cannot bind to mTORC1, which remains inactive in the cytosol. In low nutrients GATOR1, the GTPase-activating protein (GAP) for RagA/B, promotes the accumulation of GDP-bound RagA/B, thereby blocking the recruitment of mTORC1. When growth factors are absent the tuberous sclerosis

complex (TSC), which functions as GAP toward Rheb, catalyzes the conversion from the active GTP-bound Rheb to inactive GDP-bound form.

(B) In response to nutrients and growth factors, respectively, GATOR1 and TSC become inactive, thus promoting the transition of RagA/B and Rheb, respectively, to the GTP bound form. GTP-loaded RagA/B then physically recruits mTORC1 to the lysosomal membrane, whereas GTP-loaded Rheb promotes mTORC1 kinase activation. Cytoplasmic pools of leucine, arginine and methionine signal to the Rag GTPases via their dedicated sensors, Sestrin1/2, CASTOR1/2 and SAMTOR, respectively. Amino acid abundance within the lysosomal lumen, particularly arginine, is detected by the SLC38A9 amino acid permease. mTORC1 activation by cholesterol also requires SLC38A9 in a manner that is separable from its arginine sensing function. Oxysterol binding protein (OSBP) transfer cholesterol from ER to the lysosomal membrane, thus favoring mTORC1 activation. The lysosomal cholesterol transporter NPC1 negatively regulates cholesterol-mTORC1 signaling by promoting export of cholesterol to acceptor compartments, such as ER, Golgi and plasma membrane.

Inactive mTORC1 is shown in grey and active mTORC1 is in yellow. Positive regulators of the mTORC1 pathways are in green, while negative regulators of mTORC1 are shown in red.