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The Lysosome as a Regulatory Hub

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Abstract

The lysosome has long been viewed as the ‘trash can’ of the cell. However recent discoveries have challenged this simple view and established a central role of the lysosome in nutrient dependent signal transduction. The degradative role of the lysosome and its newly discovered signaling function are not in conflict, rather they cooperate extensively to mediate fundamental cellular activities such as nutrient sensing, metabolic adaptation and quality control of proteins and organelles. Moreover, lysosome-based signaling and degradation are subject to reciprocal regulation. Transcriptional programs of increasing complexity control the biogenesis, composition and abundance of lysosomes, and fine-tune their activity to match the evolving needs of the cell. Alterations in these essential activities are, not surprisingly, central to the pathophysiology of an ever-expanding spectrum of conditions including storage disorders, neurodegenerative diseases and cancer. Thus unraveling the functions of this fascinating organelle will contribute to our understanding of the fundamental logic of metabolic organization and point to novel therapeutic avenues in several human diseases.

Keywords

mTORC1; nutrient sensing; TFEB; lysosomal adaptation; autophagy; neurodegeneration; cancer metabolism

1. INTRODUCTION

Lysosomes are best known as the primary degradative compartment of eukaryotic cells. These dynamic organelles were discovered in 1955 by the Belgian biochemist, Christian De Duve, at the University of Lieges. While investigating the mechanism of action of insulin, De Duve detected a biochemical fraction enriched in hydrolytic activities toward proteins and lipids (de Duve 2005, De Duve & Wattiaux 1966). Numerous follow-up studies using both biochemical and ultrastructural methods led to the identification of a membrane-bound compartment, named ‘lysosome’, that specializes in the breakdown and

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recycling of complex cellular components (Farquhar et al 1972, Klumperman & Raposo 2014, Novikoff et al 1956). A defining property of lysosomes is the acidic internal pH (ranging between 4.5 and 5.5), established by the vacuolar H^+ ATPase (v-ATPase) aided by a counterflux of other ion species such as Cl^-, Na^+ and K^+ (Forgac 2007, Ishida et al 2013, Mindell 2012). The low pH provides an optimal environment for the function of luminal hydrolases, and thus facilitates the degradation of a vast array of macromolecules leading to the production of amino acids, monosaccharides and free fatty acids, which are exported to the cell via dedicated permeases (Jezegou et al 2012, Liu et al 2012, Rong et al 2011, Sagne et al 2001). Multiple endocytic pathways, including phagocytosis and macropinocytosis, clathrin- and caveolin-dependent and independent endocytosis, import macromolecules from the extracellular space and from the cell's own limiting membrane and feed them to the lysosomal system for degradation (Conner & Schmid 2003, Di Fiore & von Zastrow 2014, Goldstein & Brown 2015). Furthermore, a 'self-catabolic' process known as autophagy captures cytoplasmic macromolecules, damaged or misfolded proteins and even entire organelles and delivers them to the lysosome (Kaur & Debnath 2015, Mizushima & Komatsu 2011).

Hence, lysosomal processing of a variety of cargo is essential for efficient removal of toxic cellular components, elimination of worn out organelles, termination of signal transduction and maintenance of metabolic homeostasis. These functions highlight a fundamental role for lysosomes in maintenance of cellular health. Accordingly, mutations that affect key lysosomal proteins such as hydrolases and permeases lead to a group of hereditary syndromes known as lysosome-storage disorders (LSD), characterized by metabolic dysfunction, neurodegeneration and severely impaired growth (Ballabio & Gieselmann 2009, Bellettato & Scarpa 2010, Platt et al 2012). Progressive loss of lysosome efficiency that occurs over the lifespan of virtually all multicellular organisms and has been implicated in age-dependent decline of the regenerative capacity of organs and tissues (Rodriguez-Navarro et al 2012, Zhang & Cuervo 2008). In particular, lysosomal dysfunction deriving from genetic or environmental factors is strongly associated with an increased risk of neurodegeneration and impaired cognition (Filimonenko et al 2007, Nixon 2013, Ravikumar et al 2005, Spinosa et al 2008). On the other hand, rapidly dividing cancer cells rely on efficient lysosome function for stress response and nutrient scavenging during the course of tumor progression (DeNicola & Cantley 2015, Perera & Bardeesy 2015, Piao & Amaravadi 2015, Rabinowitz & White 2010)

In the past 7–8 years, an increasing body of work has radically shifted our view of the lysosome as merely a terminal degradative station to the central node of a sophisticated network for cellular adaptation. The components of this network include ion and nutrient transporters, protein kinases and phosphatases, as well as transcription factors and transcriptional regulators. Together, these components integrate important cellular parameters such as nutrient abundance, energy levels and stressors, and translate them into instructions that steer cellular metabolism toward proliferation or quiescence (Chantranupong et al 2015, Settembre et al 2013b, Zoncu et al 2011b). The focus of this review is on the recent studies that have raised the status of the lysosome from a catabolic 'dead end' to a key signaling node, with far-reaching implications for our understanding of the logic of metabolic regulation both in health and in disease.

2. STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE LYSOSOME

2A. BIOGENESIS OF LYSOSOMES

Lysosomes are found in all eukaryotic cells. However, their shape, size, number and function vary greatly depending on species, cell type and context. For example whereas most metazoan cells contain hundreds of lysosomes with diameters ranging between 0.1 um and 1um, yeast and plant cells contain one or few lysosome-like vacuoles of several microns in diameter (Baba et al 1994, Novikoff et al 1956). Enlarged lysosomes are also found in disease settings, such as lysosome storage disorders and neurodegenerative diseases (Nixon 2013, Platt et al 2012). Lysosome-related compartments such as melanosomes in melanocytes and lytic granules in lymphocytes share some common features of classical lysosomes but also contain specialized components not found in classical lysosomes, such as pigments and membrane-perforating proteins. Several reviews on the biogenesis and function of these cell-type specific compartments have been published elsewhere (Marks et al 2013, Stinchcombe & Griffiths 2007, Watts 2012).

The limiting outer membrane of the lysosome is composed of a single phospholipid bilayer of 7–10nm that is decorated with transmembrane proteins – the most abundant being the lysosome-associated membrane protein (LAMP) -1 and -2 which together total 80% of lysosome membrane proteins (Saftig & Klumperman 2009). LAMP proteins and others such as lysosomal integral membrane protein (LIMP)-2 and CD63 are heavily glycosylated on their luminal side and form the glycocalyx – a protective barrier against auto-digestion of the limiting membrane by the resident lytic enzymes within the lysosome lumen (Kornfeld & Mellman 1989, Saftig & Klumperman 2009, Settembre et al 2013b). There are ~60 resident acid hydrolases within the lysosome that serve to digest all classes of macromolecules including proteins, lipids, nucleic acids and carbohydrates. These enzymes are tuned to function optimally at the acidic pH within the internal environment of the lysosome and can be broadly categorized based on substrate preference (lipases, proteases, glycosidases, acid phosphatases, nucleases and sulfatases). The list of currently known luminal and membrane-associated lysosomal proteins is likely not exhaustive. For example, recent proteomic-based studies of purified lysosomes have identified novel candidate lysosomal proteins of unknown function that may be involved in macromolecular degradation, metabolite transport, vesicle trafficking and signal transduction (Chapel et al 2013, Schroder et al 2007, Sleat et al 2013).

Ultimately, the end products of lysosome-mediated digestion are actively or passively transported by integral membrane proteins of the lysosomal membrane to the cytoplasm, where they are utilized in biosynthetic reactions (Jezegou et al 2012, Liu et al 2012, Rabinowitz & White 2010, Rong et al 2011, Sagne et al 2001, Singh & Cuervo 2011). Emerging evidence indicates that the lysosome can also function as a cellular storage site, akin to the yeast vacuole (discussed below), where catabolic intermediates are exchanged with the cytoplasm in a regulated manner in response to changing cellular needs.

Lysosomes arise from the merger between vesicles derived from post-Golgi traffic and cargo-filled vesicles generated at the plasma membrane and traveling along the endocytic pathway. Clathrin-mediated endocytosis (CME) involves budding of cargo-filled vesicles from the cell surface that merge with Rab5-positive early endosomes. Early endosomes then

undergo multiple rounds of fusion and accretion, eventually giving rise to late endosomes (Ohya et al 2009, Rink et al 2005, Zoncu et al 2009). The progression from early to late endosomes is marked by changes in the external protein and lipid coat (Di Paolo & De Camilli 2006, Rink et al 2005, Zoncu et al 2009), progressive acidification of the lumen, as well as morphological changes such as budding of intraluminal vesicles mediated by the ESCRT complex (Henne et al 2011, Wollert & Hurley 2010). At several points along this path, a full complement of hydrolytic enzymes are delivered to the maturing late endosome via fusion with post-Golgi vesicles carrying these components (FIG. 1A).

Thus, a seamless conversion along the endocytic pathway transforms a cargo-filled early endosome into a late endosome and finally a lysosome. However, lysosomes are not simply end-points of the endocytic pathway: rather, they exist as a stable population, functioning as the terminal hub of multiple trafficking routes that carry cargo destined for degradation. These routes include macroautophagy, phagocytosis and macropinocytosis, and chaperone mediated autophagy. Microtubule-based motility enables lysosomes to participate in these diverse trafficking events. In live cell microscopy experiments, lysosomes appear to frequently and randomly switch their direction of motion. This remarkable pattern is due to the ability of lysosomes to freely switch between kinesin-mediated, plus-end directed motion and dynein-mediated, minus-end directed motion (Burkhardt et al 1997, Harada et al 1998, Nakata & Hirokawa 1995). A recently described protein complex, BLOC-1 Related Complex (BORC), mediates the physical association of lysosomes with kinesins via the small GTPase Arl8, and is essential for lysosomal trafficking to the cell periphery (Pu et al 2015). The factors that govern the association of lysosomes with dyneins include the oxysterol binding protein ORP1L, which promotes association of dynein-dynactin to the lysosomal surface under cholesterol-rich conditions, and a lysosomal calcium-sensing complex centered on the TRPML1 channel and the EF-containing protein ALG-2 (Li et al 2016, Rocha et al 2009). How these kinesin- and dynein-anchoring complexes determine the direction of motion is currently not understood, but their reciprocal regulation may affect important functions such as lysosomal acidification, cell motility and nutrient homeostasis (Johnson et al 2016, Korolchuk et al 2011, Pu et al 2015). Following their fusion with other organelles, lysosomes need to be regenerated. Membrane sorting events recycle lysosome-specific components out of hybrid organelles such as autophagolysosomes (autolysosomes), and thus contribute to maintaining a stable population of lysosomes over time (Li et al 2016, Maejima et al 2013, Yu et al 2010).

2B. AUTOPHAGY

Intracellular components are recycled, repurposed and reused via a set of self-digestive processes collectively known as autophagy. In mammalian cells, two types of autophagy have been identified, macroautophagy and chaperone-mediated autophagy (CMA) (He & Klionsky 2009, Mizushima et al 2008, Singh & Cuervo 2011). Yeast cells operate both macroautophagy and microautophagy. During CMA, proteins harboring specific recognition motifs are directly transported by chaperones to the lysosome for degradation (Kaushik & Cuervo 2012). Microautophagy involves direct engulfment of cytoplasmic constituents by the lysosome limiting membrane (Dubouloz et al 2005, Li & Kane 2009). During macroautophagy (herein referred to as autophagy), a double membrane vesicle, the

autophagosome, forms and encapsulates cytoplasmic content, which fuse with lysosomes (forming autolysosomes) in order for the cargo to be degraded (He & Klionsky 2009, Hurley & Schulman 2014, Mizushima et al 2008). Thus all three forms of autophagy are strictly dependent on lysosome function for degradation of cellular constituents.

Autophagy is functional at low baseline levels in most tissues (Hara et al 2006, Komatsu et al 2006, Mizushima & Komatsu 2011). However in response to numerous stimuli, the most potent being amino acid starvation, autophagy is induced above baseline levels via changes in the activity of master nutrient responsive kinases Mechanistic Target of Rapamycin Complex 1 (mTORC1) and Adenosine monophosphate-activated protein kinase (AMPK) (Egan et al 2011, Noda & Ohsumi 1998). Under nutrient replete conditions, activated mTORC1 phosphorylates key proteins controlling autophagy initiation, namely ULK1/2 and ATG13, to suppress autophagosome formation (Hosokawa et al 2009, Hurley & Schulman 2014, Kamada et al 2000). In contrast, phosphorylation of these same proteins on alternative residues by AMPK activates autophagosome biogenesis in response to starvation and cellular stress (Egan et al 2011, Shang et al 2011). Thus, changes in extracellular and intracellular nutrient levels directly impinge on the autophagy machinery to provide an adaptive response that, through recycling of products of autolysosome digestion back to the cytoplasm or to other organelles, attempts to restore cellular homeostasis. In addition, autophagy functions to remove misfolded proteins, damaged organelles, protein aggregates and foreign pathogens and therefore provides the cell with an important defense and quality control mechanism (Gutierrez et al 2004, Watson et al). Deregulation of these essential protective functions of autophagy have been implicated in the pathogenesis of cancer, degenerative and immune disorders as well as in aging (Gutierrez et al 2004, Hara et al 2006, Komatsu et al 2006).

Historically, the study of autophagy has focused on the molecular events that govern autophagosome formation and maturation (He & Klionsky 2009, Hurley & Schulman 2014, Mizushima & Komatsu 2011). In contrast, comparatively little attention has been given to the late steps in this process, particularly to membrane fusion and membrane sorting events that define the life cycle of the hybrid autolysosome. However, a number of recent studies have revealed that the formation of the autolysosome and its ultimate disposal are complex and highly regulated processes (Shen & Mizushima 2014). Moreover the correct execution of these processes is key to the ability of the cell to sustain additional rounds of autophagy over time.

The fusion of lysosomes with late endosomes has been characterized in detail and it provides a blueprint to understand autolysosome formation. This begins with lysosome-endosome vesicle tethering, which is mediated by the Homotypic fusion and Vacuolar Sorting (HOPS) complex in concert with the small GTPase Rab7 (Kummel & Ungermann 2014). The next step in lysosome-endosome fusion occurs when soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins on each vesicle form a parallel four-helix bundle composed of three Q-SNAREs on the late endosome, namely syntaxin 7 (Stx7) (Qa), Vti1b (Qb) and Syntaxin8 (Qc), and an R-SNARE on the lysosome, typically VAMP7 (Luzio et al 2007). A conformational change in the trans-SNARE complex, likely triggered

by Ca^{2+}, brings the two bilayers within a critical distance, resulting in their fusion and formation of a hybrid organelle.

In the context of autophagosome-lysosome fusion, the Plekstrin Homology Domain Containing Protein 1 (PLEKHM1) protein was proposed to function as a tethering factor by simultaneously binding to LC3 on autophagosomal membranes via an LC3-Interacting Region (LIR), and to the HOPS complex on the lysosomal membrane (McEwan et al 2015). The Atg14 protein also appears to function as a tethering factor between autophagosomes and lysosomes. Atg14 interacts with the Q-SNAREs, Stx17 and SNAP-29 (see below) on the autophagosomal membrane, and it forms homo-oligomers that favor the subsequent fusion reaction both in cells and *in vitro* (Diao et al 2015).

Stx17 has been identified as the putative Qa-SNARE that mediates autophagosome-lysosome fusion (Diao et al 2015, Guo et al 2014, Itakura et al 2012). Stx17 normally resides on the membranes of the Endoplasmic Reticulum (ER), but it translocates to the outer membrane of autophagosomes once the latter have progressed to fully enclosed structures. On the autophagosome, Stx17 binds to SNAP-29, which serves as the Qbc SNARE. Stx17 and SNAP-29 then form a trans-SNARE complex with VAMP8 on the lysosomal membrane, leading to fusion (Itakura et al 2012). The ability of Stx17 to distinguish complete autophagosomes from incomplete ones is intriguing and remains unexplained, although the necessary information appears to reside in the hairpin-like structure formed by the two trans-membrane domains of the protein (Itakura et al 2012).

Another report suggests that autophagosome-lysosome fusion is a regulated process that is linked the metabolic state of the cell. Under high glucose conditions, O-linked β-N-acetylglucosamine (O-GlcNAc) Transferase (OGT) modifies SNAP-29 via attachment of O-GlcNAc groups (generated via the hexosamine pathway) on four critical Ser/Thr residues. This modification inhibits the formation of the trans-SNARE complex between Stx17 and VAMP8 (Guo et al 2014). In contrast, glucose starvation leads to a drop in O-GlcNAc modification on SNAP29, allowing the formation of trans-SNARE complexes and accelerating autolysosome biogenesis. This mechanism provides a further layer of complexity and specificity to nutrient-directed regulation of autophagy (FIG. 1B).

Following cargo breakdown inside the autolysosome, primary lysosomes need to be reformed in order to sustain additional rounds of autophagy. The process of autophagic lysosome reformation (ALR) involves sorting of lysosome-specific components in LAMP1/2-positive tubular structures that separate from the hybrid organelle via a process that requires Rab7 and microtubule polymerization (Rong et al 2012, Yu et al 2010). These proto-lysosomal structures eventually acidify and acquire all the features of mature lysosomes. Interestingly, ALR requires active mTORC1, as this process was inhibited by treatment with rapamycin. It was proposed that nutrient buildup inside the autolysosome leads to mTORC1 reactivation, thus setting the timing for ALR initiation and the eventual disposal of hybrid organelles (Yu et al 2010). The machinery that mediates ALR has been partially elucidated by proteomic-based analysis of purified LAMP1/2 positive tubules. Interestingly, this process was similar to clathrin-mediated endocytosis, as it involved many of the same components including clathrin itself, $PI(4,5)P_2$ and the AP2 complex (Rong et

al 2012). Ongoing studies focusing on the molecular mechanisms governing autolysosome formation, maturation and resolution will provide important insight into specific roles for this process in both health and disease.

2C. LYSOSOMAL ION CURRENTS IN SIGNALING, EXOCYTOSIS AND MEMBRANE REPAIR

The lysosomal membrane contains several ion channels that transport important ions such as Na^+, K^+, Ca^{2+} and Cl^-. Movement of these ion species across lysosomal channels establishes a resting lysosomal membrane potential $\Delta\psi_{lyso}$ between −20 and −40mV (cytoplasmic side negative) (Xu & Ren 2015). This membrane potential helps regulate the proton gradient established by the v-ATPase (Cang et al 2013, Steinberg et al 2010, Xu & Ren 2015). It also plays a role in the release of Ca^{2+} during lysosomal exocytosis, as well as in the regulation of amino acid transport from the lysosomal lumen during starvation. Thus, similar to the mitochondrial membrane potential, modulation of $\Delta\psi_{lyso}$ is an emerging aspect of lysosomal function (Xu & Ren 2015).

Among the ion channels that establish $\Delta\psi_{lyso}$ are Two Pore Complex 1 and 2 (TPC1 and 2), 12-transmembrane spanning Na^+ channels that are responsible for large Na^+ currents detected in lysosomal patch-clamp experiments (Cang et al 2013). Na^+ is the most abundant cation in the lysosomal lumen, and its movement toward the cytoplasm can significantly affect the value of $\Delta\psi_{lyso}$. The activity of TPC1 and 2 is nutrient-regulated: high ATP levels and active mTORC1 inhibit TPC1/2. During nutrient starvation, decreasing ATP levels and mTORC1 inactivation de-inhibit TPC1/2, increase Na^+ currents out of the lysosome and cause the lysosome's interior to depolarize over the fed state. Interestingly, TPC1 and 2-mediated depolarization appeared to support the release of cationic amino acids, arginine and lysine, from the lysosomal lumen during starvation. In turn, the lysosome-derived flux of amino acids may be important to sustain energy levels during starvation (Cang et al 2013).

TPC1 and 2 are also regulated by phosphatidylinositol (3,5) bisphosphate [$PI(3,5)P_2$], a phosphoinositide specifically found in the endolysosomal system (Wang et al 2012). $PI(3,5)P_2$ was initially detected as a low-abundance phosphoinositide that rapidly accumulates on the vacuolar membrane of yeast cells challenged with hyper-osmotic buffers. In fact, $PI(3,5)P_2$ is key to adaptation of yeast cells to osmotic stress by activating several channels and permeases that release ions and solutes from the lumen of the vacuole. The resulting increase in cytoplasmic osmolarity counters water efflux and ensures cell survival (Efe et al 2005, Li & Kane 2009).

In mammalian cells, $PI(3,5)P_2$-induced ion currents may regulate the fusogenic potential of lysosomes. $PI(3,5)P_2$ also activates mucolipin 1 (MCOLN1, also known as TRPML1), a Ca^{2+}, Fe^{2+} and Zn^{2+}-permeable channel that selectively localizes to the lysosomal membrane (Dong et al 2008). MCOLN1-mediated Ca^{2+} release from the lysosomal lumen triggers lysosomal exocytosis. Lysosomes often dock and subsequently fuse with the plasma membrane and upon regulated Ca^{2+} release secrete their contents into the extracellular space(Cheng et al 2014, Medina et al 2011, Polishchuk et al 2014). Several physiological processes including bone resorption by osteoclasts, immune cell function during parasitic

attack, melanocyte function during pigmentation and fertilization all depend on lysosome exocytosis (Settembre et al 2013b). However, a more general function for this process that is common to all cell types is to mediate plasma membrane repair upon mechanical injury (Cheng et al 2014, Reddy et al 2001). MCOLN1-mediated Ca^{2+} release is also important for the ability of lysosomes to reform following their fusion with other organelles such as endosomes and phagosomes. Mutations in MCOLN1 result in mucolipidosis type IV, a lysosomal storage disorder characterized by morphological and functional defects of lysosomes (Ballabio & Gieselmann 2009, Vergarajauregui & Puertollano 2008). Imbalances in lysosomal $PI(3,5)P_2$ levels lead to lysosomal enlargement and neurodegeneration, possibly through dysregulation of MCOLN1 and TPC1/2 (Chow et al 2007).

In conclusion, regulated ion flux through the lysosomal membrane is a driving force in a number of processes ranging from nutrient homeostasis to osmotic adaptation to tissue remodeling. Future work using state-of-the-art, single organelle patch-clamp techniques, combined with fluorescent biosensors, will likely bring to light additional functions for this important process.

3. EMERGING ROLES OF THE LYSOSOME IN SIGNALING AND METABOLISM

The degradative functions of the lysosome are essential to a number of cellular processes including nutrient scavenging during starvation, elimination of damaged cellular components, termination of mitogenic signals, elimination of intracellular and extracellular pathogens and cell and tissue remodeling. Each of these processes has been described in detail in a number of excellent reviews (Deretic & Levine 2009, Nixon 2013, Sorkin & von Zastrow 2009). Here we focus on emerging aspects of lysosomal function that have projected this organelle to the center stage of metabolic regulation both in normal and disease states.

3A. THE LYSOSOME IN NUTRIENT SENSING AND mTORC1 REGULATION

The lysosome is a key node for nutrient sensing and metabolic regulation via its physical and functional association with the master growth regulator mTORC1. mTORC1 is one of two protein kinase complexes built around the ancient serine-threonine kinase mTOR (Laplante & Sabatini 2012, Wullschleger et al 2006, Zoncu et al 2011b). By integrating positive and negative signals from nutrients, growth factors, energy levels, and stress, mTORC1 drives the processes of mass accumulation and size-doubling that are obligate prerequisites for cell division (Ben-Sahra et al 2016, Duvel et al 2010, Ma & Blenis 2009). A major advance in the field was the identification of the lysosome as the cellular location where two main inputs to mTORC1, nutrients and growth factors, converge to trigger downstream programs that lead to cell growth and proliferation (Sancak et al 2010, Zoncu et al 2011b).

Initiation of mTORC1-driven programs requires the physical association of mTORC1 to the lysosomal surface, a process that is subjected to sophisticated regulation. The signal that drives the binding of mTORC1 to the lysosomal surface is provided by amino acids. In addition to serving as building blocks for protein synthesis and substrates for energy

production, amino acids also play numerous signaling roles, from control of chemotaxis in bacteria to neurotransmission in the nervous system (Durr et al 2014, Dyer et al 2009, Ottemann et al 1999, Wu et al 2006). At first, the association of a pro-growth signaling kinase to a catabolic organelle may seem puzzling. However, its meaning becomes clear when one considers the ancestral role of the vacuole/lysosome as a cellular nutrient depot, a function that is evolutionarily conserved from fungi to higher eukaryotes (Chantranupong et al 2015, Efeyan et al 2012, Li & Kane 2009). mTORC1 is found on the surface of the lysosome/vacuole both in yeast and metazoan cells, and the mechanisms that regulate mTORC1 association with this organelle present many parallels but also intriguing differences between the two kingdoms of life.

The yeast vacuole in metabolic homeostasis—Unlike most metazoan cells, in which lysosomes are small (200–500nm) and number in the hundreds, yeast cells contain a few large structures, and often a single one, known as the vacuole (Efe et al 2005, Li & Kane 2009, Luzio et al 2007). Despite the morphological differences, lysosomes and vacuoles share many of the same components, including luminal hydrolases, membrane transporters and the vacuolar proton pump. The yeast vacuole was recognized early on as a storage site for ions such as phosphate, calcium and zinc, along with nutrients, including amino acids (Li & Kane 2009). Basic amino acids arginine, lysine, and histidine preferentially accumulate in the vacuole, where their concentration can reach in the high millimolar range (Kitamoto et al 1988). Neutral amino acids also display varying degrees of vacuolar accumulation, whereas acidic amino acids aspartate and glutamate are virtually absent (Kitamoto et al 1988, Wiemken & Durr 1974). Selective accumulation of vacuolar metabolites is dependent on transport and exchange processes powered by the proton gradient, which is established by the vacuolar H^+ ATPase (Forgac 2007, Zhao et al 2015). Several amino acid transporters, belonging to the Vba and Avt families among others, exploit the proton gradient in order to establish and maintain the vacuolar amino acid pool (Russnak et al 2001, Sekito et al 2008). The presence of this large internal reservoir plays a fundamental role in fungal physiology. At several points in their life cycle, yeast cells experience drastic fluctuations in external nutrient concentration and osmolarity. Thus by mobilizing their vacuolar nutrient pools, yeast cells can support life-sustaining activities that take place in the cytoplasm independent of external nutrients levels. Mobilization of the vacuolar stores also ensures rapid recovery from quiescent states triggered by prolonged starvation (Efe et al 2005, Li & Kane 2009).

During starvation, the vacuole increases its internal nutrient pool via autophagic degradation of non-essential cellular components. As in mammalian cells, macroautophagy involves the sequestration of cytoplasmic components including large protein complexes and organelles in autophagosomes, followed by their delivery to the vacuole for degradation (Mochida et al 2015) (Kraft & Peter 2008, Lazarou et al 2012, Mizushima et al 2008, Wild et al 2014). In microautophagy, invagination of the vacuolar limiting membrane allows direct capture of cytoplasmic components and their rapid degradation inside the lumen (Dubouloz et al 2005, Sahu et al 2011). An additional mechanism for nutrient replenishment involves targeting of integral plasma membrane proteins to the vacuole via endocytosis and intraluminal protein sorting mediated by the ESCRT complex (McCullough et al 2015, Wollert & Hurley 2010). This process, which leads to the degradation of several amino acid permeases (Jones et al

2012, Lin et al 2008, MacGurn et al 2011, Muller et al 2015), serves a two-fold purpose of preventing ‘leakage’ of cytoplasmic amino acids out of the cell, and to scavenge and degrade the corresponding transporters as sources of amino acids. Both autophagy and endocytosis are post-translationally regulated by mTORC1 at multiple levels (MacGurn et al 2011, Mizushima & Komatsu 2011). Thus, mTORC1 not only associates with the vacuole physically, but also regulates its nutrient outupt. Evidence discussed below reveals a feedback mechanism by which, localizing to the lysosomal surface, mTORC1 integrates nutrient information from the lysosome’s interior and from the surrounding cytoplasm to direct cell metabolism toward biosynthetic or catabolic pathways.

Mechanisms of mTORC1 recruitment to the lysosome—In mammals, many regulatory inputs originating at the plasma membrane, such as growth factor signals, converge upstream of mTORC1 on a large protein complex known as the Tuberous Sclerosis Complex (TSC), which negatively regulates the kinase activity of mTORC1 (Inoki et al 2003, Tee et al , Zoncu et al 2011b). In contrast, amino acids signal independently of TSC, via the Rag GTPases, a family of small G-proteins related to the Ras family of GTPases (Kim et al 2008, Sancak et al 2008). Unlike Ras and most other small G-proteins, the Rags exist as heterodimers where RagA or RagB, which are highly similar to each other, bind to either RagC or RagD. Thus, four dimer combinations are possible. Mutational analysis of key catalytic residues (identified through homology with Ras) led to a model in which the Rags are thought to exist in opposite nucleotide states: when RagA or B is bound to GDP, RagC or D is loaded with GTP (Kim et al 2008, Sancak et al 2008). Crucially, amino acids cause the Rags to switch to an active conformation that is competent for mTORC1 activation, in which RagA/B is GTP-loaded and RagC/D is GDP-loaded (FIG. 2). The same model has been proposed in yeast, where GTP-loaded Gtr1 (the RagA/B ortholog) and GDP-loaded Gtr2 (the RagC/D ortholog) maximally activate yeast mTORC1 (Binda et al 2009, Dubouloz et al 2005).

In response to amino acids, the Rag GTPases activate mTORC1 not by turning on its kinase activity, but by inducing its relocalization from the cytoplasm to prominent vesicular structures, which possess all the features of lysosomes based on their morphology, trafficking pattern and expression of multiple late endosomal/lysosomal markers such as LAMP1 and LAMP2(Sancak et al 2010, Sancak et al 2008, Zoncu et al 2011a). Lysosomal localization is essential for mTORC1 activity, because it enables it to interact with Rheb, a small GTPase that turns on mTORC1 kinase activity (Demetriades et al 2014, Menon et al 2014, Sancak et al 2010, Sancak et al 2008, Zoncu et al 2011b). In summary, by modulating the nucleotide state of the Rag GTPases, amino acids cause mTORC1 to translocate to the lysosome. There, Rheb unlocks the kinase activity of mTORC1 and enables it to phosphorylate downstream substrates (FIG. 2).

These findings were paralleled by studies in *S. Cerevisiae*, in which TOR1, KOG1 (the Raptor ortholog) and LST8, tagged with GFP at the endogenous locus, display a prominent localization to the surface of the vacuole. However, an important difference between mammalian and yeast cells is that, in the latter, mTORC1 appears to remain bound to the vacuole even following amino acid withdrawal (Binda et al 2009). This important mechanistic difference could reflect evolutionary divergence. *S. Cerevisiae* lacks a clear

functional homolog of Rheb, thus in this organism Gtr1/2 may regulate TORC1 kinase activity rather than its vacuolar targeting (Efeyan et al 2012).

Because the nucleotide state of the Rag GTPases is key to their ability to bind to mTORC1, a major effort has been directed toward identifying guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) that may translate amino acid abundance into mTORC1 recruitment to the lysosome. The first such factor to be identified is a protein complex called Ragulator, which is specifically localized to the lysosome and is composed of 5 proteins known as LAMTOR 1–5 (Bar-Peled et al 2012, Nada et al 2009, Sancak et al 2010, Teis et al 2002). Ragulator has GEF activity toward RagA/B, promoting its loading with GTP and therefore driving mTORC1 recruitment and activation at the lysosome (Bar-Peled et al 2012). Interestingly, Ragulator is also a scaffold that anchors the Rag GTPases to the lysosomal surface. Unlike Ras (to which they display the highest homology) the Rag GTPases lack any lipid modification and thus cannot directly bind to membranes. When Ragulator components are deleted, the Rags become cytoplasmic and are no longer able to recruit mTORC1 in response to amino acids (Sancak et al 2010) (FIG. 2).

The second event in Rag GTPase transition to the active state is GTP hydrolysis by RagC/D. The tumor suppressor protein folliculin (FLCN) in complex with folliculin interacting protein (FNIP) 1 or 2 was shown to function as a GAP for RagC/D (Petit et al 2013, Tsun et al 2013). FLCN and FNIP reside together at the lysosomal surface, and their acute depletion blocks mTORC1 activation by amino acids. Recent studies in yeast identified a potential ortholog of FLCN/FNIP, the Lst4-Lst7 complex, which also has GAP activity toward Gtr2 and is required for full TORC1 activation by amino acids (Peli-Gulli et al 2015).

Amino acid withdrawal is thought to induce the transition of the Rag GTPases to the inactive state, in which RagA/B is GDP-loaded, RagC/D is GTP-loaded and the heterodimer cannot bind to mTORC1. Studies in mammalian cells led to the identification of the GATOR1 complex as the GAP that inactivates RagA/B. Depletion of any of the three GATOR1 subunits leads to constitutive lysosomal localization and activation of mTORC1 irrespective of amino acid status (Bar-Peled et al 2013). A second 5-subunit complex known as GATOR2, binds to GATOR1 and blocks its GAP activity toward Rag A/B. *S. Cerevisiae* has homologs of all GATOR1 and GATOR2 subunits, which are all part of one stable protein complex known as Seh1-associated (SEA) that functions in a similar way to GATOR1 and GATOR2 (Dokudovskaya et al 2011, Panchaud et al 2013) (FIG. 2).

Despite various candidates, a clear RagC/D GEF has not been identified. It is likely that, as investigations continue, additional factors controlling the nucleotide loading state of Rag/Gtr GTPases will be discovered. Moreover, there are likely species-specific differences in the identity of these factors. For instance, in *S. Cerevisiae* but not in mammals Vam6, a component of the HOPS complex, was shown to be required for mTORC1 activation at the vacuole via its GEF activity toward Gtr1 (Binda et al 2009). Thus the discovery of the Rag GTPases and the upstream machinery responsible for their activation and regulation of mTORC1 has launched a new era where the lysosome takes center stage as a metabolic regulatory hub.

Convergence of amino acid sensing pathways at the lysosome—As Ragulator, FLCN and GATOR1/2 are essential for the ability of mTORC1 to respond to amino acids, it is reasonable to assume that they function downstream of one or more amino acid-sensing proteins. Even prior to the discovery of the Rag GTPases, the prevalent view in the field was that mTORC1 senses the intracellular and not the extracellular pool of amino acids. Inside the cell, at least two pools of amino acids may be important for mTORC1 activation, the cytoplasmic pool along with amino acids derived form degradation within the lysosome/vacuole.

A possible role for the lysosomal amino acid pool in mTORC1 activation was identified using a cell-free system, in which a preparation of intact lysosomes was mixed with exogenous mTORC1, and binding of mTORC1 to these lysosomes was measured. In this assay, entry of amino acids inside the lysosome was sufficient to induce mTORC1 binding to the Rag GTPases, suggesting an 'inside-out' model of amino acid sensing (Zoncu et al 2011a). A search for proteins that may relay luminal amino acid abundance to Ragulator and Rag GTPases led to the involvement of the v-ATPase. The v-ATPase fits some of the criteria for a positive transducer of amino acid availability as it physically binds to Ragulator and Rag GTPases in an amino acid-regulated manner, and its inactivation by drugs or RNAi prevents mTORC1 recruitment to the lysosome in response to amino acids (Jewell et al 2015, Zhang et al 2014, Zoncu et al 2011a). Three recent reports implicated a second lysosomal transmembrane protein, SLC38A9, in the regulation of mTORC1 by amino acids (Jung et al 2015, Rebsamen et al 2015, Wang et al 2015a). SLC38A9 is a Na^+-dependent amino acid permease that is thought to transport amino acids between the cytoplasm and the lysosomal lumen. SLC38A9 interacts with Rag GTPases and Ragulator via its 119 aa N-terminal domain, located in the cytoplasm, and it also binds to the v-ATPase via its C-terminal transmembrane region. The transmembrane region of SLC38A9 appears to harbor an amino acid-sensing function, as its presence renders binding of the N-terminal cytoplasmic portion to Ragulator and Rags amino acid-dependent. When expressed in proteoliposomes, SLC38A9 behaved as a low-affinity transporter for various polar amino acids, especially arginine (Rebsamen et al 2015, Wang et al 2015a). Together, these results indicate that SLC38A9 may be a dedicated sensor for lysosomal arginine levels upstream of mTORC1.

Because the composition of the lysosomal amino acid pool appears to be different from the cytoplasmic pool, one would predict that additional pathways should convey information about cytoplasmic amino acid levels to the Rag GTPases. Evidence for one of these pathways has recently emerged with the identification of the Sestrin 1–3 proteins as upstream regulators of mTORC1 (Budanov & Karin 2008, Chantranupong et al 2014, Parmigiani et al 2014, Wolfson et al 2016). By binding to GATOR2, the Sestrins appear to block its ability to inhibit GATOR1, and thus indirectly promote GTP hydrolysis by RagA/B (Chantranupong et al 2014, Parmigiani et al 2014). Follow-up work on Sestrin2 has made a strong case that these proteins are dedicated leucine sensors upstream of mTORC1 (Wolfson et al 2016). Leucine but not arginine disrupts the interaction between Sestrin2 and GATOR2 in vitro. Furthermore, elegant binding assays showed that recombinantly expressed Sestrin2 binds to radiolabeled leucine with a 20uM Kd, which fits well with the half-maximal leucine

concentration for mTORC1 activation. Mutants of Sestrin2 that failed to bind to GATOR2 were unable to suppress mTORC1 signaling upon leucine deprivation; conversely, Sestrin2 mutants that fail to bind to leucine but still retain the ability to bind to GATOR2 suppressed mTORC1 in a constitutive manner (Wolfson et al 2016).

Through these studies, the lysosomal surface emerges as a metabolic signaling center that integrates nutritional information from the lysosome's interior and the cytoplasm to promote mTORC1-mediated signals that regulate the balance between biosynthetic and catabolic programs. The discovery of both cytoplasmic and lysosomal amino acid sensors poses intriguing questions such as whether detection of different amino acid species is compartment-specific, how cross talk between these compartment-specific sensors occurs, and how each sensing system contributes to metabolic adaptation within different tissues and organs.

3B. TRANSCRIPTIONAL REGULATION OF LYSOSOMAL FUNCTION

The CLEAR genome and the MiT/TFE factors—The correct execution of cellular catabolism relies on the concerted action of multiple components such as lysosomal hydrolases and transporters, the autophagic machinery, mediators of substrate selection and capture as well as the membrane trafficking pathways that connect autophagosomes with lysosomes. The inducible nature of autophagy had been appreciated for some time as discussed above. Phosphorylation of the Ulk1-Atg13-FIP200 complex by mTORC1 or AMPK is a key checkpoint in autophagy initiation (Egan et al 2011, Hosokawa et al 2009, Kamada et al 2000, Noda & Ohsumi 1998). Transcriptional control of autophagy gene subsets has been demonstrated for multiple transcription factors, including Activating Transcription Factor 4 (ATF4) (Pike et al 2013, Rouschop et al 2010), p53 (Kenzelmann Broz et al 2013), and the Forkhead Box O (FOXO) proteins (Lapierre et al 2015, Mammucari et al 2007, Warr et al 2013). In contrast, evidence for dynamic regulation of lysosomal function was lacking until recently, leading to a general perception of the lysosome as an end-point catabolic compartment, incapable of adapting to changing metabolic conditions.

This long-held view has been radically altered by the discovery that entire classes of lysosomal genes, including hydrolases, lysosomal membrane permeases and lysosome-associated proteins, are under coordinated transcriptional control (Palmieri et al 2011, Sardiello et al 2009, Settembre et al 2013b). Indeed bioinformatic analysis identified a shared E box-related consensus element present in the promoter region of many lysosomal genes, which was aptly named Coordinated Lysosomal Expression and Regulation (CLEAR) element. The CLEAR element is the target of a family of basic Helix-Loop-Helix (bHLH) transcription factors known as the MiT/TFE proteins, whose members are TFEB, TFEC, TFE3 and MITF (Palmieri et al 2011, Sardiello et al 2009, Settembre et al 2013b). MITF had previously been associated with the biogenesis of melanosomes (which are lysosome-related organelles), and is frequently amplified in melanoma, whereas the function of the other three factors was less well understood. Chromatin immunoprecipitation (ChIP) experiments showed that TFEB (and later MITF and TFE3) directly bind to CLEAR elements and stimulate the expression of their downstream target genes. Strikingly, TFEB

overexpression was sufficient to induce a dramatic expansion of the lysosomal compartment, as judged by the size, number and protein content of lysosomal vesicles (Sardiello et al 2009).

Subsequent investigations showed that TFEB also drives the expression of numerous proteins involved in multiple steps in autophagy, including autophagosome initiation (BECN1, NRBF2), elongation (GABARAP, WIPI2, ATG9b), substrate capture (SQSTM1) and autophagosome trafficking and fusion with lysosomes (UVRAG) (Palmieri et al 2011, Settembre et al 2011). Accordingly, TFEB overexpression promoted all of these steps, increasing the number of autophagosomes, the frequency of their fusion with lysosomes as well as the rate of substrate degradation (Settembre et al 2011). These results indicated that TFEB is a true master regulator of cellular catabolism, which coordinately expands the ability of the cell to select and capture substrates via autophagy and then to degrade them via the lysosome. This pro-catabolic activity occurs in most cell types, but also has tissue- and organ-specific specialization. For example, in the liver TFEB strongly promotes lipid catabolism via activation of a gene expression program that includes the master metabolic transcription factor PGC1-alpha and many of its downstream genes involved in fatty acid oxidation and mitochondrial biogenesis (Settembre et al 2013a). Liver-specific deletion of TFEB rendered mice hyper-sensitive to the effects of high fat diet, whereas its overexpression promoted resistance to lipid accumulation in an autophagy-dependent manner. The regulatory action of TFEB in lipid metabolism is also conserved in *C. Elegans* (O'Rourke & Ruvkun 2013, Settembre et al 2013a). These studies provided strong evidence that regulation of the autophagic-lysosome system may be a key aspect of metabolic adaptation both at the cellular and the organismal level.

Control of lysosomal biogenesis by pro-growth signaling pathways—A key discovery came from the realization that pathways involved in nutrient sensing and growth control regulate the MiT/TFE factors. Initial studies detected TFEB both in the nucleus of cells or in their cytoplasm. Intriguingly, TFEB nuclear accumulation was prompted by nutrient withdrawal (Sardiello et al 2009, Settembre et al 2011). Consistent with this pattern, it was subsequently found that mTORC1 has a major role in controlling the nuclear-cytoplasmic shuttling of TFEB (Martina et al 2012, Roczniak-Ferguson et al 2012, Settembre et al 2012). Acute inhibition of mTORC1 via starvation or catalytic inhibitors (e.g. Torin1) led to rapid translocation of TFEB to the nucleus, leading to activation of its target lysosomal-autophagic genes (FIG. 3). Conversely, in full nutrient conditions active mTORC1 directly phosphorylates TFEB on two critical residues, Serine 142 and Serine 211, causing its binding to 14-3-3 proteins and its retention in the cytoplasm. Dynamic imaging experiments showed that, in full nutrients, TFEB continuously cycles between the cytoplasm and the lysosomal surface, where it physically binds to mTORC1 and, upon being phosphorylated, is released into the cytoplasm (Settembre et al 2012). The Rag GTPases also play important roles in this process. Constitutively active Rag mutants (which activate mTORC1 regardless of nutrient status) strongly suppress TFEB nuclear translocation under amino acid-starved conditions, whereas dominant-negative Rag GTPases, which prevent mTORC1 binding and activation at the lysosome, cause massive accumulation of TFEB in the nucleus (Settembre et al 2012). Moreover, the Rag GTPases directly bind to TFEB

and were proposed to physically bridge its interaction with mTORC1, leading to its phosphorylation (Martina & Puertollano 2013). Other MiT/TFE family members, TFE3 and MITF, are regulated in a similar manner and therefore mediate a nutrient-dependent lysosomal response (Martina et al 2012, Roczniak-Ferguson et al 2012). Along with mTORC1 other growth-regulating kinases such as MEK/ERK and Glycogen Synthese Kinase 3 (GSK3) also affect MiT/TFE nuclear localization (Marchand et al 2015, Ploper et al 2015, Settembre et al 2011).

Dephosphorylation of TFEB is also highly regulated and represents a parallel mechanism to control its activity. The calcium-regulated serine-threonine phosphatase calcineurin was shown to be required for starvation-induced translocation of TFEB to the nucleus (Medina et al 2015). The mechanism through which calcineurin becomes activated is especially intriguing, as it involves release of lysosomal calcium stores via the lysosomal calcium channel, mucolipin 1 (MCOLN1). Starvation triggers MCOLN1-dependent elevation in cellular calcium levels, most likely via release of the lysosome's own internal stores. This is a key event, as knock down of MCOLN1 or treatment with calcium-chelating agents strongly suppress TFEB nuclear translocation and prevented upregulation of lysosomal and autophagic gene expression (Medina et al 2015, Wang et al 2015b). Thus, dynamic release of lysosomal calcium may represent a novel homeostatic response to starvation. In mice undergoing exercise, TFEB accumulated in the nuclei of muscle fibers, suggesting that dynamic control of TFEB activation is part of the normal energy replenishment process that accompanies physical activity.

Altogether, these studies help illuminate the inner logic of metabolic control. Nutrients and growth factors not only stimulate pro-growth kinases such as mTORC1 and Erk but, via these same kinases, also actively suppress catabolic programs that antagonize cell mass accumulation, an obligate prerequisite for proliferation. Conversely, low nutrients and stress trigger transcriptional programs that generate, in a coordinated manner, all the components required for nutrient scavenging and survival, including autophagic and lysosomal vesicles and their biochemical constituents.

This regulatory logic goes beyond the mTORC1-TFEB axis. ZKSCAN3 is a zinc-finger transcription factor that harbors a Kruppel-associated box and a SCAN domain (Chauhan et al 2013). Like TFEB, ZKSCAN3 binds to the promoter region of several lysosomal genes, however, unlike TFEB, ZKSCAN3 is a potent suppressor of these genes. Accordingly, nutrients and growth-control pathways regulate ZKSCAN3 oppositely from TFEB: under full nutrients, ZKSCAN3 is found in the nucleus, whereas starvation and mTOR inhibition drive ZKSCAN3 out of the nucleus, allowing activation of catabolic programs that mediate cellular response to starvation. Whether ZKSCAN3 is directly phosphorylated by mTORC1 or other nutrient responsive protein kinases is currently unknown (FIG.3).

Other studies are uncovering a broader transcriptional network that controls autophagy-lysosome function. For instance, when nutrients are high the nuclear receptor farnesoid X receptor (FXR), which is activated by bile acids upon feeding, binds to the promoters of numerous autophagic and lysosomal genes, as well as to the TFEB promoter, and suppresses their expression both in cells and in mice (Lee et al 2014, Seok et al 2014). FXR

exerts its inhibitory action by competing with two transcriptional activators of autophagy. During fasting cAMP response element-binding protein (CREB) transactivates autophagic-lysosomal genes by recruiting the transcriptional co-activator CRTC2 to their promoters. Upon refeeding, FXR binds to CREB and displaces CRTC2, effectively blocking CREB activity (Seok et al 2014) (FIG. 3). FXR also inhibits peroxisomal proliferator-activated receptor-α (PPARα), which drives the expression of genes involved in autophagy and lipid degradation (Lee et al 2014). Other seemingly tissue specific regulators of autophagy have also emerged. For instance, in the context of muscle atrophy Foxk1/2 transcription factors were shown to be negative regulators of autophagic gene expression. Interestingly, nuclear localization of Foxk1/2 proteins was positively regulated by mTORC1, thereby switching off autophagy in nutrient-rich conditions (Bowman et al 2014). Thus, FXR and Foxk1/2 proteins emerge as novel master regulators that, similar to ZKSCAN3, keep catabolic programs at bay and favor growth when nutrients are abundant in diverse tissue settings.

4. ALTERED LYSOSOME ACTIVITY IN DISEASE

Changes in endocytic traffic, autophagy and lysosome function have been implicated in a number of disorders associated with alterations in the processing and turn over of cellular constituents. Mutations in key lysosomal enzymes are implicated in inherited monogenic diseases collectively known as lysosomal storage disorders (LSDs), many of which are associated with severe neurodegenerative phenotypes and described in detail in a number of comprehensive reviews (Ballabio & Gieselmann 2009, Bellettato & Scarpa 2010, Cox & Cachon-Gonzalez 2012, Platt et al 2012). Interestingly, the effects of lysosome dysfunction in these disease settings appear to manifest more profoundly in the central nervous system than in other parts of the body. Moreover, endo-lysosomal gene mutations, some associated with LSD, have emerged as risk factors for late onset neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease and frontotemporal dementia (FTD) (Metcalf et al 2012, Ramirez et al 2006, Skibinski et al 2005). The central role of lysosomes in cellular trafficking and clearance may be especially important for maintenance of neuronal health particularly given the limited regenerative capacity and the postmitotic status of this cell population. Thus the lysosome functions at the nexus of a collection of disorders intimately associated with neurological dysfunction. In contrast, it is these same functions of the lysosome, namely the clearance of damaged organelles and aggregate prone macromolecules and recycling of cellular building blocks to maintain overall cellular fitness, that contribute to the pathogenesis of cancer. The features of these disorders resulting from opposing alterations in lysosome function will be discussed below.

4A. LINKS BETWEEN LYSOSOMAL STORAGE DISORDERS AND NEURODEGENERATION

The common endpoint to all lysosome-mediated activities is the production and release of simple metabolites to the cytoplasm or to other cellular compartments. Failure to degrade lysosomal cargo or export lysosomal catabolites resulting from deficiency of specific lysosome hydrolases or permeases underlies LSDs which are individually rare inherited metabolic disorders, with a cumulative incidence of 1 in 5000. At the whole body level, LSDs are associated with major developmental delays, neurological defects,

behavioral abnormalities and metabolic imbalance often leading to early death. At the ultrastructural level these disorders display common hallmarks associated with alterations in lysosome morphology, motility and number, abnormalities in intracellular trafficking and the accumulation of undigested substances within the lysosome lumen leading to enlarged (>1000 nm) dysfunctional lysosomes. Approximately 60 different LSDs have been identified and the specific hydrolase or permease that is mutated generally dictates the nature of the accumulated substance in the lysosome (ie - mucopolysaccharides, sphingolipids, glycoproteins, and lipofuscins) (Ballabio & Gieselmann 2009, Cox & Cachon-Gonzalez 2012, Platt 2014).

A number of LSDs are characterized by progressive neurodegeneration. For instance in Niemann-Pick type C (NPC) disease, loss of a cholesterol export system composed of two lysosomal proteins, NPC1 and NPC2, leads to massive accumulation of de-esterified cholesterol within lysosomes (Carstea et al 1997, Kwon et al 2009, Xu et al 2007). NPC patients show progressive cerebellar ataxia and, interestingly, develop neuropathological features typical of Alzheimer's disease patients, including accumulation of neurofibrillary tangles and amyloid β-peptide in the absence of mutations in Alzheimer's related genes (Nixon 2013). Gaucher disease, the most common LSD, results from homozygous loss of function mutations in the lysosomal enzyme β-glucocerebrosidase (GBA), leading to accumulation of glucosylceramide. Patients manifest multi-organ dysfunction particularly in the spleen, skeletal muscle and hematopoietic system and a subset develop parkinsonism during their lifetime (Type I). A separate cohort of patients undergoes severe progressive neurodegeneration (Type II and III) (Bultron et al 2010, Tayebi et al 2003). Moreover, unaffected family members of Gaucher patients carrying heterozygous mutations of GBA have a five fold increased risk of developing Parkinson's disease and an eight fold risk for developing Lewy body dementia (Platt 2014). Hence heterozygous mutation of GBA is the most common known risk factor for Parkinson's disease identified to date (Aharon-Peretz et al 2004, Goker-Alpan et al 2008, Neudorfer et al 1996). The underlying causative roles of mutant GBA in driving neurodegenerative phenotypes remain unclear. However, accumulation of glucosylceramide was shown to be sufficient to promote the formation of α-synuclein-positive assemblies associated with Parkinson's disease (Mazzulli et al 2011). Moreover, secondary accumulation of other lysosomal substrates can occur as a result of global lysosome dysfunction due to the primary mutational defect. For instance, in NPC extensive accumulation of sphingolipids parallels that of cholesterol, strongly suggesting that the transport of these two classes of lipids is functionally connected (Lloyd-Evans et al 2008).

Consistent with a protective role for autophagy in the brain, mice harboring neuronal specific knockout of ATG5 accumulate intracellular protein aggregates and inclusions and show progressive neurodegeneration in the absence of any additional disease associated mutations (Hara et al 2006, Karsli-Uzunbas et al 2014, Komatsu et al 2006). Similarly, a number of cellular and mouse models of LSD also show defective autophagy in several tissues including the brain (Ballabio & Gieselmann 2009, Lieberman et al 2012, Osellame et al 2013, Settembre et al 2008). Accumulation of autophagosomes was noted in LSD and may imply either a defect in autophagosome maturation or futile upregulation of autophagy to counter the decreased lysosomal degradative capacity. The net effect however is the

accumulation of autophagic substrates such as defective mitochondria and polyubiquitylated protein aggregates, which further exacerbate neurodegenerative phenotypes (Bjorkoy et al 2005). It is clear that a greater dependence on efficient autophagy/endosomal/lysosomal activity is required for neuronal function. However the mechanisms underlying the unique vulnerability of neuronal populations to impaired autophagy/endosomal/lysosomal activity still remains obscure. Furthermore, whether mutations in other lysosomal genes could also represent risk factors for development of neurodegenerative disorders is yet to be determined.

Treatment of LSDs focuses on restoration of lysosome function via a number of strategies, including gene therapy and enzyme replacement therapy (ERT). However, reversal of abnormalities in some but not all effected organs, particularly central nervous system manifestations, limits the clinical benefit of these agents (Ballabio & Gieselmann 2009, Jeyakumar et al 2005). More recently, over-expression of TFEB was shown to promote autophagosome-lysosome fusion, lysosome trafficking and exocytosis leading to a dramatic reduction in intra-lysosomal content in multiple cellular models of LSD (Decressac et al 2013, Medina et al 2011, Song et al 2013, Spampanato et al 2013). Novel strategies aimed at inhibiting mTORC1 could be employed in the setting of LSDs to promote nuclear localization of MiT/TFE factors and unlock ULK1 to promote autophagy induction. Future studies delineating the mechanisms and links between lysosomal and autophagic dysfunction and neurodegeneration may also highlight new nodes for development of novel therapeutic agents (Efeyan et al 2012, Nixon 2013, Ravikumar et al 2004).

4B. LYSOSOMES IN CANCER METABOLISM

Upregulation of catabolic processes is emerging as a driving force in cancer progression (Kaur & Debnath 2015, Rabinowitz & White 2010). Rapidly proliferating cancer cells rely on high rates of synthesis of new proteins, membrane lipids, DNA and RNA(Lunt & Vander Heiden 2011). However, the ability to recycle and reuse internal cellular constituents becomes critically important when cancer cells are denied access to a ready supply of external nutrients. This is particularly relevant in cancers that must contend with extreme fluctuations in *in vivo* microenvironment conditions due to poor vascularization, which limits nutrient and oxygen levels, and infiltration of stromal cells that compete for available nutrients (Davidson et al 2016, DeNicola & Cantley 2015, Perera & Bardeesy 2015, Yang et al 2011). Under these conditions, nutrient scavenging pathways such as autophagy and macropinocytosis, which converge on the lysosome, aid in the generation of necessary building blocks for production of all classes of cellular macromolecules.

Macropinocytosis, the process of bulk uptake and lysosome degradation of extracellular material, has emerged as an important nutrient delivery route that fuels metabolic and biosynthetic reactions in cancer cells harboring oncogenic KRAS mutations (Commisso et al 2013, Kamphorst et al 2013, Kamphorst et al 2015, Palm et al 2015). Carbon tracing of ^{13}C-labeled albumin taken up via macropinocytosis in PDA cell lines and *in vivo* tumors revealed specific labeling of multiple metabolite species, indicating that lysosome-mediated digestion of albumin is followed by the utilization of the resulting free amino acids in the cytoplasm (Commisso et al 2013, Kamphorst et al 2015). These studies highlighted the

importance of lysosomal catabolism in supplying essential building blocks for tumor growth. Indeed, there is presently considerable interest in fully understanding the contributions of lysosome-based catabolism to tumor metabolism in PDA and potentially other cancer types.

Established tumors also exploit the macromolecular recycling and detoxifying functions of autophagy to gain a growth advantage. Studies in lung and pancreatic tumor models have shown that these tumors are reliant on constitutive activation of autophagy for growth (Guo et al 2011, Karsli-Uzunbas et al 2014, Perera & Bardeesy 2015, Perera et al 2015, Rao et al 2014, Strohecker et al 2013, White 2015, Yang et al 2011). Additionally, several studies employing genetic inactivation of essential autophagy components in mouse models of melanoma and breast cancer have identified context- and stage-specific roles of autophagy in tumor initiation and progression (Lock et al 2011, Wei et al 2011, Xie et al 2015). Moreover, a recent study showed that autophagy activation is coordinated with increased lysosome biogenesis and function in highly aggressive pancreatic adenocarcinoma (PDA). Importantly, the MiT/TFE proteins were found to be upregulated in PDA and required for maintaining high levels of autophagy and lysosome function. Boosted lysosome-mediated catabolic activity in PDA tumors was required for maintenance of intracellular amino acid levels and likely serves to fuel specific biosynthetic and bioenergetic pathways required for tumor growth (Perera et al 2015). Studies addressing whether a similar reliance on autophagy or lysosome mediated catabolism occurs in other tumor types is ongoing. It will be interesting to determine whether autophagy-lysosome gene programs are activated in subsets of soft tissue sarcoma and renal cell carcinoma harboring genomic translocations of TFE3 and TFEB and melanomas having genetic amplifications of MITF (Davis et al 2003, Garraway et al 2005, Goodwin et al 2014, Haq & Fisher 2011, Kauffman et al 2014, Kuiper et al 2003, Ladanyi et al 2001) in addition to lineage specific signatures. Certainly, knowledge gained from analysis of autophagy-lysosome regulation in PDA could aid in unraveling disease mechanisms in a broader cohort of tumor types that similarly show dependence on autophagy for growth.

Lysosomes have also been implicated in resistance to cancer drug treatment or following inactivation of oncogenes. For instance, therapy-induced autophagy serves as a tumor survival pathway and has been linked to resistance to radiotherapy and cytotoxic chemotherapy in several cancer settings (Sui et al 2013). Similarly, in PDA resistance to mutant Kras ablation is mediated in part by upregulation of autophagy-lysosome activity (Viale et al 2014). It is likely that the quality-control mechanisms of autophagy and the upregulation of lysosome derived nutrients may serve to enhance overall cellular fitness and enable sustained tumor cell survival under these conditions. Therefore, much effort has been placed on development of lysosome-based inhibitors that can turn off autophagy and additional nutrient scavenging pathways. Correspondingly, treatment with inhibitors of lysosome function leads to pronounced decreases in tumorigenicity in multiple *in vitro* and *in vivo* tumor models (White 2015). Hydroxychloroquine (HCQ) is the most widely used lysosome inhibitor currently being tested in over 40 clinical trials against a diverse array of tumor types and in multiple treatment settings (Kroemer 2015, Piao & Amaravadi 2015). To date, little is known about the precise mechanism of action of HCQ at the lysosome however treatment of mammalian cells leads to a number of phenotypes consistent with lysosome dysfunction (lysosome enlargement, build up of autophagosomes, defective digestion of

cargo material). While use of HCQ as a single agent has not shown significant therapeutic efficacy (Wolpin et al 2014), results of 5 combination therapy early phase clinical trials in gliobastoma, myeloma, melanoma and other solid tumors showed high doses of HCQ were well tolerated and could block autophagy in tumors cells (Barnard et al 2014, Mahalingam et al 2014, Vogl et al 2014). Some striking responses and prolonged stable disease were observed in patients with a diverse array of tumor types suggesting that combination therapy with lysosome inhibitors may be broadly efficacious. Challenges associated with drug delivery and in vivo activity of HCQ has spurred the development of more potent autophagy-lysosome inhibitors. One example is Lys05, a novel dimeric derivative of chloroquine (CQ) currently in pre-clinical development (McAfee et al 2012). Preliminary results show significant anti-tumor activity in melanoma models as a single agent and in combination with a BRAF inhibitor (Ma et al 2014, McAfee et al 2012). Another avenue currently being explored are treatments that induce lysosomal membrane permeabilization (LMP). This process is thought to induce necrotic cell death (necroptosis) following release of factors that are normally sequestered inside the lysosomal lumen, such as cathepsins and metal ions, into the cytoplasm (Aits & Jaattela 2013, Nylandsted et al 2004, Petersen et al 2013). Thus targeting of lysosomes may be a potent therapeutic strategy that could prevent or delay therapy resistance or increase the effectiveness of anticancer drugs in several tumor settings. As the functional roles of the lysosome in the context of tumor metabolism evolve, it is likely that this key organelle will emerge as a central regulator of metabolic rewiring in a broad range of malignancies. Defining the most beneficial context in which to deploy lysosome inhibitors in the clinic is an important ongoing goal

5. CONCLUSION AND PERSPECTIVES

The lysosome has emerged as a key metabolic signaling center for the cell via its ability to transport nutrients, gauge their availability and communicate this information to growth regulatory pathways such as mTORC1. The molecular events initiated at the lysosomal surface reach all the way to the nucleus, where they control transcriptional programs that steer metabolism down biosynthetic or catabolic paths. More than likely, the studies described herein have only begun to scratch the surface of a vast and interconnected network for cellular decision-making consisting of vesicles, transporters, metabolites, signaling factors and chromatin regulators. An exciting direction will be to understand how the lysosomal signaling network operates in the context of whole organism physiology and aging. From yeast to mammals, inhibition of mTORC1 is a major lifespan-extending strategy, and recent work in *C. Elegans* shows that the resulting upregulation of TFEB activity is an important contributing factor (Lapierre et al 2015, O'Rourke & Ruvkun 2013). It is also increasingly clear that lysosomes play organ- and tissue-specific functions. For example, lysosomal biogenesis has been shown to be critical for lipid homeostasis both in worms and mice (O'Rourke & Ruvkun 2013, Settembre et al 2013a). Moreover, age-specific changes in lysosomal composition and function may impact metabolic homeostasis and degrade cellular quality control (Rodriguez-Navarro et al 2012, Zhang & Cuervo 2008). Thus, a new direction of investigation will be to apply high throughput proteomic and lipidomic techniques to investigate whether organ-specific lysosomes exist, and how their molecular makeup evolves with the age and metabolic state of the organism.

Another developing area is to understand how the lysosome interacts with other cellular compartments, both physically and functionally. For instance, a recent study showed that lysosome derived metabolites can influence transcriptional events in the nucleus (Folick et al 2015). Indeed, both in yeast and mammalian cells, there is increasing evidence of physical contacts between the lysosome/vacuole and other organelles such as mitochondria or peroxisomes, which appear to mediate or facilitate lipid transport (Chu et al 2015, Elbaz-Alon et al 2014, Honscher et al 2014). It is likely that this fertile area of investigation will continue to develop as additional modes of inter-organelle communication are discovered.

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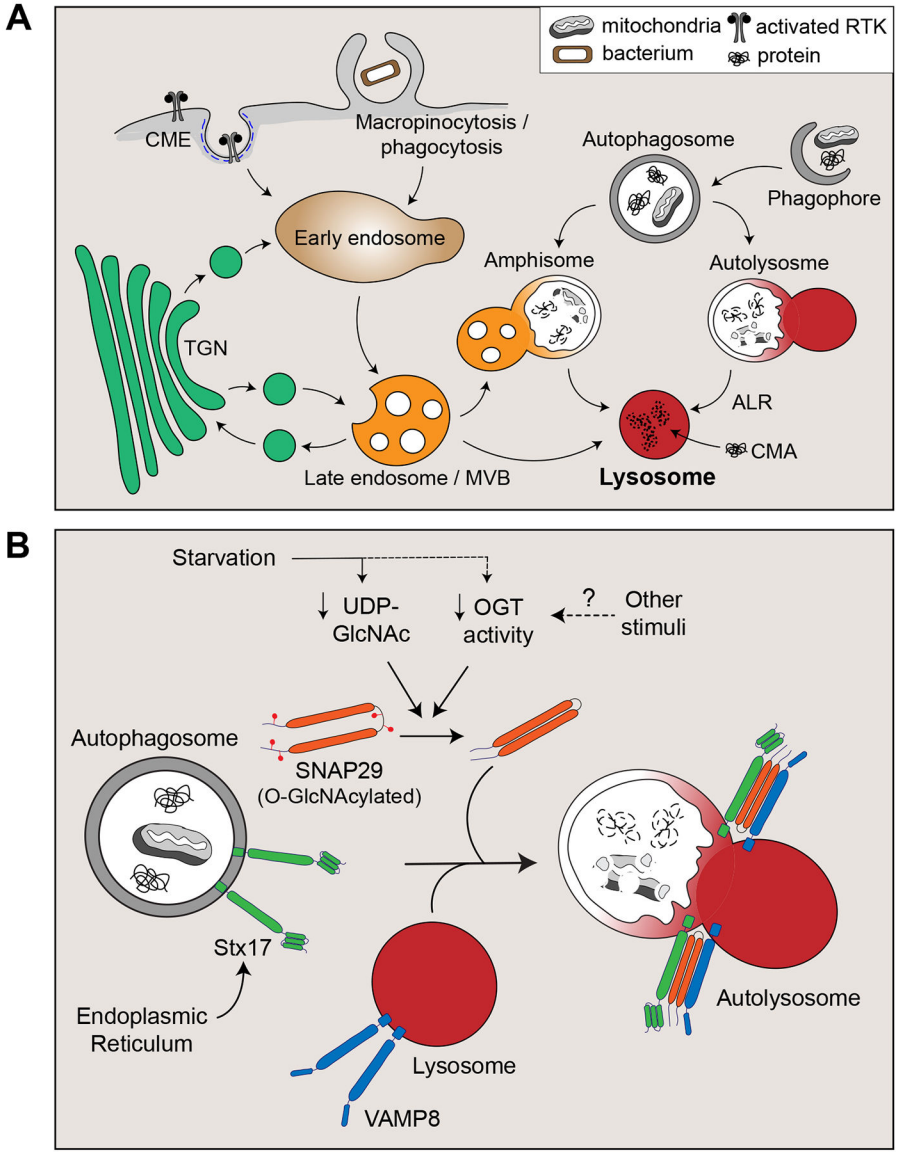

Figure 1.

A) The endolysosomal system. The lysosome is the terminal degradative station of multiple trafficking routes including endocytic and scavenging pathways. Extracellular material or pathogens are endocytosed through macropinocytosis or phagocytosis while plasma membrane localized proteins such as signaling receptors are internalized via clathrin mediated endocytosis (CME). Endocytosed material is trafficked to intracellular sorting stations – early endosomes – where cargo can be re-routed back to the plasma membrane or retained for degradation. Through progressive maturation, early endosomes convert to late endosomes that contain intraluminal vesicles. Intracellular constituents (protein aggregates, worn out organelles) are targeted for degradation via the process of autophagy. Autophagosomes containing cargo material fuse with lysosomes to mediate degradation. Autophagosomes can also fuse with late endosomes to form amphisomes – an intermediary station between classical endocytic routes and autophagic degradation.

Additional mechanisms of degradation include chaperone-mediated autophagy (CMA), which involves direct translocation of cytosolic protein across the lysosome membrane in order to be degraded. During autophagic lysosome reformation (ALR), sorting of lysosome-specific components from the hybrid autolysosome regenerates a full complement of primary lysosomes. **B)** Snare mediated fusion of autophagosomes and lysosomes in mammalian cells. During starvation-induced autophagy, the Qa-SNARE, syntaxin 17 (Stx17) present on autophagosomes interacts with the cytosolic Qbc-SNARE SNAP-29 and the R-SNARE VAMP8 present on the lysosomal membane. In nutrient replete conditions, SNAP-29 is *O*-GlcNAcylated (red symbols) by *O*-GlcNAc transferase (OGT), which inhibits the formation of the SNARE complex and subsequent fusion between the autophagosome and lysosome.

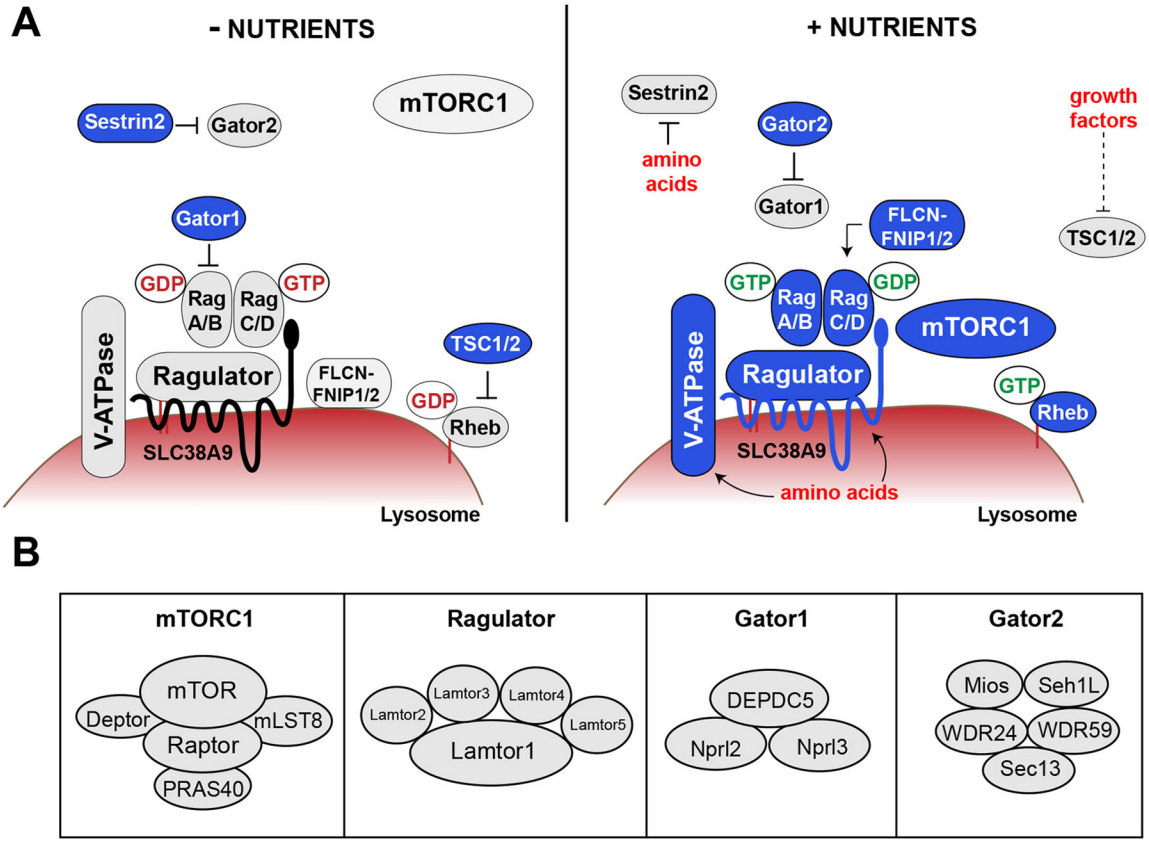

Figure 2.

(Left) mTORC1 activation requires the simultaneous presence of amino acids and growth factors. Under low amino acid conditions, the GATOR1 complex stimulates GTP hydrolysis by RagA/B. Moreover the Ragulator complex, which functions in concert with SLC38A9 and the v-ATPase, is unable to promote GTP loading of RagA/B. As a consequence, the Rag GTPases are locked in an A/B^{GDP}-C/D^{GTP} nucleotide state and cannot bind to mTORC1, which remains inactive in the cytoplasm. Absence of insulin or growth factors increases the GAP activity of the Tuberous Sclerosis Complex (TSC) toward Rheb, blocking its ability to stimulate the kinase activity of mTORC1. (Right) In the presence of amino acids, the Rag GTPase heterodimer becomes competent to physically bind to mTORC1. Amino acids within the lysosome signal through SLC38A9 and the v-ATPase and enable Ragulator to promote loading of RagA/B with GTP. Amino acids in the cytoplasm (specifically leucine) cause the dissociation of Sestrin2 from GATOR2, and enable GATOR2-mediated inhibition of GATOR1. Moreover, amino acids activate the FLCN/FNIP complex, which stimulates GTP hydrolysis by RagC/D. In the resulting A/B^{GTP}-C/D^{GDP} nucleotide state, the Rag heterodimer recruits mTORC1 to the lysosomal surface. Growth factor signals originating at the plasma membrane lead to the inhibition of TSC, switching Rheb toward the GTP bound state and enabling it to turn on the kinase activity of mTORC1.

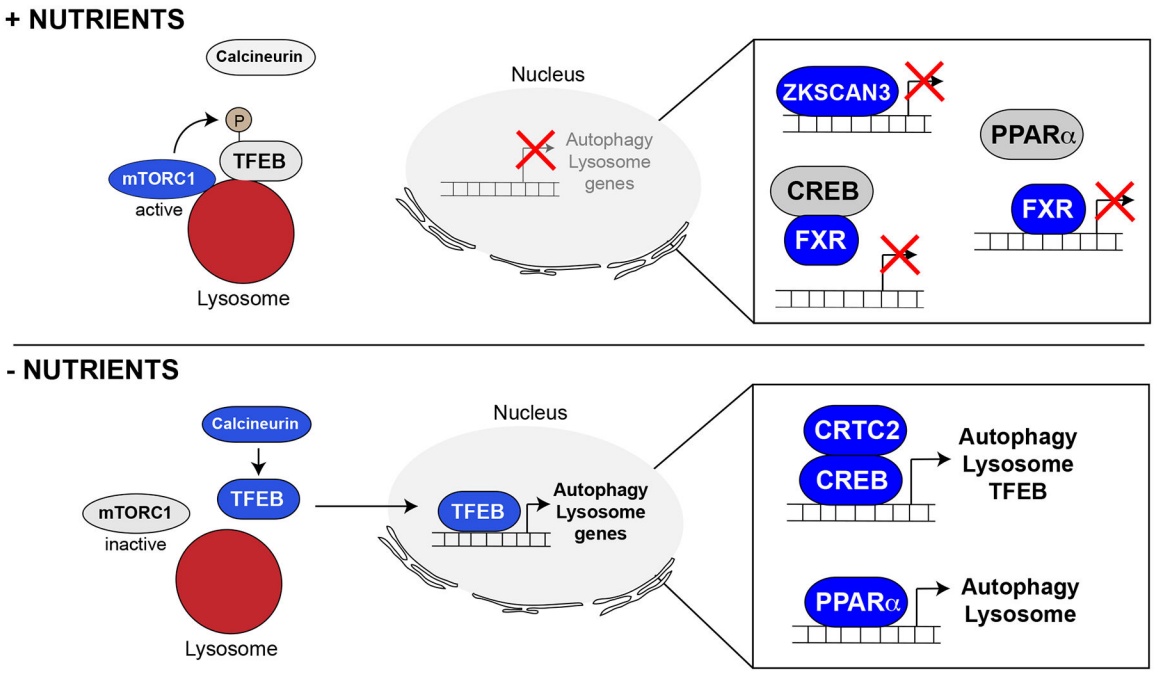

Figure 3.

Transcriptional regulation of autophagy and lysosome biogenesis. Under nutrient replete conditions, TFEB is phosphorylated by mTORC1 on conserved serine residues, which inhibits its nuclear translocation and activation. Negative transcriptional regulators of autophagosome and lysosome biogenesis are also activated including ZKSCAN3, and Farnesoid X Receptor (FXR). ZKSCAN3 binds to the promoters of lysosomal and autophagic genes and blocks their expression. FXR binds and displaces positive transcriptional regulators such as cAMP response element-binding protein (CREB) by disrupting formation of a complex between CREB and its co-activator CRTC2. FXR also displaces peroxisome proliferator activator receptor-a (PPARα) from binding to promoter regions upstream of autophagy and lysosome genes. Hence the cumulative effect is suppression of autophagy and lysosome gene induction under nutrient replete conditions. In contrast, starvation results in de-phosphorylation of TFEB via the combined action of calcineurin and inactivation of mTORC1. This allows for nuclear translocation of TFEB and binding to CLEAR elements present within the promoters of target genes. Starvation also inactivates FXR, enabling formation of the CREB-CRTC2 complex, which in turn activates TFEB transcription. Similarly suppression of FXR allows PPARα to activate autophagy and lysosme gene expression.