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The Lysosome as a Regulatory Hub

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Abstract

The lysosome has long been viewed as the recycling center of the cell. However, recent discoveries have challenged this simple view and have established a central role of the lysosome in nutrient-dependent signal transduction. The degradative role of the lysosome and its newly discovered signaling functions are not in conflict but rather 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 will point to novel therapeutic avenues in several human diseases.

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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 Leuven (Louvain). 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 the lysosome, that specializes in the breakdown and 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) that is established by the vacuolar H⁺ ATPase (v-ATPase) and that is 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, as well as 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, through a self-catabolic process known as autophagy, cytoplasmic macromolecules, damaged or misfolded proteins, and even entire organelles are captured and delivered to the lysosome (Kaur & Debnath 2015, Mizushima & Komatsu 2011).

Hence, lysosomal processing of a variety of cargo is essential for the efficient removal of toxic cellular components, the elimination of worn-out organelles, the termination of signal transduction, and the maintenance of metabolic homeostasis. These functions highlight a fundamental role for lysosomes in the 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 lysosomal storage disorders (LSDs), which are characterized by metabolic dysfunction,

neurodegeneration, and severely impaired growth (Ballabio & Gieselmann 2009, Bellettato & Scarpa 2010, Platt et al. 2012). Progressive loss of lysosomal efficiency occurs over the life spans 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, Spinoso et al. 2008). In contrast, rapidly dividing cancer cells rely on efficient lysosomal function for stress response and nutrient scavenging during the course of tumor progression (DeNicola & Cantley 2015, Perera & Bardeesy 2015, Piao & Amaravadi 2016, 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 a view in which the lysosome is the central node of a sophisticated network for cellular adaptation. The components of this network include ion and nutrient transporters, protein kinases and phosphatases, and 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.

STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE LYSOSOME

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 μm and 1 μm , yeast and plant cells contain one or a few lysosome-like vacuoles of several micrometers in diameter (Baba et al. 1994, Novikoff et al. 1956). Enlarged lysosomes are also found in disease settings, such as LSDs 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 lysosomes, such as pigments and membrane-perforating proteins. Several reviews on the biogenesis and function of these cell type-specific compartments have been published (Marks et al. 2013, Stinchcombe & Griffiths 2007, Watts 2012).

The limiting outer membrane of the lysosome is composed of a single phospholipid bilayer of 7–10 nm that is decorated with transmembrane proteins; the most abundant such proteins are the lysosome-associated membrane protein (LAMP)1 and LAMP2, which together total 80% of lysosomal membrane proteins (Saftig & Klumperman 2009). LAMP proteins and others, such as lysosomal integral membrane protein 2 (LIMP2) and CD63, are heavily glycosylated on their luminal side and form the glycocalyx, a barrier protecting against autodigestion of the limiting membrane by the resident lytic enzymes within the lysosomal lumen (Kornfeld & Mellman 1989, Saftig & Klumperman 2009, Settembre et al. 2013b). The ~ 60 resident acid hydrolases within the lysosome 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 on the basis of 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 proteomics-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 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), by progressive acidification of the lumen, and by 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 (**Figure 1a**).

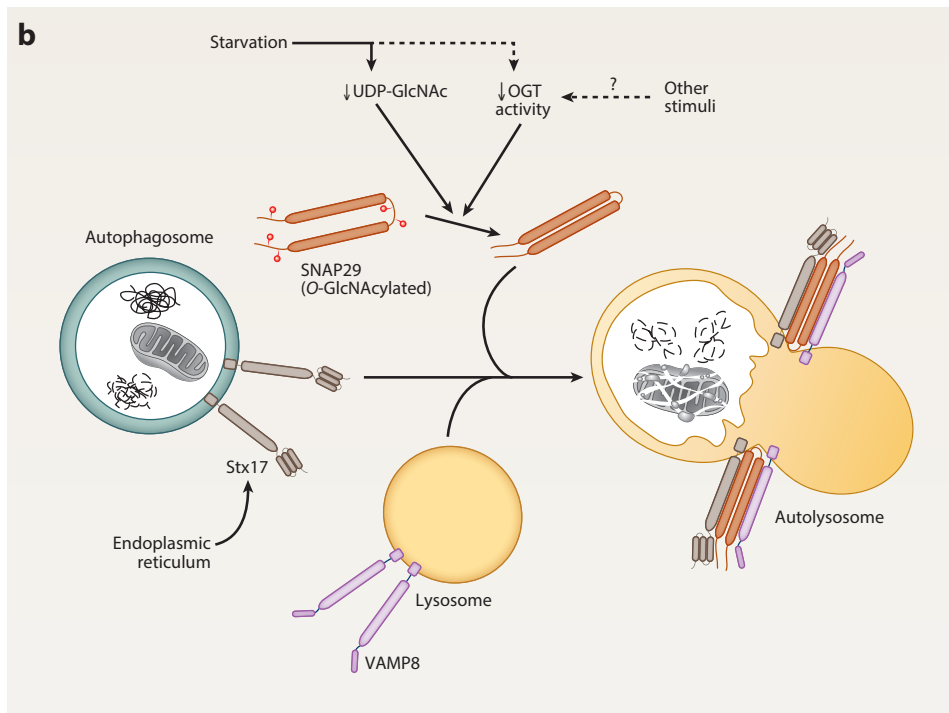
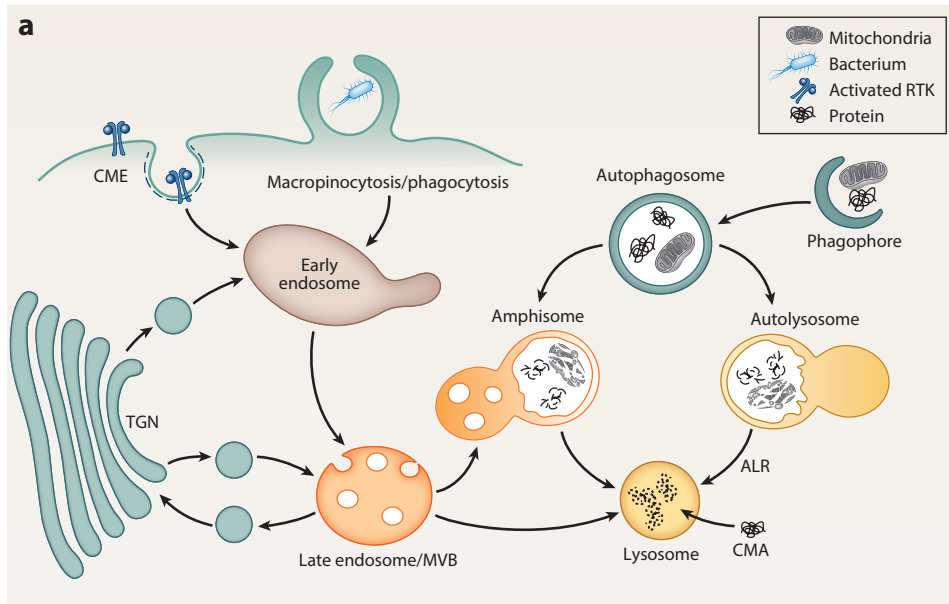
Thus, a seamless conversion along the endocytic pathway transforms a cargo-filled early endosome into a late endosome and finally a lysosome. However, lysosomes are not simply end points

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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, whereas 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—after which cargo can be rerouted 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 lysosomal membrane to be degraded. During autophagic lysosomal 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 SNAP29 and the R-SNARE vesicle-associated membrane protein (VAMP8) present on the lysosomal membrane. In nutrient-replete conditions, SNAP29 is O-GlcNAcylated (*red symbols*) by O-GlcNAc transferase (OGT), which inhibits the formation of the SNARE complex and fusion between the autophagosome and lysosome. Other abbreviations: MVB, multivesicular body; RTK, receptor tyrosine kinase; TGN, *trans*-Golgi network.

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 (CMA). 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 the association of dynein-dynactin with the lysosomal surface under cholesterol-rich conditions, and a lysosomal Ca^{2+} -sensing complex centered on the mucolipin 1 (MCOLN1) channel (also known as 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).

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—macroautophagy and CMA—have been identified (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 lysosomal 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 fuses with lysosomes (forming autolysosomes) so that the cargo can be degraded (He & Klionsky 2009, Hurley & Schulman 2014, Mizushima et al. 2008). Thus, all three forms of autophagy are strictly dependent on lysosomal 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 to 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 autophagic initiation, namely ULK1/2 and ATG13, to suppress autophagosomal 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 autophagosomal 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 autophagic machinery to provide an adaptive response that, through recycling of products of autolysosomal digestion back to the cytoplasm or to other organelles, attempts to restore cellular homeostasis. In addition, autophagy serves 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. 2012). Deregulation of these essential protective functions of autophagy has been implicated in the pathogenesis of cancer and 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 autophagosomal formation and maturation (Ge et al. 2014, 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 provides a blueprint to understand autolysosomal formation. Fusion begins with lysosomal-endosomal 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 lysosomal-endosomal 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) (a Qa-SNARE), Vti1b (a Qb-SNARE), and Stx8 (a Qc-SNARE), and an R-SNARE on the lysosome, typically vesicle-associated membrane protein 7 (VAMP7) (Luzio et al. 2007). A conformational change in the *trans*-SNARE complex, likely triggered by Ca²⁺, brings the two bilayers within a critical distance, resulting in their fusion and in the formation of a hybrid organelle.

In the context of autophagosomal-lysosomal fusion, pleckstrin homology domain-containing protein 1 was proposed to function as a tethering factor by simultaneously binding both to LC3 on autophagosomal membranes via an LC3-interacting region 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 SNAP29 (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 autophagosomal-lysosomal fusion (Diao et al. 2015, Guo et al. 2014, Itakura et al. 2012). Stx17 normally resides on the membranes of the endoplasmic reticulum, but it translocates to the outer membrane of autophagosomes once the latter have progressed to fully enclosed structures. On the autophagosome, Stx17 binds to SNAP29, which serves as the Qbc-SNARE. Stx17 and SNAP29 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 transmembrane domains of the protein (Itakura et al. 2012).

Another report suggests that autophagosomal-lysosomal fusion is a regulated process that is linked to the metabolic state of the cell. Under high-glucose conditions, *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT) modifies SNAP29 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 for the formation of *trans*-SNARE complexes and accelerating autolysosomal biogenesis. This mechanism provides a further layer of complexity and specificity to nutrient-directed regulation of autophagy (**Figure 1b**).

Following cargo breakdown inside the autolysosome, primary lysosomes need to be reformed to sustain additional rounds of autophagy. The process of autophagic lysosomal 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 protolysosomal 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 proteomics-based analysis of purified LAMP1/2-positive tubules. Interestingly, this process is similar to clathrin-mediated endocytosis, as it involves many of the same components, including clathrin, phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂], and the AP2 complex (Rong et al. 2012). Ongoing studies focusing on the molecular mechanisms governing autolysosomal formation, maturation, and resolution will provide important insight into the specific roles for this process in both health and disease.

Lysosomal Ion Currents in Signaling, Exocytosis, and Membrane Repair

The lysosomal membrane contains several ion channels that transport important ions such as Na⁺, K⁺, Ca²⁺, and Cl⁻. Movement of these ion species across lysosomal channels establishes a resting lysosomal membrane potential $\Delta\psi_{\text{lyso}}$ of between -40 and -20 mV (lumen positive) (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 Ca²⁺ during lysosomal exocytosis, as well as in the regulation of amino acid transport from the lysosomal lumen during starvation. Thus, similar to the role played by mitochondrial membrane potential, modulation of $\Delta\psi_{\text{lyso}}$ is an emerging aspect of lysosomal function (Xu & Ren 2015).

Among the ion channels that establish $\Delta\psi_{\text{lyso}}$ are two-pore complex 1 and 2 (TPC1 and -2), 12-transmembrane-spanning Na⁺ channels that are responsible for the 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 $\Delta\psi_{\text{lyso}}$. The activity of TPC1 and -2 is nutrient regulated: High ATP levels and active mTORC1 inhibit TPC1 and -2. During nutrient starvation, decreasing ATP levels and mTORC1 inactivation de/inhibit TPC1 and -2, increase Na⁺ currents out of the lysosome, and cause the lysosome's interior to depolarize relative to the fed state. Interestingly, TPC1- and TPC2-mediated depolarization appeared to support the release of the cationic amino acids arginine and lysine from the lysosomal lumen during starvation. In turn, the lysosome-derived flux of amino acids may be important for sustaining energy levels during starvation (Cang et al. 2013).

TPC1 and -2 are also regulated by phosphatidylinositol 3,5-bisphosphate [PI(3,5)P₂], a phosphoinositide specifically found in the endolysosomal system (Wang et al. 2012). PI(3,5)P₂ was initially detected as a low-abundance phosphoinositide that rapidly accumulates on the vacuolar membrane of yeast cells challenged with hyperosmotic buffers. In fact, PI(3,5)P₂ 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)P₂-induced ion currents may regulate the fusogenic potential of lysosomes. PI(3,5)P₂ also activates MCOLN1, a Ca²⁺-, Fe²⁺-, and Zn²⁺-permeable channel that selectively localizes to the lysosomal membrane (Dong et al. 2008). MCOLN1-mediated Ca²⁺

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—depend on lysosomal 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 mucopolipidosis type IV, an LSD characterized by morphological and functional defects of lysosomes (Ballabio & Gieselmann 2009, Vergarajauregui & Puertollano 2008). Imbalances in lysosomal $\text{PI}(3,5)\text{P}_2$ 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.

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 in both normal and disease states.

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 (Laplanche & 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, which are 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. Amino acids are the signal that drives the binding of mTORC1 to the lysosomal surface. In addition to serving as building blocks for protein synthesis and as substrates for energy production, amino acids play numerous signaling roles ranging 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 with 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 surfaces of lysosomes and vacuoles in both yeast and metazoan cells, and the mechanisms that regulate mTORC1 association with these organelles present many similarities 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 (200–500 nm in diameter) 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). The 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 the 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 v-ATPase (Forgac 2007, Zhao et al. 2015). Several amino acid transporters—belonging to the Vba and Avt families, among others—exploit the proton gradient 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 independently of external nutrient 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 nonessential cellular components. As in mammalian cells, macroautophagy in yeast involves the sequestration of cytoplasmic components, including large protein complexes and organelles in autophagosomes, followed by their delivery to the vacuole for degradation (Kraft & Peter 2008, Lazarou et al. 2012, Mizushima et al. 2008, Mochida et al. 2015, Wild et al. 2014). In microautophagy, invagination of the vacuolar limiting membrane allows for 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 twofold purpose of (a) preventing leakage of cytoplasmic amino acids out of the cell and (b) allowing the corresponding transporters to be scavenged and degraded as sources of amino acids. mTORC1 posttranslationally regulates both autophagy and endocytosis at multiple levels (MacGurn et al. 2011, Mizushima & Komatsu 2011). Thus, mTORC1 not only physically associates with the vacuole, but also regulates its nutrient output. The evidence discussed below reveals a feedback mechanism by which, upon 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. 2003, 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: RagA and RagB are highly similar to each other, and each binds 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 RagB is bound to GDP, RagC or RagD 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 (**Figure 2**). The same model has been proposed in yeast, in which 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 with regard to morphology, trafficking pattern, and expression of multiple late endosomal/lysosomal markers such as LAMP1 and LAMP2 (Sancak et al. 2008, 2010; Zoncu et al. 2011a). Lysosomal localization also enables interaction with Rheb, a small GTPase essential for mTORC1 kinase activity (Demetriades et al. 2014; Menon et al. 2014; Sancak et al. 2008, 2010; 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 (**Figure 2a**).

These findings were paralleled by studies in *Saccharomyces cerevisiae*, in which TOR1, KOG1 (the Raptor ortholog), and LST8, tagged with GFP at the endogenous locus, prominently localize to the surface of the vacuole. However, an important difference between mammalian cells and yeast cells is that, in the latter, mTORC1 appears to remain bound to the vacuole even after amino acid withdrawal (Binda et al. 2009). This important mechanistic difference may reflect evolutionary divergence. *S. cerevisiae* lacks a clear functional homolog of Rheb, and 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 termed Ragulator, which is specifically localized to the lysosome and is composed of five proteins known as LAMTOR1–5 (Bar-Peled et al. 2012, Nada et al. 2009, Sancak et al. 2010, Teis et al. 2002) (**Figure 2b**). 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 the Rag GTPases 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) (**Figure 2**).

The second event in the transition of Rag GTPases to the active state is GTP hydrolysis by RagC/D. The tumor suppressor protein folliculin (FLCN) in complex with folliculin-interacting

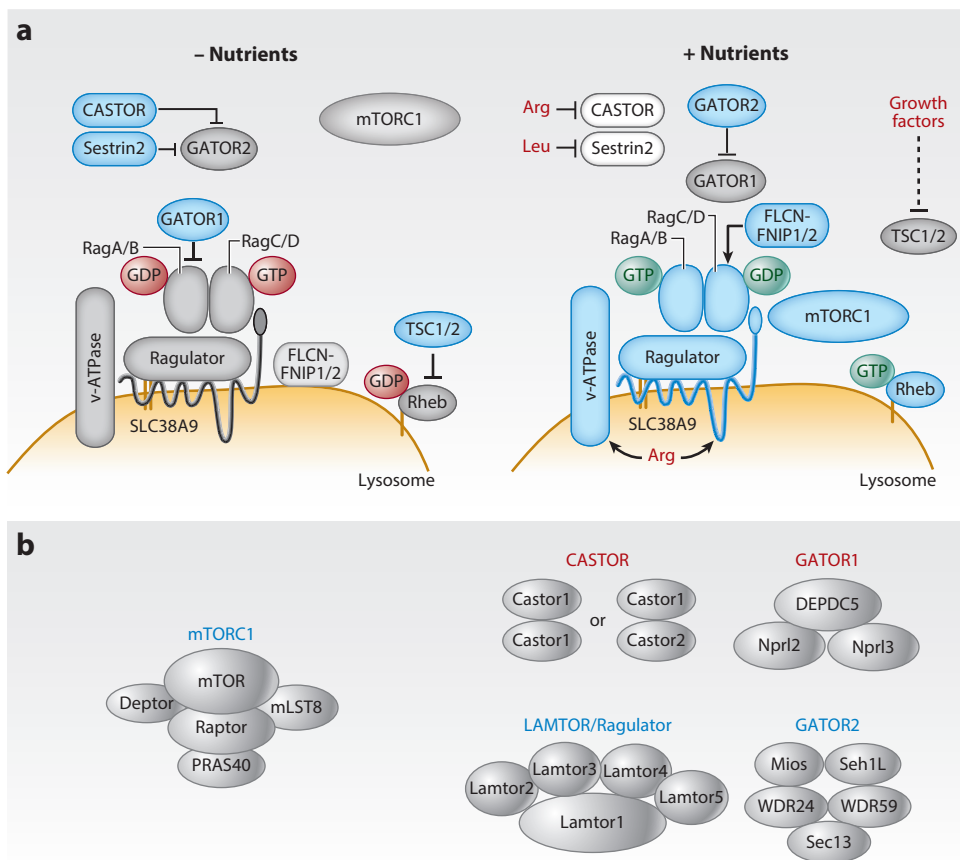


Figure 2

(a) mTORC1 activation requires the simultaneous presence of amino acids and growth factors. (*Left*) 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. Leucine and arginine in the cytoplasm cause the dissociation of Sestrin2 and CASTOR1/2, respectively, from GATOR2 and enable GATOR2-mediated inhibition of GATOR1. Moreover, amino acids activate the folliculin (FLCN)-folliculin-interacting protein (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. (b) Schematic summarizing the subunit composition of key multiprotein complexes: mTORC1; its positive regulators, GATOR2 and Ragulator; and its negative regulators, GATOR1 and CASTOR1/2.

protein (FNIP)1 or FNIP2 functions 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 to activation of mTORC1 irrespective of amino acid status (Bar-Peled et al. 2013). A second five-subunit complex known as GATOR2 binds to GATOR1 and blocks its GAP activity toward RagA/B. *S. cerevisiae* has homologs of all GATOR1 and GATOR2 subunits, all of which are part of one stable protein complex known as SEA (Seh1 associated) that functions similarly to GATOR1 and GATOR2 (Dokudovskaya et al. 2011, Panchaud et al. 2013) (Figure 2).

Despite various candidates, a clear RagC/D GEF has not been identified. As investigations continue, additional factors controlling the nucleotide loading state of Rag/Gtr GTPases will likely be discovered. Moreover, these factors may differ by species. For instance, in *S. cerevisiae*, but not in mammals, Vam6, a component of the HOPS complex, is 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 in which 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 pool, and not the extracellular pool, of amino acids. Inside the cell, at least two pools of amino acids may be important for mTORC1 activation: (a) the cytoplasmic pool and (b) the pool of amino acids derived from 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, S. Wang et al. 2015). 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-amino-acid N-terminal cytoplasmic domain and 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 regulated. When expressed in proteoliposomes, SLC38A9 behaved as a low-affinity transporter for various polar amino acids, especially arginine (Rebsamen et al. 2015, S. Wang et al. 2015). 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 that of 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 Sestrin1–3 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 20 μM K_d , which fits well with the half-maximal leucine concentration for mTORC1 activation. Crystallographic studies led to the identification of Sestrin2 mutants that failed to bind to GATOR2 and were therefore unable to suppress mTORC1 signaling upon leucine deprivation; conversely, Sestrin2 mutants that failed to bind to leucine but still retained the ability to bind to GATOR2 suppressed mTORC1 signaling in a constitutive manner (Saxton et al. 2016, Wolfson et al. 2016). Recently, two cytoplasmic proteins known as CASTOR1 and -2, which assemble into CASTOR1 homodimers or CASTOR1 and -2 heterodimers, were shown to function as arginine sensors for mTORC1 (Chantranupong et al. 2016). The mechanism of action of CASTOR1/2 is remarkably similar to that of Sestrins. In the absence of arginine, CASTOR1/2 binds to GATOR2 and blocks its ability to inactivate GATOR1, resulting in GTP hydrolysis by RagA/B and suppression of mTORC1 signaling. Arginine binds to CASTOR1/2 with physiological affinity ($K_d = 30 \mu\text{M}$) and breaks its interaction with GATOR2, thereby allowing mTORC1 activation (Chantranupong et al. 2016).

Through these studies, the lysosomal surface is emerging 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.

Transcriptional Regulation of Lysosomal Function

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, and 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 autophagic initiation (Egan et al. 2011, Hosokawa et al. 2009, Kamada et al. 2000, Noda & Ohsumi 1998). Transcriptional control of autophagic gene subsets has been demonstrated for multiple transcription factors, including activating transcription factor 4 (Pike et al. 2013, Rouschop et al. 2010), p53 (Kenzelmann Broz et al. 2013), and the Forkhead box O 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.

The CLEAR genome and the MiT/TFE factors. The long-held view of the lysosome as a static compartment has been radically altered by the discovery that entire classes of lysosomal genes, including those encoding 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, bioinformatics analysis identified a shared E box–related consensus element that was present in the promoter regions of many lysosomal genes and that was aptly named the CLEAR (coordinated lysosomal expression and regulation) element. The CLEAR element is the target of a family of basic helix–loop–helix 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 until recently less well understood. Chromatin immunoprecipitation (ChIP) experiments showed that TFEB directly binds to CLEAR elements and stimulates the expression of their downstream target genes; the same was later shown for MITF and TFE3. 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 autophagosomal initiation (BECN1, NRBF2), elongation (GABARAP, WIPI2, ATG9b), substrate capture (SQSTM1), and autophagosomal trafficking and fusion with lysosomes (UVRAG) (Palmieri et al. 2011, Settembre et al. 2011). TFEB overexpression promoted all these steps, increasing the number of autophagosomes, the frequency of their fusion with lysosomes, and the rate of substrate degradation (Settembre et al. 2011). These results indicated that TFEB is a true master regulator of cellular catabolism and coordinately expands the ability of the cell to select and capture substrates via autophagy and then to degrade them via the lysosome. This procatabolic 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- α 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 hypersensitive 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 *Caenorhabditis elegans* (O'Rourke & Ruvkun 2013, Settembre et al. 2013a). These studies provided strong evidence that regulation of the autophagic-lysosomal system may be a key aspect of metabolic adaptation at both the cellular and organismal levels.

Control of lysosomal biogenesis by progrowth 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 in both the nucleus and cytoplasm of cells. 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 (**Figure 3**). Conversely, in full-nutrient conditions, active mTORC1 directly phosphorylates TFEB on two critical residues,

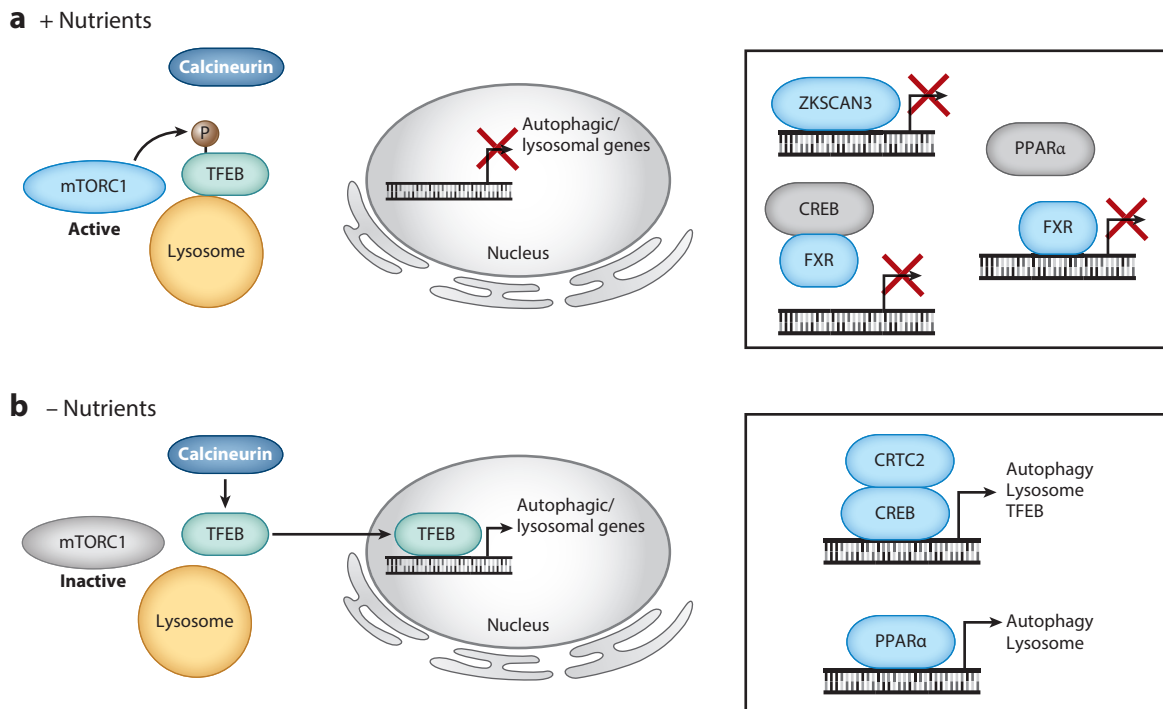


Figure 3

Transcriptional regulation of autophagic and lysosomal biogenesis. (a) Under nutrient-replete conditions, TFEB is phosphorylated by mTORC1 on conserved serine residues, which inhibits nuclear translocation and activation of TFEB. Negative transcriptional regulators of autophagosomal and lysosomal biogenesis, including ZKSCAN3 and farnesoid X receptor (FXR), are also activated. 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 coactivator, CRTC2. FXR also displaces peroxisome proliferator-activated receptor α (PPAR α) from binding to promoter regions upstream of autophagic and lysosomal genes. Hence the cumulative effect is suppression of autophagic and lysosomal gene induction under nutrient-replete conditions. (b) In contrast, starvation results in dephosphorylation of TFEB via the combined action of calcineurin and inactivation of mTORC1. Such dephosphorylation 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 PPAR α to activate autophagic and lysosomal gene expression.

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-nutrient conditions, 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). The 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, Rocznik-Ferguson et al. 2012). In addition to mTORC1, other growth-regulating kinases such as MEK/ERK and glycogen synthase kinase 3 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 by which to control its activity. The Ca^{2+} -regulated serine-threonine phosphatase calcineurin is 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 Ca^{2+} stores via the lysosomal Ca^{2+} channel MCOLN1. Starvation triggers MCOLN1-dependent elevation in cellular Ca^{2+} levels, most likely via release of the lysosome's own internal stores. This is a key event, as knockdown of MCOLN1 or treatment with Ca^{2+} -chelating agents strongly suppressed TFEB nuclear translocation and prevented upregulation of lysosomal and autophagic gene expression (Medina et al. 2015, W. Wang et al. 2015). Thus, dynamic release of lysosomal Ca^{2+} 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 progrowth kinases such as mTORC1 and Erk but also, via these same kinases, actively suppress catabolic programs that antagonize cell mass accumulation, a 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 regions of several lysosomal genes. However, unlike TFEB, ZKSCAN3 is a potent suppressor of these genes. Accordingly, nutrient and growth control pathways regulate ZKSCAN3 oppositely from TFEB: Under full-nutrient conditions, ZKSCAN3 is found in the nucleus, whereas starvation and mTOR inhibition drive ZKSCAN3 out of the nucleus, allowing for activation of catabolic programs that mediate the cellular response to starvation. Whether ZKSCAN3 is directly phosphorylated by mTORC1 or by other nutrient-responsive protein kinases is currently unknown (**Figure 3**).

Other studies are uncovering a broader transcriptional network that controls autophagic-lysosomal 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 coactivator CRTC2 to their promoters. Upon refeeding, FXR binds to CREB and displaces CRTC2, effectively blocking CREB activity (Seok et al. 2014) (**Figure 3**). FXR also inhibits peroxisomal proliferator-activated receptor α (PPAR α), 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, like ZKSCAN3, keep catabolic programs at bay and favor growth when nutrients are abundant in diverse tissue settings.

ALTERED LYSOSOMAL ACTIVITY IN DISEASE

Changes in endocytic traffic, autophagy, and lysosomal function have been implicated in a number of disorders associated with alterations in the processing and turnover of cellular constituents. Mutations in key lysosomal enzymes are implicated in inherited monogenic diseases collectively known as LSDs, many of which are associated with severe neurodegenerative phenotypes and are 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 lysosomal dysfunction in these disease settings appear to manifest more profoundly in the central nervous system than in other parts of the body. Moreover, endolysosomal gene mutations, some associated with LSDs, have emerged as risk factors for late-onset neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and frontotemporal dementia (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 the 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, these same functions of the lysosome, namely the clearance of damaged organelles and aggregate-prone macromolecules and the recycling of cellular building blocks to maintain overall cellular fitness, contribute to the pathogenesis of cancer. The features of these disorders resulting from opposing alterations in lysosomal function are discussed below.

Links Between Lysosomal Storage Disorders and Neurodegeneration

The common end point of 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 lysosomal hydrolases or permeases underlies LSDs, each of which is a rare, inherited metabolic disorder; the cumulative incidence is 1 in 5,000. 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 lysosomal morphology, motility, and number; with abnormalities in intracellular trafficking; and with the accumulation of undigested substances within the lysosomal lumen, leading to enlarged (>1,000-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 (e.g., 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 the 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 disease-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 the accumulation of glucosylceramide. Patients manifest multiorgan dysfunction, particularly in the spleen, skeletal muscle, and

hematopoietic system, and a subset of patients develop parkinsonism during their lifetime (type I Gaucher disease). A separate cohort of patients undergo severe progressive neurodegeneration (types II and III Gaucher disease) (Bultron et al. 2010, Tayebi et al. 2003). Moreover, unaffected family members of Gaucher patients carrying heterozygous mutations of GBA have a fivefold-increased risk of developing Parkinson's disease and an eightfold-increased risk of 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 role of mutant GBA in promoting neurodegenerative phenotypes remains unclear. However, accumulation of glucosylceramide is 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 lysosomal 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 neuron-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 LSDs 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 LSDs and may imply either a defect in autophagosomal 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). A greater dependence on efficient autophagic/endosomal/lysosomal activity is required for neuronal function. However, the mechanisms underlying the unique vulnerability of neuronal populations to impaired autophagic/endosomal/lysosomal activity remain obscure. Furthermore, whether mutations in other lysosomal genes could also represent risk factors for the development of neurodegenerative disorders is yet to be determined.

Treatment of LSDs focuses on restoration of lysosomal function via a number of strategies, including gene therapy and enzyme replacement therapy. However, reversal of abnormalities in some but not all affected organs, particularly central nervous system manifestations, limits the clinical benefit of these agents (Ballabio & Gieselmann 2009, Jeyakumar et al. 2005). More recently, overexpression of TFEB was shown to promote autophagosomal-lysosomal fusion, lysosomal trafficking, and exocytosis, leading to a dramatic reduction in intralysosomal content in multiple cellular models of LSDs (Decressac et al. 2013, Medina et al. 2011, Song et al. 2013, Spanpanato 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 induce autophagy. Future studies delineating the mechanisms and links between lysosomal and autophagic dysfunction and neurodegeneration may also highlight new nodes for the development of novel therapeutic agents (Efeyan et al. 2012, Nixon 2013, Ravikumar et al. 2004).

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 ability 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 the building blocks necessary for production of all classes of cellular macromolecules.

Macropinocytosis, the process of bulk uptake and lysosomal 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, 2015; Palm et al. 2015). Carbon tracing of ^{13}C -labeled albumin taken up via macropinocytosis in pancreatic ductal adenocarcinoma (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 building blocks essential 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 autophagic 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 autophagic activation is coordinated with increased lysosomal biogenesis and function in highly aggressive PDA. Importantly, the MiT/TFE proteins are upregulated in PDA and are required for maintaining high levels of autophagy and lysosomal function. Boosted lysosome-mediated catabolic activity in PDA tumors is required for the maintenance of intracellular amino acid levels and likely fuels specific biosynthetic and bioenergetic pathways required for tumor growth (Perera et al. 2015). Studies addressing whether there is a similar reliance on autophagy or lysosome-mediated catabolism in other tumor types is ongoing. It will be interesting to determine whether autophagic-lysosomal 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. The knowledge gained from analysis of autophagic-lysosomal regulation in PDA will 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 is 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 autophagic-lysosomal activity (Viale et al. 2014). The quality-control mechanisms of autophagy and the upregulation of lysosome-derived nutrients may enhance overall cellular fitness and enable sustained tumor cell survival under these conditions. Therefore, much effort

has been placed on the development of lysosome-based inhibitors that can turn off autophagy and additional nutrient-scavenging pathways. Correspondingly, treatment with inhibitors of lysosomal 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 lysosomal inhibitor; it is currently being tested in more than 40 clinical trials against a diverse array of tumor types and in multiple treatment settings (Kroemer 2015, Piao & Amaravadi 2016). 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 lysosomal dysfunction (e.g., lysosomal enlargement, buildup of autophagosomes, defective digestion of cargo material). Although use of HCQ as a single agent has not shown significant therapeutic efficacy (Wolpin et al. 2014), results of five combination therapy early-phase clinical trials in glioblastoma, myeloma, melanoma, and other solid tumors showed that high doses of HCQ were well tolerated and could block autophagy in tumor 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 lysosomal inhibitors may be broadly efficacious. Challenges associated with drug delivery and in vivo activity of HCQ have spurred the development of more potent autophagic-lysosomal inhibitors. One example is Lys05, a novel dimeric derivative of chloroquine currently in preclinical development (McAfee et al. 2012). Preliminary results show significant antitumor activity in melanoma models as a single agent and in combination with a BRAF inhibitor (Ma et al. 2014, McAfee et al. 2012). Treatments that induce lysosomal membrane permeabilization are another avenue being explored. This process is thought to induce necrotic cell death (necroptosis) following the 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 by which to prevent or delay therapy resistance or to increase the effectiveness of anticancer drugs in several tumor settings. As the functional roles of the lysosome in the context of tumor metabolism evolve, this key organelle will likely emerge as a central regulator of metabolic rewiring in a broad range of malignancies. Defining the most beneficial context in which to deploy lysosomal inhibitors in the clinic is an important ongoing goal.

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. 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 life span-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 is critical for lipid homeostasis in both 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

proteomics and lipidomics 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, in both yeast and mammalian cells, there is increasing evidence of physical contacts between the lysosome/vacuole and other organelles such as mitochondria and peroxisomes, which appear to mediate or facilitate lipid transport (Chu et al. 2015, Elbaz-Alon et al. 2014, Honscher et al. 2014). This fertile area of investigation will continue to develop as additional modes of interorganellar communication are discovered.

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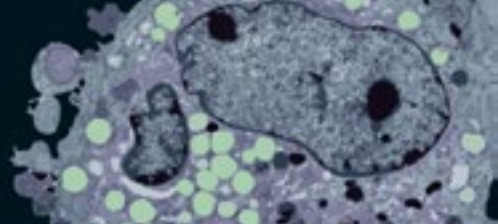
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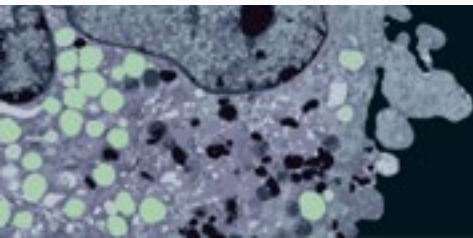
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