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## Lysosome: The Metabolic Signaling Hub

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### Abstract

For the past five decades the lysosome has been characterized as an unglamorous cellular recycling center. This notion has undergone a radical shift in the last ten years, with new research revealing that this organelle serves as a major hub for metabolic signaling pathways. The discovery that master growth regulators, including the protein kinase mTOR (mechanistic Target Of Rapamycin) make their home at the lysosomal surface has generated intense interest in the lysosome's key role in nutrient sensing and cellular homeostasis. The transcriptional networks required for lysosomal maintenance and function are just being unraveled and their connection to lysosome-based signaling pathways revealed. The catabolic and anabolic pathways that converge on the lysosome connect this organelle with multiple facets of cellular function; when these pathways are deregulated they underlie multiple human diseases, and promote cellular and organismal aging. Thus, understanding how lysosome-based signaling pathways function will not only illuminate the fascinating biology of this organelle but will also be critical in unlocking its therapeutic potentials.

### Synopsis

After decades of being known as just the cellular recycling center, the lysosome has recently emerged as a central hub for metabolic signaling. Signaling pathways that call the lysosome home connect this organelle to key anabolic and catabolic processes that control many facets of cellular metabolism. Metabolic signals emanating from the lysosome are contributors to multiple human diseases, emphasizing the key role of the lysosome as a cellular site for the regulation of human health.

### Keywords

lysosome; mTORC1; autophagy; metabolic signaling

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## Introduction

As the degradative endpoint of the endosomal pathway, the lysosome has gained much notoriety as the ‘recycling center’ of the cell. Its characterization as a major catabolic center traces its origins to its discovery in the early 1950s. While investigating the mechanism of action of insulin, Christian de Duve made the serendipitous discovery of ‘sac-like structures’ that contained lytic activity<sup>1,2</sup>. Ultrastructural characterization of these compartments by Alex Novikoff<sup>3</sup> led de Duve to rename them lysosomes. Following de Duve’s work, Werner Strauss traced the fate of radiolabeled extracellular proteins and discovered that these proteins localized to the lysosome and were found fragmented rather than intact<sup>4</sup>. These pioneering studies cemented the lysosome as the cell’s degradative organelle.

The acidic environment of the lysosome maintained by the lysosomal v-ATPase<sup>5</sup>, combined with a pantheon of luminal hydrolases, results in an organelle that is perfectly suited for the breakdown of major macromolecules, including lipids, polysaccharides and proteins<sup>6</sup>. Once degraded, free fatty acids, monosaccharides and amino acids are transported to the cytoplasm by specific permeases, where they can be reused in anabolic processes<sup>7</sup>. The degradative functions of the lysosome are key to maintaining cellular homeostasis, with perturbations in these processes leading to an array of human disorders collectively cataloged as lysosomal storage diseases<sup>8</sup>. In addition to the basal level degradative processes undertaken by the lysosome, environmental stressors trigger a massive upregulation of degradation through the self-catabolic pathways known as autophagy. These pathways are critical in restoring homeostasis during times of metabolic imbalance and not surprisingly are also deregulated in multiple human maladies<sup>9</sup>.

Our notion of the lysosome as a simple recycling center has undergone a dramatic reevaluation over the last decade. With the discovery that the master regulator of cell growth, mTORC1 is localized to the lysosomal surface, we’ve now come to appreciate that the lysosome also functions as a platform for metabolic signaling. In this mini-review we focus on the lysosome as a metabolic signaling hub which integrates different environmental signals to regulate core anabolic and catabolic pathways critical in the maintenance of cellular homeostasis. We also touch on how deregulation of these pathways lead to human pathologies.

## Metabolic signaling at the lysosomal surface

The mechanistic Target of Rapamycin (mTOR) is an evolutionarily conserved phosphatidylinositol-3-kinase (PI3K)-like serine/threonine protein kinase that is inhibited by rapamycin, a small molecule originally discovered in the soil of Easter Island<sup>10</sup>. The history of rapamycin’s development as a pharmaceutical is recounted in full elsewhere; but briefly, rapamycin’s potential as pharmaceutical was immediately apparent due to its ability to slow or arrest cell proliferation. Soon after, it and several derivatives – “rapalogs”<sup>11</sup> – were developed as immunosuppressants and later trialed as anti-cancer agents.

The 1990’s saw an intense search by several laboratories to discover the molecular mechanism by which rapamycin acts. Discoveries in yeast and in mammalian cells revealed

that rapamycin acts by binding to the immunophilin FKBP12, forming a complex that then binds to and inhibits the mTOR protein kinase<sup>12–14</sup>. Later work identified two distinct mTOR complexes<sup>15</sup>, with shared as well as unique protein subunits and different substrates. mTOR complex 1 (mTORC1), which is acutely sensitive to rapamycin, is defined by the association of mTOR with the adaptor proteins Raptor and mLST8<sup>16–18</sup>; while mTORC2, which is acutely rapamycin-resistant, is defined by the association of mTOR with the adaptor proteins Rictor, mLST8, and mSin1<sup>19–22</sup>. The list of proteins that physically associate with mTORC1 or mTORC2 continues to grow, although it is likely that some of these proteins interact with mTORC1 or mTORC2 only in particular cell types or environmental conditions.

The two mTOR complexes have different cellular roles. mTORC1 functions as a key integrator of environmental and hormonal cues, sensing the availability of amino acids, glucose, cholesterol; cellular energy status; and hormones including insulin, IGF-1, leptin and adiponectin<sup>23–28</sup>. When these states combine to form a permissive environment for cell growth and anabolism, mTORC1 localizes to the lysosome and interacts with its obligate activator Rheb-GTP to phosphorylate substrates including S6K1, 4E-BP1, and ULK1 to promote ribosomal biogenesis, translation, and lipogenesis while suppressing autophagy<sup>19,29–32</sup>. Conversely, if one or more of these environmental or hormonal cues is not permissive for growth, mTORC1 remains inactive and these anabolic activities are inhibited, while autophagy is activated.

In contrast, mTORC2 is primarily an effector of the insulin/IGF-1/PI3K signaling pathway. While the molecular mechanism by which PI3K regulates mTORC2 has proven difficult to pin down, it was recently shown that phosphatidylinositol (3,4,5)-trisphosphate (PIP<sub>3</sub>) directly activates mTORC2 by relieving an inhibitory interaction with mSIN1<sup>33</sup>. mTORC2 activity is also responsive to fatty acids<sup>34</sup>, and may require interaction with ribosomal protein subunits<sup>35</sup>. When activated, mTORC2 phosphorylates numerous AGC kinases, including AKT, SGK, and PKC $\alpha$ ; these phosphorylations have typically been shown to promote both the activity and stability of the substrate proteins<sup>36–38</sup>.

Work done over the last decade, and in particular over the last five years, have demonstrated that the activation of mTORC1 requires the coordinated interaction of many proteins at a previously unsuspected location, the lysosome. While previously conceived of as simply the “recycling center” of the cell, it is now clear that this hitherto unglamorous organelle serves as a critical platform for coordinating environmental and hormonal cues with mTORC1 and mTORC2 activation. The logic of this placement can be understood through an evolutionary context<sup>39</sup>; in yeast, the TOR pathway is localized to the vacuole, the lysosomal orthologue<sup>7</sup>, which functions as a major storage repository for nutrients. Recent technological advances<sup>40,41</sup> have determined that the mammalian lysosome is likewise selectively enriched in certain nutrients, including certain amino acids. Thus, the localization of mTORC1 to the lysosome allows mTORC1 to immediately gauge the nutrient status of the cell and rapidly shift its activity as required to maintain homeostasis.

mTORC1 is particularly sensitive to cellular and lysosomal levels of amino acids. mTORC1 is recruited to the lysosome in response to amino acids by the Rag family of heterodimeric

small GTPases, orthologs of the yeast small GTPases Gtr1 and Gtr2<sup>42–45</sup>. When amino acid levels drop within a cell, RagA/B bind GDP and RagC/D bind GTP (RagA<sup>GDP</sup>/RagC<sup>GTP</sup>), a state that does not interact with mTORC1. Upon amino stimulation, Rags flip their nucleotide bound state (RagA<sup>GTP</sup>/RagC<sup>GDP</sup>) and strongly interact with mTORC1 through its Raptor subunit, thus activating mTORC1<sup>44,45</sup>. Over the last five years it has been shown that amino acids regulate mTORC1 activity by interacting with sensor proteins at the lysosomal surface that control the nucleotide loading state of the Rags (Figure 1).

## Regulators of the Rag GTPases

The lysosomal platform upon which mTORC1 activation occurs is known as the Ragulator, a protein complex composed of the proteins MP1, p14, HBXIP1, C7ORF59 and p18 (encoded by *MAPKSP1*, *ROBLD3*, *HBXIP*, *c7orf59* and *c11orf59*, respectively), and is anchored to the lysosome by p18<sup>46–48</sup>. The Ragulator, which is orthologous to the yeast EGO complex<sup>49,50</sup>, tethers the Rag GTPases to the lysosomal surface and has guanine nucleotide exchange factor (GEF) activity for two of the Rag proteins, RagA and RagB<sup>47,51</sup>, with the G-domains of the Rag proteins projecting away from the Ragulator surface<sup>51–54</sup>. Control of Ragulator activity is one mechanism by which amino acids regulate mTORC1. Amino acids control the Ragulator in part via the lysosomal vacuolar ATPase (v-ATPase), which interacts with Ragulator in a manner sensitive to the availability of lysosomal amino acids and v-ATPase activity<sup>55</sup>.

The importance of lysosomal metabolic signaling to human physiology has come to light by the identification of patients with partial defects in Ragulator function. Mutation in the 3'UTR of the p14 component of Ragulator leads to an introduction of an aberrant splice site<sup>56</sup> which reduces both p14 transcript and protein levels. Children bearing this mutation are below the 3<sup>rd</sup> percentile for height and suffer from a complex immune disorder that is marked by B-cell and cytotoxic T-cell (CTL) deficiency, neutropenia and partial albinism<sup>57</sup>. The short stature and immune disorders of these patients are characteristic of animal models where mTORC1 signaling is attenuated<sup>58,59</sup>. Correspondingly, patient fibroblasts have a marked reduction in amino acid induced activation of mTORC1 signaling<sup>46</sup>. The clinical phenotype associating albinism and immunodeficiency is similar to other disorders caused by defects in specialized secretory lysosomes such as melanomes and cytotoxic granules<sup>60</sup>. Furthermore, in patients with reduced Ragulator activity late-endosome/lysosomal distribution was altered, with an increased distance from the nucleus. These results posit a lysosome-mTORC1 feedback loop, wherein inactivation of mTORC1 leads to altered distribution and function of the late-endosome/lysosomal compartment in multiple cell types including CTLs and melanocytes. Whether lysosomal function is compromised in other mTORC1 driven diseases or how mTORC1 activity is altered in lysosomal storage disorders (LSDs) represents an exciting area of research.

The nucleotide loading state of RagA and RagB are also controlled by the GATOR complexes<sup>61,62</sup>. GATOR1, which consists of the proteins DEPDC5, NPRL2 and NPRL3, functions as a GTPase activating protein (GAP) for RagA and RagB. In contrast, GATOR2, which consists of the proteins Mios, WDR24, WDR59, Seh1L, and Sec13, acts to inhibit GATOR1 activity by unknown mechanisms. Both GATOR1 and GATOR2 are conserved in

yeast (as SEACIT and SEACAT, respectively) as well as higher eukaryotes<sup>49</sup>. The structure and regulation of the GATOR complexes is an area of active research; recent studies have taken a cryo-EM approach to shed light on how GATOR1 functions<sup>63</sup>, and have identified a new protein complex dubbed KICKSTOR (KPTN, ITFG2, C12ORF66 and SZT2) which recruits GATOR1 to the lysosomal surface and enables its interaction with the Rag proteins<sup>64,65</sup>. KICKSTOR is required for the negative regulation of mTORC1 by amino acid or glucose deprivation, and its loss leads to hyperactivity of mTORC1; however, it is unknown if the recruitment of GATOR1 by KICKSTOR is an important mTORC1 regulatory mechanism.

While much effort has focused on understanding the regulation of RagA and RagB, the nucleotide loading state of RagC and RagD are essential for this complex's interaction with mTORC1<sup>66</sup>. The FLCN complex, composed of FLCN, FNIP1 and FNIP2, has emerged as a positive regulator of RagC and RagD through its GAP activity for these GTPases, converting them from the GTP bound state to the GDP<sup>66</sup>. FLCN is required for amino acids to recruit mTORC1 to the lysosome, and is itself recruited to the lysosomal surface by amino acid depletion<sup>67</sup>. FLCN is recruited to the lysosome by interaction with RagA/B<sup>GDP</sup> and requires GATOR1 GAP activity; FLCN recruitment to the lysosome is thus likely regulated by the same set of amino acid sensors upstream of GATOR1/2 that act to regulate mTORC1<sup>68</sup>. One possible explanation for the localization of FLCN to the lysosome following amino acid depletion was recently discovered: FLCN sequesters lysosomal leucine when amino acids are limited by blocking PAT1, a lysosomal amino acid transporter, thus helping to preserve mTORC1 activity during nutrient limitation<sup>69</sup>.

Complicating our understanding of the role of FLCN is that it is mutated in a devastating disease, Birt-Hogg-Dube, that is characterized by large benign tumors which are the hallmark of other cancers driven by loss of negative regulators of the mTORC1 pathway. A possible explanation for this paradox may be that the function of FLCN as a tumor suppressor is context dependent. One recent study reports that FLCN is a ciliary protein that recruits LKB to activate AMPK – an inhibitor of mTORC1 signaling – in response to flow stress<sup>70</sup>. Thus, cell type, local environmental conditions, and possibly, the subcellular compartment being investigated may impact the net effect of FLCN loss on mTORC1 activity.

## Amino acid sensors, from hypothesis to reality

The greatest recent advances in understanding the response of mTORC1 to amino acids have come from identifying specific proteins which bind to amino acids and regulate the function of the GATOR complexes. The existence of these 'amino acid sensors' has long been hypothesized, but it has only been in the last several years that this hypothesis came to fruition with discovery of four sensing complexes. The Sestrin family of proteins (Sestrin1, Sestrin2, Sestrin3) are highly conserved and were recently found to function as negative regulators of mTORC1<sup>71,72</sup>. Originally, the Sestrins were proposed to function as guanine dissociation inhibitors (GDI) for RagA/B, locking them in the GDP bound state<sup>73</sup>. However, the key residues required for GDI activity are found buried in the crystal structure of Sestrin2<sup>74–76</sup>. Purification experiments and subsequent biochemical investigation has

demonstrated that Sestrins bind to and inhibit the function of GATOR2. The interaction between Sestrin1/2 and GATOR2 is regulated by leucine; however the interaction of Sestrin3 with GATOR is constitutive, suggesting another mode of regulation. *In vitro* binding assays demonstrate that Sestrin2 binds directly to leucine at an affinity similar to the concentrations sensed by mTORC1. Furthermore, the crystal structure of Sestrin2 prominently displays leucine bound to critical residues required for sensing, which when mutated blocks leucine sensing by mTORC1. Leucine binding relieves the inhibitory action of Sestrin2 upon GATOR2 and thus mTORC1 <sup>76,77</sup>.

The CASTOR proteins (CASTOR1 and its homolog CASTOR2) have recently been described as arginine sensors. The CASTOR proteins are found to bind to GATOR2 and inhibit its function during arginine withdrawal. *In vitro* binding assays confirmed that CASTOR1 can bind arginine at levels similar to media concentrations required for activation of mTORC1 and mutational studies demonstrated that CASTOR mutants that cannot bind arginine block arginine induced activation of mTORC1, designating the CASTOR proteins as bona-fide amino acid sensors <sup>78,79</sup>.

A low-affinity lysosomal amino acid transporter, SLC38A9 interacts with both Ragulator and the v-ATPase, and acts as an amino acid sensor upstream of mTORC1 for asparagine, arginine, glutamine, histidine, and lysine <sup>80-82</sup>. The exact mechanism by which SLC38A9 regulates mTORC1 activity is not clear; while it was initially suggested that SLC38A9 might regulate the GEF activity of Ragulator, it was shown that in response to arginine, SLC38A9 regulates the lysosomal efflux of many amino acids that then stimulate mTORC1 from the cytoplasm <sup>40</sup>. However, this model does not explain the significance of the physical interactions between SLC38A9, the Ragulator and the v-ATPase. Curiously, SLC38A9 also enables mTORC1 activation by cholesterol via recruitment of the Niemann-Pick C1 (NPC1) protein, which acts to inhibit mTORC1 in the absence of cholesterol <sup>83</sup>. Understanding how the amino acid sensing and transport functions of SLC38A9 regulates mTORC1 activity and the sensitivity of SLC38A9 to other environmental cues, is an important area for future research.

Finally, the SAMTOR protein was recently discovered as an evolutionarily conserved negative regulator of mTORC1 amino acid sensing that binds to GATOR1 directly to modulate its activity by an unknown mechanism. Instead of directly sensing amino acid levels, SAMTOR binds to the metabolite S-adenosylmethionine (SAM) which disrupts its interaction with GATOR1, positioning SAMTOR as an indirect sensor of methionine <sup>84</sup>. Intriguingly, the discovery of SAMTOR provides the first molecular mechanism by which methionine restriction, a potent dietary intervention that improves metabolic health and extends rodent lifespan <sup>85-87</sup>, may mediate mTORC1 activity <sup>88-90</sup>. This study not only provides a key link between one carbon metabolism and the mTORC1 pathway but raises the question of whether additional intermediate metabolites in amino acid catabolism are also sensed by mTORC1. For example, mTORC1 has been shown to sense  $\alpha$ -ketoglutarate, a product of glutaminolysis <sup>91,92</sup>.

## Rheb and Tuberous sclerosis complex

Recruitment of mTORC1 to the lysosome is a prerequisite for mTORC1 activation, because only at the lysosomal surface is mTORC1 able to interact with the Rheb-GTPase. A recent Cryo-EM structure has revealed that Rheb-GTP binds to the mTOR protein kinase and allosterically realigns and activates kinase-site residues<sup>93</sup>. Rheb-GTP is regulated by at least two mechanisms; the first is that lysosomal recruitment of Rheb is regulated by amino acids. Amino acids stimulate the binding of Rheb to microspherule protein 1 (MCRS1), which promotes its localization to the lysosome, although the molecular mechanisms which regulate this process remain uncertain.

The second mechanism by which Rheb-GTP is regulated is the tuberous sclerosis complex (TSC), which is comprised of the proteins TSC1, TSC2, and TBC1D7<sup>94</sup>. The TSC complex acts as a GAP for Rheb, and acts as a “mini” signaling hub upstream of mTORC1<sup>95</sup>. Many different kinases, including AKT, AMPK, ERK, GSK3, and IKK $\beta$  phosphorylate distinct residues of TSC1 and TSC2, and thereby serve to regulate mTORC1 by altering the GAP activity of TSC towards Rheb<sup>95–101</sup>. While it was originally thought that these posttranslational modifications directly altered the activity of TSC, it was recently shown that much like mTORC1 and Rheb, TSC is also regulated by localization. In the absence of insulin signaling, TSC is localized to the lysosome, where it can directly inhibit Rheb. However, when TSC is phosphorylated by AKT in response to insulin/PI3K signaling, TSC departs from the lysosome, removing its ability to inhibit Rheb, and permitting the activation of mTORC1<sup>102</sup>. Another report suggests that TSC lysosomal localization is nutrient dependent, and may be recruited to the lysosome by the Rag GTPases in the absence of amino acids<sup>103</sup>. The mechanism by which this process is mediated remain to be determined; also unknown is if relocalization of TSC is a generalized mechanism by which post-translational modifications of TSC regulate the GTP-loading of Rheb.

## mTORC2

As noted above, mTORC2 is acutely resistant to rapamycin treatment, and this effect, along with the salt-sensitivity of mTORC2, led to a delay in its discovery<sup>15,20,21</sup>. Even after its discovery, the lack of specific chemical inhibitors of mTORC2 have slowed our understanding of the importance of this complex to a diverse set of cellular processes. The sub-cellular localization of mTORC2 in particular has been something of a mystery; while mTORC2 is an effector of PI3K signaling and sensitive to PIP<sub>3</sub>, and thus one might suspect that it is localized to the plasma membrane, work published in 2011 demonstrated that mTORC2 is physically associated with the ribosome, an interaction that is stimulated by insulin<sup>35</sup>. While this agrees well with a model in which mTORC2 phosphorylates some motifs of AKT co-translationally, the concept that mTORC2 is associated with the ribosome also received a later boost with determination that mTORC2 localizes to the mitochondria-associated endoplasmic reticulum, interacting with a specific mitochondrial tethering complex<sup>104</sup>. Recent studies have also placed mTORC2 at the lysosome (see below) and in immune cells chronic mTORC2 activity inhibits lysosomal acidification<sup>105</sup>. The effect of mTORC2 on lysosomal acidification in a more acute context, such as in response to physiological levels of PI3K signaling, is unknown.

## Lysosomal functions downstream of mTOR complexes

While mTORC1 was first characterized as regulator of ribosomal biogenesis and protein translation, an ever-growing set of identified substrates, both of the mTOR protein kinase itself as well as effectors such as S6K1 has linked mTORC1 to a growing list of cellular processes. While we will not attempt to enumerate all of the processes downstream of the mTOR protein kinase, we will note broadly that these processes include the regulation of apoptosis, amino acid and ion homeostasis, metabolism, and stress resistance downstream of mTORC2<sup>19,106–109</sup>. Downstream of mTORC1, the list has grown from ribosomal biogenesis and translation to include adipogenesis, amino acid transport, ketogenesis, lipogenesis, and nucleotide synthesis<sup>19,110–114</sup>.

In all of these processes, both mTORC1 and mTORC2 act in a common theme: to promote anabolic processes. However, both mTOR complexes also play a critical role in regulating catabolic processes. One recently discovered activity is that both mTORC2 and mTORC1 act to promote anaplerosis – the refilling of the citric acid cycle – by promoting the formation of  $\alpha$ -ketoglutarate from glutamine<sup>92</sup>. Here, we will focus on the lysosomal pathways that lie downstream of the mTOR complexes.

## Transcriptional control of lysosomal function

Exemplifying the interdependent role of lysosome as both a catabolic and anabolic signaling hub are recent discoveries linking the transcriptional control of lysosome function to mTORC1. In work published in 2009<sup>115</sup>, the basic helix-loop-helix transcription factor, TFEB, was found to bind to genes containing the CLEAR element, which is enriched in the promoters of lysosomal genes<sup>115,116</sup>. TFEB is a member of the MiT/TFE transcription factor family<sup>117</sup>, whose other members MiTF and TFE3 are now appreciated to have similar functions to TFEB. Activation of TFEB drives a major expansion of the lysosomal compartment and multiple steps in autophagy, denoting TFEB as the master transcriptional regulator of lysosomal function<sup>118</sup>. The regulation of TFEB activity is nutrient dependent<sup>119</sup>. During nutrient replete conditions, mTORC1 binds to TFEB at the lysosomal surface and phosphorylates two key residues<sup>119,120</sup> leading to 14–3–3 binding and cytoplasmic sequestration. Upon nutrient starvation, calcium is released by the lysosomal calcium channel MCOLN1, leading to the activation of a  $\text{Ca}^{2+}$ -dependent phosphatase, calcineurin, which dephosphorylates TFEB<sup>121</sup> allowing its nuclear localization and activation of target genes. To suppress lysosomal gene expression, cells rely on the zinc-finger transcription factor, ZSCAN3, which sits at the promoters of multiple lysosomal genes and inhibits their expression during nutrient replete conditions<sup>122</sup>. Upon nutrient starvation or mTORC1 inhibition, ZSCAN3 is driven from the nucleus into the cytoplasm by an unknown mechanism. Other transcription pathways including FXR also sense cellular nutrient conditions (bile acids) and coordinate lysosomal gene expression<sup>123,124</sup>. A key challenge for future studies will be to provide an integrated portrait of how multiple nutrient sensitive transcriptional pathways coordinate lysosomal gene expression in concert with anabolic signaling pathways.



## Autophagy

In response to nutrient limitation and damage to proteins or organelles, eukaryotic cells activate a highly regulated set of pathways collectively known as autophagy. Because generation of energy and metabolic intermediates under starvation and the clearance of toxic proteins and damaged organelles is a prerequisite for any cell, defects in autophagy pathways underlie human diseases ranging from neurodegenerative disorders to cancer <sup>125</sup>.

At the heart of autophagy lies the lysosome— serving as the degradative endpoint for these pathways by providing key enzymes and a harsh environment necessary for the breakdown of toxic protein aggregates, damaged lipids or complex carbohydrates. Studies initiated in yeast have provided a solid genetic foundation for autophagy pathways with the discovery of ATG (autophagy related genes) <sup>126</sup> and their mammalian homologs. In mammalian cells two autophagy pathways exist: macroautophagy and chaperone mediated autophagy (CMA). Macroautophagy (herein referred to as autophagy) is the degradation of cytosolic components through a double-membrane vesicle known as the autophagosome. CMA is characterized by the degradation of specific proteins marked with a charged recognition motif. Both forms of autophagy are highly regulated processes which sense general cell stress and are sensitive to nutrient deprivation. While autophagy functions as a rapid response to starvation and is induced within minutes of nutrient withdrawal, CMA reaches its maximal activation after 1.5 days. In addition to autophagy and CMA, specialized forms of selective autophagy which target organelles (mitophagy) <sup>127</sup> or complexes (ribophagy) <sup>128–130</sup> have recently emerged.

Initiation of autophagy occurs within minutes of nutrient deprivation and central to its regulation are mTORC1 and the energy sensing AMPK pathway. During nutrient replete conditions, lysosomal mTORC1 phosphorylates sites on key components of the master autophagy regulator ULK complex, composed of ULK1/2, ATG13, FIP200 and ATG101. mTORC1 phosphorylation of the serine/threonine kinase ULK1/2 at S757 <sup>30</sup> and ATG13 at S258 <sup>131</sup> inactivates the ULK complex to block autophagy induction. Upon starvation, mTORC1 is inactivated, in part due to a lack of free amino acids, and AMPK phosphorylates different sites on ULK1/2, necessary for its kinase activity <sup>30,132</sup>. Active ULK translocates to the ER <sup>133</sup> and phosphorylates the class III phosphatidylinositol-3 phosphate (PI3P) kinase, VPS34 <sup>134</sup>, a component of VPS34 complex I (BECN1, VPS34, ATG14L, and VPS15). Once activated, VPS34, deposits PI3P at the omegasome, a subdomain of the ER believed to give rise to the isolation membrane.

The increase in PI3P levels recruits additional ATG proteins <sup>135</sup>, leading to the nucleation of the isolation membrane (IM) at the omegasome. IMs initially form a cup-shaped double membrane structure that is readily resolved by electron microscopy and signal autophagy induction. IM expansion is driven by vesicular traffic from different compartments (golgi <sup>136</sup>, mitochondria <sup>137</sup> and plasma membrane <sup>138</sup>), which provide a source of membranes to the growing structures. As IMs continue to expand, the ATG16L1 complex (ATG16L, ATG12 and ATG5), attaches the molecule LC3 to the IM. LC3 conjugation with phosphatidylethanolamine is a critical for downstream effector protein recognition and autophagosome closure <sup>139</sup> and is often used as a molecular marker for autophagy induction.

Eventually the IM pinches off from the omegasome and as it closes to form the autophagosome it swallows cytosolic components.

After closure, autophagosomes merge with lysosomes to form autolysosomes, wherein the luminal contents of autophagosomes are degraded in the lysosomal lumen. While the molecular mechanisms governing autolysosome formation are not as well characterized as autophagosome initiation, it is now clear that autolysosome formation shares many components with from the lysosome-endosome fusion pathway<sup>140</sup>. One of the best characterized components in autophagosome-lysosome fusion is the SNARE Stx17<sup>141,142</sup>. SNARE proteins are required for membrane fusion, with a SNARE (Q-SNARE) on a vesicle forming a coiled-coil structure with a SNARE (R-SNARE) on a target membrane generating a trans-SNARE complex and driving fusion of the vesicle with the target membrane. In the context of autophagy, Stx17 is recruited to the closed autophagosome from the ER by a poorly defined mechanism and binds to another Q-SNARE, SNAP29. The Stx17/SNAP29 complex is further stabilized by ATG14<sup>142</sup> and interacts with the lysosomal R-SNARE, VAMP8, leading to membrane fusion and autolysosome formation<sup>141</sup>. Additional components required for autolysosome formation are the small GTPase Rab7<sup>143</sup>, required for late endosome/lysosome fusion events its effector the homotypic fusion and vacuolar sorting (HOPS)<sup>144</sup> and its GEF the Mon1-Ccz1 complex<sup>145</sup>. In addition to the molecular recognition between Stx17 and Vamp8, PLEKHM1 serves as a tether between lysosomal HOPS and LC3, ensuring correct autophagosome-lysosome fusion<sup>146</sup>.

Following the degradation of its contents, the lysosome needs to reform from the autolysosome to support additional rounds of autophagy through a process known as autophagic lysosome reformation (ALR)<sup>147,148</sup>. ALR initiates with the formation of tubules in the autolysosome driven by clathrin, PI(4,5)P<sub>2</sub> and the motor protein KIF5B<sup>149,150</sup>. Sorting of lysosomal proteins is thought to be mediated by the lipid kinase PI4KIIIβ<sup>151</sup> in a poorly understood manner. Curiously, ALR is regulated by mTORC1, as treatment with rapamycin inhibits this process, suggesting a model in which ALR functions as a stop-gap for autophagy once autophagy-generated nutrient levels are sufficient to reactivate mTORC1<sup>152</sup>.

## Chaperone mediated autophagy

In contrast to the degradation of cytosolic components under autophagy, CMA leads to the lysosomal degradation of only a subset of proteins that contain CMA motifs. CMA is a highly orchestrated process involving multiple steps: substrate recognition and lysosomal targeting, substrate binding and unfolding, substrate translocation and degradation in the lysosomal lumen. Key to CMA is the recognition of substrates by the cytosolic chaperone and heat shock protein HSC70<sup>153</sup>. HSC70 binds to protein substrates with a consensus pentapeptide motif KFERQ<sup>154</sup> that is recognized based on its charge. Additionally, incomplete motifs may be complemented through post-translational modification of adjacent residues by phosphorylation or acetylation<sup>155</sup>. These PTMs thus provide the necessary charge recognition by HSC70<sup>156</sup>.

Once bound by HSC70, the protein substrate is targeted to the lysosomal surface where it interacts with the cytoplasmic tail of LAMP-2A. LAMP-2A functions as the receptor for CMA substrates<sup>157</sup> and multimerizers upon contact with the substrate to facilitate lysosomal entry<sup>158</sup>. To enter the lysosomal lumen the substrate must unfold in a process mediated by HSC70 and its co-chaperones at the lysosomal membrane. Translocation of the substrate further requires a resident lysosomal luminal chaperone lys-HSC70<sup>159</sup>. lys-HSC70 facilitates substrate entry by directly pulling on the incoming substrate or by actively blocking its departure from the lysosomal lumen. After substrate translocation, the LAMP-2A multimers are disassembled so the CMA cycle can begin again.

One regulatory point in CMA is the assembly/disassembly of LAMP-2A multimers. Stabilization of LAMP-2A multimers is enhanced by the protein GFAP in its unphosphorylated state. Recent work from the Cuervo lab has demonstrated that AKT phosphorylates GFAP, leading to the destabilization of LAMP2a multimers. This study connects CMA to a balance of AKT activation at the lysosomal surface and inhibition by mTORC2 and a protein phosphatase, PHLPP1<sup>160</sup>. While conceivably mTORC2 at other cellular locations could also be involved in this process, Cuervo and colleagues found that mTORC2 associated with a subset of lysosomes; the mTORC2 component Rictor was found only in the subset of lysosomes engaged in chaperone mediated autophagy. In contrast, the mTORC1 subunit Raptor was enriched in lysosomes not engaged in chaperone mediated autophagy<sup>160</sup>. In addition to being regulated by mTORC2, CMA is also regulated transcriptionally through the expression of LAMP-2A which can be dynamically modulated during hypoxia, oxidative stress, genotoxic stress or prolonged starvation. This process is mediated in part by TFEB pathway<sup>161</sup>.

## The lysosome in aging and disease

Lysosomal function is essential for the healthy functioning of both individual cells and for an entire organism. Aging represents the end result of accumulating deficits at the molecular, cellular, and organismal level, and aberrant lysosomal signaling during aging contributes to the degradation of these processes. Loss of proteostasis has been proposed as one of the nine hallmarks of aging; while many different processes contribute to the maintenance of proteostasis, an important part of this process involves the degradation of proteins by the lysosome during both CMA and macroautophagy. Proteostasis declines with aging in most organisms, with the possible exception of the exceptionally long-lived naked mole rat<sup>162,163</sup>.

Just a decade ago, it was demonstrated that rapamycin extends the lifespan of mice, a result that has been widely reproduced<sup>164,165</sup>. Indeed, even intermittent or transient treatment with rapamycin can extend the lifespan of mice and rejuvenate specific tissues, including the hematopoietic system, heart, immune system and kidney<sup>166-172</sup>. In part, this may be due to the ability of rapamycin to block age-associated increases in mTOR signaling in certain tissues<sup>173,174</sup>. However, the specific molecular and physiological mechanisms by which rapamycin extends lifespan are difficult to examine in mammals; while it is clear that inhibition of S6K1 signaling extends lifespan, and that inhibition of translation downstream of 4E-BP1 is beneficial for metabolic health, the contribution of autophagy to the effects of

rapamycin on mammalian longevity have not been examined in detail <sup>175–177</sup>. However, there is reason to believe that many of the beneficial effects of rapamycin on aging may be driven in part by increased autophagy.

First, both CMA <sup>178</sup> and autophagy naturally decline with aging <sup>179</sup>. Evidence from model organisms suggests that this is detrimental. Overexpression of chaperones extends the lifespan of worms and flies <sup>180,181</sup>. Worms and flies with defective autophagy have reduced lifespan, while conversely overexpression of autophagy genes such as *Atg8a* extends lifespan <sup>182,183</sup>. Finally, experiments in both worms and flies have found that an inhibition of autophagy genes blunts the effects of reduced mTOR signaling on lifespan <sup>184,185</sup>. In mammals, mice with genetic impairment of CMA have reduced lifespan, with an increase in age-related pathology and accelerated cellular senescence <sup>186</sup>. While it is not yet known if upregulation of CMA can extend mammalian lifespan, an increase in hepatic CMA can rejuvenate the aging mouse liver <sup>178</sup>. However, overexpression of *Atg5*, which enhances autophagy in mice, increases resistance to oxidative stress and increases lifespan <sup>187</sup>.

Discovering safe and effective ways to regulate lysosomal function and to boost autophagy is likely to be essential to promote healthy aging and to treat a variety of age-associated diseases. Many neurodegenerative diseases, including Alzheimer's disease, is thought to be driven in part by misfolded proteins; promoting proteostasis, either by increasing lysosomal function directly or by boosting autophagy through other means is therefore a potential mechanism to delay or treat the disease <sup>188–190</sup>. In Alzheimer's disease, which is thought to be partly precipitated by amyloid aggregation, the hope is that stimulating autophagy will clear these protein aggregates <sup>191</sup>. Conversely, autophagy is believed to be beneficial for tumorigenesis and for the survival of many types of cancer cells that are under nutrient stress <sup>192</sup>, and inhibiting this process to disrupt proteostasis has emerged as a potential therapeutic option for some malignancies, with recent pre-clinical studies targeting core autophagy proteins showing promise <sup>193,194</sup>. Thus, autophagy has been catapulted to the forefront as a therapeutic avenue for multiple disease states, however, caution must be applied as targeting any metabolic signaling pathway must be considered in a context-dependent manner.

## Summary

As a newly described center for the integration of major catabolic and anabolic pathways the lysosome is positioned as a key sensor of cellular nutrient levels. With a growing appreciation for its diverse functions within the cell, many questions still loom large. At the biochemical level, understanding whether additional nutrients such as nucleotides and lipids are sensed at the lysosome and their corresponding sensors will be critical in deciphering the inner workings of metabolic signaling at this organelle. At the cellular level, providing a systems level overview of nutrient signaling at the lysosome and exploring the crosstalk between the lysosome and other organelles will allow us to predict how different cellular stressors change nutrient flux and signaling. At the organismal level, the future lies at understanding how lysosome-based metabolic signaling translates to different tissues and organ systems. Underlying the desire to study metabolic signaling at the lysosome is the immediate realization that disruption of these pathways leads to major human pathologies. While the molecular mechanisms of lysosomal signaling in cancer, aging and immune

disorders remain at their infancy, the last decade has seen the development of a numerous inhibitors targeting multiple parts of this pathway (<sup>195</sup>). While the utility of rapamycin in a handful disorders is clear, its overall success in preventing cancer growth remains poor. Whether this is due to an incomplete understanding of mTORC1's requirement for rapid cell proliferation, the ability of cancers to acquire rapamycin-resistance <sup>196</sup>, the reactivation of PI3K signaling due to systemic glucose-insulin feedback on most diets <sup>197</sup>, or its mechanism of action as an allosteric inhibitor that blocks the phosphorylation of only some of the mTORC1 substrates, remains to be seen. However, there is a palpable excitement that the new generation of therapeutic agents targeting metabolic signaling at the lysosome will have substantial benefits for human health.

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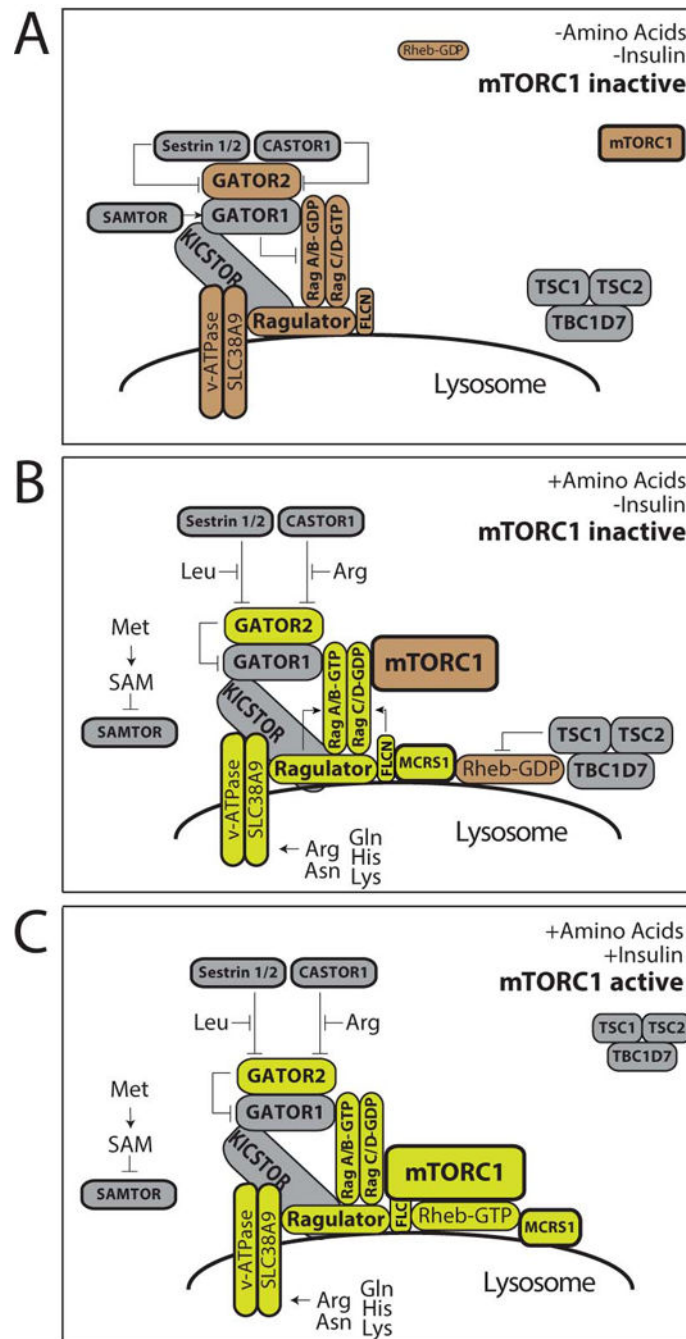
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**Figure 1. Regulation of mTORC1 Activity at the Lysosome by Amino Acids and Growth Factors.** (A) In the absence of amino acids the lysosomal platform comprised of the Rag GTPases and its scaffold the Ragulator complex are inactive resulting in the cytoplasmic localization of mTORC1. GATOR1, which is localized to the lysosome by KICKSTOR and activated by SAMTOR, inactivates RagA/B. GATOR2, a negative regulator of GATOR1, is inactivated by the Sestrin and CASTOR complexes. The absence of growth factor (e.g. insulin) signaling promotes the localization of the tuberous sclerosis complex (TSC) to the lysosome. (B) Amino acid stimulation results in the inhibition of GATOR1 through multiple pathways.

First, methionine catabolism raises cellular levels of SAM, which promotes the disassociation of SAMTOR from GATOR1. Secondly, binding of leucine by Sestrin and arginine by CASTOR results in the dissociation of these inhibitors from GATOR2, which is therefore free to inhibit GATOR1 activity. Amino acids also stimulate the Ragulator/SLC38A9/v-ATPase complex and FLCN to activate RagA/B and RagC/D, respectively. The active Rag heterodimer recruits mTORC1 to the lysosomal surface. MCRS1 recruits the small GTPase Rheb in an amino acid dependent manner. At the lysosome, TSC's GAP activity inhibits Rheb, and thus mTORC1 remains inactive. (C) Insulin induces TSC to leave the lysosome, permitting Rheb to bind to GTP. GTP-bound Rheb activates the kinase activity of mTORC1. Adapted and updated from (18) with permission from Elsevier.