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Autophagy in Cellular Growth Control

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Abstract

Cell growth is regulated by two antagonistic processes: TOR signaling and autophagy. These processes integrate signals including growth factors, amino acids, and energy status to ensure that cell growth is appropriate to environmental conditions. Autophagy responds indirectly to the cellular milieu as a downstream inhibitory target of TOR signaling and is also directly controlled by nutrient availability, cellular energy status, and cell stress. The control of cell growth by TOR signaling and autophagy are relevant to disease, as altered regulation of either pathway results in tumorigenesis. Here we give an overview of how TOR signaling and autophagy integrate nutritional status to regulate cell growth, how these pathways are coordinately regulated, and how dysfunction of this regulation might result in tumorigenesis.

Introduction

In order to reproduce efficiently, eukaryotic cells must grow in a manner appropriate to the extracellular milieu. For single-celled organisms, the decision to grow and divide is simple. In the presence of appropriate nutrients (glucose and amino acids), the cell will increase its size and mass and ultimately divide. Though the decision to grow and divide in multicellular organisms is complicated by the need for responsiveness to cell-to-cell signals and hormonal cues, much of the biological machinery required to respond to those cues remains highly conserved. One such conserved pathway is autophagy, which represents the major mechanism by which a cell catabolizes biological macromolecules (Levine and Klionsky, 2004). This can happen for small amounts of cytosol or specific proteins through direct fusion with the lysosome (i.e. microautophagy), the direct receptor-mediated import of cytosolic proteins containing specific recognition motif sequences into the lysosome (i.e. chaperone-mediated autophagy) or it can occur through the de novo formation of vesicles

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that engulf entire organelles and long-lived proteins (i.e. macroautophagy). The focus of this review will be the role of macroautophagy in the regulation of cellular growth in metazoans.

Although both increased cell size and cell proliferation contribute to cell growth, the rate of cell division is itself regulated by changes in cell size. Thus, the regulation of cell size is of paramount importance in regulating cellular growth. Increasing the mass of a cell requires the net synthesis of macromolcules including proteins, DNA, and RNA, which ultimately requires energy input. In contrast, the response to starvation or stress usually involves macromolecular remodeling, energy production, and the catabolism of existing macromolecules. The phosphatidylinositol 3-kinase (PI3K)/TOR pathway has emerged as the central conduit for integrating a variety of signaling pathways to promote cell growth and protein synthesis; autophagy is the primary pathway for catabolic activities (He and Klionsky, 2009; Hietakangas and Cohen, 2009). From a teleological perspective, an efficient system would couple the control of autophagy with the control of cell growth, and cues that promote one would inhibit the other, thus preventing inefficient use of energy and nutrients. Indeed, both autophagy and the PI3K signaling pathways have evolved to respond to a variety of cellular growth and nutrient signals including growth factors, the presence of amino acids, and the presence of glucose and energy. In addition, autophagy limits cellular growth and promotes survival during cellular stress. Given the tightly intertwined processes that are governed by TOR signaling and autophagy, previously postulated coordination and crosstalk between these pathways are now being confirmed.

Insulin/Growth factors

Anabolic hormones (e.g. insulin and growth factors) regulate cell growth by positively activating TOR signaling and inhibiting autophagy. Not surprisingly, many key signaling molecules are conserved between both pathways (e.g. AMPK, TSC2, and Vps34). Although TOR exists in two signaling complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2), this discussion focuses on the rapamycin-sensitive TORC1 which constitutes the primary complex responsible for assessing the presence of nutrients and growth factor signals to control protein translation and cell growth (Loewith 2002; Hietakangas 2009). Insulin is a well-characterized growth factor that signals through the canonical PI3K-TOR pathway. In the presence of appropriate growth factors, receptor tyrosine kinases phosphorylate targets (e.g. IRS1) to stimulate Class 1 PI3Ks to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2). The accumulation of PIP3 is inhibited by PTEN, phosphatase and tensin homolog deleted on chromosome ten, an important negative regulatory protein in the PI3K-TOR pathway. The availability of PIP3 at the membrane results in the activation of Akt which inhibits the tuberous sclerosis complex (TSC1/TSC2). This heterodimeric complex promotes the GTPase activity of Rheb, a Ras-related GTPase, which causes Rheb-GTP to become Rheb-GDP. Rheb-GTP activates TORC1 kinase activity while Rheb-GDP inhibits the complex. The net effect of growth factor stimulation is the activation of Rheb-GTP and subsequently TORC1. In addition to autophagy inhibition, some important downstream effects of the TORC1 pathway include the activation of ribosomal S6 protein kinase (S6K1), inhibition of elongation factor 4E binding protein (4E-BP1), and subsequent activation of the eukaryotic translation initiation factor 4E (eIF4E). These downstream effectors are critical for ribosome production, protein translation, and, ultimately, cell growth (Lum et al., 2005b; Wang and Proud, 2009).

The central role of the PI3K-TOR pathway in regulating growth control is relevant to human disease as the loss of TORC inhibitors (e.g. PTEN, LKB1, TSC1/2) or constitutive activation of TORC (e.g. hyperactive PI3K or Ras signaling) results in both sporadic cancers and cancer predisposition syndromes (Samuels et al., 2004; Shaw and Cantley, 2006). For

example, PTEN is mutated in Cowden's disease, a disease characterized by mucocutaneous lesions (tricholemmomas, oral papillomas, and acral keratotic papules), and mutations in PTEN also predispose to a variety of other human cancers (Keniry and Parsons, 2008). The tuberous sclerosis complex 1 or 2 are mutated in the eponymous syndrome characterized by cutaneous fibromas and hamartomatous growth of a variety of tissues (Schwartz, 2007).

While growth factors are required for TORC activation, it is growth factor *withdrawal* that induces autophagy. Early evidence for a role for receptor tyrosine kinase signaling in autophagy inhibition came from genetic studies in *C. elegans*. The loss of *C. elegans daf-2*, an ortholog of the insulin-like receptor tyrosine kinase, induces autophagy to mediate constitutive dauer formation, lifespan extension, pathogen resistance and increased degradation of β-amyloid peptide (Florez-McClure et al., 2007; Hansen et al., 2008; Hars et al., 2007; Jia et al., 2009; Melendez et al., 2003). Similarly, in *Drosophila*, the deletion of its Insulin-Like Peptides (DILPs) induces autophagy and severe growth retardation (Zhang et al., 2009). Besides insulin signaling, the loss of other growth factor signaling also induces autophagy. For example, when bound to its receptor, interleukin-3 (IL-3) induces tyrosine phosphorylation and activates the MAPK cascade within hematopoietic cell lines, and IL-3 withdrawal from immortalized, apoptosis-deficient ($Bax^{-/-}Bak^{-/-}$) cells results in the induction of autophagy (Lum et al., 2005a). In addition, the loss of EGFR signaling or substrate detachment results in the induction of autophagy in breast epithelial cells (Fung et al., 2008).

The identification of mechanisms by which growth factor withdrawal induces autophagy provided one of the earliest links between autophagy regulation and TORC signaling; evidence from evolutionarily diverse model sytems has consistently demonstrated that the inhibition of autophagy by growth factors occurs through the activation of the Class I PI3K-TORC signaling pathway (Arico et al., 2001; Petiot et al., 2000; Rusten et al., 2004; Scott et al., 2004). Although TORC1 activation is necessary and sufficient for the repression of autophagy in the presence of growth factors, it has not been determined whether the de-repression of basal autophagy is adequate to explain growth-factor-withdrawal-induced autophagy, or whether additional signals (i.e. low energy status or limiting nutrient availability) that may act in parallel of TOR are required to activate autophagy more directly.

Amino acids

Perhaps even more evolutionarily conserved than their response to growth factors, TORC and autophagy are responsive to the presence of environmental nutrients. Even in the presence of adequate growth factor signals, TORC signaling requires the presence of intracellular amino acids, especially branched chain amino acids like leucine, for maximal TOR activation (Christie et al., 2002; Hara et al., 1998). The central role of amino acids in regulating cell growth was highlighted by the finding that L-glutamine positively regulates cell size by promoting the import of leucine and other essential amino acids through the SLC7A5/SLC3A2 bidirectional transporter (Nicklin et al., 2009). When intracellular amino acids are low, the binding of Rheb to mTORC1 is impaired in a manner that is independent of both TSC2 or even GTP binding by Rheb (Long et al., 2005; Nobukuni et al., 2005; Smith et al., 2005).

Although the exact sensor(s) through which amino acids promote TORC1 activity remains ambiguous, recent studies have begun to reveal the mechanism of its amino acid-dependent signaling. The Rag-GTPases, a heterodimer of RagA/B-GTP with RagC/D-GDP, have been identified as direct binding partners of mTORC1 through Raptor and have been implicated in the amino acid sensitivity of TORC1 signaling (Kim et al., 2008; Sancak et al., 2008).

This regulation is thought to occur not through the direct activation of mTOR, but rather through the subcellular relocalization of mTORC1 complexes to a vesicular compartment that also contains Rab7 (Sancak et al., 2008). It has been proposed that the Rag heterodimers promote the amino acid-dependent signaling of TORC1 by bringing the complex into the vicinity of farnesylated Rheb. However, the precise endomembrane localizations of both TORC1 and Rheb and the dynamics of their proposed localizations remain a matter of active investigation (Buerger et al., 2006; Drenan et al., 2004; Jiang and Vogt, 2008; Sancak et al., 2008).

An independent line of investigation has also hinted at the importance of lipid signaling and vesicle trafficking in amino acid-dependent TORC1 signaling. Vps34, a class III PI3 kinase which phosphorylates phosphatidylinositol (PI) to form phosphatidylinositol 3-phosphate (PI3P), has been identified as an amino acid-dependent activator of TORC (Byfield et al., 2005; Nobukuni et al., 2005). This role of Vps34 membrane signaling in the regulation of TORC1 activation is especially interesting given recent findings demonstrating a possible role for Beclin 1, an upstream regulator of autophagy and binding partner of Vps34 in diverse functions in vesicle sorting and membrane trafficking (Itakura et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009). Curiously, Vps34 is not required for TORC activation in *Drosophila*, so it is unclear whether this regulation is evolutionarily conserved (Juhasz et al., 2008). A high research priority will therefore be to uncover the exact role of Vps34, Beclin 1, and other components of autophagosome/vesicle trafficking in crosstalk with TORC1 regulation and to further determine whether this is conserved in all metazoans.

While amino acids are required for TORC activation, the *absence* of amino acids induces autophagy through several mechanisms. Nutrient starvation activates autophagy indirectly through the inhibition of TORC1 signaling which acts to repress a complex containing Atg13, focal adhesion kinase-interacting protein 200 (FIP200), and unc-51 like kinases 1 and 2 (ULK1/2) in nutrient rich conditions; this regulation is likely important for the coordinated regulation of autophagy and TORC1 signaling (Hosokawa et al., 2009; Jung et al., 2009). However, signaling through the the PI3K-TOR pathway is only one of many ways in which autophagy is sensitive to amino acids.

Amino acid starvation has also been implicated in regulation of autophagy through the Raf kinase signaling cascade. In this cascade, in an amino acid-dependent manner, Raf-1 activates MEK1/2 (MAPK/ERK kinase), which activates ERK1/2 (extracellular signal regulated kinase 1 and ERK2 mitogen activated kinase). Erk kinases phosphorylate GAIP, a Gα interacting protein, whose phosphorylation promotes its GAP activity on the α subunit of the G_{i3} protein, which ultimately promotes autophagy (Ogier-Denis et al., 2000; Pattingre et al., 2003; Shaw and Cantley, 2006). However, Raf is also downstream of Ras, which inhibits autophagy through the Class I PI3K-TOR signaling pathway (Furuta et al., 2004). It remains to be determined whether the contradictory regulation of autophagy by Ras through its Raf-1 and PI3K-TOR effector arms represents a biological checkpoint control or whether it is an artifact of particular cell lines.

Amino acid deprivation also activates autophagy by signaling through the eIF2α and ER stress/integrated stress response (Ron and Walter, 2007; Talloczy et al., 2002). In a pathway conserved from yeast to mammals, a limiting supply of amino acids results in uncharged tRNAs and the activation of Gcn2 (general control non-derepressible-2) and phosphorylation of eukaryotic translation initiation factor-2 on its α subunit (eIF2α) (Wek et al., 1995; Zhang et al., 2002). Phosphorylated eIF2α in turn inhibits eIF2B, a pentameric guanine nucleotide exchange factor, from recycling eIF2 to its active GTP-bound state. Phosphorylation of eIF2α also results in the upregulation of ATF4 (activating transcription factor 4), a transcription factor which in turn activates a transcriptional program to respond

to diverse cellular stresses through the induction autophagy genes, amino acid transporters, anti-oxidant response proteins, and chaperones (Harding et al., 2000; Hinnebusch and Natarajan, 2002; Milani et al., 2009; Natarajan et al., 2001; Vattem and Wek, 2004). Given its broad role in response to cellular stress, it is not surprising that eIF2α is also a target for other stress kinases (e.g. PKR during viral infection, PERK during the unfolded protein response) and thus forms a common pathway for the activation of autophagy in response to amino acid starvation and other cellular insults (Talloczy et al., 2002).

Energy sensing/Glucose

In addition to amino acids, cells must also be supplied with glucose, fatty acids, or pyruvate to maintain a constant supply of ATP (Lum et al., 2005b). Both positive growth signaling and autophagy are closely linked to available energy in the cell. This is largely sensed through cellular levels of ATP. The primary energy-sensing pathway of the cell is the well-characterized AMPK pathway (Hardie, 2007). AMPK is an essential heterotrimeric kinase that is activated during times of energy depletion by increased ratios of AMP to ATP and inhibited by the presence of glycogen. When AMP is bound to the regulatory γ-subunit, LBK1/STK11 can phosphorylate and activate the catalytic α-subunit of AMPK. In contrast, the regulatory β-subunit can bind to glycogen to inhibit AMPK activity. The net effect of AMPK activation is the upregulation of the energy-producing pathways (e.g. Glut4 receptors, insulin sensitivity) and inhibition of energy storage pathways (e.g. glycogen synthesis, lipid synthesis). AMPK inhibits the TORC1 complex by phosphorylating TSC2, which then activates the GAP activity of TSC1/2 on Rheb-GTP favoring the formation of the Rheb-GDP, thereby inactivating TORC1 (Inoki et al., 2003). In a parallel inhibitory pathway, AMPK also phosphorylates and inhibits the TORC1-defining component, Raptor, leading to an interaction between Raptor and 14-3-3 that disrupts its binding to TOR (Gwinn et al., 2008). Mutations in LKB1/STK11 cause Peutz-Jeghers, which is characterized by pigmentary abnormalities and a predisposition to malignancy (Hardie, 2007; Inoki et al., 2003).

In a manner similar to growth factor withdrawal, the inhibition of TORC1 signaling by active AMPK can indirectly induce autophagy through a de-repression of the upstream regulators of the catabolic process. Evidence from yeast suggests that AMPK also has a more direct role in the induction of autophagy. Snf1, the yeast homolog of AMPK, promotes autophagy by acting on Atg1 and Atg13, yeast homologs of ULK and Atg13, respectively (Wang et al., 2001). However, a direct role for AMPK in autophagy activation in mammalian cells has not been demonstrated. In fact, early work in mammalian cells suggested that AMPK inhibits autophagy (Samari and Seglen, 1998). However, more recent work suggests that, similar to yeast, AMPK induces autophagy (Matsui et al., 2007; Meley et al.).

Cell stress

In addition to being responsive to cellular growth cues, autophagy is also critical for limiting cell growth and promoting cell survival in times of stress. Much of the cellular stress response has converged on signaling through the ER through the unfolded protein response. Mammalian cells possess three ER transmembrane receptors (IRE1α, ATF6, and PERK) that are responsible for transducing stress responses (Bernales et al., 2006; Ron and Walter, 2007). IRE1α and PERK are best characterized with respect to their ability to regulate autophagy.

IRE1α, inositol-requiring protein-1α, represents the most conserved core of the unfolded protein response. Upon activation by ER stress, IRE1α autophosphorylation induces a conformational change allowing it to bind to the adaptor protein tumour necrosis factor-α

(TNF-α)-receptor-associated factor 2 (TRAF2) through its cytoplasmic domain (Urano et al., 2000). The recruitment of TRAF2 has been shown to be important in stimulating autophagy in response to the UPR via its activation of c-Jun NH(2)-terminal kinase (JNK) (Ogata et al., 2006). In turn, JNK-mediated phosphorylation of Bcl-2 and disruption of the Bcl-2/Beclin 1 complex, and JNK-mediated upregulation of Beclin 1 transcription likely contribute to the induction of autophagy (Li et al., 2009; Pattingre et al., 2009; Wei et al., 2008).

In a parallel pathway to JNK activation, IRE1α–TRAF2 complexes also recruit IκB kinase (IKK) (Hu et al., 2006). Active IKK induces autophagy in a nuclear factor-κB (NF-κB) transcription-independent manner (Criollo et al., 2009). Strikingly, the absence of IKK results in impaired induction of autophagy suggesting that IKK-mediated induction of autophagy is not redundant with other ER stress signaling pathways. Although IKK activation appears to inhibit TOR signaling and induce AMPK and JNK, it is still unclear precisely how IKK activation promotes autophagy.

In contrast to IRE1α, the induction of autophagy by PERK appears to be transcription-dependent. In response to unfolded proteins, PERK can phosphorylate eIF2α which then activates the transcriptional upregulation of autophagy as noted previously (Kouroku et al., 2007). As already noted, other kinases capable of sensing cell stress, including GCN2 and interferon-induced double-stranded RNA-dependent protein kinase R (PKR), can also activate eIF2α in response to conditions like amino acid deprivation and viral infection (Kouroku et al., 2007; Talloczy et al., 2002). Finally, as the major site of intracellular calcium storage, ER stress also results in the release of intracellular calcium stores. This release may result in the activation of autophagy through the CaMKKβ-dependent activation of AMPK (Hoyer-Hansen et al., 2007).

Because the ER has the ability to integrate diverse cell stress signals including starvation, hypoxia, misfolded proteins, and infection, it is teleologically well-placed as a sensor to limit cell growth during times of stress. A prominent role for ER stress in limiting cell growth is consistent with a current model in which the ER is thought to be the source of membranes for autophagosomal structures (Axe et al., 2008). Although it is known that Bcl-2 inhibits autophagy through a direct interaction with the BH3 domain of Beclin 1, the precise mechanistic details of this inhibition are unclear (Pattingre et al., 2005; Sinha et al., 2008). Given that ER-localized Bcl-2 is the form that inhibits Beclin 1 autophagy function, and the recent finding that preautophagosomal structures originate from the ER membrane, it is possible that Bcl-2 sequesters Beclin 1 in ER membrane subdomains away from preautophagosomal structures in the ER (Axe et al., 2008). According to this model, in response to a variety of cell stressors (e.g. nutrient starvation, unfolded proteins, viral proteins, etc.), the phosphorylation of Bcl-2 and its subsequent dissociation from the Beclin 1/Vps34 complex may contribute to the initiation of autophagosomal membrane formation.

Crosstalk between autophagy and TOR signaling

Direct interactions between the TORC1 and autophagy pathways help to coordinate the respective anabolic and catabolic processes. As noted above, part of the coordinate regulation occurs through TORC1-dependent phosphorylation and inactivation of a complex containing Atg13, FIP200, and ULK1/2 (Hosokawa et al., 2009; Jung et al., 2009). When TOR signaling is suppressed (e.g. growth factor withdrawal, starvation, or pharmalogical treatment), ULK becomes activated and phosphorylates and activates Atg13 and FIP200 to induce autophagy. As the ULK-Atg13-FIP200 complex functions upstream in autophagy, it is likely that the direct inhibitory phosphorylation of this complex by TORC1 represents an important signaling step. The precise mechanism through which the ULK-Atg13-FIP200 complex induces autophagy in metazoans remains to be determined.

Although the regulation of autophagy is clearly downstream of TOR signaling, there is increasing evidence that autophagy may also regulate TOR signaling. Positive regulators of autophagy inhibit activation of the TOR pathway (e.g. ULK1, Atg13) as assessed by the phosphorylation of downstream targets of TOR like S6K1 (Jung et al., 2009; Lee et al., 2007). The finding that Vps34 may be important for the amino acid sensitive signaling through TORC suggests additional coordination in the regulation of autophagy and cellular growth (Byfield et al., 2005; Nobukuni et al., 2005). At first glance, it would appear that the cell growth-promoting properties of Vps34 would seem to conflict with its role in initiating autophagic vesicle nucleation to regulate autophagy. However, this apparent discrepancy may represent the function of distinct complexes of Beclin 1/Vps34 with different functions. For example, Vps34 forms a complex with Beclin 1 and Atg14 (also known as Atg14L, Barkor) to positively regulate autophagosome formation and maturation (Itakura et al., 2008; Matsunaga et al., 2009). Beclin 1 also forms Atg14-independent distinct complexes with UVRAG and Rubicon with possible roles in the regulation of autophagosome/endosome maturation; however, the precise functions of these complexes is unclear (Liang et al., 2006; Liang et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009).

Another interesting interface between PI3K-TOR signaling and autophagy resides in the regulation of PI and its derivatives by different classes of PI3Ks. Class I and Class III PI3Ks share structural similarities and both exist as a heterodimers of a catalytic (p110 and Vps34) and a regulatory (p85 and Vps15) subunit, respectively. Both PI3Ks are crucial upstream regulatory kinases in their respective signaling cascades. In PI3K-TOR signaling, the conversion of PIP2 to PIP3 is essential for the downstream activation of the PIP3-dependent kinases, PDK and Akt. Similarly, in autophagy, the conversion of PI to PI3P by the Beclin 1/Vps34 complex appears to be one of the earlier, if not the earliest, signaling event in the budding of the omegasome from the endoplasmic reticulum and the eventual formation of the autophagosome (Axe et al., 2008). In another interesting parallel, both signaling pathways possess lipid phosphatases (PTEN and Jumpy), which inhibit the activation of their respective pathways (Vergne et al., 2009). Although the role of PIs in PI3K-TOR signaling and in autophagy has been studied, little is known about how the two pathways may interface at the level of lipid signaling. For example, it will be a critical to determine whether the activity of Beclin 1/Vps34 at autophagosomes in generating PI3P ultimately affects the generation of PIP3 by Class I PI3Ks. Given the known crosstalk between Vps34 and TORC signaling, the possible role of PI derivatives in this crosstalk demands further attention.

Distinct from its activity in generating PI3P, Beclin 1/Vps34 may function in other roles to affect TORC signaling. For example, given the recent the recent finding that TORC1 relocalizes to Rab7-containing vesicles upon activation, it is possible that autophagy and cell growth could be coordinated through the action of different Beclin 1/Vps34 complexes on different subsets of the endomembrane system. In amino acid and growth factor replete conditions, the Beclin 1/Vps34 complexes would hypothetically act on endosomes/lysozomes to regulate TOR signaling complexes by ensuring the appropriate maturation and recycling of TORC1-, Rheb-GTP-, Rag-GTPase-containing vesicles. In contrast, upon depletion of amino acids, the Beclin 1/Vps34 complexes might localize to specific sites in the endoplasmic reticulum (ER) membranes where they would promote the generation of PI3P to initiate the formation of the autophagic isolation membrane. Clearly, more experiments are necessary to understand the role of the Beclin 1/Vps34 complex in regulation of endomembrane trafficking and how it may affect TORC signaling.

Emerging functions of autophagy in cell cycle control

As cell division contributes to cell growth, cell cycle checkpoints are a critical regulatory point in the growth control of eukaryotes. The decision to enter S phase from G1 is promoted by cyclin-dependent kinase (CDK)/cyclin complexes (e.g. cyclin D/Cdk4 and cyclin E/Cdk2) which are inhibited by CDK inhibitors (CDKIs) including p16 and p27. The levels of both CDK/cyclins and CDKIs are regulated through ubiquitination (Sherr and Roberts, 1999). TORC1 signaling has been shown to contribute to cell cycle regulation through affecting the levels of both cyclins and CDKIs (Wang and Proud, 2009).

Less is known about the relationship between the cell cycle and autophagy. Experiments using pharmacologically synchronized cells have demonstrated that autophagy is most active in the G1 and S phases of the cell cycle, while it is inhibited in mitosis (Eskelinen et al., 2002; Tasdemir et al., 2007). Specific regulators of the cell cycle have also been shown to affect autophagy. For example, $p14^{ARF}$, an alternative reading frame product of the p16 locus and inhibitor of G1 cell cycle progression, binds to Bcl-X_L, a Bcl-2 anti-apoptotic family member; this binding disrupts Beclin 1/ Bcl-X_L binding and induces autophagy (Pimkina et al., 2009). Also, the overexpression of the CDKI, p27, is sufficient to induce autophagy (Liang et al., 2007). Interestingly, both low energy status (via AMPK) and amino acid starvation have both been shown to stabilize p27 (Leung-Pineda et al., 2004; Liang et al., 2007) suggesting a possible role for p27 in the physiological regulation of autophagy. However, our understanding of the impact of the cell cycle on autophagy is in its infancy, and the mechanism through which cell cycle regulators, like $p14^{ARF}$ and p27, inhibit autophagy remain an important area of continued research.

In addition to ties to the canonical cell cycle, there is also evidence for a direct role for autophagy in executing cellular senescence. Cellular senescence is a form of irreversible cell cycle arrest; it can be induced by exogenous DNA damage, telomere depletion, or oncogene (e.g. Ras or MEK) overexpression. This pathway is proposed to contribute to tumor suppression by inhibiting the proliferation of otherwise damaged cells. Oncogene expression induces the transcriptional upregulation of many mediators of autophagy, and the inhibition of autophagy delays the onset of senescence (Young et al., 2009).

Although there is no direct evidence that the genetic disruption or pharmacologic inhibition of autophagy has strong effects on cell cycle regulation, experimental mouse models indicate that deficiency of some autophagy proteins such as Beclin 1 and Ambra1 results in increased cell proliferation (Fimia et al., 2007; Qu et al., 2003). Given the emerging important role for autophagy in the degradation of ubiquitinated targets through specific receptor molecules (e.g. p62/SQSTM1), it is possible that autophagy plays a role in the degradation of cell signaling molecules subject to ubiquitination (Kirkin et al., 2009). The loss of autophagy might result in the abnormal persistence of these cell cycle regulators (e.g. CDKIs) and result in aberrant cell cycle entry. While there is growing evidence for a role of autophagy in the control of cell proliferation, more work is required to define the mechanism(s) by which this occurs.

Autophagy and tumor suppression

One clinically important sequelae of dysfunction of cellular growth control is tumorigenesis. Although the role of hyperactivation of the PI3K/TOR pathway in promoting tumorigenesis is well-established, the contribution of autophagy to tumor suppression is only now being established (Shaw and Cantley, 2006). Activators of TOR signaling (e.g. Class I PI3K, Akt, and Ras) function as oncogenes while inhibitors (e.g. TSC1/2, PTEN, LKB1/AMPK) function as tumor suppressors. In contrast, activators of autophagy (e.g. LKB1/AMPK, p27, DAPk, PTEN, TSC1/2) function as tumor suppressors while inhibitors of autophagy (e.g.

Bcl-2, Akt, activated Class I PI3K) function as oncogenes. Of note, the relationship of autophagy to the established tumor suppressor p53 is less clear. Stress-induced (e.g. DNA damage) activation of p53 induces autophagy, while basal levels of p53 appear to inhibit autophagy (Feng et al., 2005; Levine and Abrams, 2008; Tasdemir et al., 2008). This apparent discrepancy may relate to the diverse, context-specific functions of p53 in multiple signaling pathways that influence autophagy or to differing nuclear and cytoplasmic functions of p53 (Levine and Abrams, 2008). Interestingly, about 1/3 of colon cancer-associated mutated forms of p53 accumulate in the cytoplasm, and inhibit autophagy, suggesting a mechanism by which mutation of p53 in human cancer may impair autophagy (Morselli et al., 2008). Thus, despite some conflicting data regarding p53-dependent control of autophagy, taken together, the preponderance of evidence suggests that autophagy may be a critical downstream effector of multiple signaling pathways relevant to tumorigenesis.

Several studies in mammalian systems have confirmed the importance of autophagy execution genes in tumor suppression. Beclin 1 and UVRAG (a potential activator of Beclin 1-dependent autophagy) inhibit tumor cell growth *in vitro* and tumor xenograft formation *in vivo* (Liang et al., 1999 Nature; Koneri K et al. 2007; Liang et al. 2006). Mouse models demonstrate that the monoallelic loss of *beclin 1* or *Ambra1* or bi-allelic loss of *Bif-1* results in increased spontaneous tumorigenesis (Qu et al., 2003; Takahashi et al., 2007) (personal communication, F. Cecconi) and *Atg4c*$^{-/-}$ null mice demonstrate increased chemically-induced fibrosarcomas. (Marino et al., 2007). Furthermore, monoallelic deletions of UVRAG are common in human colon cancer (Ionov et al., 2004) and monoallelic deletions of *beclin 1* are common in human breast, ovarian, and prostate cancer (Aita et al., 1999; Ionov et al., 2004). In addition, decreased Beclin 1 expression has been linked to advanced tumor grade and/or poor survival prognosis in several types of human cancer, including gastric carcinoma, hepatocellular carcinoma, colon cancer, ovarian cancer, and brain tumors (Ahn et al., 2007; Miracco et al., 2007; Shen et al., 2008; Won et al., 2010). Thus, there is emerging data suggesting that loss of autophagy execution gene function may contribute to tumorigenesis.

While the tumor suppressor function of autophagy is undisputed, the mechanistic details of how autophagy functions in tumor suppression are still unclear. The role of autophagy in tumor suppression can be explained, in part, by its ability to prevent chromosome instability. The loss of autophagy genes (e.g. monoallelic loss of *beclin 1*, or biallelic loss of *Atg5*) promotes DNA damage, gene amplification, and aneuploidy in cell culture and tumor xenograft models (Mathew et al. 2007; Karantza-Wadsoworth etal., 2007). It is postulated that autophagy protects cells from genomic instability by promoting the degradation of p62 and damaged organelles (e.g. mitochondria), and by decreasing the accumulation of damaging ROS (Karantza-Wadsworth et al., 2007; Mathew et al., 2009; Mathew et al., 2007). It remains to be determined whether autophagy also regulates cellular proteins that directly function in DNA damage repair. Regardless of the mechanism by which autophagy prevents genomic instability, this function of autophagy likely represents a critical part of its ability to prevent tumorigenesis.

However, in addition to preventing genomic instability, there is evidence that autophagy may function in additional parallel pathways to prevent tumorigenesis. Specifically, increased proliferation of mammary epithelial cells and splenic lymphocytes has been noted in *beclin 1* heterozygous-deficient mice and increased neural cell proliferation has been noted in *Ambra1*-deficient embryonic mice (Fimia et al., 2007; Qu et al., 2003). The mechanism by which autophagy inhibits cell proliferation is not clear. However, several known functions of autophagy could theoretically contribute. First, autophagy has been proposed to play a role in executing cellular senescence (Young et al., 2009), so the loss of autophagy might impair the induction of this tumor suppressor mechanism in response to

inappropriate mitogenic signals or DNA damage. Second, a postulated role for autophagy in limiting cell cycle progression could also be a tumor suppressor mechanism. Third, as noted above, autophagy or specific autophagic proteins may function in limiting TOR signaling, so the partial loss of autophagy could result in inappropriate signaling through the TOR pathway, which is known to occur in tumorigenesis. Finally, the loss of autophagy may also promote tumorigenesis by providing selective pressures for cells that inappropriately amplify cellular growth signals. In a setting of nutrient deprivation, individual cells of a multicellular organism usually survive through the preferred method of autophagy and the catabolism of endogenous macromolecules for the generation of energy and nutrients. Alternatively, individual cells could mutate and survive by developing a competitive advantage in nutrient uptake to the detriment of its adjacent sibling cells. Mutations that improve nutrient uptake have been shown to be tumorigenic (e.g. BRAF or KRAS mutations) (Yun et al., 2009). In this speculative model, autophagy-deficient cells would be under constant selective pressure for oncogenic mutations to overcome their nutrient limitation.

Conclusion

Given its central role in integrating environmental signals like the presence of sufficient nutrients, energy, and growth factors, the PI3K/TOR signaling pathway is often viewed as the principal mediator of cellular growth control. More recently, autophagy has emerged as a crucial player in the negative regulation of cellular growth. Although autophagy is regulated as a downstream target of TORC signaling, autophagy can also regulate cell growth in response to distinct stimuli like amino acid depletion, energy deprivation, and ER stress, independently of PI3K/TOR signaling. Thus, TORC signaling and autophagy actually represent parallel, but contrasting pathways that function together in a coordinated manner to maintain homeostasis and growth control. The loss of such control by either dysfunction of TOR signaling and/or autophagy likely underlies the pathogenesis of most human cancers. However, the precise mechanisms by which autophagy acts in cell growth control and tumor suppression require further discovery and “digestion.”

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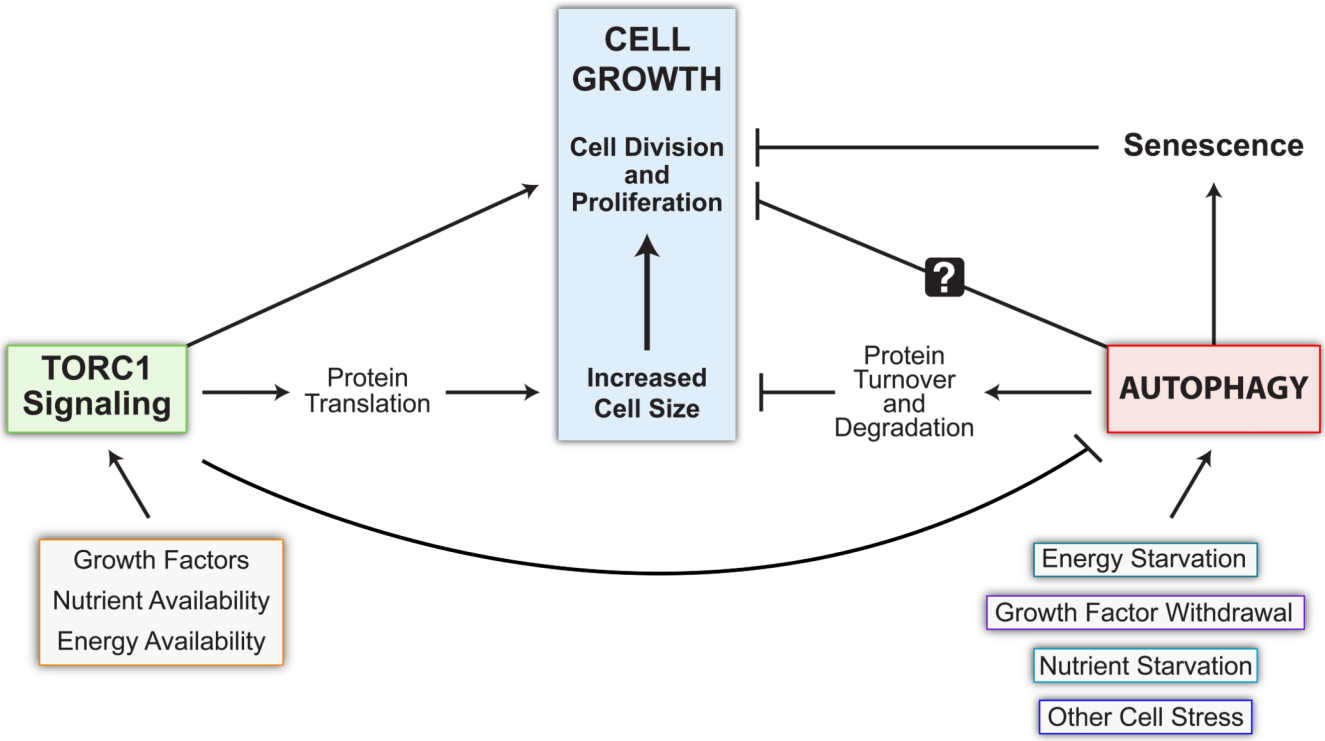

FIGURE 1. Interrelations between TOR complex signaling, autophagy, and cell growth control

TORC1 signaling promotes cell growth through its effects both on increasing cell proliferation and increasing cell size and potentially through its inhibitory effects on autophagy. TORC1 increases protein synthesis and cell size through intermediates such as 4E-BP and S6K; it promotes cell proliferation at least in part through its effects on cyclin and cyclin-dependent kinase (CKD) inhibitors. In contrast, autophagy is believed to inhibit cell growth at least in part by promoting protein and/or organelle turnover. In addition, autophagy may have indirect effects on inhibiting cell proliferation through promoting senescence. Additional, not yet defined, mechanisms may also contribute to the inhibitory effects of autophagy on cell proliferation. Note that the presence of growth factors, nutrients, and sufficient energy are all required for the full activation of TORC1 signaling, whereas the absence of any of these factors or other types of cellular stressors are sufficient to induce autophagy.

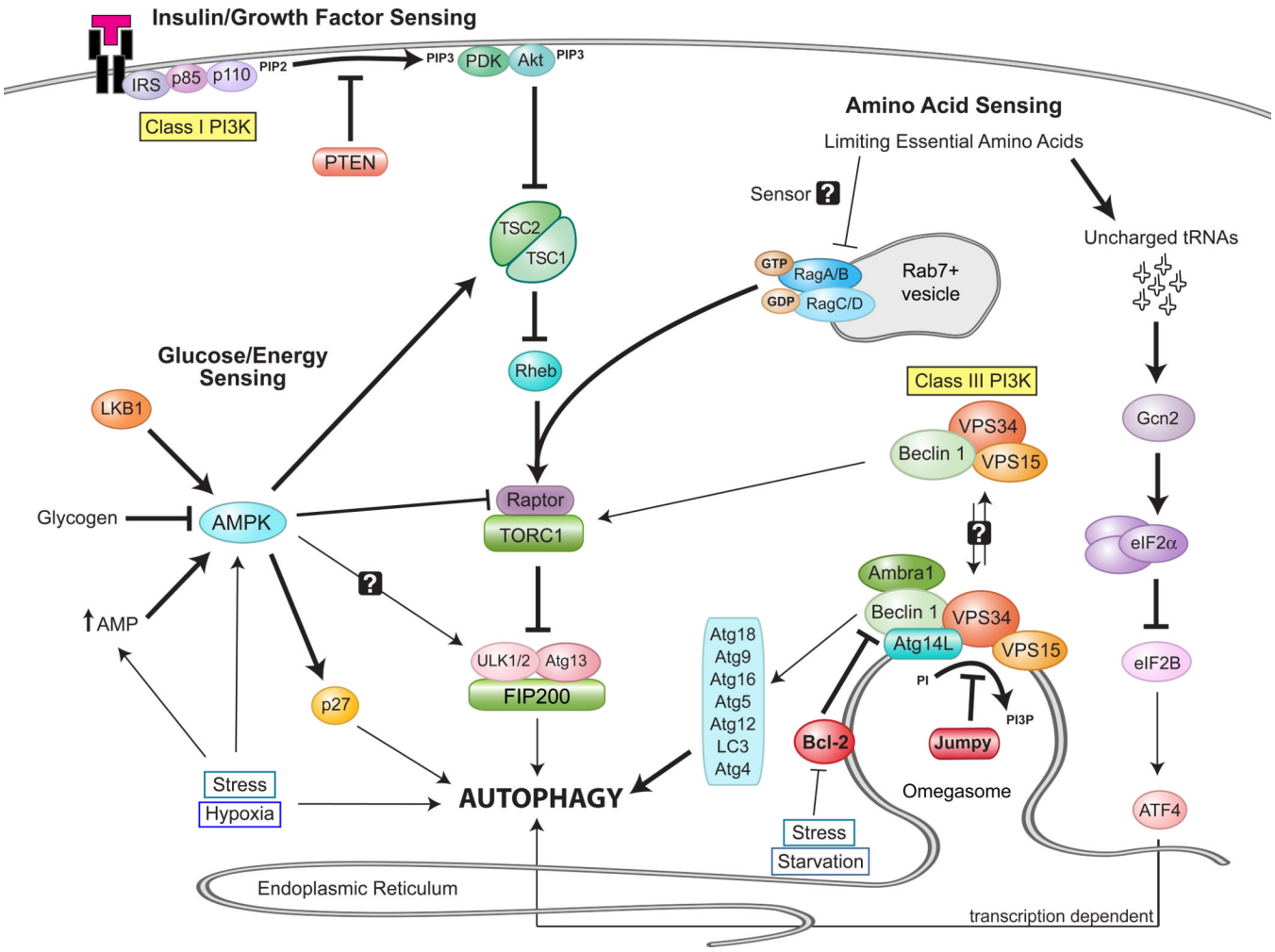

FIGURE 2. Coordinated regulation of TORC1 signaling and autophagy

Schematic diagram depicting the different inputs that contribute to the regulation of TORC signaling and autophagy and, ultimately, cell growth. Bold lines represent an activation or inhibition for which there is molecular evidence for a direct interaction. Thin lines represent activation steps that are likely indirect. Some activation steps which have not yet been demonstrated in mammlian systems cells are marked with a question mark.

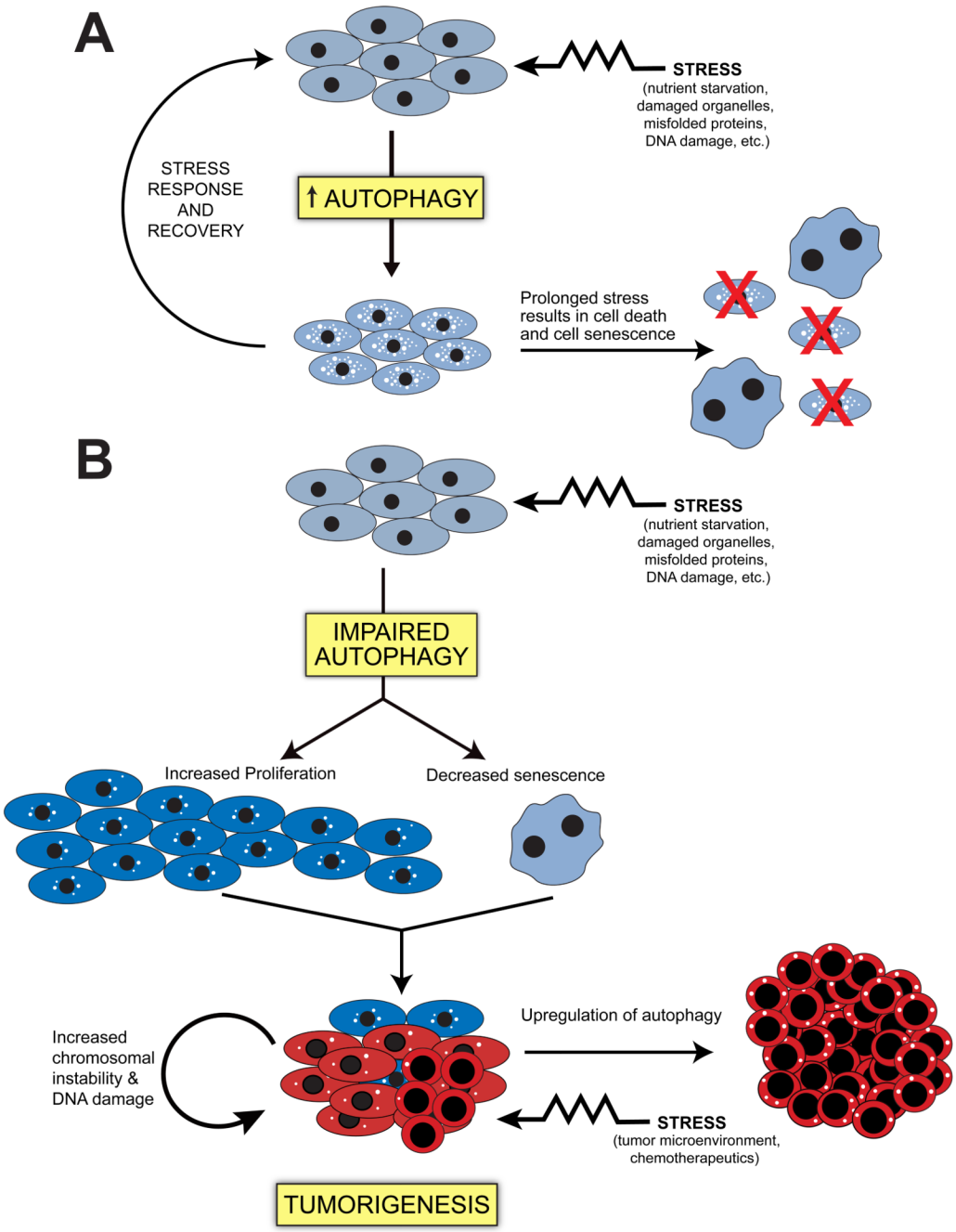

FIGURE 3. Possible mechanisms by which impaired autophagy promotes tumorigenesis

(A) In normal tissues, autophagy functions to promote cell survival in response to a variety of cellular insults including viral infection, DNA damage, nutrient starvation, or misfolded proteins. If the stressor is irreparable, the cell has a number of mechanisms to prevent the proliferation of damaged cells including apoptotic cell death, non-apoptotic cell death, and senescence. (B) In autophagy-deficient tissues, a cell is unable to respond to stressors, and several mechanisms have been proposed to contribute to tumorigenesis. (1) Autophagy has a role in the induction of senescence (Young et al., 2009) and non-apoptotic cell death (Yu et al., 2004), so its loss may result in a decreased ability to execute these potential tumor suppressor mechanisms; (2) Decreased autophagy has also been shown to increase cell

proliferation through as-of-yet unknown mechanisms (Fimia et al., 2007; Qu et al., 2003); (3) Decreased autophagy results in increased chromosomal instability and mutagenesis possibly through the accumulation of damaged organelles and increased ROS (Karantza-Wadsworth et al., 2007; Mathew et al., 2009; Mathew et al., 2007). In contrast, the upregulation of autophagy in established tumors may promote the survival of tumor cells in response to metabolic stress in the tumor microenvironment. In this figure, normal cells are depicted as light blue; cells with decreased autophagy that are hyperproliferative are depicted as dark blue; and transformed cells are depicted as red. In general, there is an inverse correlation between levels of autophagy and cell size (Hosokawa et al., 2006; Lum et al., 2005a) which is depicted in this figure; however, multiple factors other than levels of autophagy contribute to the increased nuclear/cytoplasmic ratio of tumor cells. Senescent cells display a large and flattened morphology.

Table 1

Summary of Roles of Autophagy Genes in Cellular Growth Control

Organism	Relevant Gene(s) Mutation*	Phenotype	Reference
C. elegans	*unc-51* (*Atg1/ULK*); *bec-1 (Atg6/beclin 1)*	Decreased worm length**	(Aladzsity et al., 2007)
	daf-2 (*Igf-1*)	Increased cell size rescued by *unc-51* or *bec-1* mutants	(Aladzsity et al., 2007); (McCulloch and Gems, 2003)
Drosophila	*Atg1* (*ULK*) overexpression	Decreased cell size	(Scott et al., 2007)
	$Atg1^{-/-}$	Normal cell size in well-fed animals; increased size in rapamycin-treated animals	(Scott et al., 2007)
	Atg1−/−, *dTOR*	$Atg1^{-/-}$ partially rescues the decreased cell size of *dTOR* mutants	(Lee et al., 2007)
Mammalian	$Atg5^{-/-}$ (mouse cell lines)	Increased cell size of starved fibroblasts; no change in cell cycle profile; no impairment of entry into quiescence	(Hosokawa et al., 2006); (Valentin and Yang, 2008)
	beclin $1^{+/-}$ (mice)	Increased proliferation in mammary epithelial cells and splenic germinal center lymphocytes	(Qu et al., 2003)
	$Ambra1^{-/-}$ (mice)	Increased cell proliferation in fetal brain	(Fimia et al., 2007)
	Atg7 shRNA, *Atg5* shRNA (human cell lines)	Delayed onset of senescence	(Young et al., 2009)

* Gene names in other organisms listed in parentheses

** Decreased cell size in autophagy-deficient *C. elegans* is proposed to occur through impaired ability to utilize cytosolic materials for cell remodeling and elongated cell shape.