Autophagy and cell growth – the yin and yang of nutrient responses

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Abstract

As a response to nutrient deprivation and other cell stresses, autophagy is often induced in the context of reduced or arrested cell growth. A plethora of signaling molecules and pathways have been shown to have opposing effects on cell growth and autophagy, and results of recent functional screens on a genomic scale support the idea that these processes might represent mutually exclusive cell fates. Understanding the ways in which autophagy and cell growth relate to one another is becoming increasingly important, as new roles for autophagy in tumorigenesis and other growth-related phenomena are uncovered. This Commentary highlights recent findings that link autophagy and cell growth, and explores the mechanisms underlying these connections and their implications for cell physiology and survival. Autophagy and cell growth can inhibit one another through a variety of direct and indirect mechanisms, and can be independently regulated by common signaling pathways. The central role of the mammalian target of rapamycin (mTOR) pathway in regulating both autophagy and cell growth exemplifies one such mechanism. In addition, mTOR-independent signaling and other more direct connections between autophagy and cell growth will also be discussed.

This article is part of a Minifocus on Autophagy. For further reading, please see related articles: ‘Ubiquitin-like proteins and autophagy at a glance’ by Tomer Shpilka et al. (J. Cell Sci. 125, [093757]) and ‘Autophagy and cancer – issues we need to digest’ by Emma Liu and Kevin Ryan (J. Cell Sci. 125, [093708]).

Key words: Autophagy, Cell growth, Mammalian target of rapamycin (mTOR), Protein synthesis, Lysosome, Cell cycle

Introduction

Cells have an intrinsic drive to increase their mass, which is illustrated by the ravenous growth of embryos and juveniles during development, and the logarithmic proliferation of cells in culture. Even in adult organisms, whose size has reached a steady state, continued growth and proliferation of differentiated and stem cells allows replacement of damaged and senescent cells, and many cells and organs are capable of considerable growth through hypertrophy. Cell growth can thus be considered a default state for many cell types, requiring only the presence of permissive factors such as nutrients and appropriate hormonal signals. The process of cell growth, however, has enormous energetic requirements and is rapidly abandoned when conditions become unfavorable. In response to nutrient limitation, DNA damage, excessive oxidation, viral infection and other cell stresses, the ATP-consuming processes that underlie cell growth are rapidly turned off, allowing the cell to conserve or marshal its resources to deal with the stressor.

In addition to switching off energetically demanding growth processes, cells induce a variety of responses to stress that enable them to survive in suboptimal conditions. Central among these is macroautophagy (herein referred to as autophagy), whereby portions of cytoplasm are sequestered into vesicles known as autophagosomes and degraded by hydrolytic enzymes following fusion of autophagosomes with the lysosome. This process supports cell survival by eliminating damaged and potentially harmful cellular structures, and by releasing the breakdown products as nutrients that can be re-used by the cell or exported for use by other cells.

On a fundamental level, autophagy and cell growth are mirror images of one another. If cell growth is defined as the process of mass accumulation through the net uptake and conversion of nutrients into macromolecules, autophagy can be considered to act in opposition to these biosynthetic processes through the catabolic breakdown of biomolecules. Thus, under conditions conducive to cell growth, the coupling of high rates of biosynthesis with low rates of autophagy allows cellular resources to be channeled towards growth with maximal efficiency. This synthetic flow is reversed in response to nutrient limitation or other stresses that halt cell growth and induce autophagy. This inverse correlation between autophagy and growth is widely observed under a variety of environmental conditions. For example, growth inhibition and autophagy induction are coordinated responses to nutrient starvation, growth factor withdrawal, cellular stresses such as oxidative damage, protein misfolding, viral infection, and substrate detachment or contact inhibition of cultured cells (Fig. 1) (reviewed by Neufeld, 2004).

Although the coupling of autophagy and growth might appear intuitive and logical, the potential cause-and-effect relationships between these two processes and the underlying mechanisms that link them are just beginning to be unraveled. In this Commentary, I highlight key regulatory steps and signaling pathways involved in autophagy and growth control, and discuss the regulatory relationships by which these processes are coordinated in the cell. Understanding the physiological benefits of this coordination and the consequences of its disruption should provide insight into potential autophagy-based therapeutic approaches.
Autophagy and the cell growth machinery

Formation of autophagosomes and their fusion with lysosomes are the defining events of autophagy. Whereas autophagosome–lysosome fusion largely exploits general factors of the endocytic pathway, autophagosome formation is driven by the coordinated activity of distinct sets of components that are dedicated to this process. Many of these components were first identified through pioneering genetic screens in yeast, and have clear homologs in metazoan cells. These include: (1) two ubiquitin-like molecules, Atg12 and Atg8 [microtubule-associated protein 1 light chain 3 alpha (LC3) and additional family members in mammals], and the E1-, E2- and E3-like processing machinery that regulates their conjugation to control autophagosome formation and size; (2) a multi-component protein complex containing the class III phosphatidylinositol 3-kinase Vps34 (PIK3C3 in mammals), which promotes autophagosome nucleation from cytosolic membranes; and (3) a complex containing the serine/threonine protein kinase Atg1 (ULK1 and ULK2 in mammals), which integrates signals from multiple upstream regulators to control the localization or activity of Vps34 and other autophagy components. One such regulator, the protein kinase target of rapamycin [Tor1 and Tor2 in yeast; mammalian TOR (mTOR) in mammals], has a central role in controlling Atg1 and ULK1 activity in response to nutrients and growth conditions. Together, these three systems act in concert, along with a number of accessory proteins, to form autophagosomes at a rate that is appropriate for specific environmental conditions (reviewed by Mizushima et al., 2011).

The biosynthetic pathways that promote the accumulation of mass to drive cell growth are also highly coordinated. The best understood of these processes is the control of protein synthesis, which is generally rate limiting for cell growth and sufficient to drive cell transformation (reviewed by Proud, 2007). The activity of translation initiation and elongation factors [e.g. eukaryotic translation initiation factors (eIF) 2, 3 and 4 and eukaryotic translation elongation factor 2 (eEF2)] are tightly regulated by protein kinases such as mTOR and Gcn2 (for general control non-repressed 2, also known as EIF2AK4) in response to nutrient and growth factor levels. The production of ribosomes is similarly sensitive to growth conditions, and this regulation encompasses all three RNA polymerases, as well as post-transcriptional controls, to ensure a balanced production of ribosomal proteins and RNAs. More recently, we have begun to define the signals that link cell growth to lipid synthesis and organelle biogenesis, and these appear to involve a complex interplay of transcriptional, translational and post-translational control (reviewed by Zoncu et al., 2011b). It is crucial that the synthesis rates of proteins, lipids and other biomolecules are regulated in parallel, and that these, in turn, are linked to the rates of nutrient uptake and, finally, to the overall growth and division rates of the cell and its organelles.

Together, these regulatory steps represent nodes of control at which interactions between cell growth and autophagy can occur.
Coordination of these processes can thus be achieved either through common signaling pathways that intersect at multiple independent control points, or through autophagy- or growth-dependent effects and signals (Fig. 2). Here, each of these models is considered in turn.

**Coordinated regulation of growth and autophagy by common signals**

An efficient means of achieving coordination between autophagy and cell growth rates is through the joint regulation of these processes by shared upstream signaling pathways. Although the molecules that act directly on autophagy and biosynthesis are distinct, a number of regulators have been shown to control both processes in parallel (Fig. 3).

**mTOR-dependent regulation of autophagy and growth**

As alluded to above, the kinase mTOR has a central role in controlling both cell growth and autophagy. mTOR controls the translation initiation step through direct phosphorylation of two key targets, EIF4E-binding protein 1 (EIF4EBP1) and S6 kinase (S6K, also known as RPS6KB1), which, in turn, regulate eIF3- and eIF4-dependent interactions between the mRNA 5′ cap, the poly(A)-tail and the 40S and 60S ribosomal subunits (Zoncu et al., 2011b). mTOR also has a major impact on protein synthesis and cell growth by promoting ribosome biogenesis. In yeast, this involves inhibitory phosphorylation of three transcription factors, Srb3, Dot6 and Tod6, which act as repressors of ribosome biogenesis (Rib1) and ribosomal protein (Rp) gene expression (Huber et al., 2011). This phosphorylation is mediated by the protein kinase Sch9, a yeast homolog of S6K and AKT, and a direct TOR substrate. mTOR also contributes to ribosome biogenesis by promoting expression of ribosomal RNA. Recent studies indicate that there is a nucleolar role for mTOR complexes directly at the promoters of rRNA and tRNA genes, and have identified the RNA polymerase I and III factors TIF1A (also known as TRIM24), TFIIC (also known as GTF3) and MAF1 as downstream targets (Kantidakis et al., 2010; Mayer et al., 2004; Vazquez-Martín et al., 2011).

Independent of these effects on protein synthesis, mTOR regulates the autophagic machinery and suppresses autophagosome formation under conditions that are favorable for growth. Autophagy inhibition by mTOR largely occurs independently of the targets known to be involved in growth regulation, such as EIF4EBP1 or S6K. Instead, mTOR directly interacts with and phosphorylates components of the Atg1 protein kinase complex, including Atg1 (ULK1), Atg13 and FIP200 (also known as RB1CC1) (reviewed by Mizushima, 2010). In yeast, Atg13 is phosphorylated on multiple serine and threonine residues under growth conditions, and this disrupts its association with Atg1. Ohsumi and co-workers recently found that Atg13 is phosphorylated directly by TOR in vitro, and showed that Atg13 lacking these phosphorylation sites stably associates with, and activates, Atg1 and is sufficient to promote autophagy under growth conditions, independent of TOR activity (Kamada et al., 2010). This ability of non-phosphorylatable Atg13 to bypass TOR signaling highlights the Atg1 complex as the crucial downstream target mediating the effects of TOR on autophagy.

How TOR-dependent phosphorylation of Atg13 promotes Atg1 activation and induction of autophagy remains poorly understood. Interestingly, Herman and colleagues have demonstrated that Atg1 forms dimers or oligomers under conditions that favor autophagy, and that this self-association is promoted by Atg13 (Yeh et al., 2011). Forced dimerization of Atg1 increases its kinase activity and the level of autophosphorylation, which is a prerequisite for inducing autophagy (Kijanska et al., 2010; Yeh et al., 2010). However, this is not sufficient to induce autophagy, suggesting that Atg13 has additional functions. Regulation of metazoan Atg1 (ULK1) appears to differ from that in yeast, as mTOR inhibitsULK1 without affecting its interaction with Atg13. A functional role for Atg13 phosphorylation has not yet been demonstrated in mammals, and mTOR-mediated phosphorylation of other components of the ULK1 complex might have important roles. Recently, Ser757 of ULK1 was identified as an mTOR-dependent phosphorylation site that influences the association of ULK1 with the AMP-activated protein kinase (AMPK), which is an important activator of autophagy in response to cellular energy levels (Kim et al., 2011a; Shang et al., 2011) (see below).

The potential role of mTOR to regulate later steps of autophagosome maturation, movement and lysosomal fusion is less well characterized. At least in some cell types, mTOR activation can promote autophagosome–lysosome fusion, in part by facilitating the interaction of Rab7 with its lysosomal effector Rab-interacting lysosomal protein (RILP) (Bains et al., 2009; Bains et al., 2011; Yamamoto et al., 2006). Furthermore, reactivation of mTOR during sustained nutrient starvation is required to allow re-formation and recycling of lysosomes following the massive delivery of autophagic membrane and cargo. This recovery of mTOR activity and lysosomal integrity is dependent on autophagic degradation and efflux, suggesting that nutrients derived from the autolysosome are able to promote mTOR activation (Rong et al., 2011; Shin and Huh, 2011; Yu et al., 2010). Nutrient efflux from autolysosomes also appears to promote TOR signaling required for secretory activity in senescent cells (Narita et al., 2011).

Recent investigations into the nutrient-dependent regulation and localization of mTOR are consistent with this model. In response to an increase in nutrient levels, mTOR is recruited to the surface of lysosomes by members of the Rag family of heterodimeric GTPases (Sancak et al., 2010). The lysosomal translocation of mTOR requires nutrient-dependent GTP loading of the Rag complex, and this recruitment is sufficient to bypass the nutrient input to mTOR activation. Interestingly, nucleotide...
loading of Rag GTPases is sensitive to the amino acid concentrations within the lysosome, and subunits of the vacuolar ATPase protein pump are required for this signaling (Zoncu et al., 2011a). The microtubule-dependent positioning of lysosomes within the cell has also been shown to influence mTOR activity, and this appears to be mediated by nutrient-dependent changes in intracellular pH (Korolchuk et al., 2011).

Importantly, lysosome-mediated regulation of mTOR influences multiple, if not all, aspects of mTOR function, including its effects on targets involved in cell growth and autophagy. This implies that mTOR might retain its activation state for some time after its departure from the lysosomal surface and diffusion or transport to other cellular compartments. Indeed, activation of mTOR might be required for its release from the lysosome (Ohsaki et al., 2010). Compartmentalization of mTOR from its targets, such as ULK1, has been suggested to promote autophagy during senescence, but the extent to which similar mechanisms contribute more generally to mTOR signaling is unclear (Narita et al., 2011). Live imaging and fluorescence recovery after photobleaching (FRAP)-based analysis of mTOR complexes might help to clarify this issue. This centralized control of mTOR activation status probably makes a substantial contribution to coordinating its diverse functions in autophagy and cell growth.

mTOR-independent control of autophagy and growth

Despite the multiple inputs to mTOR signaling and its pervasive effects on autophagy and cell growth, a surprisingly large number of signaling pathways and regulators have been shown to affect these processes independently of changes in mTOR activity. Small-molecule screens have identified several compounds that induce autophagy without affecting mTOR. These chemicals inhibit a variety of intracellular targets and processes, including ion channels, Ca$^{2+}$ homeostasis and G-protein signaling (Williams et al., 2008; Zhang et al., 2007). A strong correlation has been observed between the ability of these compounds to promote long-lived protein degradation and to increase cellular levels of phosphatidylinositol 3-phosphate [PtdIns(3)P], the product of Vps34, thus implicating this kinase as an ultimate autophagic effector. Despite some reports linking Vps34 to mTOR activation (Byfield et al., 2005; Nobukuni et al., 2005), no effect on the phosphorylation of mTOR substrates has been found for these compounds (Williams et al., 2008; Zhang et al., 2007). Similarly, a genomic siRNA screen for autophagy regulatory genes has found little correlation between autophagy induction and mTOR activity, whereas nearly half of the 236 identified hits had a substantial effect on PtdIns(3)P levels (Lipinski et al., 2010). Gene ontology analysis of these hits has revealed a
substantial enrichment in signaling molecules and transcription factors, including multiple genes involved in growth factor and cytokine signaling that have well-characterized effects on cell growth. Taken together, these results indicate that the ability of signaling pathways to regulate autophagy and cell growth in parallel is not confined to the mTOR pathway and might be widespread.

The oncogenic Ras–RAF–MAPK cascade stimulates cell growth through multiple transcription factors including MYC, JUN (also known as transcription factor AP-1) and ETS1, which target genes that regulate cell cycle progression, ribosome biogenesis and cytokine signaling (McCubrey et al., 2007). Ballabio and colleagues recently identified the mammalian transcription factor EB (TFEB) as a master regulator of a large number of autophagy and lysosomal genes that facilitate the coordination of autophagosome formation, fusion and degradation (Settembre et al., 2011). Furthermore, this group showed that TFEB can be negatively regulated through direct phosphorylation by extracellular-signal-regulated kinase 2 (ERK2), which is independent of mTOR activity. Similarly in yeast, Ras and its downstream target cAMP-dependent protein kinase (PKA) inhibit the starvation-induced expression of autophagy genes, such as ATG8, independently of TOR, and PKA promotes expression of growth gene networks such as the RiBi regulon through inactivation of the repressor Tod6 (Graef and Nummari, 2011; Lippman and Broach, 2009). Thus both mammalian and yeast Ras signaling pathways use a coordinated transcriptional mechanism to link growth stimulation and autophagy inhibition in response to nutrients.

This transcriptional response is probably shared by many other factors that regulate growth in response to multiple stimuli. Botstein and colleagues found that 25% of all yeast genes display a similar transcriptional response to changes in nutrient conditions regardless of carbon source, a pattern they termed a ‘universal’ growth rate response (GRR) (Slavov and Botstein, 2011). Growth-related genes involved in ribosome biogenesis and translation display a positive universal GRR (i.e. increased transcription in rich medium), whereas autophagy- and vacuolar-associated genes have a negative GRR. Although such widespread responses certainly reflect the activity of multiple transcriptional regulators, individual transcription factors can also generate coordinated effects on growth and autophagy. For example, the yeast transcriptional regulator Gcn4 responds to amino acid starvation by promoting expression of autophagy genes and repressing genes encoding ribosome proteins and translation factors (Natarajan et al., 2001). The synthesis of Gcn4 protein itself increases under conditions of general translation inhibition, providing an additional layer of connection between autophagy and cell growth (Hinnebusch, 1997).

In metazoans, the FOXO family of transcription factors suppresses cell growth through the expression of inhibitors of translation and proliferation (Jünger et al., 2003; Stahl et al., 2002). Concurrently to this, they stimulate autophagy by activating the expression of a large number of autophagy-associated genes (Mammuccari et al., 2007; Zhao et al., 2007). Interestingly, FOXO1 also has a transcription-independent capacity to promote autophagy. This process involves deacetylation of cytoplasmic FOXO1 in response to starvation or oxidative stress, which promotes its association with ATG7 and leads to autophagy through an, as yet, unclear mechanism (Zhao et al., 2010). Dual nuclear and cytoplasmic effects have also been described for p53, which induces autophagy in response to DNA damage through the activation of target genes such as those encoding the lysosomal protein DNA-damage regulated autophagy modulator 1 (DRAM1) and ULK1, as well as factors, such as sestrin 2, phosphatase and tensin homolog (PTEN) and tuberous sclerosis 2 (TSC2), that inhibit mTOR signaling (Crighton et al., 2006; Feng et al., 2005; Gao et al., 2011; Maiuri et al., 2009). These inductive effects on autophagy through p53-mediated transcription are opposed by a cytoplasmic function of p53, which increases mTOR activity and suppresses autophagy under basal conditions (Tasdemir et al., 2008).

Other factors also regulate cell growth and autophagy through a combination of mTOR-dependent and -independent mechanisms. For example, in addition to its transcriptional effects noted above, PKA also inhibits autophagy in yeast by directly phosphorylating Atg1 and Atg13, which prevents their localization to the sites of autophagosome assembly (Stephan et al., 2009). At the same time, PKA activation increases the sensitivity of cells to rapamycin and exacerbates the growth defects of tor mutants, which indicates that PKA has antagonistic effects on TOR (Ramachandran and Herman, 2011). Taken together, these results suggest that PKA sends both positive and negative signals to the autophagy network, and that the inhibitory effect is dominant under most conditions. Similarly, the energy-sensing AMPK regulates autophagy both upstream and downstream of mTOR. By inhibiting mTOR activity under conditions of low energy, AMPK indirectly promotes activation of the ULK1 complex. In addition, AMPK can directly phosphorylate ULK1, in this case leading to further activation and induction of autophagy (Egan et al., 2011; Kim et al., 2011b; Shang et al., 2011).

**Regulation of autophagy by cell growth**

As discussed above, the inverse relationship between cell growth and autophagy can result in part from their co-regulation. However, this close correlation is also consistent with a cause-and-effect connection between these processes, and suggests that autophagy and cell growth might inhibit each other independently of shared upstream signaling components.

We can examine this issue from the viewpoint of protein synthesis and cell cycle progression, two processes that are central to cell growth and proliferation. The first obligate step in increasing cellular protein mass is the import of amino acids. The regulation of this process in metazoan cells is poorly defined, but it is clear that growth factor signaling promotes the expression of a variety of nutrient transporters on the cell surface, in part by blocking their endocytic degradation (Edinger, 2007; Edinger and Thompson, 2002; Hennig et al., 2006). Amino acid uptake in growing cells might directly lead to mTOR activation and hence suppression of autophagy. However, rapid incorporation of amino acids into growing peptide chains might limit their free intracellular concentration and thus mTOR activation. Transport of amino acids into the cell can also have secondary consequences, such as changes in osmolarity and cell membrane polarization, which have been shown to influence autophagic signaling (Häussinger et al., 1990). In addition, there is evidence that some amino acid transporters might act as ‘transceptors’ with dual transport and signaling functions, and mTOR and PKA have been implicated as targets of such molecules (Taylor, 2009).
Subsequent steps in protein synthesis can also have substantial regulatory effects on autophagy. Following their transport into the cell, amino acids are ligated to their cognate tRNAs by aminoacyl-tRNA synthetases. Disruption of this process causes accumulation of uncharged tRNAs, which directly activates the aminoacyl-tRNA synthetases. Disruption of this process causes the cell, amino acids are ligated to their cognate tRNAs by regulatory effects on autophagy. Following their transport into activity, which indicates that tRNAs can influence multiple signaling pathways.

In rapidly growing cells, the elongation of polypeptide chains might also inhibit autophagosome formation. Recent studies have implied that PtdIns(3)P-rich domains of the rough endoplasmic reticulum (ER) are central staging areas of autophagosome initiation (Axe et al., 2008). The tight juxtaposition of the growing autophagic membrane with the ER surface (Hayashi-Nishino et al., 2009; Ylä-Anttila et al., 2009) suggests that engagement of active ribosomes with the ER translocon might be incompatible with this initiation process. In rapidly growing cells, high occupancy of the surface of the ER with translating ribosomes might therefore limit its availability as a platform for autophagosome formation (Blommaart et al., 1995).

The cell division cycle of proliferating cells provides another stage for the interaction between autophagy and growth control. It has been known for some time that cells in mitosis are resistant to a variety of autophagic stimuli, including starvation and mTOR inhibition (Eskelinen et al., 2002), and this might provide a crucial barrier to autophagic degradation of spindle components, genetic material and other exposed cellular structures. A study using multiple cell cycle markers and a variety of autophagy inducers found that each stimulus had maximal effects in the G1 and S phases of the cell cycle, with little activity in the G2 and M phases (Tasdemir et al., 2007). This autophagy timecourse corresponds inversely with activity of the mitotic protein cyclin-dependent kinase 1 (CDK1). Yuan and colleagues recently identified Vps34 as a substrate of CDK1–cyclin-B during mitosis in human cells (Furuya et al., 2010). That study found that CDK1–mediated phosphorylation of Vps34 on Thr159 disrupts its association with beclin 1, an essential core component of Vps34 complexes, thereby reducing the lipid kinase activity of Vps34. Thr159 phosphorylation of Vps34 increases during mitosis, correlating with a reduction in autophagy. These findings suggest that suppression of autophagy during mitosis can result from CDK1-dependent inhibition of Vps34 activity. By contrast, yeast Cdk1 appears to have a positive function in autophagy because loss of Cdk1 activity leads to G1 arrest and autophagy inhibition (Yang et al., 2010). The region of Vps34 that surrounds Thr159 is not well conserved in yeast, which indicates that other Cdk1 substrates probably mediate its effects on autophagy.

Regulation of autophagy by CDKs might be a general phenomenon. The CDKN1B (also known as p27 and KIP1) and CDKN2A (also known as p16 and INK4) families of G1 CDK inhibitors are important regulators of cell cycle and growth in response to stress, hormonal and developmental signals. Recent studies have revealed that these factors have a similarly crucial role in regulating autophagy by integrating signals from multiple upstream pathways. Liang and co-workers have shown that autophagy is inhibited in mouse embryonic fibroblasts that lack p27, and that overexpression of p27 or depletion of CDK2 or CDK4 induces autophagy in breast adenocarcinoma cells (Liang et al., 2007). Both p27 and p16 are linked to autophagy through the tumor suppressor retinoblastoma 1 (RB1, also known as pRb), which is a central downstream target of CDK4 in cell cycle regulation. RB1 is required for the induction of autophagy by p27 and p16, and can trigger autophagy when overexpressed (Jiang et al., 2010). RB1 controls cell cycle progression, cell growth and survival by binding to the transcription factor E2F and inhibiting expression of E2F target genes. Similarly, induction of autophagy by RB1 requires binding of RB1 to E2F, and this process can be antagonized by overexpression of E2F. The effects of RB1 and E2F are complex, however, and might be influenced by cell type and stress conditions. For example, E2F is required for autophagy induction by DNA-damaging agents in U2OS cells, and overexpression or activation of E2F can promote autophagy and increase expression of Atg1 (ULK1), ATG5, LC3 and DRAM1 in these cells (Polager et al., 2008). In addition, RB1–E2F complexes have a negative effect on autophagy in response to hypoxia, in this case by inhibiting expression of BNIP3 (for BCL2/adenovirus E1B 19kDa interacting protein 3), an activator of VPS34–beclin-1 complexes and inhibitor of mTOR (Tracy et al., 2007).

Even a single CDK can have both positive and negative effects on autophagy. Klionsky and co-workers have found that the yeast CDK Pho85 has both positive and negative effects on autophagy induction, depending on the cyclin partner it is associated with (Yang et al., 2010). The relative expression levels of these cyclins vary with cell cycle phase and in response to environmental signals, and each phase probably directs Pho85 to different cellular compartments or downstream targets, thereby allowing Pho85 to provide an integrated autophagic response to multiple growth and cell cycle cues.

In addition to these molecular links, processes that are intrinsic to cell growth and proliferation might themselves be incompatible with autophagy. For example, periodic reorganization of microtubules into a mitotic spindle in dividing cells would be expected to disrupt transport of autophagic vesicles or of components required for their formation. Similarly, dedicating vesicular trafficking pathways towards active synthesis and secretion might preclude their use in autophagy. Growth and autophagy might also compete for raw materials, such as membrane lipids, or for regulators that function in both processes. For example, Vps34 can assemble into at least three complexes with distinct subunits and functions, but only one them (which is defined by the presence of Atg14) is dedicated to autophagosome formation (Simonsen and Tooze, 2009). Such competition-based mechanisms could prevent autophagy from causing damage during vulnerable cell cycle phases.

**Regulation of cell growth by autophagy**

Bone marrow cells derived from mice that are deficient for the proapoptotic BCL2 family proteins BAX and BAK are able to survive in culture for several weeks in the absence of growth factors. This survival is autophagy-dependent and coincides with a dramatic reduction in cell mass (Lum et al., 2005). In vivo, starvation of Drosophila can result in a 90% decrease in the size of larval fat body cells, which is accompanied by a massive induction of autophagy (Butterworth et al., 1965). Genetic
disruption of autophagy in this system suppresses the observed reduction in growth, and overexpression of Atg1, which induces autophagy, is sufficient to reduce cell size in the fat body (Scott et al., 2007; Scott et al., 2004). In starved mice, the intracellular protein degradation rate of hepatocytes approaches 40% per day in vivo, and starvation-induced size reduction of mouse embryonic fibroblasts can be substantially inhibited by disrupting Atg5 (Conde and Scornik, 1976; Hosokawa et al., 2006). Together, these examples illustrate the intrinsic ability of autophagy to directly reduce cellular mass through the degradation of bulk cytoplasm (Fig. 4A).

In addition to these presumably non-selective effects in growth-arrested cells, autophagy might further reduce the growth rate of actively growing cells through targeted elimination of growth-promoting molecules, complexes and organelles. The protein p62 (also known as sequestosome 1) is an intriguing candidate for such a factor. p62 is a multifunctional adapter protein that binds LC3 and other Atg8 family members through its LC3-interacting region (LIR), and thereby becomes incorporated into autophagosomes (Bjørkøy et al., 2005). This results in efficient autophagy-dependent degradation of p62, and, indeed, p62 protein levels are widely used as indicators of autophagic flux (Klionsky et al., 2008). Recently, an important role for p62 in mTOR activation has been described. In cells where p62 has been depleted or knocked out, mTOR fails to be activated in response to amino acids, which leads to a decrease in cell growth. Remarkably, p62 has been shown to stimulate mTOR signaling by supporting the interaction between Rag GTPases, the mTOR partner Raptor and the lysosomal surface, which promotes amino-acid-dependent recruitment of mTOR to the lysosome and its subsequent activation (Duran et al., 2011). These results imply that autophagy can impair mTOR-dependent cell growth through selective degradation of p62. In addition, p62 contains a C-terminal ubiquitin-associated (UBA) domain and can promote selective autophagy of ubiquitylated soluble or aggregated proteins (Bjorkoy et al., 2005; Kim et al., 2008), which potentially include other factors involved in cell growth regulation. In a proteomic study tracing the progressive autophagic degradation of proteins in breast cancer cells, it has been found that proteins involved in translation, including mTOR, ribosomal protein S6 and tRNA synthetases, are among the most rapidly depleted factors (Kristensen et al., 2008). Whether these and other growth-promoting factors are selectively targeted for autophagy in a p62-dependent manner remains to be shown.

Autophagy can also be employed by cells to selectively degrade larger complexes and organelles that are required for cell growth, such as ribosomes and mitochondria. In yeast, starvation results in rapid sequestration and digestion of ribosomes in the vacuole. This process is essential for survival under starvation conditions, is highly selective and involves the ubiquitin protease Ubp3, its activator Bre5, and ubiquitylation of specific ribosomal proteins (Kraft et al., 2008). Interestingly, the mammalian orthologs of Ubp3 and Bre5 interact with ULK1, which is required for the clearance of ribosomes from the cytoplasm of reticulocytes (Behrends et al., 2010; Kundu et al., 2008). Ribosomal degradation represents a potent mechanism to directly reduce cellular growth capacity. In addition, large macromolecular complexes such as P granules in C. elegans and midbody rings of dividing cell populations can indirectly promote growth by acting as stem cell factors, and these complexes have also been shown to be selective autophagy substrates (Kuo et al., 2011; Pohl and Jentsch, 2009; Zhang et al., 2009).

Mitochondria can be selectively degraded by autophagy in a process referred to as mitophagy recently (reviewed in Youle and Narendra, 2011). Both starvation and mitochondrial damage can trigger mitophagy, and this requires LIR-motif-containing proteins [Atg32 in yeast, Nix (also known as BNIP3L) and possibly p62 in mammalian cells], which are thought to guide expansion of autophagosomal membranes over the mitochondrial surface through interaction with Atg8 family members. In yeast, this interaction is regulated by phosphorylation of Atg32 (Aoki et al., 2011). It was recently demonstrated that mitochondria can be protected from starvation-induced mitophagy by a PKA-dependent process of fusion and elongation, which somehow spares mitochondria from engulfment into autophagosomes (Gomes et al., 2011; Rambold et al., 2011). Similarly, in yeast that are grown under conditions in which mitochondria are required for metabolism, mitophagy is not induced, not even by severe starvation (Kanki and Klionsky, 2008). In starved mammalian cells, degradation of mitochondria occurs subsequent to degradation of cytosolic proteins and ribosomes (Kristensen et al., 2008). Thus, mitochondria appear to be the last

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**Fig. 4. Negative effects of autophagy on cell growth.** (A) Multiple forms of autophagy can contribute to growth suppression. From left to right: bulk autophagy of cytoplasmic contents including cytosol and organelles; mitophagy, which utilizes adapter molecules such as Atg32, Nix and p62; ribophagy, which requires the ubiquitin protease Ubp3 and its activator Bre5; and p62-dependent degradation of ubiquitylated (Ub) signaling molecules and of p62 itself, which can promote mTOR signaling. (B) Activation of autophagy factors such as ULK1 and WIPI1 might lead to downregulation of mTOR through multiple mechanisms, possibly including ULK1-mediated phosphorylation (P) of Raptor (indicated by the blue oval) and WIPI1-dependent effects on maturation of endocytic vesicles.
resort for nutrients, which probably reflects their essential role in both growing and stationary cells.

Several components of the autophagic machinery have been shown to have important autophagy-independent roles in regulating apoptotic cell death, and it is interesting to speculate that similar dual functions might be at work in controlling cell growth. One possible example of this is a feedback signaling loop from ULK1 to mTOR, which involves mTOR inhibition by its downstream kinase (Fig. 4B). In Drosophila and mammalian cells, Tor (or mTOR)-dependent phosphorylation of its substrates S6K and EIF4E/EBP1 is increased in Atg1 (or ULK1)-depleted cells, and decreased in response to the overexpression of Atg1 (Lee et al., 2007; Scott et al., 2007). These effects correlate with changes in the cytoplasmic localization and trafficking of TOR, and with ULK1-dependent phosphorylation of the TORC1 component Raptor (Chang and Neufeld, 2009; Dunlop et al., 2011). Feedback signaling from Atg1 to mTOR might help to amplify weak or variable signals into a stable biphasic switch, and might allow pathways that regulate ATG1 and autophagy independently of mTOR to influence growth in an mTOR-dependent manner. Other autophagy regulators can also affect mTOR signaling independently of their effects on autophagy. A recent study identified the Atg18 ortholog WD repeat domain, phosphoinositide interacting 1 (WIPI1) as an inhibitor of mTOR in human melanocytes (Ho et al., 2011). WIPI1 has an autophagy-independent role in the biogenesis of melanosomes, which are lysosome-related organelles that develop through an endosome-like maturation process. WIPI1 has been shown to function in part by suppressing mTOR activity, which negatively regulates transcription of melanogenic proteins in these cells. As mTOR activation is sensitive to disruption of endocytic maturation (Flinn et al., 2010; Li et al., 2010), it will be interesting to see whether WIPI1 also regulates TOR in other contexts through its effects on endocytic trafficking.

Conclusions

The separation of cell growth and autophagy into non-overlapping states appears to provide several advantages, including maximal resource efficiency and protection against cellular damage. However, although autophagy and cell growth tend to display an inverse response to many stimuli, it should be noted that this correlation is not absolute. High levels of autophagy can be observed in some rapidly growing cells, and in some cases this might actually contribute to cell growth and biosynthesis. Deeper investigation into these apparent ‘exceptions to the rule’ might prove particularly fruitful. For example, exercise induces both autophagy and cell growth of muscle fibers (Ogura et al., 2011), and this might involve a temporal separation into distinct phases of fiber breakdown and rebuilding. Similarly, senescent cells display high levels of autophagy despite showing highly active mTOR signaling and protein synthesis. In this case, these processes are separated spatially within the cell, with autophagy components being insulated within an ‘mTOR-autophagy spatial coupling compartment’ that protects them from mTOR-mediated downregulation (Narita et al., 2011). In other cases, such as in Ras-transformed cells, autophagy might provide intermediate metabolites that are specifically required to fuel the altered metabolic pathways of these cells and to facilitate their rapid growth (Guo et al., 2010; Kim et al., 2011b; Lock et al., 2011).

As new roles of autophagy continue to be revealed, the view that emerges is that this ancient process is deeply entwined with the basic cellular functions of growth, proliferation and survival. Disentangling these connections will be important to our understanding of the molecular regulation of each of these processes. Furthermore, as autophagy-based therapies against a number of diseases are pursued, it will be important to consider how potential treatments might impact other processes such as cell growth that are linked to autophagy. This will be aided by a better understanding of growth-dependent and -independent mechanisms that regulate autophagy.

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