The cell biology of autophagy in metazoans: a developing story

Alicia Meléndez¹ and Thomas P. Neufeld²

The cell biological phenomenon of autophagy (or ‘self-eating’) has attracted increasing attention in recent years. In this review, we first address the cell biological functions of autophagy, and then discuss recent insights into the role of autophagy in animal development, particularly in C. elegans, Drosophila and mouse. Work in these and other model systems has also provided evidence for the involvement of autophagy in disease processes, such as neurodegeneration, tumorigenesis, pathogenic infection and aging. Insights gained from investigating the functions of autophagy in normal development should increase our understanding of its roles in human disease and its potential as a target for therapeutic intervention.

Introduction

Autophagy is a ubiquitous catabolic process that involves the bulk degradation of cytoplasmic components through a lysosomal pathway. This process is characterized by the engulfment of part of the cytoplasm inside double-membrane vesicles called autophagosomes. Autophagosomes subsequently fuse with lysosomes to form an autophagolysosome in which the cytoplasmic cargo is degraded (Fig. 1).

Although this process was initially described over 40 years ago (de Duve, 2005), two developments in the past decade have led to a substantial increase in interest and activity in the field of autophagy research. The first of these developments was a series of genetic screens in yeast that led to the discovery of the autophagy-related (ATG) genes that control this process (Harding et al., 1995; Thumm et al., 1994; Tsukada and Ohsumi, 1993). Orthologs of most of these ATG genes have now been identified in higher eukaryotes (Table 1), and genetic analyses have begun to decipher the functions of autophagy in multicellular organisms, such as nematodes, flies and mice.

A second factor drawing investigators to this field is the growing awareness of the potential impact of autophagy on specific aspects of human health and disease, and the potential opportunity to develop novel therapies based on the manipulation of autophagy (Levine and Kroemer, 2008). Together, research into the basic cell biology of autophagy and its potential disease functions has revealed a substantial influence of autophagy on cellular physiology. For example, it is clear that autophagy plays an essential role in ridding the cell of damaged or superfluous organelles and proteins, in generating nutrients essential for cell survival under starvation and other stressful conditions, and in some cases acting as a cell death effector.

Given its role in these crucial cellular functions, it would seem reasonable to expect that autophagy will have a significant impact on animal development, a process ultimately driven by changes in individual cell activity. Here, we discuss current concepts and recent findings regarding the cellular and developmental functions of autophagy. Although its role in development has yet to be fully defined, studies in model organisms have begun to describe a growing number of developmental processes that are influenced by autophagy, and are showing how this fundamental cellular process affects other cellular activities crucial for development.

The molecular machinery of autophagy

The autophagic process can be divided into several distinct steps: signaling and induction; autophagosome nucleation; membrane expansion and vesicle completion; autophagosome targeting, docking and fusion with the lysosome; and, finally, degradation and re-export of materials to the cytoplasm (Fig. 1). The molecular cascade that regulates autophagy and the mechanisms by which autophagy occurs have been the subject of recent comprehensive reviews (Klionsky, 2007; Maiuri et al., 2007b; Mizushima and Klionsky, 2007; Yorimitsu and Klionsky, 2005) and thus are described only briefly below.

Autophagy occurs at basal levels in normal growing conditions, but can be dramatically upregulated by a number of stimuli, including starvation, hypoxia, intracellular stress, high temperature, high culture density, hormones and growth factors deprivation. The best characterized of these stimuli is nutrient starvation, which induces autophagy in part through the inactivation of the protein kinase target of rapamycin (Tor) (Fig. 2). In yeast, this inductive step includes the Atg1, Atg13 and Atg17 proteins, the association of which is regulated by TOR-dependent signaling (Fig. 3). Atg1 also appears to be a target of TOR signaling in higher eukaryotes (Scott et al., 2007), suggesting that this mechanism is widely conserved.

A second functional complex involved in the vesicle nucleation step consists of the class III phosphatidylinositol 3 kinase Vps34, Atg6/Vps30 and several associated factors (Fig. 3B). Two distinct Vps34/Atg6 complexes have been described in yeast (Kametaka et al., 1998): an autophagy-specific complex that is thought to localize other autophagy proteins to the pre-autophagosomal structure or phagophore assembly site (PAS); and a second complex involved in vacuolar protein sorting. Likewise in metazoans, Vps34 participates in both autophagy and other vesicle trafficking processes, most probably via its assembly into distinct complexes (Zeng et al., 2006). Interestingly, C. elegans bec-1 and the human beclin 1 gene, the ATG6 orthologs, can complement the autophagy, but not the VPS, function of ATG6 in yeast (Meléndez et al., 2003), suggesting that there are functional differences between metazoan and yeast ATG6.

Two novel and highly conserved ubiquitin-like conjugation pathways, the Atg12 conjugation system (consisting of a complex of Atg12p, Atg5p, and Atg16p), and the Atg8 lipidation system
(consisting of Atg8p, Atg3p, and Atg7p) (Fig. 3C) mediate vesicle expansion and vesicle completion (Mizushima, 2007; Ohsumi, 2001; Suzuki and Ohsumi, 2007). These conjugation systems are widely conserved in eukaryotes and have an essential role in autophagy.

Once the autophagosome is completed, it is transported to the lysosome (or the vacuole in yeast) in a dynein-dependent manner, and the outer membrane of the autophagosome fuses with the lysosomal membrane using fusion machinery that is not specific to the autophagic pathway. The subsequent breakdown of the internal autophagosomal membrane allows acidic lysosomal hydrolases to access the cytosolic cargo, leading to its degradation and, ultimately, to its recycling.

**Cellular functions of autophagy**

**Starvation survival**

One of the best understood and perhaps most fundamental cellular roles of autophagy is to provide an internal source of nutrients under starvation conditions. In most cell types, nutrient withdrawal elicits a robust stimulation of autophagy, and this can significantly extend the survival time of yeast and cultured mammalian cells in the absence of nutrients. Indeed, many yeast ATG genes were first identified through screens for starvation sensitivity (Tsukada and Ohsumi, 1993). Apoptosis-deficient Bax−/− Bak−/− mouse bone marrow cells can remain viable for several weeks in the absence of IL3, which is essential for nutrient uptake in these cells; disruption of autophagy under these conditions results in rapid cell death (Lum et al., 2005a). In mammalian cells with an intact apoptotic system, such as in mouse embryonic fibroblasts or HeLa cells, the genetic or pharmacological inhibition of autophagy also significantly accelerates starvation-induced death (Boya et al., 2005). Under normal conditions in vivo, autophagy probably acts as a buffer against fluctuations in exogenous nutrient availability, maintaining intracellular nutrient concentrations at a relatively constant level.

How do the nutrients liberated by autophagy promote survival during starvation? The death of autophagy-deficient IL3-deprived Bax−/− Bak−/− mouse cells can be prevented by the addition of methylpyruvate (Lum et al., 2005b), a cell-permeable substrate of the tricarboxylic acid (TCA) cycle in mitochondria, indicating that autophagy-derived nutrients have a crucial role in ATP production. In addition, in starved ATG mutant yeast cells, the intracellular level of free amino acids drops significantly, becoming limiting for protein synthesis (Onodera and Ohsumi, 2005). Thus, nutrients derived from autophagy can be essential for both energetic and biosynthetic functions.

In addition to providing these functions cell autonomously, autophagy-derived nutrients can be exported out of the cell to support peripheral tissues. Accordingly, fasting induces a more severe reduction of plasma amino acid concentrations in Atg5−/− mice than in controls (Kuma et al., 2004). Thus, in multicellular organisms, autophagy helps to maintain extracellular nutrient levels within a narrow limit compared with the wider range faced by single celled organisms or cells grown in culture. Nonetheless, the finding that fasting leads to the induction of autophagy in most of the tissues of transgenic mice that express the autophagosome marker GFP-Atg8 (a notable exception being cells of the CNS) (Mizushima et al., 2004) indicates that, despite the buffering effects of autophagy, the magnitude of extracellular nutrient fluctuation in animals can be considerable.

**Fig. 1. Schematic representation of autophagy progression in metazoans.** (A) In response to starvation or other inductive cues, a membranous sac referred to as the phagophore or isolation membrane is nucleated from a poorly characterized structure known as the pre-autophagosomal structure or phagophore assembly site (PAS). (B) Expansion and curvature of the isolation membrane leads to engulfment of cytosolic material within the double membrane-bound autophagosome. The source of lipid contributing to this membrane growth has not been established. (C) Fusion of the autophagosomal outer membrane with lysosomes results in hydrolytic digestion of the inner membrane and the sequestered material, and export of the breakdown products into the cytoplasm. The prior fusion of autophagosomes with early or late endosomes (forming a structure known as an amphisome, not shown) may be required for autophagosome-lysosome fusion. (D, E) Electron micrographs of corresponding structures, including (D) a nearly completed autophagosome engulfing a mitochondrion, and (E) an auto-lysosome containing several degraded organelles and an intact mitochondrion.
Organelle turnover and cellular renewal

Although autophagy is most evident following starvation, a basal level of constitutive autophagy appears to be a universal feature of nearly all eukaryotic cells. One important function of basal autophagy is to rid the cell of defective or superfluous organelles, and autophagy would appear to be the sole cellular process by which this occurs. Mitochondria accumulate oxidative damage with age, and, in cells with defective autophagy, such damaged mitochondria fail to be eliminated and accumulate to high levels (Kim and Sun, 2007; Zhang et al., 2007). Defective mitochondria also appear to be a significant source of reactive oxygen species, leading to genotoxic damage in *atg* mutant cells, which may partially explain the potential tumor-suppressive effects of autophagy (Mathew et al., 2007). Both the random and selective incorporation of mitochondria into autophagosomes can contribute to this process (Kissova et al., 2007), sometimes referred to as mitophagy. Conditions that lead to mitochondrial damage cause a strong induction of mitophagy, and, indeed, mitophagy might have a confounding effect on chemotherapeutic agents that function through mitochondria-dependent damage in metabolically active cells (Amaravadi et al., 2007).

The life cycle of other organelles can be similarly influenced by autophagy. Induction of the unfolded protein response pathway in the endoplasmic reticulum (ER) results in the activation of autophagy and in the engulfment of regions of the ER (Hoyer-Hansen and Jaattela, 2007). This autophagic response is crucial for surviving ER stress, although sequestration of the ER into autophagosomes without subsequent lysosomal degradation might be sufficient for protection in some cases (Bernales et al., 2006). Selective autophagy also plays a key role in eliminating excess peroxisomes in yeast and mammalian cells following removal of peroxisome-proliferating drugs or media (Iwata et al., 2006; Sakai et al., 2006).

In addition to these effects on organelles, basal autophagy also plays a key role in eliminating misfolded, aggregated or otherwise damaged proteins, which may be resistant to degradation by the ubiquitin-proteosome system. Indeed, ubiquitinated protein aggregates accumulate in *atg* mutant mouse hepatocytes and neuronal cells (Hara et al., 2006; Juhász et al., 2007; Komatsu et al., 2005), and autophagy can suppress neurodegenerative phenotypes caused by the expression of aggregate-prone proteins in various fly, nematode and mouse neurodegeneration models (Florez-McClure et al., 2007; Jia et al., 2007; Ravikumar et al., 2004). Interestingly, the inhibition of proteosome function can lead to an increased rate of autophagy, indicating that communication occurs between these two, major, degradative pathways (Ding et al., 2007). Together, the turnover of organelles and macromolecules through basal autophagy plays a major role in cell renewal and viability, and the upregulation of this process is crucial for surviving cellular insults and stresses. The activation of autophagy in response to fasting may further boost this renewal effect, and may contribute to the anti-aging effects of calorific restriction (Bergamini et al., 2004).

Cell death

Seemingly at odds with its role in promoting cell survival, autophagy is often observed at high levels in dying cells, and in some cases might actively contribute to cell death. Also referred to as type II programmed cell death, autophagic cell death is distinguished from type I (apoptotic) cell death by the presence of abundant autophagic structures in the dying cell, by a lack of phagocyte recruitment and, in some instances, by caspase independence (Baehrecke, 2005; Schweichel and Merker, 1973). It is unclear whether autophagy provides the killing mechanism in such cells, or whether it represents a failed attempt at survival or the elimination of cell debris. Nonetheless, the disruption of autophagy can block the death of cultured cells with disabled apoptotic machinery (Shimizu et al., 2004; Yu et al., 2004), and can impair or delay developmental cell death in *Drosophila* (Berry and Baehrecke, 2007; Juhász et al., 2007), as discussed further below. In addition, the induction of high levels of autophagy can be sufficient to cause cell death (Kang et al., 2007; Pattingre et al., 2005; Scott et al., 2007). Although the mechanisms by which autophagy leads to cell death are unclear in most cases, they may involve the targeted destruction of factors required for cell survival (Yu et al., 2006). Future studies are required to understand the different contexts under which autophagy can promote either cell survival or destruction.

Cell growth

It has long been noted that there exists an inverse correlation between rates of autophagy and cellular growth. For example, most conditions that stimulate autophagy, such as starvation, growth factor withdrawal, and rapamycin treatment, result in reduced cell growth (Neufeld, 2004). Furthermore, many negative regulators of autophagy are growth-promoting oncoproteins, whereas positive regulators are often tumor suppressors (Botti et al., 2006). Although these correlative data may simply reflect regulatory pathways that...
Table 1. Mutant phenotypes of autophagy-related genes in metazoans

<table>
<thead>
<tr>
<th>Gene (S. cerevisiae)</th>
<th>Biochemical function</th>
<th>C. elegans ortholog</th>
<th>Drosophila ortholog</th>
<th>Mammalian ortholog</th>
<th>Loss-of-function phenotypes*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of induction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOR1, TOR2</td>
<td>PIK-family Ser/Thr protein kinase, rapamycin target</td>
<td>let-363</td>
<td>Tor</td>
<td>Frap1 (also known as mTOR)</td>
<td>AA, G, L, LL</td>
<td>(Blommaart et al., 1995; Hansen et al., 2008; Hansen et al., 2007; Hentges et al., 2001; Jia et al., 2004; Kapahi et al., 2004; Long et al., 2002; Noda and Ohsumi, 1998; Oldham et al., 2000; Scott et al., 2004; Vellai et al., 2003; Zhang et al., 2000)</td>
</tr>
<tr>
<td>ATG1</td>
<td>Ser/Thr protein kinase</td>
<td>unc-51</td>
<td>Atg1</td>
<td>Ulk1, Ulk2</td>
<td>Aut, D, Egl, G, L, Nd, Scd, St, Unc</td>
<td>(Berry and Baehrecke, 2007; Chan et al., 2007; Hedgecock et al., 1985; Matsuura et al., 1997; Meléndez et al., 2003; Ogura et al., 1994; Rubinsztein, 2006; Scott et al., 2007; Scott et al., 2004)</td>
</tr>
<tr>
<td>ATG13</td>
<td>Phosphoprotein component of Atg1 complex</td>
<td>D2007.5</td>
<td>KIAA0652</td>
<td>Harbi1</td>
<td>?</td>
<td>(Funakoshi et al., 1997; Meijer et al., 2007)</td>
</tr>
<tr>
<td>Vesicle nucleation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG6</td>
<td>Component of Vps34 complex, BH3-like domain, Bcl-2 interacting protein</td>
<td>bec-1</td>
<td>Atg6</td>
<td>Becn1 (beclin 1)</td>
<td>Ap, Aut, D, G, Nd, L, Scd, SL, Ster, T</td>
<td>(Berry and Baehrecke, 2007; Boya et al., 2005; Hansen et al., 2008; Jia et al., 2007; Jia and Levine, 2007; Kametaka et al., 1998; Kang et al., 2007; Meléndez et al., 2003; Qu et al., 2003; Scott et al., 2004; Seaman et al., 1997; Takacs-Vellai et al., 2005; Yue et al., 2003)</td>
</tr>
<tr>
<td>VPS34</td>
<td>Class III Ptdins 3 kinase</td>
<td>let-512</td>
<td>Vps34/ Pi3K59F</td>
<td>Pik3c3 (also known as Vps34)</td>
<td>Aut, L, SL</td>
<td>(Hansen et al., 2008; Juhász et al., 2008; Kihara et al., 2001; Petiot et al., 2000; Roggo et al., 2002; Seglen and Gordon, 1982)</td>
</tr>
<tr>
<td>VPS15</td>
<td>Ser/Thr protein kinase, component of Vps34 complex</td>
<td>ZK930.1</td>
<td>Vps15lird1</td>
<td>Pik3r4 (also known as Vps15)</td>
<td>Aut, I</td>
<td>(Juhász et al., 2008; Kihara et al., 2001; Lindmo et al., 2008; Wu et al., 2007)</td>
</tr>
<tr>
<td>–</td>
<td>Component of Vps34 complex, coiled-coil domain</td>
<td>–</td>
<td>–</td>
<td>Uvrag</td>
<td>Aut, G, T</td>
<td>(Liang et al., 2006)</td>
</tr>
<tr>
<td>–</td>
<td>Endophilin B1, BAR domain, component of Vps34 complex</td>
<td>erp-1</td>
<td>endophilin B</td>
<td>Sh3glb1 (also known as Bif1)</td>
<td>Aut, Scd, T</td>
<td>(Takahashi et al., 2007)</td>
</tr>
<tr>
<td>–</td>
<td>WD40 domain, component of Vps34 complex</td>
<td>–</td>
<td>–</td>
<td>Ambra1</td>
<td>Ap, Aut, G, L</td>
<td>(Fimia et al., 2007)</td>
</tr>
<tr>
<td>–</td>
<td>Negative regulator of apoptosis, inhibits Beclin 1-Vps34 interaction</td>
<td>ced-9</td>
<td>buffy</td>
<td>Bcl2</td>
<td>AA, Ap</td>
<td>(Hengartner and Horvitz, 1994; Pattingre et al., 2005; Quinn et al., 2003; Saeki et al., 2000)</td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>S. cerevisiae gene</th>
<th>Biochemical function</th>
<th>C. elegans ortholog</th>
<th>Drosophila ortholog</th>
<th>Mammalian ortholog</th>
<th>Loss-of-function phenotypes*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vesicle expansion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG3</td>
<td>E2-like enzyme, conjugates PE to Atg8</td>
<td>Y55F3AM.4</td>
<td>Atg3 (previously Aut1)</td>
<td>Atg3</td>
<td>Aut, L, Scd</td>
<td>(Berry and Baehrecke, 2007; Ichimura et al., 2000; Juhász et al., 2003; Scott et al., 2007; Tanida et al., 2002b)</td>
</tr>
<tr>
<td>ATG4</td>
<td>Cysteine protease cleaves Atg8 C-terminus</td>
<td>ZK792.8, Y87G2A.3</td>
<td>Atg4</td>
<td>Atg4a-Atg4d (autophagin 1-4)</td>
<td>Aut, N, T</td>
<td>(Hemelaar et al., 2003; Kirisako et al., 2000; Marino et al., 2007; Thumm and Kadowaki, 2001)</td>
</tr>
<tr>
<td>ATG5</td>
<td>Conjugated to Atg12 through internal Lys</td>
<td>atgr-5</td>
<td>Atg5</td>
<td>Atg5</td>
<td>Ap, Aut, B, Cm, G, N, Pl, Nd, Scd</td>
<td>(Boya et al., 2005; Hara et al., 2006; Hosokawa et al., 2006; Kametaka et al., 1996; Kuma et al., 2004; Miller et al., 2008; Nakagawa et al., 2004; Nakai et al., 2007; Qu et al., 2007; Scott et al., 2007; Scott et al., 2004)</td>
</tr>
<tr>
<td>ATG7</td>
<td>E1-like enzyme, activates Atg8 and Atg12</td>
<td>atgr-7</td>
<td>Atg7</td>
<td>Atg7</td>
<td>Aut, D, Nd, Ox, Scd, St, Std</td>
<td>(Berry and Baehrecke, 2007; Jia and Levine, 2007; Juhász et al., 2007; Komatsu et al., 2006; Komatsu et al., 2005; Komatsu et al., 2007; Meléndez et al., 2003; Scott et al., 2004; Yu et al., 2004)</td>
</tr>
<tr>
<td>ATG8</td>
<td>Ubiquitin-like protein conjugated to PE</td>
<td>lgg-1, lgg-2</td>
<td>Atg8a, Atg8b</td>
<td>Lc3, Gabarap, GabarapI2 (previously Gate16)</td>
<td>Aut, D, Ox, Scd, St, Std</td>
<td>(Berry and Baehrecke, 2007; Hemelaar et al., 2003; Kirisako et al., 1999; Meléndez et al., 2003; Scott et al., 2007; Simonsen et al., 2008)</td>
</tr>
<tr>
<td>ATG10</td>
<td>E2-like enzyme, conjugates Atg5 and Atg12</td>
<td>D2085.2</td>
<td>CG12821</td>
<td>Atg10</td>
<td>Ap, Aut</td>
<td>(Boya et al., 2005; Meléndez et al., 2003; Mizushima et al., 1998; Shintani et al., 1999)</td>
</tr>
<tr>
<td>ATG12</td>
<td>Ubiquitin-like protein conjugated to Atg5</td>
<td>lgg-3</td>
<td>Atg12</td>
<td>Atg12</td>
<td>Ap, Aut, Scd, St</td>
<td>(Boya et al., 2005; Hars et al., 2007; Mizushima et al., 1998; Scott et al., 2004; Tanida et al., 2002a)</td>
</tr>
<tr>
<td>ATG16</td>
<td>Component of Atg5-Atg12 complex</td>
<td>K06A1.5, F02E8.5</td>
<td>CG31033</td>
<td>Atg16I, Atg16I2</td>
<td>Aut</td>
<td>(Mizushima et al., 2003; Mizushima et al., 1999; Rioux et al., 2007)</td>
</tr>
<tr>
<td><strong>Recycling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG2</td>
<td>Peripheral membrane protein, interacts with Atg9</td>
<td>K0TG5.10</td>
<td>Atg2</td>
<td>Atg2a, Atg2b</td>
<td>Aut, L, Scd</td>
<td>(Berry and Baehrecke, 2007; Scott et al., 2004; Shintani et al., 2001; Wang et al., 2001)</td>
</tr>
<tr>
<td>ATG9</td>
<td>Integral membrane protein, interacts with Atg2</td>
<td>T22H9.2</td>
<td>Atg9</td>
<td>Atg9a, Atg9b</td>
<td>Aut</td>
<td>(Noda et al., 2000; Young et al., 2006)</td>
</tr>
<tr>
<td>ATG18</td>
<td>Peripheral membrane protein, PI(3,5)P binding</td>
<td>atgr-18, Y39A1A.1</td>
<td>Atg18 (previously CG7986, CG11975, CG8678)</td>
<td>Wipi1 (previously WIP149, Atg18)</td>
<td>Aut, D, L, Nd, Scd</td>
<td>(Barth et al., 2001; Berry and Baehrecke, 2007; Jia et al., 2007; Meléndez et al., 2003; Proikas-Cezanne et al., 2006; Scott et al., 2004)</td>
</tr>
</tbody>
</table>

*Phenotypes of gene inactivation: AA, autophagy activation; Ap, high incidence of cell apoptosis; Aut, Autophagy dysfunction; B, defective B cell development; Cm, cardiomyopathy; D(+), normal dauer morphogenesis; D, abnormal dauer; Egl, egg laying defective; G, cell growth or proliferation; I, defective immune response; L, lethal; LL, long lifespan; M, modified Notch signaling; Nd, increased susceptibility to neurodegeneration; Ox, sensitive to oxidative stress; Pl, postnatal lethality; Scd, suppression of cell death; SL, short lifespan; St, required for survival under starvation conditions; Ster, sterile; T, increased tumorigenesis; ?, unknown.
Functions of autophagy in development

Is autophagy essential for normal development?

It is clear that autophagy has many important cellular functions, many of which are involved in development. What is the evidence that autophagy plays a role in development and through which cellular function does autophagy influence a given developmental process? Surprisingly, genetic studies in mice, flies and worms have found that although some mutations in genes essential for autophagy result in embryonic lethality, other mutants with strong autophagy phenotypes remain viable throughout embryogenesis, with defects becoming apparent only postnatally or in adulthood.

In C. elegans, bec-1 homozygous animals, derived from bec-1 heterozygous parents, exhibit a highly penetrant lethal phenotype; they arrest at various stages of development, exhibiting the formation of cellular vacuoles, uncoordinated phenotypes and molting defects (Takacs-Vellai et al., 2005). Mutant bec-1 animals that reach adulthood are sterile. Thus, bec-1 activity is required for viability and fertility. A lethal phenotype is also observed in worms carrying a mutation in let-512, which encodes the C. elegans ortholog of the BEC-1 partner Vps34 (Roggo et al., 2002). In addition, RNAi-mediated knockdown of two other C. elegans atg orthologs (Atg8/igg-1 and Atg18/F41E6.13) result in early developmental arrest, during or before the first larval stage (Meléndez et al., 2003). By contrast, the C. elegans ATG1 ortholog unc-51 is dispensable for embryonic development, despite playing
an essential role in autophagy. Mutations in unc-51 result in adults with an uncoordinated phenotype, and axons of the mutant animals display abnormal vesicles and membrane cisternae (Hedgecock et al., 1985; McIntire et al., 1992). These results indicate that different autophagy genes may play distinct roles during embryonic development or that some genes display partial redundancy.

A similar conclusion can be drawn from genetic studies in flies and mice. In Drosophila, null mutations in Atg1 result in fully penetrant lethality late in the pupal stage, just prior to eclosion (Scott et al., 2004). Earlier lethality at the larval stage is observed in animals carrying mutations in the Drosophila Atg18 or Atg6 genes. Mutations in the beclin 1 gene also disrupt embryonic development in the mouse, resulting in severely reduced growth at embryonic day (E) 7.5 and death by E8.5 (Yue et al., 2003). By contrast, flies with mutations in Drosophila Atg7 or Atg8a develop to adulthood, and mice with mutations in Atg4C, Atg5, Atg7 or Bif-1 progress through embryonic development in normal numbers, although in each of these cases, serious defects are observed postnatally (see below) (Juhász et al., 2007; Komatsu et al., 2005; Kuma et al., 2004; Marino et al., 2007; Scott et al., 2007; Simonsen et al., 2008; Takahashi et al., 2007).

The mechanistic basis for the broad range of Atg mutant phenotypes in these genetic model organisms is poorly understood, in part due to our incomplete understanding of the cellular focus of the lethality. We also do not understand to what extent potential embryonic phenotypes may be masked by maternally contributed mRNA or protein, nor at what stages of development these maternal supplies may be depleted in each case. Overall, these findings suggest that autophagy plays somewhat subtle or redundant roles during development, or that defects in autophagy can be compensated for by the activation of other cellular processes. In addition, the range of mutant phenotypes indicates that some Atg genes have additional roles in other essential cellular functions. For example, Atg1/UNC-51 is involved in endocytic processes in the neurons of mice and worms, and mammalian beclin 1 protein interacts with members of the Bcl2 family, suggesting a potential role in apoptosis (Liang et al., 1998; Tomoda et al., 2004; Zhou et al., 2007).

In some cases, the connection between a specific cellular function of autophagy and a given developmental process is straightforward. For example, the final stages of erythrocyte maturation occur through direct autophagic elimination of mitochondria and other organelles (Fader and Colombo, 2006; Schweers et al., 2007). Other less extreme examples of this type of cellular remodeling are likely to contribute widely to cell differentiation. However, in many cases, the mechanisms by which autophagy contributes to development are less clear and may be indirect, and more than one cellular function of autophagy may be at play. Below, we discuss recent studies of development-related phenotypes of autophagy mutants in three specific areas—stress adaptation, cell death and neuronal development—emphasizing the underlying cellular mechanisms where known.

**Adaptation to starvation and stress**

A role for autophagy in C. elegans development was first provided by studies of dauer morphogenesis (Meléndez et al., 2003). C. elegans larvae respond to unfavorable environmental conditions (e.g., starvation, high population density, increased temperature) by arresting in an alternative third larval stage, referred to as the dauer diapause (Cassada and Russell, 1975; Riddle and Albert, 1997). Dauer larvae are characterized by radial constriction and elongation of the body and pharynx, resistance to detergent (sodium dodecyl sulfate or SDS) treatment, hyperpigmentation of the intestinal granules, increased fat accumulation, long lifespan and the ability to exit the dauer stage if the environmental conditions improve. The control of dauer development in C. elegans shares similarities with the induction of autophagy in other eukaryotes. Environmental cues, such as temperature, starvation, and high population, are potent inducers of autophagy in yeast, Dictostylium and mammals, and also induce dauer formation in C. elegans. Dauer development is regulated by different signaling pathways that also regulate fat storage, longevity, reproduction and stress responses, including the insulin/IGF-1, the transforming growth factor β (TGF-β) and the cyclic guanosine monophosphate (cGMP) pathways (Barbieri et al., 2003; Patterson and Padgett, 2000; Raizen et al., 2006). In dauer constitutive daf-2 (the insulin/IGF-1 receptor) mutants, an increase in autophagy occurs as noted by the localization of GFP::LGG-1 into discrete punctate structures in hypodermal seam cells (a cell required for multiple aspects of dauer morphogenesis) (Meléndez et al., 2003). This autophagy induction is required for dauer morphogenesis, as most daf-2 mutant animals that are grown at dauer-inducing temperature and in which which bec-1, unc-51/Atg1, atgr-7, lgg-1/Atg8, and atgr-18 were individually knocked down by RNAi fail to complete normal dauer development (Meléndez et al., 2003). These animals lack the characteristics associated with dauer: they do not undergo pharyngeal and total body constriction and elongation, they have less fat accumulation, they are not resistant to SDS, they die within a few days, and they fail to resume normal development when transferred to the non-dauer-inducing temperature. Autophagy is also required for dauer formation that is triggered by the lack of daf-7/TGF-β (Meléndez et al., 2003). Together, these data suggest that autophagy genes act downstream of insulin/IGF-1 and TGF-β signaling, that autophagy is required for the normal process of dauer morphogenesis, and that autophagy may be required for the cellular and tissue remodeling that permits C. elegans to adapt successfully to environmental stress. How autophagy is regulated during dauer formation is not known. Whether autophagy, in addition to its role in dauer morphogenesis, is required for dauer survival and/or dauer recovery is also unknown.

The autophagic machinery has been shown to promote organismal survival directly during starvation. Using animals that express the autophagy marker GFP::LGG-1 in the pharynx (see Box 1), Kang et al. have shown that starvation activates autophagy in the pharyngeal muscle (Kang et al., 2007). Interestingly, a deficiency in the levels of autophagy, caused by either a partial knockdown of bec-1 or of atgr-7, promotes the death of starved animals. The decrease in pharyngeal pumping that occurs in knockdown bec-1 animals can be attenuated by giving them food. A correlation between pumping efficiency and survival suggests that autophagy may provide the energy that is essential to maintain pharyngeal pumping efficiency and to ensure organismal survival during starvation. Whether autophagy is induced in other tissues in response to starvation is not yet known. In mammals, other muscle cells may also undergo autophagic catabolism to generate nutrients in order to maintain the health of neurons and other vital tissues.

Although the developmental response to starvation in Drosophila is quite distinct from that of C. elegans, autophagy has an important role in the survival of stress and starvation in the fly, and at least some of the genetic circuitry appears to be conserved. The larval fat body is a nutrient storage organ that contains large deposits of lipid and glycogen, somewhat analogous to the vertebrate liver. Drosophila larvae can survive for several days in the complete absence of nutrients, and two or more weeks in the absence of amino acids (Britton and Edgar, 1998). During these periods of starvation, nutrients are mobilized from the fat body to support the imaginal tissues, which are destined to give rise to adult structures of the fly. This starvation-induced mobilization occurs in large part through...
autophagy, and indeed autophagy-defective *Drosophila* mutants die more rapidly under starvation conditions (Juhász et al., 2007; Scott et al., 2004). Like dauer development in *C. elegans*, this starvation response occurs through the downregulation of insulin/IGF and TOR signaling, and constitutive activation of these pathways prevents the induction of autophagy by starvation and leads to starvation hypersensitivity (Britton et al., 2002; Scott et al., 2004). Paradoxically, the downregulation of TOR signaling in response to starvation leads both to the induction of autophagy and to the reduced activity of S6K, a substrate of TOR that is required for autophagy (Scott et al., 2004). This self-limiting feature of the TOR pathway activity of S6K, a substrate of TOR that is required for autophagy starvation leads both to the induction of autophagy and to the reduced incidence of spontaneous tumors (Takahashi et al., 2007).

In each of these examples, we have only a dim appreciation of the cellular functions and cell-specific requirements that are supported by autophagy. In autophagy-defective newborn mice, plasma concentrations of amino acids are significantly reduced, and these animals display electrocardiograms consistent with a shortage of respiratory substrates (Kuma et al., 2004). Thus, general defects in energy metabolism may be the immediate cause of the premature death of *Atg5*^{-/-} and *Atg7*^{-/-}null mice, although other abnormalities observed in these mutants, such as suckling defects and ubiquitin...
positive cytoplasmic inclusions (Komatsu et al., 2005), are consistent with their having additional problems earlier in development. The role of autophagy in dauer formation in C. elegans is likely to be more complicated, given the involvement of multiple cell types and physiological responses in this process. Autophagy is likely to serve as a crucial source of the nutrients and energy that are necessary for the extensive morphogenetic changes that occur during dauer development. In addition, autophagy may directly contribute to cellular remodeling by eliminating superfluous cytoplasmic components, and may influence the survival of specific cell types. Mosaic analysis of autophagy mutants may help to identify cell types with special requirements for autophagy in these developmental processes.

Neuronal development
An exclusive role for autophagy in mouse neuronal development has been reported for the Ambra1 (activating molecule in beclin 1-regulated autophagy) protein (Fimia et al., 2007). Ambra1 was identified in a gene-trap approach in mice to find genes expressed in the developing nervous system. Cecconi and colleagues have shown that Ambra1 interacts with beclin 1 protein in vivo, and regulates Vps34-dependent autophagy. As in beclin 1 in vitro studies, overexpression of Ambra1 in human fibrosarcoma cells leads to constitutively high levels of autophagy and to decreased cell proliferation. Furthermore, downregulation of Ambra1 impairs the interaction between beclin 1 and Vps34 proteins. Ambra1-null mutant mice display embryonic lethality (starting from E14.5) that is characterized by severe neural tube defects associated with an impairment in the autophagy pathway, an excess in programmed cell death, an increase in cell proliferation and an accumulation of ubiquitinated proteins. However, no defects in neuronal specification were detected (Fimia et al., 2007). Hyperproliferation is the earliest detectable abnormality in the developing neural tubes of these mutant embryos, followed by caspase-dependent apoptosis, indicating that there is a complex interplay between autophagy, the regulation of cell survival and the regulation of cell proliferation in mammals. Interestingly, hyperproliferation is observed in response to the disruption of any of several components of the beclin 1/Vps34 protein complex in mammalian cells, including beclin 1, Ambra1, Uvrag or Bif1. This phenotype is not observed in other autophagy mutants, indicating that the disruption of this complex results in a distinct autophagy defect that leads to increased proliferation, or that this complex has a growth suppressive function that is separate from its role in autophagy. Once again, the cellular basis for the developmental phenotypes of Ambra1 mutants is unclear, although the increased rate of cell proliferation and death are consistent with defective turnover of an oncogenic factor(s). The restriction of the Ambra1 phenotype to neurons suggests that other factors may supplant its role in non-neuronal tissues.

A role for autophagy in the clustering of neurotransmitter receptors in development has been reported in C. elegans (Rowland et al., 2006). The clustering of neurotransmitter receptors results from signaling events during development that are initiated when presynaptic terminals are contacted by the postsynaptic cell. In C. elegans, body-wall muscles are innervated by both GABA and non-GABA neurons (White et al., 1986). GABA terminals organize GABA_{A} receptors into synaptic clusters, which are internalized and degraded, as long as they lack presynaptic input. This degradation of GABA_{A} receptors is specifically mediated by an autophagic pathway, whereas that of acetylcholino receptors in the same cells is not (Rowland et al., 2006). Curiously, the mammalian GABA_{A} receptor-associated protein Gabarap is an ortholog of the yeast autophagy protein Atg8p (two other mammalian orthologs of Atg8p are LC3 and Gate16, see Table 1), which may hint at a potentially regulatory role of autophagy in balancing neuronal excitation and inhibition (owing to selective GABA_{A} receptor degradation). These findings show an unexpected degree of specificity and a novel function for autophagy in the degradation of neuronal cell surface receptors. Similar mechanisms of selective receptor degradation by autophagy may also be at play to regulate cellular growth, differentiation and development.

Programmed cell death
The developmental process of insect metamorphosis involves the wholesale death and elimination of a substantial part of the larval body, providing both space and nourishment for imaginal tissues as they are reorganized into their adult form inside the pupal case. Destruction of larval structures such as the Drosophila salivary gland and digestive tract is triggered by a sharp rise in the steroid hormone 20-hydroxyecdysone, and is associated with a dramatic upregulation of autophagy prior to and during cell death. This process has thus served as a valuable model for studying developmentally regulated autophagic cell death (Baehrecke, 2005). Interestingly, the death of these tissues displays characteristics of both autophagy, such as abundant cytoplasmic vacuolization, as well as apoptosis, including the upregulation of pro-apoptotic genes, caspase activation and DNA cleavage (Lee et al., 2002; Martin and Baehrecke, 2004). Elimination of larval gut and salivary glands can be partially suppressed or delayed by mutations in components of either the apoptotic or autophagic machinery (Berry and Baehrecke, 2007; Juhász et al., 2007; Muro et al., 2006). Disruption of both autophagy and apoptosis results in a more complete suppression (Berry and Baehrecke, 2007), indicating that these processes act cooperatively, and that one process may be upregulated to compensate for the lack of the other. Pupal development is delayed by ~4 hours in Atg7 mutant animals, consistent with a reduction in the efficiency of cell elimination when autophagy is defective (Juhász et al., 2007). The ultimate viability of these mutants, however, indicates that other death mechanisms are sufficient to compensate for this defect. A similar combination of apoptotic and autophagic morphologies has been described in the death of nurse and follicle cells during oogenesis in a number of insects, including silkworms, medflies and other Drosophila species (Mpakou et al., 2006; Nezis et al., 2006; Velentzas et al., 2007), although the requirement for these processes in cell death has yet to be determined experimentally in these cases.

Under what conditions and in what cell types is autophagy likely to be used as a means of cell death? Although phagocytes normally play an important role in eliminating apoptotic corpses, few phagocytes are observed in dying salivary glands. The extremely large size of polyplloid larval cells, their sequestration into inaccessible areas of the body cavity and the sheer number of cells dying during metamorphosis may preclude their efficient engulfment by phagocytes, necessitating their self-clearance by autophagy. However, although the function of autophagy in the death of these cells would appear to be direct, it is important to consider other mechanisms by which autophagy may contribute to cell elimination. In an in vitro model of mouse embryonic cavitation using embryoid bodies derived from ES cells, it was found that disruption of autophagy prevented the clearance of the inner core of ectodermal cells, which normally die by an apoptotic form of programmed cell death (Qu et al., 2007). In this case, however, autophagy was required not as an effector of death nor to degrade dying cells, but rather as an energy source to facilitate signaling from the dying cells to macrophages (Qu et al., 2007). The absence of autophagy resulted in...
decreased engulfment of apoptotic cells by macrophages, leading to accumulation of apoptotic corpses within the embryoid bodies. These results suggest that caution is warranted when interpreting effects of autophagy on apoptosis. For example, the observed increase in apoptotic corpses in *becl-1* mutant embryos in *C. elegans* (Takacs-Vellai et al., 2005) may reflect either an increased rate of apoptosis or a decrease in the clearance of dead cells.

So far, there is only limited evidence that autophagy functions as a death mechanism in cells with an intact apoptotic pathway, apart from the studies described above in *Drosophila*. In mammalian cells, most reports of the involvement of autophagy in the death execution process are in cells that are defective in the apoptotic pathways (Levine and Yuan, 2005; Mauri et al., 2007b). Furthermore, defects in autophagy, as in *becl-1/ATG6* or beclin 1 mouse knockouts, cause an increase, and not a decrease in cell death, supporting a pro-survival role for autophagy during development.

Recent studies of EGL-1 in *C. elegans*, provide interesting insights with regard to the connection between autophagy and apoptosis (Mauri et al., 2007a). EGL-1 encodes a novel protein that contains a Bcl2 homology 3 (BH3) domain. In *C. elegans*, EGL-1 functions as a positive regulator of apoptosis by interacting directly with the anti-apoptotic CED-9 to induce the release of CED-4 from CED-4/CED-9 complexes, resulting in the activation of the caspase CED-3 (Conradt and Horvitz, 1998; Conradt and Horvitz, 1999; del Peso et al., 1998; Trent et al., 1983). Although a deletion of *egl-1* compromises starvation-induced autophagy, a gain-of-function mutation of *egl-1* induces autophagy (Mauri et al., 2007a). The interaction between mammalian Bcl2/Bcl-xL (Bcl2l1 – Mouse Genome Informatics) and beclin 1 protein involves a BH3 domain within beclin 1, and this interaction is competitively blocked by the overexpression of BH3-only pro-apoptotic proteins or by pharmacological BH3 mimetics (Mauri et al., 2007b). Thus, EGL-1 might not only positively regulate programmed cell death, but might also positively regulate autophagy by interacting with CED-9 to induce BEC-1 release from CED-9/BEC-1 complexes. It is unclear whether the increase in autophagy in *egl-1* mutants contributes directly to cell death. Future studies in *C. elegans* should clarify the molecular crosstalk between the autophagic and apoptotic machinery during development.

In addition to these connections between autophagy and apoptosis, recent studies suggest that autophagy may also play a role in necrotic cell death. Gain-of-function mutations in *C. elegans* ion channel genes (*mec-4* or *deg-1*), the acetylcholine receptor channel subunit gene (*deg-3*) and the Gs protein α-subunit gene (*gsa-1*) cause a necrotic-like cell degeneration in neurons (Berger et al., 1998; Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991; Korswagen et al., 1997; Treinin and Chalfie, 1995). Studies of dying neurons in animals carrying the gain of function *mec-4(d)* allele, display extensive degradation of cellular contents during the process of necrosis (Hall et al., 1997). This ultrastructural feature is reminiscent of autophagy and does not require the caspase CED-3, which mediates programmed cell-death, nor CSP-1, CSP-2 or CSP-3 (CED-3-related proteases) (Chung et al., 2000), indicating that a distinct and non-apoptotic mechanism may function in neurodegeneration (Syntichaki et al., 2002). The knockdown of three autophagy transcripts (*unc-51/ATG1, bec-1/ATG6* and *lgl-1/ATG8*) by RNAi suppresses the degeneration of neurons with hyperactive toxic ion channels (Toth et al., 2007), suggesting a role for autophagy in cellular necrosis. Recently, Samara et al. have also shown that excessive autophagosome formation is induced early during necrotic cell death and that the autophagy pathway synergizes with lysosomal proteolytic mechanisms to facilitate necrotic cell death in *C. elegans* neurons (Samara et al., 2008). Together these results suggest that the boundaries between apoptotic, autophagic and necrotic forms of cell death are not sharply defined, and aspects of each mechanism can be used together to achieve a cell death process that is appropriate for a given developmental context.

**Autophagy and disease**

The recent surge in interest and activity in the field of autophagy research is driven in part by the impact of this process on several aspects of human health and disease, and by the potential benefits of targeting autophagy to increase lifespan or delay age-related disease. As a fundamental cellular process important for energy homeostasis and renewal, it is perhaps not surprising that defects in autophagy are being associated with an increasing assortment of human diseases. As in the case for tumorigenesis and neurodegeneration described in the text, autophagy can potentially play both beneficial and detrimental roles in these other contexts as well. For example, autophagy affects immune system function at several levels, acting to promote the degradation of a variety of pathogens, including bacteria, viruses and intracellular parasites (Andrade et al., 2006; Nakagawa et al., 2004; Tallozcy et al., 2002). But some viruses can subvert the autophagic machinery to their advantage, utilizing autophagosomal membranes to facilitate viral replication and release (Jackson et al., 2005). Autophagy also plays a central role in the processing, and MHC class II-mediated presentation of, intracellular antigens (Munz, 2006). Recently, Atg16L has been identified as a susceptibility gene for Crohn’s disease, an inflammatory disorder associated with a maladaptive immune response to intestinal flora (Rioux et al., 2007). The potential roles of autophagy in this, and other, immune-related illnesses in vivo should become clearer through future studies.

Autophagy might also play an especially prominent role in the heart, consistent with the high metabolic demand placed on contractile cardiomyocytes. As observed in the nervous system, basal levels of autophagy are crucial for normal cell function in the mouse heart (Nakai et al., 2007). In addition, autophagy is markedly upregulated in cardiac cells in response to a number of stresses, including ischemia, hypoxia and pressure overload but why is unknown. For example, pathological effects of pressure overload were reported to be either less or more severe in mice mutant for different autophagy-related genes (Nakai et al., 2007; Zhu et al., 2007). In mouse models of ischemia/reperfusion, autophagic activation plays an adaptive role at the early stage of insult but has harmful effects in the recovery phase (Matsu et al., 2007).

By contrast, autophagy might act more beneficially by promoting longevity. Treatments that induce autophagy, such as caloric restriction and reduced insulin or TOR signaling, increase lifespan across the animal kingdom and in *C. elegans* these effects depend on autophagy (Méndez et al., 2003). Direct manipulation of the autophagic machinery through increased expression of the Atg8a gene has also been shown to extend life in *Drosophila* (Simonsen et al., 2008).

Together, these types of studies can point towards specific dietary programs and pharmaceutical interventions that may provide effective therapy for a number of medical conditions. Pharmacological inhibitors of autophagy such as 3-methyladenine (3-MA) and the AMP-activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), and autophagy inducers such as rapamycin, an FDA-approved immunosuppressant, serve as proof-of-principle that drugs targeting autophagy can be effectively developed. Although these compounds are not selective for autophagy, recent small-molecule screens have begun to identify additional autophagy modulators (Sarkar et al., 2007), and an important future goal will be to develop drugs that specifically target autophagy.
opportunity to develop novel therapies involving the manipulation of autophagy (Box 2). In two areas in particular, tumorigenesis and neurodegeneration, autophagy plays an important role and developmental studies in model organisms are leading to new insights into disease mechanism and potential therapeutic strategies.

Although correlations between malignancy and defects in autophagy have long been noted, the first indication of a mechanistic connection between autophagy and cancer came from studies of the beclin 1 gene, which is monoallelically deleted in a high percentage of human breast and prostate cancer (Aita et al., 1999; Liang et al., 1999). The heterozygous disruption of the beclin 1 gene in mice was found to increase the rate of spontaneous and virally induced tumor formation, confirming the role of beclin 1 protein as a haploinsufficient tumor suppressor (Qu et al., 2003; Yue et al., 2003). Other components of the beclin 1/Vps34 protein complex, including Uvrag and Bif1, also have tumor suppressive properties (Liang et al., 2006; Takahashi et al., 2007). Recent studies by White and colleagues have demonstrated that a loss of autophagy in immortalized mouse epithelial cells leads to a marked increase in DNA damage, genomic instability and necrosis, all of which potentially contribute to tumorigenesis (Degenhardt et al., 2006; Mathew et al., 2007). In contrast to this tumor suppressive function, autophagy might promote tumorigenesis by supporting the growth and survival of solid tumors at early stages of development, prior to vascularization. Thus, autophagy appears to play both positive and negative roles at different stages of cancer development. Studies of the normal functions of autophagy during vasculogenesis in developing embryos may help to clarify these issues. Indeed, observations of increased inflammatory responses in atg5 mutant mouse embryos (Qu et al., 2007) suggest additional mechanisms by which autophagy may contribute to tumorigenesis.

An essential role for autophagy has also been found in the maintenance of axonal homeostasis and prevention of neurodegeneration (Rubinsztai, 2006). As in the case of cancer, autophagy appears to play primarily a protective role against neurodegeneration, probably stemming from the function of basal autophagy in degrading damaged organelles and aggregate-prone proteins. This neuroprotective role is exemplified by the neurodegeneration phenotypes observed in fly and mouse autophagy mutants, and by the suppression of polyglutamine-induced toxicity by autophagy in fly and worm models of Huntington’s and other neurodegenerative diseases (Hara et al., 2006; Jia et al., 2007; Juhász et al., 2007; Komatsu et al., 2006; Sarkar et al., 2007). However, in some cases, autophagy appears to play dual harmful and beneficial roles in neuronal health. For example, autophagy contributes to the production of amyloid β peptide through degradation of amyloid β precursor protein (APP)-containing organelles. Normally this toxic peptide is degraded following the fusion of autophagosomes with lysosomes; however, in Alzheimer’s disease, the fusion process of autophagosomes with lysosomes is defective, leading to the massive accumulation of autophagic vacuoles and to increased amyloid β levels in the degenerating neurons (Nixon, 2007). A similar accumulation of autophagic vacuoles has been described in an increasing number of neurodegenerative conditions, but additional investigation is necessary to determine whether this represents increased induction of autophagy or defects in autophagosome turnover.

Conclusions

In the past few years, there has been a deluge of publications identifying many of the components of the protein machinery involved in autophagic function in yeast, and providing insights as to the molecular mechanism of autophagy. Although orthologs of many of these components exist in metazoans, in some cases formal proof of their involvement in autophagy is still lacking. Another area where our understanding is still limited is that of the regulatory pathways that control autophagy, and the level of crosstalk that probably exists between these pathways. Genetic model organisms may be able to shed light on this issue. Based on our current understanding of the physiological functions of autophagy, both basal levels and stress-induced levels of autophagy can promote mammalian health. Autophagy maintains energy homeostasis and provides nutrients under conditions of stress and nutrient deprivation. In addition, autophagy rids the cell of intracellular proteins and damaged organelles that may cause cellular degeneration, genomic instability, tumorigenesis and aging.

As our appreciation of the roles of autophagy in cancer, neurodegeneration and other diseases grows, it will become increasingly important to understand the normal range of autophagic functions and control mechanisms in the healthy state. Increased insight into the developmental roles of autophagy is likely to point towards ways in which this process can be exploited for therapeutic purposes. A challenge for the future will be to identify the signaling mechanisms and regulatory steps that can be targeted for intervention, and to determine the circumstances under which autophagy plays a net beneficial versus detrimental role. Developmental studies in nematodes, flies and mice have already begun to shed light onto the variety of uses to which autophagy has been put by evolution, and how this fascinating process influences a wide spectrum of developmental events.

The work in the authors’ laboratories is supported by the NIH, and by the PSC CUNY Research Award Program (A.M.). We thank Hannes Bölow for comments and helpful suggestions in the preparation of this manuscript.

References


