The Role of Autophagy in Age-Related Neurodegeneration

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Key Words

Autophagy · Neurodegeneration · Aging · Lysosome · Protein aggregation · Ubiquitin proteasome system

Abstract

Most age-related neurodegenerative diseases are characterized by accumulation of aberrant protein aggregates in affected brain regions. In many cases, these proteinaceous deposits are composed of ubiquitin conjugates, suggesting a failure in the clearance of proteins targeted for degradation. The 2 principal routes of intracellular protein catabolism are the ubiquitin proteasome system and the autophagy-lysosome system (autophagy). Both of these degradation pathways have been implicated as playing important roles in the pathogenesis of neurodegenerative disease. Here we describe autophagy and review the evidence suggesting that impairment of autophagy contributes to the initiation or progression of age-related neurodegeneration. We also review recent evidence indicating that autophagy may be exploited to remove toxic protein species, suggesting novel strategies for therapeutic intervention for a class of diseases for which no effective treatments presently exist.

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Attendant to the process of protein synthesis is the risk of producing defective polypeptides that are prone to misfolding and aggregation. Indeed, it has been estimat-

ed that in excess of 25% of nascent peptides are faulty and rapidly removed by proteasomal degradation [1]. Even with correctly synthesized proteins, there is competition between alternative folding pathways, some terminating in kinetically trapped and incorrectly folded conformers. Postsynthetic damage, cleavage events, or an imbalance of necessary cofactors or components of multimeric complexes also contribute to the burden of misfolded proteins [2]. More than simply being functionally deficient, misfolded proteins are prone to aberrant interactions, the formation of insoluble protein aggregates and the acquisition of toxic properties. Thus, the importance of a vigilant quality control system to prevent the cytotoxic accumulation of misfolded proteins. This may be of particular importance to neurons, which are postmitotic and unable to dilute cytotoxic misfolded proteins through cell division. Protein quality surveillance takes place at many levels in the synthetic and folding processes, and this has been reviewed elsewhere [3].

In many cases, the ultimate fate of defective proteins identified by quality control systems is degradation by either of 2 intracellular catabolic pathways, the ubiquitin proteasome system (UPS) or the autophagy-lysosome system [4]. The UPS accomplishes selective degradation of short-lived proteins. Degradation by the proteasome is spatially and temporally controlled largely by highly specific targeting of proteins by conjugation with polyubiquitin chains [5]. As such, proteasomal degradation contributes to fine tuning the expression levels of select proteins and participates in the regulation of diverse cellular functions including cell cycling, signal transduction and

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Fig. 1. Intracellular protein degradation pathways. Cytosolic proteins can be degraded by the UPS or autophagy-lysosome system. Multiple forms of autophagy involve distinct routes by which cytoplasmic components are delivered for lysosome-mediated degradation. (1) Proteasome-mediated degradation involves covalent addition of ubiquitin chains to target proteins followed by proteolytic cleavage within the catalytic barrel of the proteasome. (2) Macroautophagy is a multistep process by which cytosolic com-

ponents are engulfed by an isolation membrane to form autophagosomes that are delivered to lysosomes. (3) In pexophagy, peroxisomes are surrounded by autophagic membranes and delivered to lysosomes. (4) Chaperone-mediated autophagy involves recognition of a peptide signal that induces receptor-mediated translocation into the lysosome. (5) In microautophagy, cytosolic contents are directly engulfed by lysosomes.

transcription regulation. The UPS also plays an important role in clearance of defective or misfolded proteins. Many neurodegenerative diseases are characterized pathologically by the accumulation of ubiquitin conjugates in affected neurons, suggesting that a defect in UPS function contributes to pathogenesis. This hypothesis has been bolstered by the identification of disease-causing mutations in several UPS components, but remains controversial, as has been reviewed elsewhere [6].

By contrast with the UPS, autophagy is a less selective, bulk degradation process that is largely responsible for the turnover of longer-lived proteins [7]. Autophagy plays a vital role in neuronal homeostasis by removing aged and potentially damaged proteins and providing a steady supply of macromolecules for further synthesis. Increasingly, defects in autophagy have been implicated as contributing to the initiation or progression of neurodegenerative disease. Here we will (1) describe the process of autophagy, (2) review the evidence implicating a role for autophagy in neurodegenerative disease, (3) address the controversy of whether autophagy is helpful or harmful

in the context of neurodegenerative disease, and (4) discuss the prospects of harnessing autophagy for therapeutic benefit.

The Autophagy-Lysosome System

The term autophagy describes a catabolic process in which cytoplasmic components such as organelles and proteins are delivered to the lysosomal compartment for degradation. Several specialized forms of autophagy exist, and they are distinguished by the way in which cytosolic constituents reach lysosomes (fig. 1). Microautophagy involves direct engulfment of small volumes of cytosol by lysosomes [8]. Pexophagy is a specialized form of autophagy for selective degradation of peroxisomes [9]. Chaperone-mediated autophagy is a regulated process in which proteins harboring a pentapeptide motif are specifically targeted for receptor-mediated translocation into the lysosome, and it is a significant catabolic pathway that may account for degradation of up to 30% of all cy-

Fig. 2. Macroautophagy. Macroautophagy begins with the formation of an isolation membrane (phagophore) that surrounds cytoplasmic constituents. This membrane elongates and then fuses with itself to create a sealed vesicle, termed the autophagosome, which encloses a portion of cytosol. In mammalian cells, autophagosomes, which are marked by LC3-II, undergo a maturation

process that involves fusion events with late endosomes and multivesicular bodies. The products of these fusions are termed amphisomes. Finally, amphisomes fuse with lysosomes to generate degradative autolysosomes which complete the digestion of amphisome contents. Adapted with permission from Martinez-Vicente and Cuervo [14].

tosolic proteins [10, 11]. These processes are distinguished from macroautophagy (hereafter referred to as autophagy), in which a membranous structure termed the isolation membrane or phagophore expands as a cup-shaped organelle to engulf a portion of cytoplasm, eventually fusing to form a new vacuole known as an autophagosome that compartmentalizes the cytosolic components [12]. In mammals, newly formed autophagosomes undergo a stepwise maturation process involving fusion with late endosomes and multivesicular bodies to form a structure termed an amphisome [13]. Autolysosomes are formed when amphisomes ultimately fuse with lysosomes to deliver their contents for degradation by lysosomal hydrolases (fig. 2). Finally, the breakdown products from the autolysosome are translocated back across the lysosomal membrane for reuse in metabolic processes.

Induction and Maturation of Autophagosomes

Autophagosome formation involves the coordinated activity of a large number of autophagy-related genes (Atg genes). Initially identified in yeast, the Atg genes are highly conserved in metazoans, including mammals [15]. These genes function in 2 independent ubiquitin-like conjugation systems that each culminate in the formation of membrane-associated complexes that carry out the process of autophagosome formation [16]. In one arm of

the conjugation system, sequential action of an E1 ligase-like protein (Atg7) and an E2 ligase-like protein (Atg10) generates a covalent isopeptide linkage of the C-terminal glycine of a ubiquitin-like protein (Atg12) with a lysine residue of Atg5. Multiple Atg5-Atg12 conjugates are then cross-linked by Atg16 to form large Atg5-Atg12-Atg16 complexes that contribute to phagophore membrane elongation. In the other arm of the conjugation pathway, Atg8 is proteolytically processed by Atg4 before interacting with the E1-like enzyme Atg7. Next, Atg8 is transferred to the E2-like protein Atg3 which ultimately catalyzes the conjugation of Atg8 to the lipid phosphatidylethanolamine (PE). This lipidation reaction allows Atg8-PE to associate with both the outer and inner membranes of the forming autophagosome [17]. The mammalian homolog of Atg8 is known as microtubule-associated protein 1 light chain 3 (LC3). During activation of autophagy in mammals, a processed form of LC3, denoted LC3-I, is further cleaved to generate LC3-II, which is conjugated to PE and inserted into the phagophore [17]. LC3 is the only protein in higher eukaryotes that is known to remain associated with the completed autophagosome, and consequently LC3 staining is used extensively as a histological marker of autophagosomes, and accumulation of LC3-II as an index of autophagic activity and/or flux [18]. In addition, increased LC3-II levels correlate with induction of autophagosome formation, a defect in their maturation, or both.

Regulation of Autophagy

Autophagy is an evolutionarily conserved process whose primary task in lower organisms is the maintenance of metabolic homeostasis in the face of changing nutrient availability. Induction of autophagy leads to the degradation of nonessential cytoplasmic constituents into basic materials that can be reused for anabolism or energy production. As such, autophagy is tightly regulated by the nutrient supply via nutrient signaling pathways. In yeast, autophagy is situated downstream of the nutrient sensor phosphatidylinositol 3-kinase (PI3K), and its downstream effector Akt controls the activity of the kinase target of rapamycin (TOR), a negative regulator of autophagy [19, 20]. Thus, activation of insulin-like receptors and PI3K leads to stimulation of TOR activity and suppression of autophagy. Conversely, autophagy is induced by signals that indicate decreased nutrients such as growth factor removal, serum starvation and amino acid depletion. Inhibition of TOR, either by decreased signaling through PI3K and Akt, or pharmacologically by the macrolide antibiotic rapamycin, also leads to up-regulation of autophagy [21]. In multicellular organisms, this general arrangement is conserved and nutrient sensing is subserved by Class I PI3K, although additional modes of regulation have also evolved, including activation by Class III PI3K which likely induces autophagy through interaction with Atg6 [19].

Multiple Roles for Autophagy: Matters of Life and Death

In yeast, the primary function of autophagy is to maintain viability during times of starvation. In multicellular organisms, autophagy is also critical to maintaining metabolic homeostasis, but has taken on additional, pleiotropic cellular functions. Autophagy is protective in stressful conditions such as nutrient or growth factor depletion, and also defends cells from invasion by certain pathogenic bacteria and viruses [22]. Constitutive clearance of cytosolic proteins by low-level basal autophagy is an additional important cytoprotective function, particularly to neurons, as evidenced by the accumulation of ubiquitinated proteinaceous deposits and the development of neurodegeneration in mice deficient for basal autophagy [23, 24]. Autophagy also plays a vital role in protecting cells from oxidative stress by selectively eliminating damaged mitochondria, the most important source of free radicals in the cell [25].

In addition to this cytoprotective role, induction of autophagy can be detrimental. For example, some cancer cells use autophagy for protection against radiation therapy [26] and various pathogens have evolved mechanisms to subvert autophagy for their own purposes [22]. In some paradigms, activation of autophagy has been associated with nonapoptotic cell death, termed type II programmed cell death [27–29]. Autophagy was initially implicated in cell death by work demonstrating a role for beclin-1 (a positive regulator of autophagy that is homologous to yeast ATG6) as a potential tumor suppressor. Beclin-1 directly associates with the antiapoptotic protein Bcl-2 [30], and loss of beclin-1 activity predisposes to malignant transformation in animal models [31, 32]. Furthermore, beclin-1 mutations have been identified in human breast cancers [33]. However, the precise relationship of beclin-1, induction of autophagy and cell death remains obscure.

Neuronal cell death is frequently accompanied by autophagic features [34], although evidence of a role for autophagy in mediating cell death is limited to a few studies. In the nematode *Caenorhabditis elegans*, gain-of-function mutations in genes that encode specific ion channel subunits such as the degenerins DEG-1 and MEC-4 and the acetylcholine receptor subunit DEG-3 lead to a necrotic-like degeneration of a subset of neurons which is suppressed by genetic inhibition of autophagy genes [35]. Similarly, in the Lurcher mouse model of cerebellar degeneration, autophagic neuronal death was implicated as mediating the pathological effects of mutations in the GluR2^{Lc} glutamate receptor [36]. The observation in Lurcher pathogenesis may have broad implications regarding the possibility that autophagy may be a common mediator of cell death initiated by excitotoxicity [37], but this remains to be established.

However, a general role for autophagy in neuronal death remains speculative. In most instances in which autophagic morphology has been found to accompany neuronal cell death, it remains indeterminate whether autophagy is the culprit, whether autophagy was induced secondarily to facilitate the removal of cellular components, or whether autophagy was induced as a cytoprotective response to cellular stress. It is quite possible that autophagic induction has the potential to be either protective or destructive, and the influence of autophagy depends on the type, degree and duration of the inciting cellular stress.

Decline of Autophagy with Aging

The autophagy-lysosome system undergoes striking changes in aging cells. Aging leads to reductions in autophagosome formation and autophagosome-lysosome fusion, both of which are consistent with decreased macroautophagy [38]. There are also notable changes in lysosomes themselves, including increased lysosome volume, decreased lysosomal stability, altered activity of hydrolases and intralysosomal accumulation of the indigestible material lipofuscin [39]. The precise molecular defects remain unknown, but these changes correlate with a decrease in the total capacity for degradation of long-lived proteins in many tissues of aged animals [38, 40]. Studies in aged rodents and senescent cells in culture have revealed that aging is also associated with reduced rates of translocation of substrate proteins into lysosomes through chaperone-mediated autophagy [41]. This reduction is caused by age-related changes including increased degradation of the lysosomal membrane protein LAMP-2A and reduced ability of LAMP-2A to reinsert into the lysosomal membrane [42].

The consequences of age-related decline in autophagy are diminished turnover of intracellular components and reduced ability of cells to adapt to changes in the extracellular environment [43]. Compromised clearance of old and/or damaged mitochondria by macroautophagy coupled with reduced turnover of long-lived proteins likely contribute to the intracellular accumulation of oxidized proteins in aged organisms [25]. Age-related decline in autophagy may be particularly detrimental to the nervous system, as postmitotic cells such as neurons are vulnerable to the accumulation of undegraded metabolic products over the lifetime of the organism [44]. Neuronal homeostasis uniquely depends on balanced, bidirectional trafficking of intracellular constituents between distal neurites and the cell soma. In neurons, autophagosomes and endosomes that fuse in the distal axon must be retrogradely transported, often over great distances, to the soma in order to fuse with lysosomes and degrade their contents [45, 46]. Thus, subtle disruptions of autophagosome formation, maturation or trafficking would be predicted to have dire consequences for autophagic flux and neuronal homeostasis. Indeed, evidence is mounting that autophagic pathways are vitally important for the maintenance of neuronal health, particularly in the context of degenerative disease states.

Accumulation of Autophagic Vacuoles in Patients and Models of Neurodegenerative Disease

Autophagic vacuoles are rare in neurons of the normal adult brain [47]. Increasingly, however, accumulation of autophagic vacuoles has been appreciated in the affected brain regions of a wide variety of neurodegenerative diseases. In Alzheimer's disease, autophagic vacuoles appear in neocortical and hippocampal pyramidal neurons and accumulate markedly within the dendritic arbors of these affected cells (for images and additional description of altered autophagy in Alzheimer's disease, see fig. 3) [47]. Autophagic vacuoles have also been described in melanized neurons of the substantia nigra in Parkinson's disease [49], and in affected brain regions in a variety of prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker disease and fatal familial insomnia, where prominent changes in axon terminals include the accumulation of autophagic vacuoles and features consistent with axonal degeneration [50]. Accumulation of autophagic vacuoles has also been described in pathological evaluation of tissue from polyglutamine disease patients. For example, cathepsin D immunopositive autophagic vacuoles that contain remnants of polyglutamine-expanded huntingtin protein are found in lymphoblasts derived from Huntington's disease patients, while examination of cortical neurons by immunoelectron microscopy shows huntingtin-immunoreactive bodies that resemble multivesicular bodies, but are also cathepsin D positive and may be amphisomes or autolysosomes [51].

In many cases, the accumulation of autophagic vacuoles observed in patients with neurodegeneration has been recapitulated in respective disease models. For example, exogenous expression of a variety of polyglutamine-expanded disease proteins leads to an increase in biochemical and morphological markers of autophagy *in vitro* [52–55], as well as in fly [56] and mouse [57, 58] models of polyglutamine diseases.

However, from these descriptive studies it is not possible to distinguish whether accumulation of autophagic vacuoles is due to induction of autophagosome formation or a defect in clearance of autophagic vacuoles, such as might occur with failure of autophagosome-lysosome fusion. If the accumulation of autophagic vacuoles observed in neurodegenerative disease is due to autophagy induction, descriptive studies also do not permit determination of whether increased autophagy is helpful (for example, an adaptive response to misfolded protein stress) or harmful (for example, autophagic cell death). Of course, autophagy is not necessarily performing the same

Fig. 3. Accumulation of autophagic structures in Alzheimer's disease brain. Ultrastructural appearance of dystrophic neurites from Alzheimer's disease brain (**a**) demonstrating autophagic vacuoles that are similar to autophagic vacuoles from highly purified subcellular fractions from mouse liver (**b**, **c**). Autophagic vacuole morphologies in Alzheimer's brain include large double-membrane-limited vesicles containing multiple smaller double-membrane vesicles exhibiting heterogeneous intraluminal contents (**d**). Multilamellar bodies, another variant of autophagic vacuoles, are also common in dystrophic neurites (**e**). This figure is adapted with permission from Nixon et al. [47]. Upregulation of the endosomal-lysosomal system is an early cellular event in Alzheimer's disease, evident even before amyloid deposits, and is manifest as lysosomal proliferation and increased expression of lysosomal enzymes necessary for the successful removal of aggregated and toxic proteins. In later stages of the disease, the efficiency of the lysosomal system decreases, resulting in poor

clearance of autophagic vacuoles, whose formation is apparently unaffected. Many vacuoles fail to fuse with lysosomes, while others undergo lysosomal fusion but, for unknown reasons, their contents are never degraded. Consequently, numerous autophagic vacuoles accumulate inside affected neurons. It is speculated that the presence of these enlarged compartments may interfere with normal intracellular trafficking essential for proper neuronal functioning. Alternatively, or in addition, vacuoles may leak releasing hydrolytic enzymes and toxic undigested products that could be detrimental to cells. Finally, recent observations made both in patient tissue and mouse models indicate that the autophagic vacuoles accumulating in Alzheimer's disease are highly enriched in both the components and enzyme activity of the γ -secretase complex and contain amyloid precursor protein and β -carboxy terminal fragment. The authors suggest that autophagic vacuoles may be a significant source of amyloidogenic amyloid β in Alzheimer's disease brain [48].

function in all diseases or at all stages within a single disease. Nevertheless, recognition of morphological features of altered autophagy in neurodegeneration set the stage for experimental studies of the functional role of autophagy in neurodegenerative diseases. Substantial progress has been made in sorting among these possibilities from studies of model systems of disease.

Autophagy is Neuroprotective

Evidence suggesting a protective role for autophagy in the context of disease was initially provided by a series of in vitro studies demonstrating that disease-causing pro-

teins are frequently degraded by autophagy. For example, pharmacological induction or inhibition of macroautophagy alters the rate of turnover of polyglutamine-expanded proteins, polyalanine-expanded proteins, as well as wild-type and mutant forms of α -synuclein [59, 60]. Moreover, ultrastructural analysis by immunoelectron microscopy has demonstrated delivery of polyglutamine-expanded proteins to autophagic vacuoles [52, 53]. Chaperone-mediated autophagy has also been found to contribute to the degradation of α -synuclein, a process that is impaired by disease-causing mutations [61]. An interesting observation is the recruitment of Atg proteins, including LC3, Atg5, Atg12 and Atg16, into mutant huntingtin aggregates, suggesting autophagic activity in the vicinity of

protein inclusions [62, 63]. However, as protein aggregates have recently been found to nonspecifically sequester autophagy components, these particular results should be interpreted with caution [64]. Collectively, these studies suggested that autophagy contributes to the degradation of multiple disease proteins and raised the possibility that induction of autophagy could be a protective pathway in the context of misfolded protein accumulation.

Compelling evidence that autophagy is neuroprotective was provided by a series of animal studies in which impairment of the autophagy-lysosomal system was consistently found to induce neurodegeneration. In mice, knockout of cathepsin D, a lysosomal protease highly expressed in the nervous system, caused accumulation of autophagosomes and lysosomes with accompanying neural dysfunction and degeneration [65–67]. Interestingly, signs of autophagic stress occur at very early ages in these mice and precede cell death [65]. A *Drosophila* cathepsin D mutant also shows accumulation of indigestible pigments and modest neurodegeneration [68]. The importance of autophagy in neurodegeneration was further underscored by 2 studies examining conditional central nervous system knockout of autophagy in mice. Deficiency of Atg5 or Atg7, both critical genes for autophagosome formation, caused neurodegeneration characterized by ubiquitin-positive neuropathology [23, 24]. No evidence of proteasome impairment was detected, suggesting that loss of basal autophagy alone leads to ubiquitinated protein accumulation even in the context of normal UPS function. In addition to highlighting the importance of autophagy in protecting neurons from toxic protein accumulation, these studies suggested the possibility of functional cross talk between autophagy and the UPS. Subsequent studies using *Drosophila* genetics provided direct evidence of a compensatory relationship between autophagy and the UPS [56].

But what of autophagy in the context of neurodegenerative disease in vivo? In all studies published to date, autophagy has been found to be neuroprotective in the context of disease. In several different *Drosophila* models of neurodegeneration based on misexpression of disease-related proteins, genetic inhibition of autophagy is detrimental, while pharmacological induction of autophagy with rapamycin is protective [54, 56, 69]. While the effect of TOR inhibition by rapamycin is pleiotropic, in 2 of these studies it was verified that the beneficial effect of rapamycin was autophagy dependent [56, 70]. Consistent results were obtained in a *C. elegans* model expressing a pathologic huntingtin fragment, in which degeneration was enhanced by genetic inhibition of autophagy [71].

Perhaps the most exciting result was the finding that treatment of a transgenic mouse model with CCI-779, an analog of rapamycin, ameliorated several metrics of neurodegeneration, including tremor prevalence, grip strength, body and brain weight, and rotarod performance, thus extending observations to mammals [54].

Can Autophagy Be Co-Opted for Therapeutic Benefit?

The evidence indicating that induction of autophagy is cytoprotective in neurodegenerative disease models raises the tantalizing possibility that this intracellular catabolic pathway may be exploited to clear toxic disease proteins and provide therapeutic benefit for patients. While rapamycin is an FDA-approved drug that has been used extensively in renal transplant patients, it is a less than optimal choice for therapeutic induction of autophagy as it is associated with serious adverse effects, most of which are related to long-term immunosuppression. Consequently, novel approaches to manipulating autophagy in human patients are desirable. Using a yeast screen, one recent study identified several small molecules capable of augmenting autophagy in mammalian cells and further demonstrated therapeutic benefit of these compounds in a *Drosophila* model of neurodegeneration [72]. However, it remains to be seen whether any of these compounds will have clinical benefit and a favorable adverse effect profile in mammalian neurodegeneration models. Recently, overexpression of histone deacetylase 6 (HDAC6), a cytoplasmic deacetylase containing a ubiquitin-binding domain, was found to suppress neurodegeneration in a model of polyglutamine disease and to compensate for defects in the UPS by facilitating autophagic protein degradation [56]. These results suggest that HDAC6 functions at the intersection of the UPS and autophagy and identify HDAC6 as a promising target for pharmacological manipulation in neurodegeneration. Future studies defining the molecular details of autophagic degradation of misfolded proteins will likely provide additional targets for therapeutic intervention in neurodegenerative diseases.

Unanswered Questions and Future Directions

While we now know that misfolded protein accumulation induces autophagy, and that autophagy suppresses neurodegeneration by assisting in the clearance of toxic

protein species, unresolved questions remain. For instance, why is endogenous upregulation of autophagy insufficient to protect against the accumulation of proteotoxic species? Studies of autophagy and degeneration also underscore larger questions relating to fundamental issues in neurodegenerative disease. For instance, why do mutations that affect often ubiquitously expressed proteins specifically cause the demise of specific subpopulations of neurons? And finally, why is the onset of many neurodegenerative diseases age dependent in spite of expression of toxic protein species throughout the lifespan? While the answers to these questions are sure to be multifactorial and complex, perhaps some of the answers are related to the changing nature of autophagy with age. It is possible that upregulation of autophagy is sufficient to effectively clear misfolded proteins and forestall neurodegeneration early in life, but that diminished autophagic protein degradation with age might allow toxic protein accumulation and late-onset development of neurodegenerative pathology. Postmitotic cells such as neurons are likely to be particularly vulnerable to subtle disruptions in protein degradation pathways and membrane homeostasis as they require massive coordination of long-distance trafficking of vesicles and trophic factors. Therefore, a neuronal age-related decline in autophagy combined with the unique homeostatic demands of neurons might largely contribute to the selective sensitivity of neurons to the accumulation of proteotoxins. Support for these notions has come from manipulation of aging pathways in model organisms. Inhibition of insulin signaling pathways prolongs lifespan in multiple species [73]. Strikingly, these pathways involve repression of PI3K and Akt signaling, manipulations which lead to upregulation of autophagy. Furthermore, autophagy is required for extension of lifespan in worms that are deficient in insulin-like signaling pathways, suggesting that mutations that extend lifespan might work by activating autophagy [74]. Recent evidence has now directly linked neurodegeneration to aging by demonstrating that aging pathways strongly influence the onset of disease in *C. elegans* models of neurodegeneration. Expression of amyloid β (A β) in muscle causes the progressive development of motor degeneration, but by delaying the aging process, aggregation of A β and the onset of symptoms is also delayed [75]. In addition, knockdown of autophagy blocks the protective effects of delayed aging, suggesting that neuroprotection associated with manipulation of aging pathways is autophagy dependent [76]. These results are consistent with the notion that protein aggregation and accompanying neuronal dysfunction might occur coin-

cidentially with age-related decline in cellular mechanisms to deal with misfolded protein species. Thus, the age-related onset of pathology in neurodegenerative conditions might be correlated with a decline in autophagic capacity beyond a critical threshold. However, additional studies will be required to define the precise relationship of aging, autophagy and neurodegeneration.

The result suggesting that autophagy contributes to basal turnover of ubiquitinated proteins is a fascinating finding that provokes additional questions. Presently, it is not known whether autophagy contributes nonspecifically to bulk degradation of ubiquitinated proteins, whether a certain subset of ubiquitinated proteins is selectively identified for autophagic degradation, or whether undigested autophagy substrates are futilely ubiquitinated after deposition. And how precisely does central nervous system knockout of autophagy lead to degeneration? Defects in basal autophagy could lead to altered neuronal homeostasis and degeneration through impaired utilization of nutrients or an imbalance of vesicular biogenesis and turnover. Alternatively, neuronal dysfunction might be a more direct result of failed protein degradation with resultant accumulation of ubiquitinated protein aggregates. A final question: is autophagy universally protective in the context of neurodegenerative disease? A recent provocative study implicating autophagy as a contributor to the production of toxic A β species in Alzheimer's disease suggests that this is not necessarily so [48]. Perhaps in the case of some diseases autophagy is initially induced as a neuroprotective response in stressed or injured neurons, but is subsequently overwhelmed or impaired by disease-related factors. This could partly account for evidence that autophagy seems to be both induced and impaired in several major neurodegenerative diseases.

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