Autophagy, amyloidogenesis and Alzheimer disease

Ralph A. Nixon

Center for Dementia Research, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962, Departments of Psychiatry and Cell Biology, NYU School of Medicine, New York, NY 10016, USA

e-mail: nixon@nki.rfmh.org

Accepted 30 October 2007
Journal of Cell Science 120, 4081-4091 Published by The Company of Biologists 2007
doi:10.1242/jcs.019265

Summary

Autophagy is the sole pathway for organelle turnover in cells and is a vital pathway for degrading normal and aggregated proteins, particularly under stress or injury conditions. Recent evidence has shown that the amyloid β peptide is generated from amyloid β precursor protein (APP) during autophagic turnover of APP-rich organelles supplied by both autophagy and endocytosis. Aβ generated during normal autophagy is subsequently degraded by lysosomes. Within neurons, autophagosomes and endosomes actively form in synapses and along neuritic processes but efficient clearance of these compartments requires their retrograde transport towards the neuronal cell body, where lysosomes are most concentrated. In Alzheimer disease, the maturation of autophagolysosomes and their retrograde transport are impeded, which leads to a massive accumulation of 'autophagy intermediates' (autophagic vacuoles) within large swellings along dystrophic and degenerating neurites. The combination of increased autophagy induction and defective clearance of Aβ-generating autophagic vacuoles creates conditions favorable for Aβ accumulation in Alzheimer disease.

Key words: Autophagy, Amyloidogenesis, AD

Introduction

In his report of the first case of Alzheimer disease (AD) in 1907, Alois Alzheimer described the two pathologic lesions that have remained the diagnostic hallmarks of the disease (Fig. 1). One lesion, the neurofibrillary tangle, is present inside affected neurons and later was found to be composed mainly of a form of the microtubule-associated protein tau that is abnormally phosphorylated and aggregated into paired helical filaments (PHFs). A second lesion, termed the senile plaque, consists of small patches of deposited extracellular material interspersed among clusters of axons and dendrites (neurites), many of which are grossly swollen or atrophic. These extracellular deposits were identified some fifty years later as a specific type of amyloid (amyloid-β) composed of fibrillar aggregates of amyloid-β peptide (Aβ) (Glenner and Wong, 1984; Masters and Beyreuther, 2006). Derived by proteolysis from a larger amyloid-β precursor protein (APP), Aβ comprises a set of 40-43-residue polypeptides that have poorly understood biological functions (Pearson and Peers, 2006; Small et al., 2001). In its pathologic oligomeric state, Aβ exerts a range of cytotoxic actions considered important to the evolution of neurodegeneration in AD (Selkoe, 2001; Watson et al., 2005; Small et al., 2001; Laferla et al., 2007; Lee et al., 2006).

Viewed at the ultrastructural level, swollen (dystrophic) neurites in the AD brain contain tau filaments but are much more abundantly filled with collections of vacuolar structures, including acid-phosphatase-positive lysosome-dense bodies enriched in cathepsins (Suzuki and Terry, 1967) (Fig. 1). Moreover, proliferation of lysosomes in the cell bodies of affected neurons (Fig. 1C,D), another obvious feature of the cellular pathology in AD, reflects a strongly upregulated synthesis of components of the lysosomal system (Cataldo et al., 1995; Cataldo et al., 1991; Cataldo et al., 1990). The contribution of the lysosomal system to AD pathogenesis is beginning to be clarified and may be substantial. Recent clues come from detailed ultrastructural analyses showing that the vesicles that accumulate in dystrophic neurites are not actually lysosomes but are mainly autophagic vacuoles (AVs) (Nixon et al., 2005) (Fig. 1E, Fig. 2). These AVs represent intermediate stages in macroautophagy, the lysosomal pathway responsible for degrading cytoplasmic constituents, including organelles. Among the implications of this pathology (Nixon, 2006) is its possible major contribution to the hallmark amyloid lesions of AD, a possibility supported by observations that AVs generate Aβ and fuse with endosomes – another Aβ-generating compartment.

In this Commentary, I consider autophagy in the larger perspective of disturbed endosomal-lysosomal system dysfunction in AD and its relevance to the production and removal of Aβ. Recent reviews of other aspects of Aβ and APP biology are available (Zheng and Koo, 2006; De Strooper and Annaert, 2000), and these are covered here only briefly for background information.

APP biology in brief

APP is a member of a family of conserved type 1 membrane proteins, which includes in mammals APP-like protein (APLP) 1 and APLP2 (Coulson et al., 2000; Senechal et al., 2006). Although its function remains uncertain, putative physiological roles in trafficking, neurotrophic signaling, cell adhesion and cell signaling have been proposed (Reinhard et al., 2005; Zheng and Koo, 2006; Hoareau et al., 2006).

After APP is synthesized, the mature glycosylated form of APP in the trans-Golgi network (TGN) is delivered to the plasma membrane, where it is fairly rapidly turned over by either of two mechanisms (Fig. 3). An aspartyl protease at the cell surface, tumor necrosis factor α converting enzyme

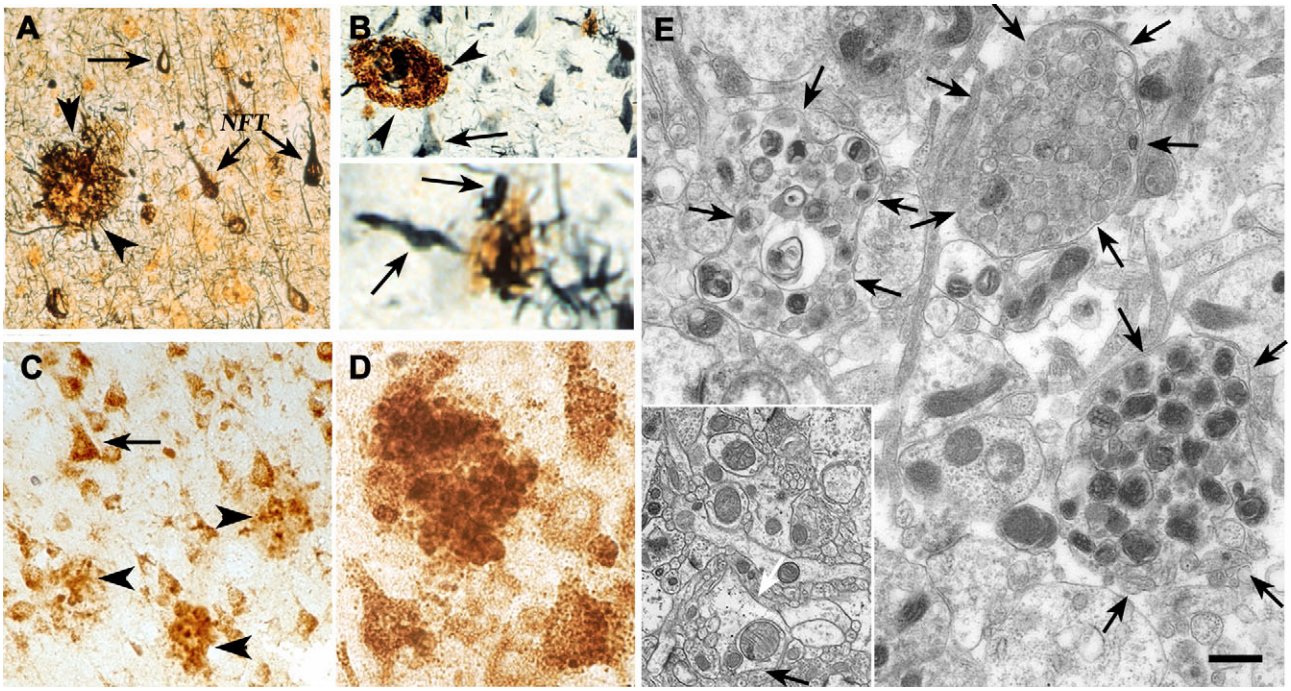

Fig. 1. Pathology of Alzheimer disease. (A) The two hallmark features of Alzheimer disease, β-amyloid plaques (arrowheads) and neurofibrillary tangles (arrows) in AD brain are revealed by the Bielschowsky silver stain. (B) Antibodies against paired-helical-filament (PHF) tau (arrows) and β-amyloid (arrowheads) label PHF-containing neurites associated with amyloid deposits. (C,D) Cathepsin D antibodies decorate lysosomes in cell bodies of (C, arrow) pyramidal neurons and in dystrophic neurites associated with plaques (C, arrowheads; and D). (E) Dystrophic neurites (arrows) are grossly enlarged compared with neurites in normal brain (inset) by electron microscopy. Abnormal swollen neurites contain predominantly AVs of varying morphologies. By contrast, AVs are rare in normal brain (inset). Bar, 500 nm. Panel E reprinted by permission (Nixon et al., 2005).

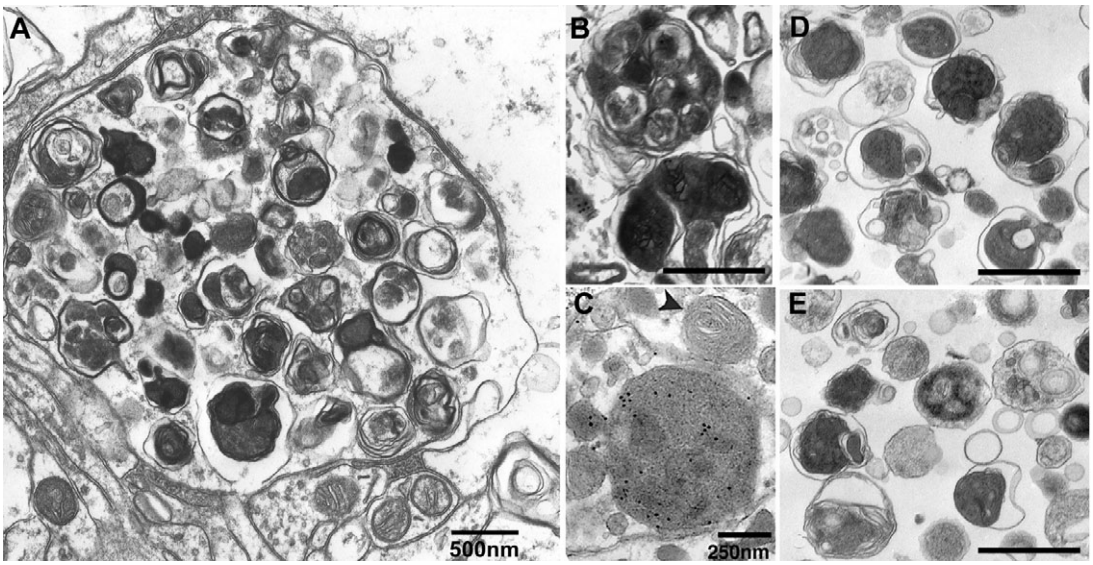

Fig. 2. Ultrastructure of AVs in AD brain. (A-C) Dystrophic neurites are filled predominantly with (A) AVs, including large double-membrane limited immature AVs, such as autophagosomes containing multiple smaller compartments (B) or multilamellar structures (C, arrowhead), and single-membrane vesicles containing electron-dense intraluminal materical, which correspond to late AVs (autophagolysosomes, amphisomes) or lysosomes (C, arrow). The latter structures are immunogold labeled by antibodies to cathepsin D, which identifies them as autophagolysosomes. AVs in AD brain are similar to AVs isolated from livers of mice treated with vinblastine to slow autophagosome-lysosome fusion (panels D,E). Bars, 500 nm. Reprinted with permission (Nixon et al., 2005).

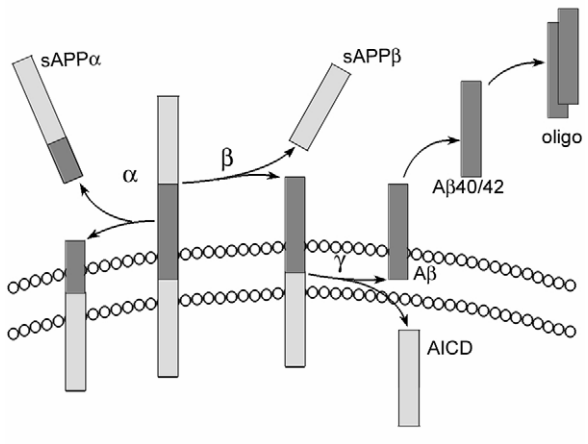

Fig. 3. Functional domains of APP and major sites of proteolytic cleavage (α, β, γ) by APP secretases. sAPP, soluble APP fragment; AICD, APP intracellular domain.

(TACE) or a distintegrin and metalloproteinase 10 (ADAM10) (Lopez-Perez et al., 2001; Buxbaum et al., 1998), also referred to as α-secretase, can mediate α-cleavage of APP within its lumenal/extracellular domain to generate a large soluble N-terminal fragment (sAPPα), which is released from the cell, and a C-terminal fragment (CTF) that remains membrane associated. Alternatively, cell surface APP is internalized within early endosomes, where cleavage at a more distal site along the lumenal/extracellular domain by β-site-APP-cleaving enzyme (BACE, β-secretase) releases a soluble APP fragment (sAPP β) and generates a membrane-associated 99-residue CTF (βCTF) that contains the whole Aβ peptide. A transmembrane aspartyl protease that has optimal activity at low pH distributes predominantly to endosomes (Vassar et al., 1999; Capell et al., 2000; Walter et al., 2001), where βCTF has been shown to be generated (Grbovic, 2003; Mathews et al., 2002), although additional BACE is found in the TGN (von Arnim et al., 2006) (Fig. 3).

Aβ is generated from βCTF by an intramembrane γ-cleavage that yields predominantly a 40-mer peptide (Aβ40) and smaller amounts of a 42-mer peptide (Aβ42). The γ-cleavage of APP is mediated by a γ-secretase complex composed of the proteins presenilin (PS), nicastrin, APH1 and PEN2 (St George-Hyslop, 2000; Edbauer et al., 2003; Go et al., 2004). Components of the complex have been detected in many cellular locations, including at the plasma membrane and in early endosomes (Runz et al., 2002), late endosomes, autophagic vacuoles (Yu et al., 2005; Yu et al., 2004) and lysosomes (Pasternak et al., 2003; Pasternak et al., 2004; Cupers et al., 2001) (Fig. 3).

Aβ generated within cells is the suspected source of most Aβ in extracellular diffuse or fibrillar β-amyloid plaque deposits. Before Aβ is deposited extracellularly, the intracellular pool of soluble Aβ rises substantially in endosomal-lysosomal compartments (Cataldo et al., 2004a; Takahashi et al., 2004) and remains substantial even in the heavily plaque-laden AD brain (Naslund et al., 2000). Cognitive deficits have been reported in AD models in which intracellular Aβ levels are elevated in the absence of plaque deposition (Koistinaho et al., 2001; Laferla et al., 2007), supporting other evidence that Aβ is toxic intracellularly before it is released (McGowan et al., 2005). Once it is released, extracellular Aβ in soluble or aggregated form has been proposed to interact pathologically with surface receptors (Hseih et al., 2006; Snyder et al., 2006), affect the membrane lipid bilayer directly (Marchesi, 2005) or act in endosomal-lysosomal compartments after re-internalization (Almeida et al., 2006).

Aβ degradation in vivo is mediated by several proteases whose overexpression or deletion alters brain Aβ levels appropriately in mice (Guenette, 2005; Eckman and Eckman, 2005). The best-characterized proteases in this group are all zinc metallopeptidases: neprilysin (Iwata et al., 2000); insulin-degrading enzyme (Kurochkin and Goto, 1994; McDermott and Gibson, 1997) and the endothelin-converting enzymes ECE1 and ECE2 (Eckman et al., 2001; Eckman et al., 2003). Given their intracellular localizations and pH optima, these proteases are unlikely to operate in highly acidic compartments such as late endosomes and autolysosomes. Although less well studied, the cysteine protease cathepsin B in lysosomes also degrades Aβ peptides, especially the aggregation-prone species Aβ1-42. Cathepsin B deletion increases Aβ1-42 levels and worsens plaque deposition in mice expressing familial AD-mutant human APP (Mueller-Steiner et al., 2006), whereas virus-mediated overexpression of the enzyme has the opposite effect.

The lysosomal system in neurons

The lysosomal system (Fig. 4) is defined broadly as a family of communicating acidic compartments pH (3.5-6.0), which contain varying levels of >80 'lysosomal' acid hydrolases. These include nearly two dozen proteases (cathepsins) of varying catalytic classes and peptide-bond specificities. Collectively, the cathepsins, which act across a broad range of acidic pH values, degrade most proteins rapidly to their component amino acids, although post-translational enzymatic and chemical modifications of proteins during aging and in disease states may render some of the proteins more resistant to degradation and promote the accumulation of partially degraded protein and lipid as lipofuscin within residual bodies (lipofusion granules) (Terman et al., 2006). Newly synthesized acidic hydrolases are extensively processed in the endoplasmic reticulum (ER) and the Golgi complex before being engaged by either of two mannose-6-phosphate receptors (MPRs), cation-dependent 46 kDa MPR (CD-MPR) and cation-independent 215 kDa MPR (CI-MPR), which deliver them to late endosomes (LEs) (Eskelinen, 2006) before recycling to the TGN (Mullins and Bonifacino, 2001).

Substrates for degradation are delivered to lysosomes by two general routes heterophagy (receptor-mediated endocytosis, pinocytosis, phagocytosis) and autophagy, which carry extracellular and intracellular constituents, respectively (Fig. 4). Both routes have relevance to APP processing and to AD pathogenesis.

APP processing in the endosomal-lysosomal pathway

Material internalized by receptor-mediated endocytosis or bulk-phase endocytosis (pinocytosis) is sorted within several types of endosome for delivery back to the plasma membrane, to the TGN for further packaging and trafficking, or to late

endosomes for lysosomal degradation (van der Goot and Gruenberg, 2006). APP reaches each of these destinations and is potentially processed at each. Aβ production slows markedly when endocytosis or APP internalization is selectively blocked and it accelerates when endocytosis is stimulated. βCTF is generated in early endosomes (Grbovic et al., 2003; Mathews et al., 2002), which contain BACE (Huse et al., 2000; Vassar et al., 1999) and PS-1 (Lah and Levy, 2000). In AD, Aβ has been detected in abnormally large endosomes carrying the early-endosomal marker Rab5 (Cataldo et al., 2004a). The

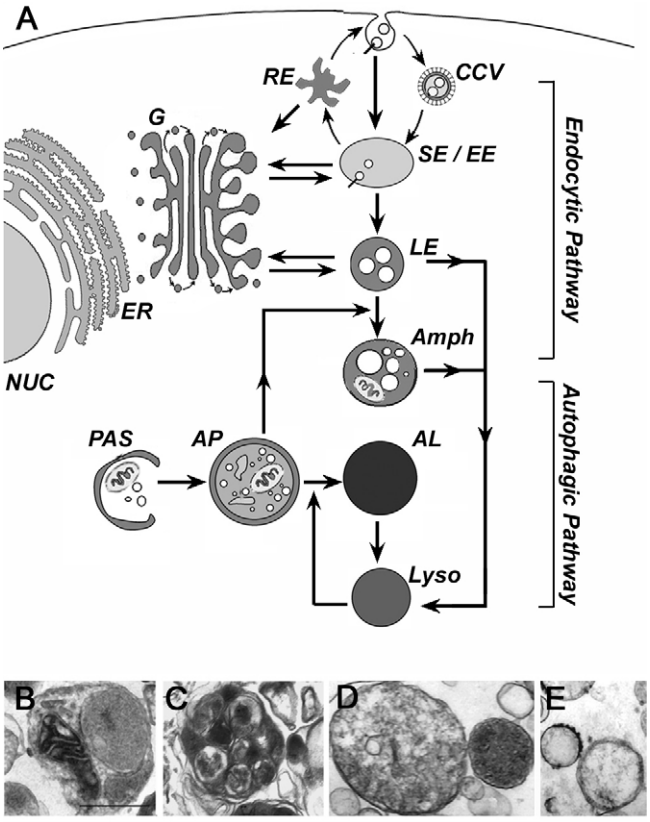

Fig. 4. (A) Schematic of the lysosomal system illustrating the endocytic and autophagic pathways to the (A) lysosome and the ultrastructure of (B-E) specific compartments. The major organelles of the autophagic pathway are a pre-autophagic structure (PAS), which sequesters large areas of cytoplasm within a double membrane-limited autophagosome (AP). This organelle receives hydrolases by fusing with either a lysosome to form an autophagolysosome (AL) or with a late endosome/multivesicular body (LE/MVB) to form an amphisome (Gordon and Seglen, 1998; Liou et al., 1997). Efficient digestion of substrates within these compartments in both cases yields a lysosome containing mainly acid hydrolases. (B) Internalized materials entering the endocytic pathway are directed to early (sorting) endosomes (EE), which mature to LE/MVB. (C) An autophagosome, which is hydrolase-negative, contains recognizable but partially digested organelle compartments. (D) Another type of immature AV is double-membrane limited and contains heterogeneous intraluminal materials, including other organelles. (E) Following further substrate digestion, the content of an autolysosomes is amorphous and less dense. Complete digestion of substrates within autolysosomes ultimately yields lysosomes, which are smaller, less dense vesicles containing mainly lysosomal hydrolases (E). Bar, 500 nm.

increased expression of Rab5 and other effectors of early endosome fusion early in AD (Cataldo et al., 1996; Cataldo et al., 2000) elevates Aβ production in endosomes when AD is modeled in cells (Grbovic et al., 2003), and leads to enlargement of late endosomes/lysosomes (A. M. Cataldo, unpublished).

Early endosomes contain a subset of cathepsins delivered by CD-MPR (Mullins and Bonifacino, 2001). At initial stages of AD, cathepsin D and B levels rise in Rab5-positive endosomes owing to the upregulated expression of CD-MPR in affected neurons. Since CD-MPR overexpression substantially elevates Aβ production in cells (Grbovic et al., 2003), this is relevant to AD pathogenesis. The effect requires targeting of overexpressed CD-MPR specifically to Rab5 endosomes, although whether cathepsins contribute indirectly or directly to this APP processing is not known. In this regard, cathepsins D and B do have β- and γ-secretase activity towards APP model peptides in vitro (Mackay et al., 1997; Ladror et al., 1994; Dreyer et al., 1994; Chevallier et at., 1997; Hook et al., 2007), although degradation of Aβ is likely to be their principal function, except possibly in some pathological circumstances.

As early endosomes become degradative late endosomes, regions of the surface membrane bud off into the endosome lumen to form a multivesicular body (MVB) (van der Goot and Gruenberg, 2006). This facilitates ubiquitin-dependent sorting of MVB cargo for degradation by lysosomal hydrolases or recycling to other cellular sites (Russell et al., 2006). The contents of late endosomes/MVBs have several different fates relevant to APP processing. Through either fusion or transient 'kiss and run' interactions, late endosomes exchange content with lysosomes, which creates a dynamic system of late-endosome–lysosome hybrids (Mullins and Bonifacino, 2001). In addition, late endosomes/MVBs fuse with autophagosomes in the autophagy pathway to create hybrid 'amphisome' structures, which mature to lysosomes upon further acidification (Berg et al., 1998). In neurons, there is substantial fusion of endosomes with these autophagy-related compartments (Larsen and Sulzer, 2002), which indicates that endocytosis is a significant entry point for APP and APP secretases into the autophagic pathway. The dynamic changes in substrate and hydrolase composition and internal pH within interacting endocytic and autophagic compartments create environments favorable for either production or degradation of Aβ, depending on the health of the cells.

MVBs and late endosomes are relatively rich in APP and APP secretases, and those in AD brain and mouse models of AD contain Aβ peptide (Gouras et al., 2000; Takahashi et al., 2002). Aβ has also been seen within Rab7-positive vesicular compartments corresponding to late endosomes/MVBs or autophagic vacuoles along neuritic processes of CNS-derived neuronal cells in culture (Muresan and Muresan, 2006). In mice overexpressing a familial mutant form of APP, Aβ42 is detected in compartments containing the late endosomal marker Tsg101, where its appearance is linked to defective ubiquitin-dependent sorting and degradation of endocytic cargo, such as the EGF receptor (Almeida et al., 2006). In Niemann-Pick disease, a mutated endosomal protein, NPC1, impairs cholesterol trafficking from late endosomes, resulting in a developmental disorder that has neuropathologic features in common with AD (Nixon, 2004), including neurofibrillary tangles and the accumulation of Aβ in enlarged endosomes (Jin

et al., 2004; Yamazaki et al., 2001). Aβ-dependent and -independent endosome dysfunction can therefore promote further abnormalities of APP processing, including increased delivery of APP-rich compartments to the autophagy pathway.

Autophagy – a new APP-processing pathway

Autophagy refers to at least three processes by which intracellular constituents enter lysosomes for degradation: chaperone-mediated autophagy (CMA), microautophagy and macroautophagy (Cuervo, 2004). In CMA, cytosolic proteins containing a KFERQ motif are selectively targeted to the lysosomal lumen for degradation. Notably APP and synuclein, a protein implicated in Parkinson disease, contain this targeting sequence (Massey et al., 2004), and impaired CMA-mediated degradation contributes to accumulation of mutant α-synuclein in the disease (Cuervo et al., 2004). In microautophagy, small quantities of cytoplasm non-selectively enter lysosomes when the lysosomal membrane invaginates and pinches off small vesicles for digestion within the lumen. Finally, macroautophagy, a pathway conserved from yeast to mammals, mediates large-scale degradation of cytoplasmic constituents (Fig. 4). This process has been reviewed in detail (Shintani and Klionsky, 2004; Levine and Klionsky, 2004; Seglen and Bohley, 1992; Dunn, 1994; Ohsumi, 2001; Mizushima et al., 2002). Hereafter, macroautophagy is referred to by the general term autophagy unless otherwise indicated.

Autophagy is initiated when an 'isolation membrane' is created from a pre-autophagosomal structure (PAS) (Fig. 4) and sequesters a region of cytoplasm to form a double-membrane-limited autophagosome. The translocation of the autophagy-gene-related (Atg) protein LC3-II to the autophagosome membrane is commonly used as a marker of autophagosome formation (Fig. 5). Digestion of sequestered material within autophagosomes is initiated when lysosomes fuse with the outer membrane of the autophagosome, although, as discussed earlier, late endosomes can also fuse with autophagosomes (Gordon and Seglen, 1988; Liou et al., 1997). Induction of autophagy is regulated by the mTOR kinase (mammalian target of rapamycin), which suppresses autophagy in response to signaling by growth factors (especially insulin), and elevated nutrient levels, which inhibit AMP-activated protein kinase (AMPK) (Meijer and Codogno, 2006). mTOR-independent induction of autophagy can also occur (Zeng et al., 2006; Furuya et al., 2005). This pathway targets beclin 1 and hVps34, two downstream effectors in the process, and is particularly activated by the

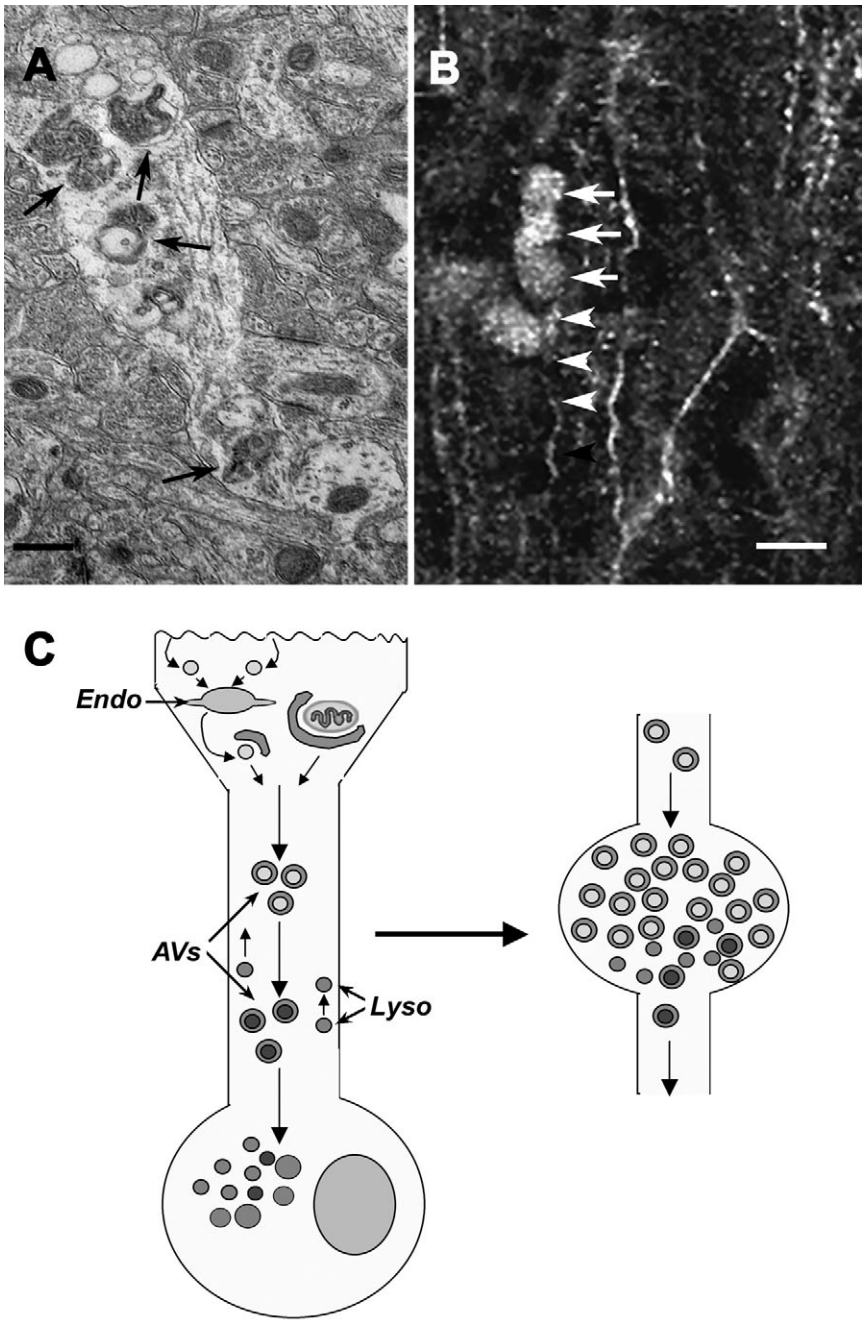

Fig. 5. Macroautophagy is impaired in the PS1-APP mouse model of AD. (A, arrows) Immature AVs accumulate during the early evolution of pathology in a dendrite. (B) Punctate structures exhibiting strong LC3 immunofluorescence can be seen in neurites of PS1-APP mice (arrows) and especially in the swollen dystrophic dendrites of cortical pyramidal neurons. (C) Development of neuritic dystrophy in AD brain. Endocytic trafficking and autophagy are both normally active in neuronal processes. Nascent AVs mature to autophagolysosomes during their retrograde transport, fusing with anterogradely transported lysosomes. Pathological AV accumulation is associated with inhibited retrograde AV transport and impaired autophagosome-lysosome fusion. Fusion of some accumulated AV compartments with the plasma membrane may contribute to local membrane expansion. Bars, 500 nm (A); 10 μm (B). Panels A and B reprinted with permission (Yu et al., 2006).

presence of aggregates of mutant huntingtin, the pathogenic protein in Huntington disease (Ravikumar et al., 2002; Sarkar et al., 2005; Yamamoto et al., 2005; Shibata et al., 2006).

Originally considered mainly an inducible process, autophagy has now been shown to play an important constitutive role maintaining cellular homeostasis by continually degrading and recycling cellular components for biosynthesis and energy production. Complementing the proteasome's role in the turnover of short-lived nuclear and cytosolic proteins (Goldberg, 2003), autophagy is most important for degrading long-lived proteins and is solely responsible for turnover of organelles and protein complexes that are too big to pass through the proteasome. Preventing autophagy in mice by conditionally deleting Atg7 blocks autophagosome formation, leading to accumulation of abnormal membranous structures, deformed mitochondria and ubiquitin-positive aggregates in hepatocytes (Komatsu et al., 2006).

Because AVs are scarce in the healthy brain, neuronal autophagy was initially believed to be relatively inactive; however, neurons accumulate ubiquitylated proteins and degenerate within weeks after macroautophagy is inactivated by knocking out *Atg7* or *Atg5* in mice. Basal levels of autophagy in neurons may, in fact, be quite active. When lysosomal cysteine and aspartyl proteases in primary neurons are inhibited, autophagic vacuoles containing undigested material build up relatively rapidly even though mTOR-dependent autophagy is not further induced (B. Boland, unpublished). These observations suggest that, although autophagy is constitutively active in neurons, AV intermediates are normally scarce because their clearance is exceptionally efficient. The extreme polar morphology of neurons, however, imposes a unique challenge to this otherwise efficient degradative process. Because lysosomes concentrate in or near the neuronal perikaryon, endosomes or autophagosomes continuously generated at the distal ends of axons and dendrites must travel retrogradely long distances before being degraded by lysosomes nearer to the cell body. The AV clearance process is, therefore, vulnerable to disruption (Nixon et al., 2005) (Fig. 5C).

Autophagy is further induced under conditions of cell stress (Terman et al., 2003; Anglade et al., 1997; Kadowaki and Kanazawa, 2003; Shigemitsu et al., 1999; Kanazawa et al., 2003), including neurodegenerative diseases (Nixon et al., 2005; Rubinsztein et al., 2005), and is considered to be a neuroprotective response in these states (Levine and Yuan, 2005; Nixon, 2006).

APP processing in the autophagy pathway

Macroautophagy is an active pathway for turning over APP and generating Aβ peptide (Yu et al., 2005; Yu et al., 2004). AVs contain immunoreactive Aβ peptide and are also enriched in its immediate precursor, βCTF (Fig. 6). Moreover, AVs isolated from several different tissue sources are highly enriched in components of the γ-secretase complex and exhibit high PS-dependent γ-secretase activity (Yu et al., 2004). Although the γ-secretase complex is a constituent of some organelles that become subject to autophagy, PS is localized mainly to the surface of amphisome-like compartments rather than the AV lumen (Fig. 6B). Moreover, this localization is consistent with evidence that certain proteins accumulate in autophagic vacuoles when the PS gene is deleted (Wilson et al., 2004; Esselens et al., 2004) and that PS1 is required for autophagic degradation (Yu et al., 2006). Another γ-secretase complex component, nicastrin, has also been reported to be highly enriched in outer membranes of isolated lysosomes along with PS1 and γ-secretase activity (Pasternak et al., 2003).

When autophagy is induced in mouse fibroblasts by inhibition of mTOR by rapamycin or by nutrient deprivation, the γ-secretase complex translocates from a predominantly endosomal/ER pool to autophagic vacuoles, which accumulate transiently and become the largest cellular pool of γ-secretase activity. Under these conditions, Aβ production rises twofold over that in autophagy-suppressed cells and Aβ immunoreactivity appears within AVs (Yu et al., 2005). In human neurons, serum starvation, which strongly induces autophagy, elevates Aβ levels threefold (LeBlanc et al., 1996). The Aβ generated in AVs is presumably delivered principally to lysosomes and degraded by cathepsins, which have the

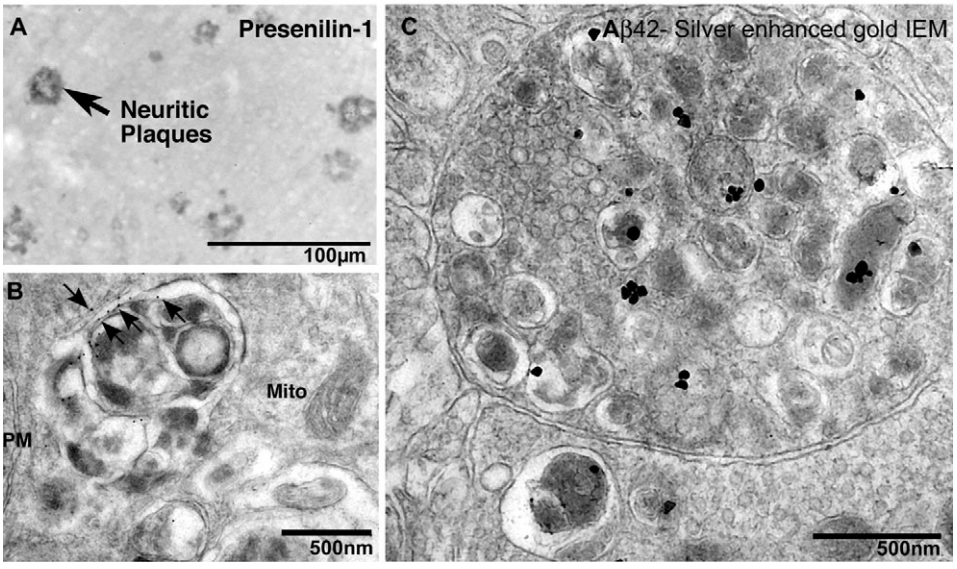

Fig. 6. Presenilin and Aβ peptide selectively immunolocalize to AVs within dystrophic neurites of PS1/APP mice. (A) Antibodies to PS1 strongly decorate neuritic plaques. (B) Immunogold-EM reveals that PS1 localizes principally to the limiting membranes of the AV, while mitochondria (Mito) or plasma membrane (PM) are unlabeled. (C) Aβ42 immunoreactivity is detected within AVs of dystrophic neurites by silver-enhanced immunogold labeling. Reprinted with permission (Yu et al., 2005).

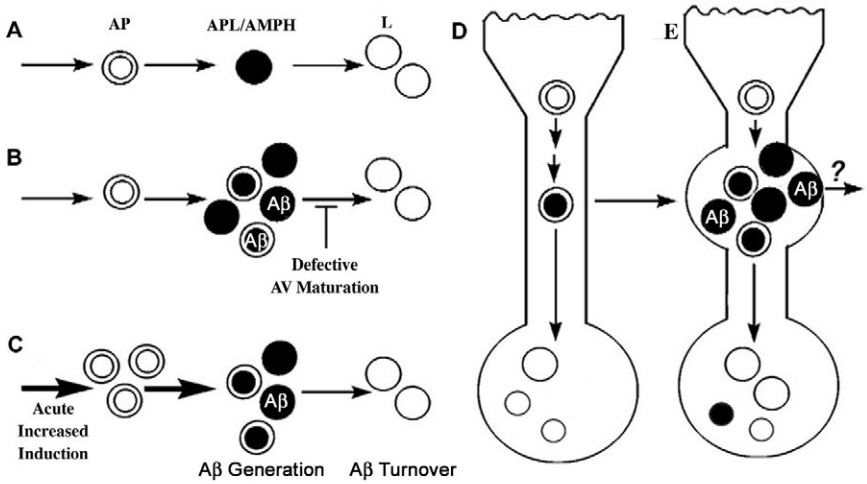

Fig. 7. (A-E) Proposed models of AV accumulation leading to elevated Aβ levels. (A) Usual progression from autophagosomes (AP) to autophagolysosomes (APL) to lysosomes (L). Conditions that result in AV build up are expected to promote Aβ generation and accumulation. (B,C) These conditions include impaired and delayed maturation of autophagosomes to (B) lysosomes or (C) acute induction of autophagy. (D,E) Within neurons, AVs normally mature to lysosomes efficiently as they reach the perikaryon and are usually rare (D). In AD, however, AVs in neurites fail to mature completely to lysosomes either as a cause or consequence of disrupted proteolytic clearance and/or retrograde transport of AVs, thereby promoting the accumulation of AVs capable of generating Aβ and the delayed degradation of Aβ by lysosomes (E). The continued capacity of immature AVs to fuse with other membranous structures, including possibly the plasma membrane, is a possible basis for slow exocytic release of AV contents, including Aβ, from the dystrophic neurite.

necessary cleavage specificity (Grbovic et al., 2003; Heinrich et al., 1999; Bahr and Bendiske, 2002; Florez-McClure et al., 2007). Cathepsin B deletion in mice, for example, elevates Aβ levels in brain, whereas increasing cathepsin B expression has the opposite effect (Mueller-Steiner et al., 2006). Interestingly, human CNS neurons, relative to those of rodents, appear to be particularly dependent on lysosomes for eliminating Aβ generated in the endocytic-autophagic pathways (LeBlanc and Goodyer, 1999).

A fraction of the Aβ generated specifically by autophagy is released extracellularly. Autophagosomes and amphisomes dynamically exchange contents with late endosomes/MVBs. Since these can communicate with endocytic recycling compartments (Luzio et al., 2000) or fuse directly with the plasma membrane and liberate their contents (Jackson et al., 2005), this may account for the Aβ release. A fraction of the Aβ may be exocytosed from cells via MVBs in association with intralumenal vesicles (exosomes), remnants of which can be detected in plaques of AD patient brains (Rajendran et al., 2006). A similar mechanism has been proposed for prion release (Fevrier, 2005).

In the healthy brain, macroautophagy may play a relatively minor role in basal Aβ production because efficient clearance of AVs and lysosomal degradation of Aβ prevent a build-up (Hamazaki, 1996a; Hamazaki, 1996b). After acute autophagy induction in fibroblasts, lysosomes appear to become rate limiting and AVs transiently accumulate (Fig. 7). The increased residence time of these AVs in cells increases the chance of Aβ production and exocytosis through several pathways. Neurons seem to be exceptionally efficient at clearing autophagic vacuoles and, even after strong autophagy induction, AVs are cleared quickly from primary cortical neurons (B. Boland, unpublished). Conditions that delay or impair maturation of AVs to lysosomes, however, would be expected to increase AV numbers and raise intracellular and extracellular Aβ levels. In the AD brain, chronic stasis of AVs within dystrophic neurites increases opportunities for them to receive more APP substrate from transported endosomes and to fuse with the plasma membrane and release Aβ extracellularly (Fig. 7).

Autophagy dysfunction in Alzheimer disease

Macroautophagy is both induced and impaired in AD brain and in a model of AD pathology (PS1-APP mice, which overexpress FAD-related mutant human PS1 and APP), which leads to the accumulation of Aβ-containing AVs within affected neurons (Nixon et al., 2005; Yu et al., 2005). In PS-APP mice, autophagosomes proliferate in dendrites at young ages before β-amyloid is deposited, which indicates that induction of macroautophagy is an early response in the disease and not a consequence of amyloid deposition. The levels of LC3-II rise and both LC3 isoforms redistribute to dendrites from a mainly perikaryal location (Yu et al., 2005) (Fig. 5B). In addition, the expression of many lysosome system components is upregulated (Cataldo et al., 1995; Cataldo et al., 1997) and this upregulation is sustained even at late stages of neuronal degeneration when the expression of most other transcripts has declined (Callahan et al., 1999; Ginsberg et al., 2000). Autophagosomes and other AV subtypes, together with hydrolase-positive dense bodies (Suzuki and Terry, 1967; Cataldo et al., 1991; Kawai et al., 1992), progressively accumulate in large numbers in grossly distended dystrophic neurites, becoming the principal organelles in these structures (Nixon et al., 2005) (Fig. 3). The extensive neuritic dystrophy in AD (Maslish et al., 1993; Schmidt et al., 1994) and characteristic grossly distended neurites filled nearly entirely with AVs are not typical in neurodegenerative diseases that do not generate β-amyloid (Benzing et al., 1993) and, therefore, constitute a uniquely large 'burden' of autophagy-related compartments in the AD brain.

Induction of macroautophagy at early stages of AD, which may increase protein/organelle turnover in the injured and regenerating neurites, diverts APP-rich substrates into the macroautophagy pathway. In fact, APP is highly concentrated in dystrophic neurites and localizes predominantly to AVs (Cras et al., 1991). AVs and APP also accumulate after insults to axons, such as traumatic brain injury, which is associated with increased local Aβ production and deposition, and is a risk factor for AD (Smith et al., 2003). Autophagy-related 'rimmed' vacuoles containing APP, Aβ, BACE and presenilin (Askanas and Engel, 1998; Askanas et al., 1998) also

accumulate in inclusion-body myositis, a rare instance in which β-amyloid is deposited outside the nervous system (Askanas et al., 1998). Upregulated endocytosis at early stages of AD also increases the delivery of APP-rich compartments to the autophagic pathway and is accelerated by *App* triplication, which causes early-onset AD in families and in Down syndrome (Margallo-Lana et al., 2004; Prasher et al., 1998), and by inheritance of the E4 isoform of the apolipoprotein E gene (apo E) (Cataldo et al., 1997), the strongest genetic risk factor for late-onset AD.

Impaired clearance of AVs and their contents by lysosomes might be an even more crucial factor than autophagy induction in the development of pathology. Immature AVs accumulate in dystrophic neurites even though acid-hydrolase-containing dense lysosomes are abundant, which suggests that autophagosomes have access to hydrolase-containing compartments but may not fuse efficiently. A relatively high proportion of double-membrane-limited 'immature' AVs contain cathepsin immunoreactivity but also abundant partially digested substrates. These observations indicate impaired maturation of AVs to lysosomes (Nixon et al., 2005). Indeed, AVs with morphologies similar to those in dystrophic neurites can be produced experimentally by impeding AV clearance either by slowing autophagosome-lysosome fusion or proteolysis within AVs (B. Boland, unpublished). Similar patterns are also observed in vivo after leupeptin administration and in mice, in which the genes encoding cathepsin D or cathepsin B and L are deleted (Koike et al., 2000).

Further evidence for impaired AV clearance in AD is the observation that mutations in PS1 cause early-onset familial AD (Levy-Lahad et al., 1995; Sherrington et al., 1995) and potentiate lysosomal system pathology, amyloidogenesis and neurodegeneration in this disorder (Cataldo et al., 2004b). Moreover, recent studies indicate that PS1 mutations in familial AD compromise macroautophagic turnover of proteins (Yu et al., 2006) and that PS1 may be required for normal lysosomal system turnover of certain proteins (Esselens et al., 2004; Wilson et al., 2004). A loss of PS function leading to altered trafficking of APP secretase or APP-rich compartments is one of several scenarios by which PS mutations could alter Aβ production or clearance and, at the same time, disrupt other neuronal processes dependent on autophagy (Nixon, 2006).

Conclusions

The role of the lysosome system in amyloidogenesis has been elusive. New observations that Aβ may be generated but also turned over at different stages along the same pathway suggest that disruption of the lysosomal system may increase Aβ levels, decrease them or have no net effect, depending on which steps in the pathway are altered. In Alzheimer disease, evidence that neuronal autophagy is induced but is also impaired at late steps in the pathway accounts for the massive accumulations of AVs within dystrophic neurites – a neuropathologic hallmark of AD – and intracellular accumulation of Aβ, which could contribute to its later extracellular deposition in plaques. A growing number of risk factors for AD, including brain aging, are known to induce autophagy, impair its efficiency or both, which probably promotes sporadic AD, the most common form of the disease. Moreover, AD-causing mutations promote autophagy disruption or increase delivery of APP-rich vesicles to the autophagy-lysosomal pathway, further supporting a role for autophagy in accelerating amyloidogenesis in familial AD. Beyond its importance to amyloidogenesis, autophagy dysfunction might promote neuronal cell death by blocking neuroprotective effects of autophagy against apoptosis and limiting accumulation of toxic proteins, including tau. Possible therapies in AD based on modulation of autophagy will require careful targeting of specific steps in the pathway to achieve more efficient digestion, which will require a deeper understanding of the regulation of these individual steps.

I am grateful to Nicole Piorkowski for assistance in manuscript preparation, Corrinne Peterhoff for assistance with graphics, and Ju-Hyun Lee for critical reading of the manuscript. Work from this laboratory has been supported by grants from the National Institute on Aging and the Alzheimer's Association.

References

Almeida, C. G., Takahashi, R. H. and Gouras, G. K. (2006). Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *J. Neurosci.* **26**, 4277-4288.

Anglade, P., Vyas, S., Javoy-Agid, F., Herrero, M. T., Michel, P. P., Marquez, J., Mouatt-Prigent, A., Ruberg, M., Hirsch, E. C. and Agid, Y. (1997). Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol. Histopathol.* **12**, 25-31.

Askanas, V. and Engel, W. K. (1998). Does overexpression of betaAPP in aging muscle have a pathogenic role and a relevance to Alzheimer's disease? Clues from inclusion-body myositis, cultured human muscle, and transgenic mice. *Am. J. Pathol.* **153**, 1673-1677.

Askanas, V., Engel, W. K., Yang, C. C., Alvarez, R. B., Lee, V. M. and Wisniewski, T. (1998). Light and electron microscopic immunolocalization of presenilin 1 in abnormal muscle fibers of patients with sporadic inclusion-body myositis and autosomal-recessive inclusion-body myopathy. *Am. J. Pathol.* **152**, 889-895.

Bahr, B. A. and Bendiske, J. (2002). The neuropathogenic contributions of lysosomal dysfunction. *J. Neurochem.* **83**, 481-489.

Benzing, W. C., Mufson, E. J. and Armstrong, D. M. (1993). Alzheimer's disease-like dystrophic neurites characteristically associated with senile plaques are not found within other neurodegenerative diseases unless amyloid beta-protein deposition is present. *Brain Res.* **606**, 10-18.

Berg, T. O., Fengsurd, M., Stromhaug, P. E., Berg, T. and Seglen, P. O. (1998). Isolation and characterization of rat liver amphisomes. Evidence for fusion of autophagosomes with both early and late endosomes. *J. Biol. Chem.* **273**, 21883-21892.

Buxbaum, J. D., Liu, K. N., Luo, Y., Slack, J. L., Stocking, K. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Cerretti, D. P. and Black, R. A. (1998). Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* **273**, 27765-27767.

Callahan, L. M., Vaules, W. A. and Coleman, P. D. (1999). Quantitative decrease in synaptophysin message expression and increase in cathepsin D message expression in Alzheimer disease neurons containing neurofibrillary tangles. *J. Neuropathol. Exp. Neurol.* **58**, 275-287.

Capell, A., Steiner, H., Romig, H., Keck, S., Baader, M., Grim, M. G., Baumeister, R. and Haass, C. (2000). Presenilin-1 differentially facilitates endoproteolysis of the beta-amyloid precursor protein and Notch. *Nat. Cell Biol.* **2**, 205-211.

Cataldo, A. M., Thayer, C. Y., Bird, E. D., Wheelock, T. R. and Nixon, R. A. (1990). Lysosomal proteinase antigens are prominently localized within senile plaques of Alzheimer's disease: evidence for a neuronal origin. *Brain Res.* **513**, 181-192.

Cataldo, A. M., Paskevich, P. A., Kominami, E. and Nixon, R. A. (1991). Lysosomal hydrolases of different classes are abnormally distributed in brains of patients with Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **88**, 10998-11002.

Cataldo, A. M., Barnett, J. L., Berman, S. A., Li, J., Quarless, S., Bursztajn, S., Lippa, C. and Nixon, R. A. (1995). Gene expression and cellular content of cathepsin D in Alzheimer's disease brain: evidence for early up-regulation of the endosomallysosomal system. *Neuron* **14**, 671-680.

Cataldo, A. M., Hamilton, D. J., Barnett, J. L., Paskevich, P. A. and Nixon, R. A. (1996). Properties of the endosomal-lysosomal system in the human central nervous system: disturbances mark most neurons in populations at risk to degenerate in Alzheimer's disease. *J. Neurosci.* **16**, 186-199.

Cataldo, A. M., Barnett, J. L., Pieroni, C. and Nixon, R. A. (1997). Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. *J. Neurosci.* **17**, 6142-6151.

Cataldo, A. M., Peterhoff, C. M., Troncoso, J. C., Gomez-Isla, T., Hyman, B. T. and Nixon, R. A. (2000). Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am. J. Pathol.* **157**, 277-286.

Cataldo, A. M., Petanceska, S., Terio, N. B., Peterhoff, C. M., Durham, R., Mercken, M., Mehta, P. D., Buxbaum, J., Haroutunian, V. and Nixon, R. A. (2004a). Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. *Neurobiol. Aging* **25**, 1263-1272.

Cataldo, A. M., Peterhoff, C. M., Schmidt, S. D., Terio, N. B., Duff, K., Beard, M., Mathews, P. M. and Nixon, R. A. (2004b). Presenilin mutations in familial Alzheimer's disease and transgenic mouse models accelerate neuronal lysosomal pathology. *J. Neuropathol. Exp. Neurol.* **63**, 821-830.

Chevallier, N., Vizzavona, J., Marambaud, P., Baur, C. P., Spillantini, M., Fulcrand, P., Martinez, J., Goedert, M., Vincent, J. P. and Checler, F. (1997). Cathepsin D displays in vitro beta-secretase-like specificity. *Brain Res.* **750**, 11-19.

Coulson, E. J., Paliga, K., Beyreuther, K. and Masters, C. L. (2000). What the evolution of the amyloid protein precursor supergene family tells us about its function. *Neurochem. Int.* **36**, 75-184.

Cras, P., Kawai, M., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B. and Perry, G. (1991). Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **88**, 7552-7556.

Cuervo, A. M. (2004). Autophagy: many paths to the same end. *Mol. Cell. Biochem.* **263**, 55-72.

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T. and Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperson-mediated autophagy. *Science* **305**, 1292-1295.

Cupers, P., Bentahir, M., Craessaerts, K., Orlans, I., Vanderstichele, H., Saftig, P., De Strooper, B. and Annaert, W. (2001). The discrepancy between presenilin subcellular localization and gamma-secretase processing of amyloid precursor protein. *J. Cell Biol.* **154**, 731-740.

De Strooper, B. and Annaert, W. (2000). Proteolytic processing and cell biological functions of the amyloid precursor protein. *J. Cell Sci.* **113**, 1857-1870.

Dreyer, R. N., Bausch, K. M., Fracasso, P., Hammond, L. J., Wunderlich, D., Wirak, D. O., Davis, G., Brini, C. M., Buckholz, T. M., Konig, G. et al. (1994). Processing of the pre-beta-amyloid protein by cathepsin D is enhanced by a familial Alzheimer's disease mutation. *Eur. J. Biochem.* **224**, 265-271.

Dunn, W. A., Jr (1994). Autophagy and related mechanisms of lysosome-mediated protein degradation. *Trends Cell Biol.* **4**, 139-143.

Eckman, E. A. and Eckman, C. B. (2005). Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. *Biochem. Soc. Trans.* **33**, 1101-1105.

Eckman, E. A., Reed, D. K. and Eckman, C. B. (2001). Degradation of the Alzheimer's amyloid beta peptide by endothelin-converting enzyme. *J. Biol. Chem.* **276**, 24540-24548.

Eckman, E. A., Watson, M., Marlow, L., Sambamurti, K. and Eckman, C. B. (2003). Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. *J. Biol. Chem.* **278**, 2081-2084.

Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H. and Haass, C. (2003). Reconstitution of gamma-secretase activity. *Nat. Cell Biol.* **5**, 486-488.

Eskelinen, E. L. (2006). Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Mol. Aspects Med.* **27**, 495-502.

Esselens, C., Oorschot, V., Baert, V., Raemaekers, T., Spittaels, K., Serneels, L., Zheng, H., Saftig, P., De Strooper, B., Klumperman, J. et al. (2004). Presenilin 1 mediates the turnover of telencophalin in hippocampal neurons via an autophagic degradative pathway. *J. Cell Biol.* **166**, 1041-1054.

Fevrier, B. (2005). Exosomes: a bubble ride for prions? *Traffic* **6**, 10-17.

Florez-McClure, M. L., Hohsfield, L. A., Fonte, G., Bealor, M. T. and Link, C. D. (2007). Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in C. elegans. *Autophagy* **3**, 569-580.

Furuya, N., Yu, J., Byfield, M., Pattingre, S. and Levine, B. (2005). The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. *Autophagy* **1**, 46-52.

Ginsberg, S. D., Hemby, S. E., Lee, V. M., Eberwine, J. H. and Trojanowski, J. Q. (2000). Expression profile of transcripts in Alzheimer's disease tangle-bearing CA1 neurons. *Ann. Neurol.* **48**, 77-87.

Glenner, G. G. and Wong, C. W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**, 885-890.

Go, Y. M., Gipp, J. J., Mulcahy, R. T. and Jones, D. P. (2004). H2O2-dependent activation of GCLC-ARE4 reporter occurs by mitogen-activated protein kinase pathways without oxidation of cellular glutathione or thioredoxin-1. *J. Biol. Chem.* **279**, 5837-5845.

Goldberg, A. L. (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature* **426**, 895-899.

Gordon, P. B. and Seglen, P. O. (1988). Prelysosomal convergence of autophagic and endocytic pathways. *Biochem. Biophys. Res. Commun.* **151**, 40-47.

Gouras, G. K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J. P., Haroutunian, V., Buxbaum, J. D. and Xu, H. (2000). Intraneuronal Abeta42 accumulation in human brain. *Am. J. Pathol.* **156**, 15-20.

Grbovic, O. M., Mathews, P. M., Jiang, Y., Schmidt, S. D., Dinaker, R., Summers-Terio, N. B., Ceresa, B. P., Nixon, R. A. and Cataldo, A. M. (2003). Rab5-stimulated up-regulation of the endocytic pathway increases intracellular bCTF levels and Ab production. *J. Biol. Chem.* **278**, 31261-31268.

Guenette, S. Y. (2005). Mechanisms of Abeta clearance and catabolism. *Neuromol. Med.* **4**, 147-160.

Hamazaki, H. (1996a). A beta-amyloid peptide variant related with familial Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis is poorly eliminated by cathepsin D. *FEBS Lett.* **397**, 313-315.

Hamazaki, H. (1996b). Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid protein. *FEBS Lett.* **396**, 139-142.

Heinrich, M., Wickel, M., Schneider-Brachert, W., Sandberg, C., Gahr, J., Schwandner, R., Weber, T., Saftig, P., Peters, C., Brunner, J. et al. (1999). Cathepsin D targeted by acid sphingomyelinase-derived ceramide. *EMBO J.* **18**, 5252-5263. Erratum: *EMBO J.* **19**, 315.

Hoareau, C., Borrell, V., Soriano, E., Krebs, M. O., Prochiantz, A. and Allinquant, B. (2006). APP cytoplasmic domain antagonizes reelin neurite outgrowth inhibition of hippocampal neurons. *Neurobiol. Aging*. In press.

Hook, V., Kindy, M. and Hook, G. (2007). Cysteine protease inhibitors effectively reduce in vivo levels of brain beta-amyloid related to Alzheimer's disease. *Biol. Chem.* **388**, 247-252.

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S. and Malinow, R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* **52**, 831-843.

Huse, J. T., Pijak, D. S., Leslie, G. J., Lee, V. M. and Doms, R. W. (2000). Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. *J. Biol. Chem.* **275**, 33729-33737.

Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Sekiguchi, M., Hosoki, E., Kawashima-Morishima, M., Lee, H. J., Hama, E., Sekine-Aizawa, Y. et al. (2000). Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat. Med.* **6**, 143-150.

Jackson, G. S., McKintosh, E., Flechsig, E., Prodromidou, K., Hirsch, P., Linehan, J., Brandner, S., Clarke, A. R., Weissmann, C. and Collinge, J. (2005). An enzyme-detergent method for effective prion decontamination of surgical steel. *J. Gen. Virol.* **86**, 869-878.

Jin, L. W., Shie, F. S., Maezawa, I., Vincent, I. and Bird, T. (2004). Intracellular accumulation, of, amyloidogenic fragments, of, amyloid-beta precursor protein, in, neurons with, Niemann-Pick type C defects is associated with, endosomal abnormalities. *Am. J. Pathol.* **64**, 975-985. Erratum: *Am. J. Pathol.* **165**, 1447.

Kadowaki, M. and Kanazawa, T. (2003). Amino acids as regulators of proteolysis. *J. Nutr.* **133**, 2052S-2056S.

Kanazawa, T., Taneike, I., Akaishi, R., Yoshizawa, F., Furuya, N., Fujimura, S. and Kadowaki, M. (2003). Amino acids and insulin control autophagic proteolysis through different signaling pathways in relation to mTOR in isolated rat hepatocytes. *J. Biol. Chem.* **279**, 8452-8459.

Kawai, M., Cras, P., Richey, P., Tabaton, M., Lowery, D. E., Gonzalez-DeWhitt, R. A., Greenberg, B. D., Gambetti, P. and Perry, G. (1992). Subcellular localization of amyloid precursor protein in senile plaques of Alzheimer's disease. *Am. J. Pathol.* **140**, 947-958.

Koike, M., Nakanishi, H., Saftig, P., Ezaki, J., Isahara, K., Ohsawa, Y., Schulz-Schaeffer, W., Watanabe, T., Waguri, S., Kametaka, S. et al. (2000). Cathepsin d deficiency induces lysosomal storage with ceroid lipofusion in mouse CNS neurons. *J. Neurosci.* **20**, 6898-6909.

Koistinaho, M., Ort, M., Cimadevilla, J. M., Vondrous, R., Cordell, B., Koistinaho, J., Bures, J. and Higgins, L. S. (2001). Specific spatial learning deficits become severe with age in beta-amyloid precursor protein transgenic mice that habor diffuse beta-amyloid deposits but do not form plaques. *Proc. Natl. Acad. Sci. USA* **98**, 14675-14680.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Kolke, M., Uchivama, Y., Kominami, E. et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441**, 880-884.

Kurochkin, I. V. and Goto, S. (1994). Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. *FEBS Lett.* **345**, 33-37.

Ladror, U. S., Snyder, S. W., Wang, G. T., Holzman, T. F. and Krafft, G. A. (1994). Cleavage at the amino and carboxyl termini of Alzheimer's amyloid-beta by cathepsin D. *J. Biol. Chem.* **269**, 18422-18428.

Laferla, F. M., Green, K. N. and Oddo, S. (2007). Intracellular amyloid-beta in Alzheimer's disease. *Nat. Rev. Neurosci*. In press.

Lah, J. J. and Levey, A. I. (2000). Endogenous presenilin-1 targets to endocytic rather than biosynthetic compartments. *Mol. Cell. Neurosci.* **16**, 111-126.

Larsen, K. E. and Sulzer, D. (2002). Autophagy in neurons: a review. *Histol. Histopathol.* **17**, 897-908.

LeBlanc, A. C. and Goodyer, C. G. (1999). Role of endoplasmic reticulum, endosomallysosomal compartments, and microtubules in amyloid precursor protein metabolism of human neurons. *J. Neurochem.* **72**, 1832-1842.

LeBlanc, A. C., Xue, R. and Gambetti, P. (1996). Amyloid precursor protein metabolism in primary cell cultures of neurons, astrocytes, and microglia. *J. Neurochem.* **66**, 2300-2310.

Lee, H. G., Zhu, X., Nunomura, A., Perry, G. and Smith, M. A. (2006). Amyloid beta: the alternate hypothesis. *Curr. Alzheimer Res.* **3**, 75-80.

Levine, B. and Klionsky, D. J. (2004). Development by self-digestion; molecular mechanisms and biological functions of autophagy. *Dev. Cell* **6**, 463-477.

Levine, B. and Yuan, J. (2005). Autophagy in cell death: an innocent convict? *J. Clin. Invest.* **115**, 2679-2688.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingell, W. H., Yu, C. E., Jondro, P. D., Schmidt, S. D., Wang, K. et al. (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus [see comments]. *Science* **269**, 973-977.

Liou, W., Geuze, H. J., Geelen, M. J. and Slot, J. W. (1997). The autophagic and endocytic pathways converge at the nascent autophagic vacuoles. *J. Cell Biol.* **136**, 61-70.

Lopez-Perez, E., Zhang, Y., Frank, S. J., Creemers, J., Seidah, N. and Checler, F. (2001). Constitutive alpha-secretase cleavage of the beta-amyloid precursor protein in the furin-deficient LoVo cell line: involvement of the pro-hormone convertase 7 and the disintegrin metalloprotease ADAM10. *J. Neurochem.* **76**, 1532-1539.

Luzio, J. P., Rous, B. A., Bright, N. A., Pryor, P. R., Mullock, B. M. and Piper, R. C. (2000). Lysosome-endosome fusion and lysosome biogenesis. *J. Cell Sci.* **113**, 1515-1524.

Mackay, E. A., Ehrhard, A., Moniatte, M., Guenet, C., Tardif, C., Tarnus, C., Sorokine, O., Heintzelmann, B., Nay, C., Remy, J. M. et al. (1997). A possible role for cathepsins D, E, and B in the processing of beta- amyloid precursor protein in Alzheimer's disease. *Eur. J. Biochem.* **244**, 414-425.

Marchesi, V. T. (2005). An alternative interpretation of the amyloid Ab hypothesis with regard to the pathogenesis of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **102**, 9093-9098.

Margallo-Lana, M., Morris, C. M., Gibson, A. M., Tan, A. L., Kay, D. W., Tyrer, S. P., Moore, B. P. and Ballard, C. G. (2004). Influence of the amyloid precursor protein locus on dementia in Down syndrome. *Neurology* **62**, 1996-1998.

Masliah, E., Mallory, M., Deerinck, T., DeTeresa, R., Lamont, S., Miller, A., Terry, R. D., Carragher, B. and Ellisman, M. (1993). Re-evaluation of the structural organization of the neuritic plaques in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **52**, 619-632.

Massey, A., Kiffin, R. and Cuervo, A. M. (2004). Pathophysiology of chaperone-mediated autophagy. *Int. J. Biochem. Cell Biol.* **36**, 2420-2434.

Masters, C. and Beyreuther, K. (2006). Pathways to the discovery of the Abeta amyloid of Alzheimer's disease. *J. Alzheimers Dis.* **9**, 155-161.

Mathews, P. M., Guerra, C. B., Jiang, Y., Grbovic, O. M., Kao, B. H., Schmidt, S. D., Dinakar, R., Mercken, M., Hille-Rehfeld, A., Rohrer, J. et al. (2002). Alzheimer's disease-related overexpression of the cation-dependent mannose 6-phosphate receptor increases Ab secretion: role for altered lysosomal hydrolase distribution in bamyloidogenesis. *J. Biol. Chem.* **277**, 5299-5307.

McDermott, J. R. and Gibson, A. M. (1997). Degradation of Alzheimer's beta-amyloid protein by human and rat brain peptidases: involvement of insulin-degrading enzyme. *Neurochem. Res.* **22**, 49-56.

McGowan, E., Pickford, F., Kim, J., Onstead, L., Eriksen, J., Yu, C., Skipper, L., Murphy, M. P., Beard, J., Des, P. et al. (2005). Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron* **47**, 191-199.

Meijer, A. J. and Codogno, P. (2006). Signalling and autophagy regulation in health, aging and disease. *Mol. Apsects Med.* **27**, 411-425.

Mizushima, N., Ohsumi, Y. and Yoshimori, T. (2002). Autophagosome formation in mammalian cells. *Cell Struct. Funct.* **27**, 421-429.

Mueller-Steiner, S., Zhou, Y., Arai, H., Roberson, E. D., Sun, B., Chen, J., Wang, X., Yu, G., Esposito, L., Mucke, L. et al. (2006). Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron* **51**, 703-714.

Mullins, C. and Bonifacino, J. S. (2001). The molecular machinery for lysosome biogenesis. *BioEssays* **23**, 333-343.

Muresan, Z. and Muresan, V. (2006). Neuritic deposits of amyloid-beta peptide in a subpopulation of central nervous system-derived neuronal cells. *Mol. Cell. Biol.* **26**, 4982-4997.

Naslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P. and Buxbaum, J. D. (2000). Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* **283**, 1571-1577.

Nixon, R. A. (2004). Niemann-Pick Type C disease and Alzheimer's disease: the APP-endosome connection fattens up. *Am. J. Pathol.* **164**, 757-761.

Nixon, R. A., Wegiel, J., Kumar, A., Yu, W. H., Peterhoff, C., Cataldo, A. and Cuervo, A. M. (2005). Extensive involvement of autophagy in Alzheimer disease: an immunoelectron microscopy study. *J. Neuropathol. Exp. Neurol.* **64**, 113-122.

Nixon, R. A. (2006). Autophagy in neurodegenerative disease: friend, foe or turncoat? *Trends Neurosci.* **29**, 528-535.

Ohsumi, Y. (2001). Molecular dissection of autophagy: two ubiquitin-like systems. *Nat. Rev. Mol. Cell Biol.* **2**, 211-216.

Pasternak, S. H., Bagshaw, R. D., Guiral, M., Zhang, S., Ackerley, C. A., Pak, B. J., Callahan, J. W. and Mahuran, D. J. (2003). Presenilin-1, nicastrin, amyloid precursor protein, and gamma-secretase activity are co-localized in the lysosomal membrane. *J. Biol. Chem.* **278**, 26687-26694.

Pasternak, S. H., Callahan, J. W. and Mahuran, D. J. (2004). The role of the endosomal/lysosomal system in amyloid-beta production and the pathophysiology of Alzheimer's disease: reexamining the spatial paradox from a lysosomal perspective. *J. Alzheimers Dis.* **6**, 53-65.

Pearson, H. A. and Peers, C. (2006). Physiological roles for amyloid beta peptides. *J. Physiol.* **575**, 5-10.

Prasher, V. P., Farrer, M. J., Kessling, A. M., Fisher, E. M., West, R. J., Barber, P. C. and Butler, A. C. (1998). Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann. Neurol.* **43**, 380-383.

Rajendran, L., Honsho, M., Zahn, T. R., Keller, P., Geiger, K. D., Verkade, P. and Simons, K. (2006). Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. USA* **103**, 11172-11177.

Ravikumar, B., Duden, R. and Rubinsztein, D. C. (2002). Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum. Mol. Genet.* **11**, 1107-1117.

Reinhard, C., Herbert, S. S. and De Strooper, B. (2005). The amyloid-beta precursor protein: integrating structure with biological function. *EMBO J.* **24**, 3996-4006.

Rubinsztein, D. C., DiFiglia, M., Heintz, N., Nixon, R. A., Qin, Z. H., Ravikumar, B., Stefanis, L. and Tolkovsky, A. (2005). Autophagy and its possible roles in nervous system diseases, damage and repair. *Autophagy* **1**, 11-22.

Runz, H., Rietdorf, J., Tomic, I., de Bernard, M., Beyreuther, K., Pepperkok, R. and Hartmann, T. (2002). Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J. Neurosci.* **22**, 1679-1689.

Russell, M. R., Nickerson, D. P. and Odorizzi, G. (2006). Molecular mechanisms of late endosome morphology, identity and sorting. *Curr. Opin. Cell Biol.* **18**, 422-428.

Sarkar, S., Floto, R. A., Berger, Z., Imarisio, S., Cordenier, A., Pasco, M., Cook, L. J. and Rubinsztein, D. C. (2005). Lithium induces autophagy by inhibiting inositol monophosphatase. *J. Cell Biol.* **170**, 1101-1111.

Schmidt, M. L., DiDario, A. G., Lee, V. M. and Trojanowski, J. Q. (1994). An extensive network of PHF tau-rich dystrophic neurites permeates neocortex and nearly all neuritic and diffuse amyloid plaques in Alzheimer disease. *FEBS Lett.* **344**, 69-73.

Seglen, P. O. and Bohley, P. (1992). Autophagy and other vacuolar protein degradation mechanisms. *Experientia* **48**, 158-172.

Selkoe, D. J. (2001). Clearing the brain's amyloid cobwebs. *Neuron* **32**, 177-180.

Senechal, Y., Larmet, Y. and Dev, K. K. (2006). Unraveling in vivo functions of amyloid precursor protein: insights from knockout and knockdown studies. *Neurodegener. Dis.* **3**, 134-147.

Sherrington, R., Rogaev, E. I., Liang, Y., Rogawva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K. et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754-760.

Shibata, M., Lu, T., Furuya, T., Degerev, A., Mizushima, N., Yoshimori, T., MacDonald, M., Yankner, B. and Yuan, J. (2006). Regulation of intracellular accumulation of mutant Huntingin by Beclin 1. *J. Biol. Chem.* **281**, 14474-14485.

Shigemitsu, K. Y., Tsujishita Hara, K., Nanahoshi, M., Avruch, J. and Yonezawa, K. (1999). Regulation of translational effectors by amino acid and mammalian target of rapamycin signaling pathways. Possible involvement of autophagy in cultured hepatoma cells. *J. Biol. Chem.* **274**, 1058-1065.

Shintani, T. and Klionsky, D. J. (2004). Autophagy in health and disease: a double-edged sword. *Science* **306**, 990-995.

Small, D. H., Mok, S. S. and Bornstein, J. C. (2001). Alzheimer's disease and Abeta toxicity: from top to bottom. *Neuroscience* **2**, 595-598.

Smith, D. H., Uryu, K., Saatman, K. E., Trojanowski, J. Q. and McIntosh, T. K. (2003). Protein accumulation in traumatic brain injury. *Neuromol. Med.* **4**, 59-72.

Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K. and Greengard, P. (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nat. Neurosci.* **8**, 1051-1058.

St George-Hyslop, P. (2000). Alzheimer's disease. *Neurobiol. Dis.* **7**, 546-548.

Suzuki, K. and Terry, R. D. (1967). Fine structural localization of acid phosphatase in senile plaques in Alzheimer's presenile dementia. *Acta Neuropathol.* **8**, 276-284.

Takahashi, R. H., Milner, T. A., Li, F., Nam, E. E., Edgar, M. A., Yamaguchi, H., Beal, M. F., Xu, H., Greengard, P. and Gouras, G. K. (2002). Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am. J. Pathol.* **161**, 1869-1879.

Takahashi, R. H., Almeida, C. G., Kearney, P. F., Yu, F., Lin, M. T., Milner, T. A. and Gouras, G. K. (2004). Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J. Neurosci.* **24**, 3592-3599.

Terman, A., Dalen, H., Eaton, J. W., Neuzil, J. and Brunk, U. T. (2003). Mitochondrial recycling and aging of cardiac myocytes: the role of autophagocytosis. *Exp. Gerontol.* **38**, 863-876.

Terman, A., Gustafsson, B. and Brunk, U. T. (2006). Mitochondrial damage and intralysosomal degradation in cellular aging. *Mol. Aspects Med.* **27**, 471-482.

van der Goot, F. G. and Gruenberg, J. (2006). Intra-endosomal membrane traffic. *Trends Cell Biol.* **16**, 514-521.

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R. et al. (1999). Beta-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735-741.

von Arnim, C. A., Spoelgen, R., Peltan, I. D., Deng, M., Courchesne, S., Koker, M., Matsui, T., Kowa, H., Lichtenthaler, S. F., Irizarry, M. C. et al. (2006). GGA1 acts as a spatial switch altering amyloid precursor protein trafficking and processing. *J. Neurosci.* **26**, 9913-9922.

Walter, J., Fluhrer, R., Hartung, B., Willem, M., Kaether, C., Capell, A., Lammich, S., Multhaup, G. and Haass, C. (2001). Phosphorylation regulates intracellular trafficking of beta-secretase. *J. Biol. Chem.* **276**, 14634-14641.

Watson, D., Castano, E., Kokjohn, T. A., Kuo, Y. M., Lyubchenko, Y., Pinsky, D., Connolly, E. S., Esh, C., Luehrs, D. C., Stine, W. B. et al. (2005). Physicochemical characteristics of soluble oligomeric Abeta and their pathologic role in Alzheimer's disease. *Neurol. Res.* **27**, 869-881.

Wilson, C. A., Murphy, D. D., Giasson, B. I., Zhang, B., Trojanowski, J. Q. and Lee, V. M. (2004). Degradative organelles containing mislocalized alpha- and beta-synuclein proliferate in presenilin-1 null neurons. *J. Cell Biol.* **165**, 335-346.

Yamamoto, A., Cremona, M. L. and Rothman, J. E. (2005). Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. *J. Cell Biol.* **172**, 719-731.

Yamazaki, T., Chang, T. Y., Haass, C. and Ihara, Y. (2001). Accumulation and aggregation of amyloid beta-protein in late endosomes of Niemann-pick type C cells. *J. Biol. Chem.* **276**, 4454-4460.

Yu, W. H., Kumar, A., Peterhoff, C., Shapiro Kulnane, L., Uchiyama, Y., Lamb, B. T., Cuervo, A. M. and Nixon, R. A. (2004.). Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for β-amyloid peptide overproduction and localization in Alzheimer's disease. *Int. J. Biochem. Cell Biol.* **36**, 2531-2540.

Yu, W. H., Cuervo, A. M., Kumar, A., Peterhoff, C. M., Schmidt, S. D., Lee, J. H., Mohan, P. S., Mercken, M., Farmery, M. R., Tjernberg, L. O. et al. (2005). Macroautophagy – a novel amyloid-b (Ab) peptide-generating pathway activated in Alzheimer's disease. *J. Cell Biol.* **171**, 87-98.

Yu, W. H., Lee, J. H., Massey, A., Peterhoff, C., Kumar, A., Mohan, P., Cuervo, A. M. and Nixon, R. A. (2006). Presenilin-1 is required for macroautophagy: PS1 mutations causing AD confer loss of macroautophagy function. *Alzheimers Dement.* **2**, S58.

Zeng, X., Overmeyer, J. H. and Maltese, W. A. (2006). Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking. *J. Cell Sci.* **119**, 259-270.

Zheng, H. and Koo, E. H. (2006). The amyloid precursor protein: beyond amyloid. *Mol. Neurodegener.* **1**, 5.