Chapter 11
Mitochondrial Degradation, Autophagy and Neurodegenerative Disease

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Abstract Mitochondria are essential for cellular and organismal health, such that mitochondrial dysfunction can contribute to ageing and age-related diseases. In particular, impairment of mitochondrial function has been shown to be an underlying cause of several human neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease. In this chapter, we outline the current understanding of various mechanisms of mitochondrial quality control with a specific focus on mitophagy, the process of mitochondrial degradation via the autophagy pathway. We describe the autophagy and mitophagy pathways and highlight the key molecular players controlling these processes. Additionally, we discuss how mutations in the components of the molecular machinery controlling mitophagy can lead to the loss of neuronal function and viability and eventually result in disease. Studies of these pathways not only produce an important insight into the mechanisms of neurodegenerative diseases but also suggest molecular components of mitochondrial quality control which could be used as targets for therapeutic interventions.

Keywords Autophagy • Mitophagy • Mitochondria • Parkin • PINK1 • Neurodegeneration • Alzheimer's disease • Parkinson's disease • Huntington's disease • Amyloid lateral sclerosis

11.1 Introduction

Amongst several cellular pathways involved in mitochondrial quality control, the process of mitochondrial degradation via the autophagosome-lysosome pathway, termed mitophagy, has emerged as a key determinant of cellular, and in particular neuronal, function. The importance of this process became particularly evident when the mutations in the proteins regulating autophagy and mitophagy pathways

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were found to be genetic determinants in an array of devastating human neurodegenerative diseases [1–3]. This chapter will describe the molecular mechanisms of these pathways and their perturbation in age-related neurodegeneration.

11.2 The Autophagy Pathway

The term autophagy refers to the cellular self-digestion process that occurs through the lysosomal degradation pathway. Autophagy can be activated by different stress conditions including starvation, oxidative stress and an accumulation of damaged proteins. Since the discovery of the autophagy degradation pathway almost 50 years ago [4], it has been implicated in ageing, immunity, development of cancer, myopathies and neurodegeneration [5].

Autophagy is the principle mechanism for the turnover of long-lived proteins, as well as the only known pathway for the degradation and recycling of cytoplasmic organelles [6]. A rapid degradation of irreversibly damaged proteins is essential to preserve normal cellular functions and to prevent the formation of abnormal intermolecular interactions. Autophagy plays this important proteostatic role, and any malfunction in this process is likely to lead to the generation of insoluble aggregates and contribute to the pathology of many human diseases [7].

Autophagy can operate in three different patterns with distinct delivery modality, specificity and regulation: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. During microautophagy, the lysosomal membrane plays an active role where it invaginates or protrudes to enclose a portion of cytoplasm [8]. In CMA, proteins presenting the targeting KFERQ motif (a pentapeptide) are recognised by the hsc70 (heat-shock cognate of 70 kDa), and the protein-chaperone complex is internalised directly into lysosomes by binding to the LAMP-2a receptor (lysosomal-associated membrane protein type 2a) [9].

Macroautophagy is the most widely studied and best characterised form of autophagy. In the macroautophagy pathway (for simplicity frequently referred to as autophagy), the formation of a double membrane structure called an autophagosome engulfs cytosolic components. The cargoes are then subsequently delivered to lysosomes via a fusion process resulting in a hybrid structure termed the autolysosome, where hydrolases can degrade trapped material (Fig. 11.1) [10]. After degradation of macromolecules, the building blocks (e.g. amino acids) are exported back to the cytoplasm for reutilisation as a source of energy or for the synthesis of new cellular components. This process is orchestrated by a set of Atg (*aut*oph*ag*y-related) proteins [11]. To date, more than 30 ATG genes have been identified in yeast, and many of them have mammalian orthologues [12]. The induction of the autophagic process is typically triggered by a lack of nutrients and growth-promoting signals. Insulin, growth factors or amino acid deprivation inhibits mTOR (mechanistic target of rapamycin), an evolutionarily conserved serine/threonine, phosphatidylinositol kinase-related protein kinase (PIKK) which is considered the master

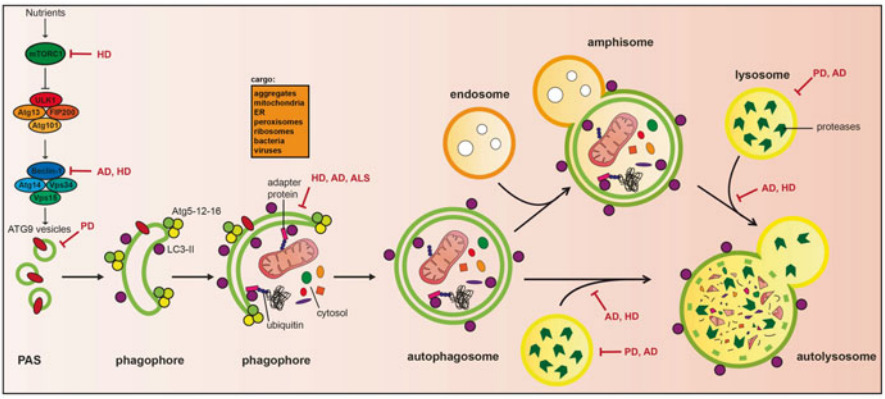

Fig. 11.1 Schematic diagram of autophagy. Mammalian autophagy is typically triggered by nutrient deprivation, which is sensed by mTORC1. The ULK complex (ULK1, Atg13, FIP200 and Atg101) is responsible for autophagy initiation and the PI3K activity of the Vps34 complex (Vps34, Vps15, Atg14 and Beclin-1) is required for the formation of the phagophore. Atg9-positive pre-autophagosomal structures (*PAS*), contribute to the delivery of membrane to the autophagosome. Next, two conjugation systems add the Atg12-Atg5-Atg16 complex and LC3-II to the expansion of the phagophore. The membrane grows and engulfs a portion of the cytosol, and cargo is selectively recruited via receptor proteins and an autophagosome is formed. This is followed by autophagosome maturation by fusion with endosomes (results in the formation of amphisomes) or lysosomes (results in the formation of autolysosomes) and degradation of the autophagosomal cargo through lysosomal proteases, and the degradation products then can be recycled. The steps that are altered in neurodegeneration are indicated in *red. HD* Huntington's disease, *PD* Parkinson's disease, *AD* Alzheimer's disease, *ALS* amyotrophic lateral sclerosis

regulator of nutrient signalling [13]. Inhibition of mTOR results in cell growth arrest and in the activation of the autophagic pathway.

11.2.1 The Molecular Machinery of Macroautophagy

The first step in the formation of autophagosomes is the sequestration of cytoplasmic components to a unique membrane called the "phagophore" that expands to enclose the substrate (see Fig. 11.1). This process, called *initiation*, is triggered by ULK1, a serine/threonine kinase that becomes active after mTOR inhibition and forms a complex with Atg13, FIP200 and Atg101 (see Fig. 11.1) [14]. Following initiation, a *nucleation event* takes place driven by Vps34, a phosphatidylinositol 3-kinase (PI3K) that phosphorylates the phosphatidylinositol (PI) generating the phosphatidylinositol 3-phosphate (PI3-P). This lipid then promotes the formation of the autophagosome membrane. The Vps34 forms a complex with Vps15/p150, Vps14 and Vps30/Atg6 (orthologous of mammalian Beclin-1), which starts the nucleation event (see Fig. 11.1) [15]. An important step in the formation of the

autophagosome membrane is the involvement of Atg9, a multispanning transmembrane protein that has a principal role in the formation of the *p*re-*a*utophagosomal *s*tructure (PAS) [16]. The *elongation* of the membrane is controlled by two ubiquitin-like conjugation systems. The first system allows the conjugation of the phosphatidylethanolamine (PE) lipid molecule to the C-terminus of Atg8 (orthologous of mammalian LC3, microtubule-associated protein – MAP – light chain 3). Atg8/LC3 is an ubiquitin-like protein synthesised as a precursor, with an additional sequence at the C-terminus that is cleaved by Atg4, a cysteine protease [17]. The cleaved form of LC3 (called LC3-I) is subsequently activated by Atg7 and transferred onto Atg3, which conjugates LC3-I to PE thus forming LC3-II, the lipidated form of LC3 that is specifically associated with the autophagosome membrane [18]. The second conjugation system involves the covalent attachment of Atg12 to Atg5 mediated by Atg7 and Atg10 enzymes [19]. The Atg5-Atg12 complex subsequently associates with Atg16 in a non-covalent way, and then the 350 kDa Atg5-Atg-12-Atg16 complex assists the binding of Atg8/LC3 to PE and the extension of the phagophore (see Fig. 11.1) [20, 21]. Finally, the last step in the autophagosome biogenesis process is termed *maturation*, where autophagosomes fuse with endosomes (thus forming the amphisomes) or directly with lysosomes forming autolysosomes. Amphisomes eventually mature into autolysosomes following their acidification driven by the proton pumps located in the endosomal membranes. Formation of autolysosomes allows the degradation of autophagy substrates (see Fig. 11.1) [22].

11.2.2 Selective Autophagy

For a long time, autophagy was thought to be a non-selective degradation pathway where cytoplasmic components were destroyed in bulk, usually under starvation conditions. However, recently the idea of autophagy as a selective degradation process, where specific substrates are recognised by cargo receptor proteins and targeted for degradation, has been firmly established [12]. Selective autophagy is commonly associated with its housekeeping role promoting the maintenance of cellular homeostasis. An important role of selective autophagy, allowing the clearance of aggregate-prone proteins (aggrephagy) and selective degradation of damaged organelles including mitochondria (mitophagy), is specifically relevant in the context of many neurodegenerative diseases [23, 24]. Other targets of selective autophagy include peroxisomes (pexophagy), endoplasmic reticulum (reticulophagy), ribosomes (ribophagy) and bacteria and viruses (xenophagy) [25–28]. Growing evidence suggests that receptor proteins of selective autophagy, such as p62, NBR1, Nix and NDP52, act to bind ubiquitinated proteins marked for autophagic degradation and shuttle them to the autophagosomal membrane. This process is mediated by the interactions of receptor proteins with both ubiquitin and the LC3-II residing on the autophagic membrane [29].

The signal that leads to the autophagic degradation of substrates appears to be predominantly ubiquitination, which acts as a signalling molecule in the autophagic

degradation of both protein aggregates and organelles [30]. It has been reported that Lys63-polyubiquitin chains are predominantly involved in this process [31]; however, other types of ubiquitination have also been found to play role in targeting substrates to autophagic vesicles. Indeed, the lack of autophagy after knocking out the ATG7 or ATG5 genes cause the formation of protein inclusions containing all types of ubiquitin linkages in mice, similar to those observed in neurodegenerative disorders [32].

11.3 Mitochondrial Quality Control

Mitochondria are the cellular power plants responsible for aerobic production of ATP. In addition to energy production, mitochondria are involved in calcium signalling and storage, metabolite synthesis and apoptosis. Mitochondria are also the main cellular source of intracellular reactive oxygen species (ROS). These highly reactive molecules, in particular superoxide anion (O2•-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•HO), are potentially toxic by-products of oxidative phosphorylation (see Chap. 1 for detail). ROS can cause oxidative damage to mitochondrial lipids, DNA and proteins, thus damaging the mitochondrial machinery involved in electrochemical reactions and contributing to further ROS production. Under normal physiological conditions, oxidative damage caused by ROS is buffered by antioxidants like thioredoxin- or glutathione-dependent peroxidases [33]. When the capacity of this antioxidant system is exceeded, cells use several other lines of defence, such as the mitochondrial unfolded protein response (UPR^{mt}), the ubiquitin-proteasome system (UPS) and mitophagy. The UPR^{mt} involves the upregulation of mitochondrial chaperones and proteases that remove misfolded and non-assembled polypeptides in mitochondria [34]. Similarly, the UPS recognises and removes mistargeted and misfolded mitochondrial proteins, including those localised to the outer mitochondrial membrane [35]. The proteases upregulated by the UPR^{mt} include mitochondrial matrix and the intermembrane space (IMS) targeted AAA^+ metalloproteases. These are of central importance for the quality control of the inner mitochondrial membrane (IMM), the site where most ROS are produced. However, the selective removal of damaged/oxidised mitochondrial proteins is not sufficient to remove large numbers of damaged proteins or whole mitochondria. Hence, cells have developed a mechanism to eliminate dysfunctional mitochondria via mitophagy. It has been shown that mitochondria can be completely renewed within 14 days in certain cell types and tissues [36].

11.3.1 Mitophagy

Mitophagy is a type of selective autophagy that mediates clearance of damaged mitochondria, as well as being important in specific cellular contexts such as during maturation of erythrocytes or for the elimination of sperm-derived mitochondria after

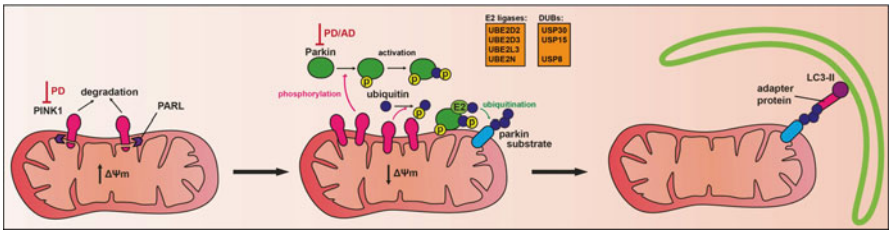

Fig. 11.2 Schematic diagram of PINK1/Parkin-dependent mitophagy. In healthy mitochondria with a high membrane potential (ΔΨm), PINK1 gets proteolytically cleaved by PARL (presenilin-associated rhomboid-like protein), leading to proteasomal degradation. However, upon loss of ΔΨm, PINK1 accumulates at the outer mitochondrial membrane and gets activated. PINK1 phosphorylates Parkin, resulting in its activation. Ubiquitin also gets phosphorylated by PINK1, which is required for the recruitment of Parkin to damaged mitochondria. Several mitochondrial membrane proteins can be ubiquitinated in a Parkin-dependent manner, thereby creating a signal for the removal of the damaged mitochondria. The presence of ubiquitin on the outer mitochondrial membrane results in the recruitment of receptor proteins, which bind to LC3 and target the mitochondria for autophagy-dependent degradation. The steps that are altered in neurodegeneration are indicated in *red*. *PD* Parkinson's disease, *AD* Alzheimer's disease

fertilisation [37–39]. Due to the importance of mitochondria in the development of various degenerative diseases, a lot of interest centres around their selective degradation through mitophagy [40]. Different signals have been shown to result in mitophagy activation, including loss of mitochondrial membrane potential [41], accumulation of misfolded mitochondrial proteins [42], loss of iron from intracellular stores [43] and ROS [44, 45]. Mitochondrial dynamics (fission and fusion (see also Chap. 7) play a critical role in mitochondrial degradation. Specifically, increased fission, resulting in smaller mitochondria that are easier to engulf by autophagosomes, favours degradation of mitochondria, whilst fusion prevents mitophagy [46, 47]. Different mitophagy pathways have been elucidated in the last decade; these will be described in the coming sections. The types of mitophagy taking place during erythrocyte differentiation and oocyte fertilisation are programmed mitochondrial degradation pathways and are relevant to the topic of this chapter due to the mechanistic insight they offer into the molecular events underlying this process, whilst the third pathway, the PINK1/Parkin-dependent mitophagy, is particularly important as it has been extensively implicated in human disease, including neurodegeneration (Fig. 11.2).

11.3.1.1 Mitochondrial Clearance During Erythrocyte Differentiation

During differentiation of reticulocytes to mature erythrocytes, mitochondria and other organelles are eliminated from the cell in a programmed fashion [48]. Nix is a BH3-domain-containing protein and was reported to be a mitochondrial receptor required for the clearance of mitochondria in reticulocytes [49]. Therefore, Nix-deficient mice develop anaemia and severe reticulocytosis caused by inefficient elimination of mitochondria [37]. The basic autophagy machinery is functional in

the absence of Nix, but the targeting of mitochondria to autophagosomes is impaired. Nix functions as an adaptor protein and recruits LC3 or *g*amma-*a*mino*b*utyric *a*cid *r*eceptor-*a*ssociated *p*roteins (GABARAP) to damaged mitochondria via a LIR (*LC*3-*i*nteracting *r*egion) motif in its N-terminus [23, 49]. Mitochondrial clearance during reticulocyte differentiation is mainly dependent on ULK1 and to a lesser extent on ATG5 [50]. Even though the molecular mechanism for mitochondrial elimination during this process is well studied, the upstream signal to target the mitochondria for degradation is unknown. The relevance of this mitophagy pathway to human pathophysiology is highlighted by the role of Nix in hypoxia-induced mitochondrial degradation [51].

11.3.1.2 Sperm-Derived Mitochondrial Clearance After Fertilisation

Another example of programmed mitochondrial degradation has been reported during oocyte fertilisation; in sexual reproduction, mitochondria are usually inherited from the mother; thus the paternal mitochondria have to be eliminated when the sperm-derived mitochondria and mitochondrial genome enters the oocyte. In *C. elegans*, mitophagy has been shown to actively target paternal mitochondria for degradation after fertilisation [38, 39]. Immediately after fertilisation, parental mitochondria are surrounded by autophagosomes, which then fuse with lysosomes. In worms with a knock-down or mutation of the homolog of LC3, paternal mitochondria are still present in late-stage embryos [38, 39]. Interestingly, no ubiquitin was detected on parental mitochondria entering the oocyte. This does not rule out that low levels of ubiquitination occur; however, it is possible that another mechanism primes the paternal mitochondria for degradation in worms. In *Drosophila melanogaster* and mice, ubiquitination has been shown to be involved in the degradation of sperm mitochondria. In all studied species, LC3, together with the receptor protein p62, were found on the surface of the ubiquitination mitochondria [39, 52, 53]. Surprisingly, despite recruitment of these autophagy proteins, they are not required for the elimination of paternal mitochondria [53]. Therefore, it remains controversial whether mitophagy is indeed the mechanism of paternal mitochondria and mtDNA elimination after fertilisation.

11.3.1.3 PINK1/Parkin-Dependent Mitophagy

Probably the most studied mitophagy pathway is dependent on the PINK1/Parkin pathway, which is an ubiquitin-dependent process (see Fig. 11.2). When the mitochondrial membrane potential ($\Delta\Psi m$) is high, PINK1, a serine/threonine kinase, binds to PGAM5 (phosphoglycerate mutase family member 5) in the inner mitochondrial membrane, where it gets proteolytically cleaved by PARL (presenilin-associated rhomboid-like protein), leading to proteasomal degradation (see Fig. 11.2) [54–56]. However, upon loss of $\Delta\Psi m$, PINK1 accumulates at the mitochondrial membrane where its activity recruits the E3 ubiquitin ligase, Parkin, from

the cytosol to the depolarised mitochondrion [57]. Parkin then ubiquitinates many mitochondrial membrane proteins, thereby creating a signal for the removal of the damaged mitochondrion (see Fig. 11.2) [58]. It is thought that the recruitment and activation of Parkin relies on PINK1 phosphorylation of Parkin itself, but also phosphorylation of mono-ubiquitin and ubiquitin chains, which are required for recruitment of Parkin to damaged mitochondria [59, 60]. Recently, several E2 ubiquitin-conjugating enzymes were identified for Parkin, such a UBE2D2, UBE2D3 and UBE2L3 and UBE2N [61, 62].

The mitochondrial anchored deubiquitylases (DUBs), USP30 and USP15, have been identified as negative regulators of Parkin-mediated mitophagy [63–65]. Another DUB, USP8 selectively deubiquitinates Parkin, but not Parkin substrates [66]. Recently, in a screen to identify positive and negative regulators of mitophagy in yeast, more DUBs were identified. Interestingly, one of the deubiquitination complexes (Ubp3-Bre5) inhibits mitophagy but promotes other types of autophagy [67].

The presence of ubiquitin on the outer mitochondrial membrane results in the recruitment of receptor proteins such as NBR1 and p62 (see Fig. 11.2) [29]. These receptor proteins contain an UBA (*ub*iquitin-*a*ssociated) domain and a LIR (LC3-interacting region) motif, which facilitates simultaneous binding of these adaptors to the ubiquitin-labelled mitochondria and the autophagosomal machinery. As a result, damaged mitochondria are engulfed by phagophores and processed for autophagic degradation.

The importance of these autophagy receptors for the process of Parkin-mediated mitophagy, however, remains controversial. Thus, p62, possibly together with *h*istone *deac*etylase 6 (HDAC6), has been suggested to work as the key mitophagy receptor [68]. Following Parkin-mediated ubiquitination, p62 and HDAC6 bind ubiquitin and drive damaged mitochondria along microtubules to the perinuclear site for degradation [68]. Similarly, Geisler et al. demonstrated that Parkin-dependent mitophagy is dependent on p62 [69]. In support of the role of p62 as a mitophagy receptor protein, studies in *Drosophila melanogaster* demonstrated that ref(2)p, the homolog of mammalian p62, is required for mitophagy [70, 71]. At the same time, Narendra et al. showed that p62 may be required for Parkin-induced mitochondrial clustering, but is not limiting for mitophagy [57, 69]. One possible explanation for these contradictory findings is functional redundancy. In the absence of p62, several intracellular proteins such as NBR1, Alfy (autophagy-linked FYVE protein), BAG3 (Bcl-2-associated athanogene 3), optineurin, NPD52 (nuclear dot protein 52 kDa) or TAX1BP (Tax1-binding protein 1) may be able to link Parkin-mediated ubiquitination to autophagic degradation [30]. Indeed, Lazarou et al. recently demonstrated that optineurin and NPD52, but not p62, are required for mitophagy. HeLa cells with a knock-out of five different autophagy receptors, including optineurin, NPD52, p62, TAX1BP and NBR1, were used, followed by the reintroduction of the different receptors to study their role in mitophagy. Optineurin and NDP52 single knock-out did not affect mitophagy, whilst double knock-out did prevent mitochondrial degradation, suggesting that optineurin and NDP52 are the primary, yet redundant, receptors [72].

Vincow et al. showed that the Pink/Parkin pathway not only promotes mitophagy, but also promotes the selective turnover of mitochondrial respiratory subunits [73]. This is interesting, because the respiratory subunits are prone to oxidation; therefore, quality control of these proteins is essential for mitochondrial function. The mechanism for the selective turnover of respiratory subunits is unknown, and it would be of great interest to further study. Suggested models at the moment are chaperone-mediated extraction of mitochondrial proteins or degradation via mitochondria-derived vesicles [73, 74]. The latter process has been demonstrated to directly deliver parts of the mitochondrion to the lysosomes for degradation in a PINK1/Parkin-dependent manner [75, 76].

The great majority of studies on PINK1/Parkin-dependent mitophagy are done in tissue culture using the acute loss of $\Delta\Psi$m model, mediated by uncouplers such as CCCP and FCCP [69]. However, depolarisation of the mitochondrial membrane is not sufficient for Parkin recruitment to mitochondria in neurons. Rather, the generation of ROS is required to trigger this mitophagy pathway [44, 77]. The relevance of PINK1/Parkin-dependent mitophagy in neurons remains controversial [78]. Thus, the PINK1/Parkin mitophagy pathway has been robustly demonstrated in immortalised cells and in *Drosophila melanogaster* [79]; however, other models fail to replicate this. For example, in Parkin knock-out mice, no increased neurodegeneration or accumulation of damaged mitochondria was observed [80]. The physiological relevance of mitophagy and the mechanism in neurons remain largely unclear, and future research should focus on mitophagy in neurons in physiologically relevant conditions. This might lead to the discovery of new therapeutic targets for Parkinson's disease and other neurodegenerative diseases.

11.3.1.4 Other Mechanisms of Mitophagy

In addition to the most studied mitophagy pathways as described above, other mechanisms have been observed. One of them involves the translocation of cardiolipin from the inner mitochondrial membrane (IMM) to the mitochondrial surface in neuronal cells, followed by the binding of cardiolipin to the autophagosomal machinery, thereby targeting the mitochondria for degradation [81]. In addition, Melser et al. identified a Rheb-dependent mitophagy pathway that is induced upon stimulation of oxidative phosphorylation [82]. The small GTPase Rheb (*R*as *h*omolog *e*nriched in *b*rain protein) is recruited to the mitochondrial outer membrane upon high oxidative phosphorylation activity, where it promotes mitophagy through an interaction with the mitochondrial adaptor protein Nix and the autophagosomal protein LC3-II. This Rheb-dependent mitophagy pathway is suggested to be important for the renewal of mitochondria and the maintenance of a healthy population of mitochondria. Intriguingly, levels of ROS are also increased upon oxidative conditions, but their role in the initiation of Rheb-dependent mitophagy is not well understood.

In conclusion, mitophagy has been a subject of intense investigations in recent years and significant advances have been made. However, the Pink/Parkin pathway

is primarily studied in a very artificial experimental setting requiring overexpression of Parkin and complete depolarisation of mitochondrial membrane; thus, the biological relevance of this pathway in human pathophysiology, specifically in neurodegenerative diseases, requires further clarification. Additionally, whilst the basal mitophagy clearly occurs in neurons [83], mechanistic studies of mitophagy have been largely performed in non-neuronal cells. It will be important to establish whether different mitophagy pathways also occur in neurons and how their impairment can result in neurodegeneration. Some of the existing links between mitochondrial quality control via mitophagy and human age-related neurodegenerative diseases are outlined below.

11.3.2 Impaired Mitophagy and Neurodegeneration

One of the side effects of an extended lifespan in the human population is the increment of age-related neurodegenerative diseases, which are chronic, incurable and terminal conditions affecting the brain, where a structural and functional decline leads to neuronal death. Well-known examples of neurodegenerative diseases are Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), as well as frontotemporal dementia (FTD), dementia with Lewy bodies (DLB) and many others. Despite identification of some hereditary cases, the overwhelming majority have a sporadic onset, and the causes of which are unknown. Mitochondrial dysfunction as well as autophagy dysregulation is strongly linked to different neurodegenerative diseases [84, 85]. Neurons are postmitotic cells, and therefore, they cannot rely on cell division to dilute the burden of damaged cellular components. It could be predicted that neurons may be particularly dependent on homeostatic processes such as autophagy and specifically mitophagy to prevent accumulation of cellular waste. Indeed, neurons with dysfunctional autophagy accumulate ubiquitinated protein aggregates and damaged mitochondria [86, 87]. In neurodegenerative diseases, autophagy dysfunction can occur at different stages of autophagy, giving rise to different pathologic outcomes. In the following subsections, we will discuss how dysregulation of autophagy and mitophagy might contribute to the pathology of several key neurodegenerative diseases (see Figs. 11.1 and 11.2).

11.3.2.1 Parkinson's Disease

Mitochondrial dysfunction has been widely implicated in Parkinson's disease (PD), which is a disorder characterised by the loss of the dopamine containing neurons of the substantia nigra and the presence of Lewy bodies (intracellular inclusions containing α-synuclein) in remaining cells. Mutations in the genes encoding *PINK1*, *Parkin* and *DJ-1* account for most autosomal-recessive cases of PD and lead to dysfunctional mitophagy and accumulation of damaged mitochondria, which are thought to contribute to neuronal cell death [88, 89].

The first gene identified to be associated with autosomal-recessive PD was *Parkin* (see Fig. 11.2). One hundred twenty-seven pathogenic PD mutations in Parkin have been identified to date, and these are spread throughout the different domains, suggesting that all domains are important for its function [90] (www.molgen.ua.ac.be/PDmutDB/). In the last few years, the structure of the different Parkin domains has become available, thus helping in the understanding of its function. Parkin has a *ub*iquitin-*l*ike domain (Ubl) and four zinc-binding RING domains (RING0, RING1, IBR and RING2) [91–94]. The Ubl is required for substrate binding, whereas RING1 is the binding site for the ubiquitin-conjugated E2 enzyme and RING2 is the catalytic domain [95]. At the same time, the function of the RING0 and IBR domains remains a mystery. In mice, loss-of-function mutations in Parkin cause mitochondrial dysfunction and oxidative damage, and this seems to precede neurodegeneration within the substantia nigra [96]; therefore, mitochondrial dysfunction might be the cause of PD and not a consequence.

The second most frequent gene linked to PD is *PINK1*, which is intimately linked to Parkin. Twenty-eight pathogenic mutations have been described for PINK1, and most of these are located in the kinase domain and result in the loss of kinase activity (www.molgen.ua.ac.be/PDmutDB/). PINK1 deficiency also leads to mitochondrial dysfunction [97], which can be explained by its role in PINK/Parkin-dependent mitophagy (see Fig. 11.2). However, PINK1 also has some Parkin-independent functions, which affect mitochondrial function as well. For example, subunit NDUFA10 of mitochondrial complex I is phosphorylated by PINK, which is important for complex I activity. Expression of a phosphomimetic NDUFA10 in neurons derived from iPS (induced pluripotent stem) cells from PD patients with PINK1 mutations rescued complex I function and mitochondrial membrane potential [98]. In addition, in *Drosophila melanogaster*, expression of the NDUFA10 homolog partially rescues complex I activity and restores locomotor function. Parkin overexpression also rescues the phenotypes of PINK1 mutations in *Drosophila*, but does not restore complex I function [99]. Together, these observations suggest that complex I dysfunction is not sufficient to cause impairment of locomotor function in PD.

The *DJ-1* gene has also been associated with PD with six pathogenic mutations identified to date. The role of DJ-1 in PD is less understood compared to PINK1 and Parkin, but it appears to function in the same pathway [89]. This is supported by the fact that loss of DJ-1 results in mitochondrial dysfunction and can be rescued by Parkin overexpression [100]. DJ-1 is a small oxidation sensing protein, but surprisingly its biochemical function is still not completely clear. What is known is that DJ-1 takes part in the oxidative stress response [101, 102].

Above-mentioned genes all have a significant role in maintaining mitochondrial homeostasis and mitophagy. In contrast, mutations in some other PD-related proteins primarily locate to the cytosol, like LRRK2 (leucine-rich repeat kinase 2) and α-synuclein, and these cause autosomal dominant forms of PD. α-synuclein is an aggregate-prone protein, and mutations or gene duplications/triplications result in the formation protein aggregates, resulting in PD [103]. The mutant form of α-synuclein is degraded via autophagy, causes the accumulation of autophagic-

vesicular structures and impairs lysosomal function (see Fig. 11.1) [104, 105]. Moreover, α-synuclein overexpression inhibits autophagy by inhibition of Rab1a, which causes mislocalisation of Atg9, leading to impaired autophagosome formation (see Fig. 11.1) [106]. Also, a PD-related mutation in *VPS35* (*v*acuolar *p*rotein sorting-associated protein 35), disrupts autophagosome formation [107], similar to LRRK2, which localises to autophagosomes and is important for autophagic induction [108, 109]. The interplay between LRRK2 and autophagy appears to be complex; in cultured cells, mutations in LRRK2 resulted in increased autophagy, whilst in knock-out mice, autophagy was reduced [110–112]. Furthermore, two other lysosomal proteins have been linked to PD, ATP13A2 (type 5 P-type ATPase) [113] and glucocerebrosidase [114]. To conclude, mutations in genes resulting in defective autophagy (and particularly mitophagy) can result in PD. Autophagosome maturation, cargo loading and lysosomal function are all implicated and thus will be important to further study how these mutations affect mitochondrial function in neuronal models of PD.

11.3.2.2 Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder and cause of dementia, characterised by neuronal loss in the cerebral cortex and accumulation of amyloid plaques and neurofibrillary tangles [115]. Mitochondrial dysfunctions have been implicated in the neuropathogenesis of AD since changes in their appearance occur when neurofibrillary tangles are not yet evident in neurons [116, 117]. Abnormalities in mitochondrial morphology in the neurons of AD brains have been suggested to cause an impairment in the ATP production, which can lead to loss of synaptic activity and cognitive decline [118]. It has been reported that AD patients possess defective mitochondrial cytochrome *c* oxidase in both temporal cortex and hippocampus, which may be the cause of reduced energy generation [119]. Importantly, amyloid β [Aβ] can also directly disrupt mitochondrial function by inhibiting the activity of respiratory enzymes such as cytochrome oxidase, α-ketoglutarate dehydrogenase and pyruvate dehydrogenase [120]. Expression of several proteins implicated in mitochondrial fission/fusion is abnormal in AD, leading to reduced mitochondrial density and increased fragmentation [121]. Thus, together with the observation of increased autophagic degradation of mitochondria in AD [122] and decreased number of mitochondria together with increased amounts of mitochondrial DNA and proteins [116], it suggests that mitophagy may be increased in AD in an attempt to degrade damaged mitochondria or that the proteolytic turnover is impaired. On the other hand, impaired macroautophagy has been also implicated in the pathogenesis of AD, where high amounts of autophagic vacuoles (AVs) accumulate in neurons [123, 124]. The increased amount of AVs in AD brains, compared to the low levels seen in healthy neurons, has been firstly connected with increased autophagic activity [124, 125]. Rather, it has become clear that impairment in the clearance of AVs in AD was instead the case, where the fusion between autophagosomes and lysosomes is not efficient (see Fig. 11.1) [123].

Additionally, the regulatory protein beclin1 which activates autophagy appears to be decreased in AD affected brains (see Fig. 11.1) [126]. Autophagy inhibition also appeared to increase the accumulation of hyperphosphorylated and fragmented tau, responsible for the formation of neurofibrillary tangles [127]. Lysosomal function was also inhibited by deposition of fragmented tau on the outer membrane, leading to lysosomal leakage and further release of toxic tau in the cytoplasm [127]. Due to its role in microtubule stabilisation, dysfunctional tau has been implied to interfere with autophagosome trafficking and clearance, as well as with mitochondrial and lysosomal mobility [128].

Parkin, involved in targeting mitochondria to the autophagosomes, has been shown to be reduced in the frontal and temporal cortex of AD brains together with increased Aβ accumulation (see Fig. 11.2) [129]. Importantly, amyloid precursor protein (APP) and Aβ have been found in AVs, together with components of the γ-secretase complex involved in the cleavage of APP [125, 130]. Thus, macroautophagy itself seems to be linked to the production of Aβ in AD brains. Presenilin1 (PS1), a component of the γ-secretase complex, has been shown to be essential for lysosomal acidification and for correct autophagic degradation [131]. AD-related mutations of PS-1 have shown lysosomal and autophagic dysfunctions in AD patients, leading to abnormal accumulation of autophagic vesicles and early-onset AD pathology [131, 132].

In conclusion, it is clear that autophagy dysfunction plays an important role in the development of AD, where accumulation of insoluble protein aggregates and dysfunctional mitochondria lead to neuronal loss.

11.3.2.3 Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder leading to cognitive and motor disorders, caused by an expanded trinucleotide CAG repeat in exon 1 of the gene encoding for the Huntingtin (Htt) protein. Htt is ubiquitously expressed in the brain, and the mutated version (mHtt) aggregates causing neuronal dysregulation and death [133]. Several lines of study suggest the involvement of respiratory chain defects in mitochondria in HD. A remarkably decreased activity in complex II/III and IV in basal ganglia samples from HD patients has been observed [134]. Also mitochondrial biogenesis dysregulation has been implied in the pathogenesis of HD, due to transcriptional repression of the mitochondrial regulator PGC-1α by mHtt [135]. In addition, mitochondrial mobility and axonal transport are thought to be impaired in HD due to mHtt aggregation [136, 137].

Amongst its numerous functions that have been described in the cell, it has recently emerged that Htt plays an important role in the autophagy process [138]. Whilst a decrease in the autophagic flux often occurs in other neurodegenerative diseases [139], this has been reported to be increased in HD, together with an enhanced number of autophagosomes [140, 141]. The induction of autophagy in HD has been attributed to the sequestration of mTOR by mHtt aggregates, leading

to the inactivation of this autophagy-inhibitory kinase [142]. In contrast, recruitment and sequestration of beclin1 by mHtt has been suggested to result in reduction of autophagy levels in HD brains, leading to defective protein turnover [143].

Although autophagosome production and clearance by lysosomes was enhanced, cargo recognition and engulfment failed to occur in HD cells, leading to impaired turnover of cytosolic components and accumulation of damaged mitochondria (see Fig. 11.1) [140]. Hence, this would contribute to the increase in protein aggregates and dysfunctional mitochondria seen in HD cells. However, a defect in the axonal transport of autophagosomes mediated by mHtt has also been connected with mitochondrial accumulation, due to defects in autophagosome/lysosome fusion events [144]. Therefore, it has been proposed that Htt acts as an autophagy scaffold in the cell, also due to its similarities with yeast's autophagy regulatory proteins and the prediction of LC3-interacting repeats in its sequence [138, 145]. Moreover, it has been recently reported that Htt positively modulates selective autophagy by interacting with p62, facilitating its interaction with LC3 and Lys63-poly-ubiquitinated substrates [146]. The interaction between Htt and ULK1, which releases it from the mTOR-negative regulation, has also been connected with the induction of selective autophagy [146].

In summary, Htt has a central role in the regulation of protein trafficking and turnover, where disruption of its function leads to the deficiency of selective autophagy and defective mitochondrial function.

11.3.2.4 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterised by motor neuron death and muscle wasting. Approximately 10 % of all the cases are familial (fALS), where 20 % of them are caused by a dominant mutation in the gene SOD1 (superoxide dismutase 1) [147, 148]. Abnormalities in mitochondrial structure and localisation have been shown in both spinal cord and muscle of ALS patients, together with defective respiration and electron transport chain [149, 150]. Interestingly, mutant SOD1 has been demonstrated to localise to the mitochondrial outer membrane, intermembrane space and matrix and appears to be concentrated in vacuolated mitochondria [151, 152]. This interaction would lead to damage of the mitochondrial outer membrane proteins as well as cytochrome *c* release and apoptosis [153, 154].

Overexpression of the G93A-SOD1 mutation in mice leads to mitochondrial degeneration and the formation of mitochondrial vacuoles, possibly triggering the onset of the disease [155]. Additionally, the same transgenic mice have shown increased autophagy levels during the presymptomatic and symptomatic stages, exhibiting increased LC3-II levels and inhibition of mTOR [156, 157]. However, the involvement of autophagy in ALS and its role in the disease progression are controversial [158]. Enhanced autophagy has been linked to increased degradation of mutant SOD1, whereas autophagy inhibition led to drastic accumulation of mutant SOD1 and increased neurotoxicity [159]. It has been also shown that

p62 accumulates in the G93A-SOD1 mouse spinal cord and mediates the poly-ubiquitination and aggregation of mutant SOD1, possibly contributing to the ALS aetiology [160]. The identification of several mutations in p62 sequence related to late-onset alS also suggests the importance of selective autophagy in the disease progression (see Fig. 11.1) [161, 162]. However, an opposite role of autophagy in ALS has been shown after treating G93A-SOD1 mice with rapamycin, an inducer of autophagy [163]. Surprisingly, activation of autophagy by rapamycin in the presymptomatic ALS increased motor neuron death and decreased the lifespan of the animals. Thus, accumulation of autophagosomes and p62 would mean impairment in the autophagic flux rather than enhanced autophagic activity.

Nevertheless, as mitochondrial abnormalities and degeneration seem to contribute with the onset of ALS, mitophagy could play an important role in the disease progression. Degradation of damaged mitochondria would possibly ameliorate the mitochondrial pool and attenuate the disease furtherance.

11.4 Conclusion

Impairment of autophagy (and specifically mitophagy), associated either with age-related decline in the pathway's efficiency or caused by the mutations in the key regulatory components, became evident as an important determinant of many human diseases. Neurons appear to be particularly susceptible to the perturbations in these mitochondrial quality control pathways, possibly due to the damage produced by undegraded toxic cellular components which accumulate over the course of decades in these extremely long-lived cells. Whilst accumulation of general cellular "junk" such as aggregate-prone toxic proteins due to their insufficient degradation via autophagy is undoubtedly contributing to the pathology of neurodegenerative diseases (hence their classification as proteinopathies), it is the perturbation of mitochondrial degradation via the mitophagy pathway which appears to be particularly damaging for the neurons [87, 164]. This, in turn, offers a novel concept for the treatment of these diseases by targeting regulatory components of the mitophagy machinery and upregulating this pathway [165]. Thus, drugs promoting the activity of the general autophagy pathway, and which would also be expected to stimulate mitophagy, have now been shown to protect against a wide range of neurodegenerative diseases with several such drugs having already reached clinical trials [166–168]. Whilst similar therapeutic strategies are yet to be established for the specific activation of the mitophagy pathway, studies in animal models show that overexpression of mitophagy proteins such as Parkin can indeed produce beneficial effect and prevent age-related neuronal decline [169–172]. Further mechanistic studies of mitophagy are likely to lead to the identification and validation of therapeutic targets and eventually identification of specific and potent drugs stimulating mitophagy and protecting neurons against senescence and degeneration.

References

1. Scarffe LA, Stevens DA, Dawson VL, Dawson TM. Parkin and PINK1: much more than mitophagy. Trends Neurosci. 2014;37(6):315–24.
2. Lee JK, Shin JH, Lee JE, Choi EJ. Role of autophagy in the pathogenesis of amyotrophic lateral sclerosis. Biochim Biophys Acta. 2015;1852(11):2517–24.
3. Cortes CJ, La Spada AR. The many faces of autophagy dysfunction in Huntington's disease: from mechanism to therapy. Drug Discov Today. 2014;19(7):963–71.
4. De Duve C, Wattiaux R. Functions of lysosomes. Annu Rev Physiol. 1966;28:435–92.
5. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069–75.
6. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science. 2000;290(5497):1717–21.
7. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol. 2000;10(12):524–30.
8. Li WW, Li J, Bao JK. Microautophagy: lesser-known self-eating. Cell Mol Life Sci. 2012;69(7):1125–36.
9. Kaushik S, Cuervo AM. Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends Cell Biol. 2012;22(8):407–17.
10. Ravikumar B, Futter M, Jahreiss L, Korolchuk VI, Lichtenberg M, Luo S, et al. Mammalian macroautophagy at a glance. J Cell Sci. 2009;122(Pt 11):1707–11.
11. Klionsky DJ, Cregg JM, Dunn Jr WA, Emr SD, Sakai Y, Sandoval IV, et al. A unified nomenclature for yeast autophagy-related genes. Dev Cell. 2003;5(4):539–45.
12. van der Vaart A, Mari M, Reggiori F. A picky eater: exploring the mechanisms of selective autophagy in human pathologies. Traffic. 2008;9(3):281–9.
13. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004;18(16):1926–45.
14. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell. 2011;147(4):728–41.
15. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ. 2011;18(4):571–80.
16. Yamamoto H, Kakuta S, Watanabe TM, Kitamura A, Sekito T, Kondo-Kakuta C, et al. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. J Cell Biol. 2012;198(2):219–33.
17. Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, et al. The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. J Cell Biol. 2000;151(2):263–76.
18. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, et al. A ubiquitin-like system mediates protein lipidation. Nature. 2000;408(6811):488–92.
19. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, et al. A protein conjugation system essential for autophagy. Nature. 1998;395(6700):395–8.
20. Kuma A, Mizushima N, Ishihara N, Ohsumi Y. Formation of the approximately 350-kDa Apg12-Apg5.Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. J Biol Chem. 2002;277(21):18619–25.
21. Mizushima N, Noda T, Ohsumi Y. Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. EMBO J. 1999;18(14):3888–96.
22. Puissant A, Fenouille N, Auberger P. When autophagy meets cancer through p62/SQSTM1. Am J Cancer Res. 2012;2(4):397–413.
23. Novak I, Dikic I. Autophagy receptors in developmental clearance of mitochondria. Autophagy. 2011;7(3):301–3.
24. Lamark T, Johansen T. Aggrephagy: selective disposal of protein aggregates by macroautophagy. Int J Cell Biol. 2012;2012:736905.

25. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS Biol. 2006;4(12):e423.
26. Kim PK, Hailey DW, Mullen RT, Lippincott-Schwartz J. Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes. Proc Natl Acad Sci U S A. 2008;105(52):20567–74.
27. Gomes LC, Dikic I. Autophagy in antimicrobial immunity. Mol Cell. 2014;54(2):224–33.
28. Kraft C, Deplazes A, Sohrmann M, Peter M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nat Cell Biol. 2008;10(5):602–10.
29. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy. 2011;7(3):279–96.
30. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. Mol Cell. 2009;34(3):259–69.
31. Tan JM, Wong ES, Kirkpatrick DS, Pletnikova O, Ko HS, Tay SP, et al. Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. Hum Mol Genet. 2008;17(3):431–9.
32. Komatsu M, Ueno T, Waguri S, Uchiyama Y, Kominami E, Tanaka K. Constitutive autophagy: vital role in clearance of unfavorable proteins in neurons. Cell Death Differ. 2007;14(5):887–94.
33. Lu J, Holmgren A. The thioredoxin antioxidant system. Free Radic Biol Med. 2014;66:75–87.
34. Hu F, Liu F. Mitochondrial stress: a bridge between mitochondrial dysfunction and metabolic diseases? Cell Signal. 2011;23(10):1528–33.
35. Karbowski M, Youle RJ. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. Curr Opin Cell Biol. 2011;23(4):476–82.
36. Menzies RA, Gold PH. The turnover of mitochondria in a variety of tissues of young adult and aged rats. J Biol Chem. 1971;246(8):2425–9.
37. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, et al. Essential role for Nix in autophagic maturation of erythroid cells. Nature. 2008;454(7201):232–5.
38. Sato M, Sato K. Degradation of paternal mitochondria by fertilization-triggered autophagy in C. elegans embryos. Science (New York, NY). 2011;334(6059):1141–4.
39. Al Rawi S, Louvet-Vallee S, Djeddi A, Sachse M, Culetto E, Hajjar C, et al. Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. Science (New York, NY). 2011;334(6059):1144–7.
40. Kubli DA, Gustafsson AB. Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res. 2012;111(9):1208–21.
41. Ye X, Sun X, Starovoytov V, Cai Q. Parkin-mediated mitophagy in mutant hAPP neurons and Alzheimer's disease patient brains. Hum Mol Genet. 2015;24(10):2938–51.
42. Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. Autophagy. 2013;9(11):1750–7.
43. Allen GF, Toth R, James J, Ganley IG. Loss of iron triggers PINK1/Parkin-independent mitophagy. EMBO Rep. 2013;14(12):1127–35.
44. Joselin AP, Hewitt SJ, Callaghan SM, Kim RH, Chung YH, Mak TW, et al. ROS-dependent regulation of Parkin and DJ-1 localization during oxidative stress in neurons. Hum Mol Genet. 2012;21(22):4888–903.
45. Rubio N, Coupienne I, Di Valentin E, Heirman I, Grooten J, Piette J, et al. Spatiotemporal autophagic degradation of oxidatively damaged organelles after photodynamic stress is amplified by mitochondrial reactive oxygen species. Autophagy. 2012;8(9):1312–24.
46. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, et al. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol. 2010;191(7):1367–80.
47. Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. Antioxid Redox Signal. 2011;14(10):1939–51.

48. Ney PA. Normal and disordered reticulocyte maturation. Curr Opin Hematol. 2011;18(3):152–7.
49. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. 2010;11(1):45–51.
50. Honda S, Arakawa S, Nishida Y, Yamaguchi H, Ishii E, Shimizu S. Ulk1-mediated Atg5-independent macroautophagy mediates elimination of mitochondria from embryonic reticulocytes. Nat Commun. 2014;5:4004.
51. Ding WX, Yin XM. Mitophagy: mechanisms, pathophysiological roles, and analysis. Biol Chem. 2012;393(7):547–64.
52. Politi Y, Gal L, Kalifa Y, Ravid L, Elazar Z, Arama E. Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in Drosophila. Dev Cell. 2014;29(3):305–20.
53. Luo SM, Sun QY. Autophagy is not involved in the degradation of sperm mitochondria after fertilization in mice. Autophagy. 2013;9(12):2156–7.
54. Yamano K, Youle RJ. PINK1 is degraded through the N-end rule pathway. Autophagy. 2013;9(11):1758–69.
55. Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. J Neurochem. 2011;117(5):856–67.
56. Fedorowicz MA, de Vries-Schneider RL, Rub C, Becker D, Huang Y, Zhou C, et al. Cytosolic cleaved PINK1 represses Parkin translocation to mitochondria and mitophagy. EMBO Rep. 2014;15(1):86–93.
57. Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy. 2010;6(8):1090–106.
58. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. Nature. 2013;496(7445):372–6.
59. Shiba-Fukushima K, Arano T, Matsumoto G, Inoshita T, Yoshida S, Ishihama Y, et al. Phosphorylation of mitochondrial polyubiquitin by PINK1 promotes Parkin mitochondrial tethering. PLoS Genet. 2014;10(12):e1004861.
60. Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, et al. Phosphorylated ubiquitin chain is the genuine Parkin receptor. J Cell Biol. 2015;209(1):111–28.
61. Fiesel FC, Moussaud-Lamodiere EL, Ando M, Springer W. A specific subset of E2 ubiquitin-conjugating enzymes regulate Parkin activation and mitophagy differently. J Cell Sci. 2014;127(Pt 16):3488–504.
62. Geisler S, Vollmer S, Golombek S, Kahle PJ. The ubiquitin-conjugating enzymes UBE2N, UBE2L3 and UBE2D2/3 are essential for Parkin-dependent mitophagy. J Cell Sci. 2014;127(Pt 15):3280–93.
63. Bingol B, Tea JS, Phu L, Reichelt M, Bakalarski CE, Song Q, et al. The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. Nature. 2014;510(7505):370–5.
64. Cunningham CN, Baughman JM, Phu L, Tea JS, Yu C, Coons M, et al. USP30 and parkin homeostatically regulate atypical ubiquitin chains on mitochondria. Nat Cell Biol. 2015;17(2):160–9.
65. Cornelissen T, Haddad D, Wauters F, Van Humbeeck C, Mandemakers W, Koentjoro B, et al. The deubiquitinase USP15 antagonizes Parkin-mediated mitochondrial ubiquitination and mitophagy. Hum Mol Genet. 2014;23(19):5227–42.
66. Durcan TM, Tang MY, Perusse JR, Dashti EA, Aguileta MA, McLelland GL, et al. USP8 regulates mitophagy by removing K6-linked ubiquitin conjugates from parkin. EMBO J. 2014;33(21):2473–91.
67. Muller M, Kotter P, Behrendt C, Walter E, Scheckhuber CQ, Entian KD, et al. Synthetic quantitative array technology identifies the Ubp3-Bre5 deubiquitinase complex as a negative regulator of mitophagy. Cell Rep. 2015;10(7):1215–25.

68. Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP. Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J Cell Biol. 2010;189(4):671–9.
69. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 2010;12(2):119–31.
70. Pimenta de Castro I, Costa AC, Lam D, Tufi R, Fedele V, Moisoi N, et al. Genetic analysis of mitochondrial protein misfolding in Drosophila melanogaster. Cell Death Differ. 2012;19(8):1308–16.
71. de Castro IP, Costa AC, Celardo I, Tufi R, Dinsdale D, Loh SH, et al. Drosophila ref(2)P is required for the parkin-mediated suppression of mitochondrial dysfunction in pink1 mutants. Cell Death Dis. 2013;4:e873.
72. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2015;524(7565):309–14.
73. Vincow ES, Merrihew G, Thomas RE, Shulman NJ, Beyer RP, MacCoss MJ, et al. The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. Proc Natl Acad Sci U S A. 2013;110(16):6400–5.
74. Margineantu DH, Emerson CB, Diaz D, Hockenbery DM. Hsp90 inhibition decreases mitochondrial protein turnover. PLoS One. 2007;2(10):e1066.
75. McLelland GL, Soubannier V, Chen CX, McBride HM, Fon EA. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. EMBO J. 2014;33(4):282–95.
76. Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA, et al. A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. Curr Biol. 2012;22(2):135–41.
77. Ashrafi G, Schlehe JS, LaVoie MJ, Schwarz TL. Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. J Cell Biol. 2014;206(5):655–70.
78. Grenier K, McLelland GL, Fon EA. Parkin- and PINK1-dependent mitophagy in neurons: will the real pathway please stand up? Front Neurol. 2013;4:100.
79. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by Parkin. Nature. 2006;441(7097):1157–61.
80. Sterky FH, Lee S, Wibom R, Olson L, Larsson NG. Impaired mitochondrial transport and Parkin-independent degeneration of respiratory chain-deficient dopamine neurons in vivo. Proc Natl Acad Sci U S A. 2011;108(31):12937–42.
81. Chu CT, Ji J, Dagda RK. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat Cell Biol. 2013;15(10):1197–205.
82. Melser S, Chatelain EH, Lavie J, Mahfouf W, Jose C, Obre E, et al. Rheb regulates mitophagy induced by mitochondrial energetic status. Cell Metab. 2013;17(5):719–30.
83. Maday S, Wallace KE, Holzbaur EL. Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. J Cell Biol. 2012;196(4):407–17.
84. Wong E, Cuervo AM. Integration of clearance mechanisms: the proteasome and autophagy. Cold Spring Harbor Perspect Biol. 2010;2(12):a006734.
85. Johri A, Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. J Pharmacol Exp Ther. 2012;342(3):619–30.
86. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006;441(7095):885–9.
87. Son JH, Shim JH, Kim KH, Ha JY, Han JY. Neuronal autophagy and neurodegenerative diseases. Exp Mol Med. 2012;44(2):89–98.
88. Corti O, Lesage S, Brice A. What genetics tells us about the causes and mechanisms of Parkinson's disease. Physiol Rev. 2011;91(4):1161–218.

89. Trempe JF, Fon EA. Structure and function of Parkin, PINK1, and DJ-1, the three musketeers of neuroprotection. Front Neurol. 2013;4:38.
90. Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. Hum Mutat. 2012;33(9):1340–4.
91. Riley BE, Lougheed JC, Callaway K, Velasquez M, Brecht E, Nguyen L, et al. Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. Nat Commun. 2013;4:1982.
92. Trempe JF, Sauve V, Grenier K, Seirafi M, Tang MY, Menade M, et al. Structure of parkin reveals mechanisms for ubiquitin ligase activation. Science (New York, NY). 2013;340(6139):1451–5.
93. Tomoo K, Mukai Y, In Y, Miyagawa H, Kitamura K, Yamano A, et al. Crystal structure and molecular dynamics simulation of ubiquitin-like domain of murine parkin. Biochim Biophys Acta. 2008;1784(7–8):1059–67.
94. Wauer T, Komander D. Structure of the human Parkin ligase domain in an autoinhibited state. EMBO J. 2013;32(15):2099–112.
95. Seirafi M, Kozlov G, Gehring K. Parkin structure and function. FEBS J. 2015;282(11):2076–88.
96. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, et al. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J Biol Chem. 2004;279(18):18614–22.
97. Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, et al. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. Mol Cell. 2009;33(5):627–38.
98. Morais VA, Haddad D, Craessaerts K, De Bock PJ, Swerts J, Vilain S, et al. PINK1 loss-of-function mutations affect mitochondrial complex I activity via NdufA10 ubiquinone uncoupling. Science (New York, NY). 2014;344(6180):203–7.
99. Pogson JH, Ivatt RM, Sanchez-Martinez A, Tufi R, Wilson E, Mortiboys H, et al. The complex I subunit NDUFA10 selectively rescues Drosophila pink1 mutants through a mechanism independent of mitophagy. PLoS Genet. 2014;10(11):e1004815.
100. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet. 2011;20(1):40–50.
101. Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, et al. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A. 2004;101(24):9103–8.
102. Taira T, Saito Y, Niki T, Iguchi-Ariga SM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep. 2004;5(2):213–8.
103. Cox D, Carver JA, Ecroyd H. Preventing alpha-synuclein aggregation: the role of the small heat-shock molecular chaperone proteins. Biochim Biophys Acta. 2014;1842(9):1830–43.
104. Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, et al. Interplay of LRRK2 with chaperone-mediated autophagy. Nat Neurosci. 2013;16(4):394–406.
105. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem. 2003;278(27):25009–13.
106. Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, et al. Alpha-Synuclein impairs macroautophagy: implications for Parkinson's disease. J Cell Biol. 2010;190(6):1023–37.
107. Zavodszky E, Seaman MN, Moreau K, Jimenez-Sanchez M, Breusegem SY, Harbour ME, et al. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. Nat Commun. 2014;5:3828.
108. Schapansky J, Nardozzi JD, Felizia F, LaVoie MJ. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. Hum Mol Genet. 2014;23(16):4201–14.
109. Schapansky J, Nardozzi JD, LaVoie MJ. The complex relationships between microglia, alpha-synuclein, and LRRK2 in Parkinson's disease. Neuroscience. 2014;302:74–88.

110. Bravo-San Pedro JM, Niso-Santano M, Gomez-Sanchez R, Pizarro-Estrella E, Aiastui-Pujana A, Gorostidi A, et al. The LRRK2 G2019S mutant exacerbates basal autophagy through activation of the MEK/ERK pathway. Cell Mol Life Sci. 2013;70(1):121–36.
111. Tong Y, Yamaguchi H, Giaime E, Boyle S, Kopan R, Kelleher 3rd RJ, et al. Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. Proc Natl Acad Sci U S A. 2010;107(21):9879–84.
112. Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, et al. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. Hum Mol Genet. 2009;18(21):4022–34.
113. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet. 2006;38(10):1184–91.
114. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med. 2009;361(17):1651–61.
115. Salawu FK, Umar JT, Olokoba AB. Alzheimer's disease: a review of recent developments. Ann Afr Med. 2011;10(2):73–9.
116. Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, et al. Mitochondrial abnormalities in Alzheimer's disease. J Soc Neurosci. 2001;21(9):3017–23.
117. Garcia-Escudero V, Martin-Maestro P, Perry G, Avila J. Deconstructing mitochondrial dysfunction in Alzheimer disease. Oxid Med Cell Longev. 2013;2013:162152.
118. Baloyannis SJ. Mitochondrial alterations in Alzheimer's disease. J Alzheimers Dis. 2006;9(2):119–26.
119. Maurer I, Zierz S, Moller HJ. A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiol Aging. 2000;21(3):455–62.
120. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem. 2002;80(1):91–100.
121. Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, et al. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. J Neurosci. 2009;29(28):9090–103.
122. Moreira PI, Siedlak SL, Wang X, Santos MS, Oliveira CR, Tabaton M, et al. Autophagocytosis of mitochondria is prominent in Alzheimer disease. J Neuropathol Exp Neurol. 2007;66(6):525–32.
123. Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, et al. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J Neurosci. 2008;28(27):6926–37.
124. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, et al. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol. 2005;64(2):113–22.
125. Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, et al. Macroautophagy – a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol. 2005;171(1):87–98.
126. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, et al. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. J Clin Invest. 2008;118(6):2190–9.
127. Wang Y, Martinez-Vicente M, Kruger U, Kaushik S, Wong E, Mandelkow EM, et al. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum Mol Genet. 2009;18(21):4153–70.
128. Funderburk SF, Marcellino BK, Yue Z. Cell "self-eating" (autophagy) mechanism in Alzheimer's disease. Mt Sinai J Med. 2010;77(1):59–68.
129. Rosen KM, Moussa CE, Lee HK, Kumar P, Kitada T, Qin G, et al. Parkin reverses intracellular beta-amyloid accumulation and its negative effects on proteasome function. J Neurosci Res. 2010;88(1):167–78.

130. Yu WH, Kumar A, Peterhoff C, Shapiro Kulnane L, Uchiyama Y, Lamb BT, et al. Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease. Int J Biochem Cell Biol. 2004;36(12):2531–40.
131. Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, et al. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell. 2010;141(7):1146–58.
132. Theuns J, Del-Favero J, Dermaut B, van Duijn CM, Backhovens H, Van den Broeck MV, et al. Genetic variability in the regulatory region of presenilin 1 associated with risk for Alzheimer's disease and variable expression. Hum Mol Genet. 2000;9(3):325–31.
133. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol. 2011;10(1):83–98.
134. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. 1997;41(5):646–53.
135. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 2006;127(1):59–69.
136. Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, et al. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. J Neurosci. 2008;28(11):2783–92.
137. Reddy PH, Mao P, Manczak M. Mitochondrial structural and functional dynamics in Huntington's disease. Brain Res Rev. 2009;61(1):33–48.
138. Martin DD, Ladha S, Ehrnhoefer DE, Hayden MR. Autophagy in Huntington disease and huntingtin in autophagy. Trends Neurosci. 2015;38(1):26–35.
139. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, et al. Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol. 2014;112:24–49.
140. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, et al. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci. 2010;13(5):567–76.
141. Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, et al. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J Neurosci. 2000;20(19):7268–78.
142. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet. 2004;36(6):585–95.
143. Shibata M, Lu T, Furuya T, Degterev A, Mizushima N, Yoshimori T, et al. Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. J Biol Chem. 2006;281(20):14474–85.
144. Wong YC, Holzbaur EL. The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. J Neurosci. 2014;34(4):1293–305.
145. Ochaba J, Lukacsovich T, Csikos G, Zheng S, Margulis J, Salazar L, et al. Potential function for the Huntingtin protein as a scaffold for selective autophagy. Proc Natl Acad Sci U S A. 2014;111(47):16889–94.
146. Rui YN, Xu Z, Patel B, Chen Z, Chen D, Tito A, et al. Huntingtin functions as a scaffold for selective macroautophagy. Nat Cell Biol. 2015;17(3):262–75.
147. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;362(6415):59–62.
148. Bruijn LI, Miller TM, Cleveland DW. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Ann Rev Neurosci. 2004;27:723–49.
149. Bacman SR, Bradley WG, Moraes CT. Mitochondrial involvement in amyotrophic lateral sclerosis: trigger or target? Mol Neurobiol. 2006;33(2):113–31.

150. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 2006;443(7113):787–95.
151. Jaarsma D, Rognoni F, van Duijn W, Verspaget HW, Haasdijk ED, Holstege JC. CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. Acta Neuropathol. 2001;102(4):293–305.
152. Vijayvergiya C, Beal MF, Buck J, Manfredi G. Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. J Neurosci. 2005;25(10):2463–70.
153. Liu J, Lillo C, Jonsson PA, Vande Velde C, Ward CM, Miller TM, et al. Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. Neuron. 2004;43(1):5–17.
154. Takeuchi H, Kobayashi Y, Ishigaki S, Doyu M, Sobue G. Mitochondrial localization of mutant superoxide dismutase 1 triggers caspase-dependent cell death in a cellular model of familial amyotrophic lateral sclerosis. J Biol Chem. 2002;277(52):50966–72.
155. Kong J, Xu Z. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. J Neurosci. 1998;18(9):3241–50.
156. Li L, Zhang X, Le W. Altered macroautophagy in the spinal cord of SOD1 mutant mice. Autophagy. 2008;4(3):290–3.
157. Morimoto N, Nagai M, Ohta Y, Miyazaki K, Kurata T, Morimoto M, et al. Increased autophagy in transgenic mice with a G93A mutant SOD1 gene. Brain Res. 2007;1167:112–7.
158. Nassif M, Hetz C. Targeting autophagy in ALS: a complex mission. Autophagy. 2011;7(4):450–3.
159. Kabuta T, Suzuki Y, Wada K. Degradation of amyotrophic lateral sclerosis-linked mutant Cu, Zn-superoxide dismutase proteins by macroautophagy and the proteasome. J Biol Chem. 2006;281(41):30524–33.
160. Gal J, Strom AL, Kilty R, Zhang F, Zhu H. p62 accumulates and enhances aggregate formation in model systems of familial amyotrophic lateral sclerosis. J Biol Chem. 2007;282(15):11068–77.
161. Rea SL, Majcher V, Searle MS, Layfield R. SQSTM1 mutations – bridging Paget disease of bone and ALS/FTLD. Exp Cell Res. 2014;325(1):27–37.
162. Teyssou E, Takeda T, Lebon V, Boillee S, Doukoure B, Bataillon G, et al. Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: genetics and neuropathology. Acta Neuropathol. 2013;125(4):511–22.
163. Zhang X, Li L, Chen S, Yang D, Wang Y, Zhang X, et al. Rapamycin treatment augments motor neuron degeneration in SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Autophagy. 2011;7(4):412–25.
164. Palikaras K, Tavernarakis N. Mitophagy in neurodegeneration and aging. Front Genet. 2012;3:297.
165. Santos RX, Correia SC, Carvalho C, Cardoso S, Santos MS, Moreira PI. Mitophagy in neurodegeneration: an opportunity for therapy? Curr Drug Targets. 2011;12(6):790–9.
166. Hochfeld WE, Lee S, Rubinsztein DC. Therapeutic induction of autophagy to modulate neurodegenerative disease progression. Acta Pharmacol Sin. 2013;34(5):600–4.
167. Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. Nat Rev Drug Discov. 2012;11(9):709–30.
168. Cheng Y, Ren X, Hait WN, Yang JM. Therapeutic targeting of autophagy in disease: biology and pharmacology. Pharmacol Rev. 2013;65(4):1162–97.
169. Rana A, Rera M, Walker DW. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. Proc Natl Acad Sci U S A. 2013;110(21):8638–43.
170. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, et al. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. Proc Natl Acad Sci U S A. 2006;103(28):10793–8.

171. Hong X, Liu J, Zhu G, Zhuang Y, Suo H, Wang P, et al. Parkin overexpression ameliorates hippocampal long-term potentiation and beta-amyloid load in an Alzheimer's disease mouse model. Hum Mol Genet. 2014;23(4):1056–72.
172. Bian M, Liu J, Hong X, Yu M, Huang Y, Sheng Z, et al. Overexpression of parkin ameliorates dopaminergic neurodegeneration induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. PLoS One. 2012;7(6):e39953.