Parkin-mediated ubiquitin signalling in aggresome formation and autophagy

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Abstract

Understanding how cells handle and dispose of misfolded proteins is of paramount importance because protein misfolding and aggregation underlie the pathogenesis of many neurodegenerative disorders, including PD (Parkinson's disease) and Alzheimer's disease. In addition to the ubiquitin–proteasome system, the aggresome–autophagy pathway has emerged as another crucial cellular defence system against toxic build-up of misfolded proteins. In contrast with basal autophagy that mediates non-selective, bulk clearance of misfolded proteins along with normal cellular proteins and organelles, the aggresome–autophagy pathway is increasingly recognized as a specialized type of induced autophagy that mediates selective clearance of misfolded and aggregated proteins under the conditions of proteotoxic stress. Recent evidence implicates PD-linked E3 ligase parkin as a key regulator of the aggresome–autophagy pathway and indicates a signalling role for Lys^{63}-linked polyubiquitination in the regulation of aggresome formation and autophagy. The present review summarizes the current knowledge of the aggresome–autophagy pathway, its regulation by parkin-mediated Lys^{63}-linked polyubiquitination, and its dysfunction in neurodegenerative diseases.

Keywords

aggresome; autophagy; misfolded protein; parkin; Parkinson's disease; ubiquitin-protein ligase

Introduction

PD (Parkinson's disease) and AD (Alzheimer's disease) as well as many other neurodegenerative disorders are often referred to as 'protein misfolding diseases' because their pathogenesis involves protein misfolding and aggregation [1,2]. The accumulation of misfolded proteins in these diseases probably occurs due to a chronic imbalance in the generation and clearance of misfolded proteins, and it suggests that the failure of cells to cope with excess misfolded proteins may be a common pathological mechanism linking these clinically distinct diseases. Protein misfolding can occur as a result of genetic mutations, environmental insults or oxidative damage [3]. Misfolded proteins are often prone to aggregation into oligomers and aggregates, and they can impair cell function and viability through a variety of mechanisms, including pore formation, proteasome inhibition and disruption of intracellular transport [1,3].

Growing evidence indicates that, when the production of misfolded proteins exceeds the capacity of the molecular chaperone system and the Ub (ubiquitin)–proteasome pathway, misfolded and aggregated proteins are actively sequestered in a microscopically visible, pericentriolar structure called an aggresome [3,4] and are subsequently degraded by

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macroautophagy (hereafter referred to as autophagy), a lysosome-dependent process that mediates bulk clearance of cytosolic proteins and organelles [5,6]. Here, we review recent evidence for the involvement of the aggresome–autophagy pathway in protection against misfolded protein accumulation and neurodegeneration and discuss the role of Lys^{63}-linked polyubiquitination by PD-linked E3 ligase parkin in regulation of misfolded protein handling by this pathway.

The aggresome–autophagy pathway

The aggresome–autophagy pathway is increasingly recognized as a key cellular defence system against accumulation of misfolded and aggregated proteins when the proteasome is overwhelmed or impaired [3,4]. In this system, misfolded and aggregated proteins are selectively recognized and delivered via dynein-mediated, microtubule-based retrograde transport towards the MTOC (microtubule-organizing centre) to form aggresomes at the pericentriolar region (Figure 1). Accumulating evidence indicates that aggresome formation not only protects cells by sequestering cytotoxic misfolded and aggregated proteins but also serves as a mechanism for concentrating misfolded and aggregated proteins for subsequent clearance by autophagy [3,7].

Autophagy is a multistep process characterized by the formation of an isolation membrane called a phagophore that expands to sequester a portion of the cytoplasm, leading to the formation of the double-membrane autophagosome, which subsequently fuses with the lysosome for degradation of the sequestered cytoplasmic cargo [5,6]. Unlike the proteasome, autophagy does not require unfolding of the substrates and is able to break down large protein complexes, protein aggregates and entire organelles. Autophagy is a highly regulated process involving the co-ordinated action of a large number of proteins encoded by Atg (autophagy-related) genes [5,6]. Recent studies have shown that autophagy is induced in response to oxidative stress or proteasome impairment and participates directly in the clearance of aggresomes [6–10].

Although autophagy is generally thought to be a non-selective, bulk degradation process, increasing evidence indicates the presence of selective autophagy that mediates clearance of specific cargos, such as mitophagy, reticulophagy, pexophagy and xenophagy [6]. Emerging evidence suggests that selective autophagy is involved in the clearance of misfolded and aggregated proteins, as inhibition of autophagy preferentially affects the degradation of several neurodegenerative disease-associated mutant proteins but not their wild-type counterparts [8, 11]. Although the mechanism underlying the selective autophagic clearance of misfolded proteins is not understood, the selective sequestration of misfolded proteins in aggresomes offers one method of preferential clearance of the abnormal proteins by autophagy. Aggresomes have also been shown to participate in the induction of autophagy by sequestering the endogenous autophagy suppressor mTOR (mammalian target of rapamycin) kinase [3,9]. Together, current data suggest that, unlike basal autophagy that mediates non-selective clearance of misfolded proteins along with normal cellular proteins, the aggresome–autophagy pathway is a specialized type of induced autophagy that mediates selective clearance of misfolded and aggregated proteins under the conditions of proteotoxic stress.

Parkin-mediated Ub signalling in regulation of the aggresome–autophagy pathway

Parkin and PD

PD is the most common neurodegenerative movement disorder, characterized by the loss of nigral dopaminergic neurons and the presence of intraneuronal cytoplasmic inclusions called

Lewy bodies [12–14]. Homozygous mutations in the gene encoding the E3 Ub–protein ligase parkin cause an autosomal recessive, early onset form of PD that is devoid of Lewy bodies [12,13]. In addition, heterozygous mutations in parkin have been implicated as a significant risk factor in the development of late-onset sporadic PD [15]. Studies in a number of cell and animal model systems have shown that parkin exerts cytoprotective action against a wide variety of cellular stresses, including oxidative stress, proteasome inhibition and proteotoxic stress induced by overexpression of aggregation-prone proteins [14]. However, the molecular mechanisms underlying the cytoprotective action of parkin remain poorly understood.

A mechanistic understanding of the cytoprotective action of parkin requires molecular characterization of parkin E3 ligase function, its substrates and the types and functional consequences of parkin-mediated ubiquitination. Parkin has been reported to regulate Lys[48]-linked polyubiquitination and proteasomal degradation of several putative substrates [13,14], although the validity of these proteins as physiological parkin substrates remains controversial [16]. Parkin has also been shown to be capable of catalysing monoubiquitination and Lys[63]-linked polyubiquitination, thereby regulating proteasome-independent cellular processes, such as endocytosis and NF-κB (nuclear factor κB) signalling [17,18]. In addition, parkin has been implicated in regulation of mitochondria dynamics [19,20], although the parkin substrate(s) on the mitochondria remains to be identified.

Parkin-mediated Lys[63]-linked polyubiquitination and aggresome formation

In a recent study [21], we investigated the specificity of parkin-mediated ubiquitination and its role in cellular management of misfolded proteins using wild-type DJ-1 and L166P mutant DJ-1 as the substrates. DJ-1 is a ubiquitously expressed protein that is mutated in an autosomal recessive, early-onset form of PD [22]. We have previously shown that wild-type DJ-1 is a compactly folded protein with a helix–strand–helix sandwich structure, whereas the PD-linked L166P mutant DJ-1 is a misfolded protein that is efficiently degraded by the Ub–proteasome system under normal conditions [23,24]. The results from our recent study [21] showed that, under conditions of proteasomal impairment, parkin co-operated with the heterodimeric E2 enzyme UbcH13–Uev1a to selectively catalyse Lys[63]-linked polyubiquitination of misfolded DJ-1, but not wild-type DJ-1 (Figure 1, step 6). Our results [21] further revealed that parkin-mediated Lys[63]-linked polyubiquitination coupled misfolded DJ-1 with the dynein motor complex via the adaptor protein HDAC6 (histone deacetylase 6) and thereby facilitated its transport to the MTOC for sequestration into the aggresome (Figure 1, steps 7 and 8).

HDAC6 is a Ub-binding protein that can simultaneously bind ubiquitinated proteins via its ZnF-UBP (zinc finger Ub-processing protease) domain and the dynein motor via another domain [25]. Depletion of HDAC6 by siRNAs (small interfering RNA) blocks aggresome formation, and this phenotype can be rescued only with a Ub-binding-competent form of HDAC6 [25]. Our previous study revealed that HDAC6 preferentially bound Lys[63]-linked polyubiquitinated proteins and that inhibition of Lys[63]-linked polyubiquitination or targeted disruption of parkin in mice impaired recruitment of misfolded DJ-1 to aggresomes [21]. Our findings suggest that Lys[63]-linked polyubiquitination by parkin serves as a signal for targeting misfolded proteins to the aggresome [21,26]. Consistent with our results, expression of mutant Ub that can only form Lys[63]-linked chains was recently shown to promote aggresome formation of several misfolded proteins, including tau protein and SOD-1 (superoxide dismutase 1) mutants [27], providing additional evidence supporting a role for Lys[63]-linked polyubiquitination in facilitating aggresome formation.

Parkin-mediated Lys[63]-linked polyubiquitination and autophagy

Our recent work suggests that parkin-mediated Lys[63]-linked polyubiquitination not only promotes sequestration of misfolded proteins into aggresomes but also facilitates their

subsequent clearance by autophagy [21,26]. We found that misfolded DJ-1-containing aggresomes stained with autophagic markers and were tightly encircled by lysosomes, indicating that aggresomes promoted by parkin-mediated Lys[63]-linked polyubiquitination are active sites of autophagy (Figure 1). Corroborating our findings, Tan et al. [27] demonstrated that the aggresomes formed in cells overexpressing the mutant Ub that can only form Lys[63]-linked polyubiquitin chains were preferentially cleared when autophagy is induced, whereas those formed in cells expressing Ub mutant that was unable to form Lys[63]-linked polyubiquitin chains were resistant to autophagic clearance. Thus emerging evidence has begun to suggest that Lys[63]-linked polyubiquitination has a signalling role in autophagic clearance of aggresomes.

A potential mechanism by which parkin-mediated Lys[63]-linked polyubiquitination may facilitate autophagic clearance of misfolded proteins is to promote the recruitment of autophagic membranes and autophagy machinery via binding to the adaptor protein p62 (Figure 1, steps 9 and 10). p62 is a Ub-binding protein that interacts with ubiquitinated proteins via its UBA (Ub-associated) domain and the autophagy machinery component LC3 via a 22-amino-acid LIR (LC3-interacting region) [28,29]. p62 shows preference for binding Lys[63]-linked polyubiquitin chains [30,31], and deletion of its UBA domain or LIR impairs the packing of ubiquitinated aggregates into autophagosomes [28,29]. Recent evidence indicates that, in addition to facilitating autophagic clearance, p62 also has a role in promoting protein aggregate formation [32]. Further studies are needed to determine whether p62 is indeed a Ub receptor for regulating the processing of Lys[63]-linked polyubiquitinated misfolded proteins by the aggresome–autophagy pathway.

Recently, parkin was shown to be selectively recruited to damaged mitochondria and promote their clearance by autophagy [20]. Autophagic clearance of mitochondria is often referred to as mitophagy [6], and there is evidence suggesting the involvement of ubiquitination in this process [33,34]. It remains to be determined whether the E3 ligase activity of parkin is required for its action in promoting mitophagy, what the parkin substrates on the mitochondria are and what type of ubiquitination is involved. A tantalizing possibility is that, similar to its action in promoting clearance of misfolded proteins by the aggresome–autophagy pathway (Figure 1), parkin may catalyse Lys[63]-linked polyubiquitination of misfolded proteins on the damaged mitochondria and this ubiquitination could be the signal for targeting damaged mitochondria for mitophagy.

Dysfunction of the aggresome–autophagy pathway and neurodegeneration

A link between dysfunction of the aggresome–autophagy pathway and neurodegeneration was first suggested by postmortem findings of the accumulation of Ub-positive protein aggregates and autophagosome-like structures in brains of patients with diverse neurodegenerative diseases, including PD and AD [3,35,36]. This link was further strengthened by recent identification of mutations in the aggresome–autophagy pathway components as the genetic defects responsible for several hereditary forms of neurodegenerative disorders (Table 1).

Loss-of-function mutations in parkin are a major cause of recessively transmitted early-onset PD [12,13]. Our finding that parkin-mediated Lys[63]-linked polyubiquitination of misfolded proteins promotes their sequestration into aggresomes and subsequent clearance by autophagy [21,26] provides evidence linking deregulation of the aggresome–autophagy pathway to PD pathogenesis. The impaired aggresome formation observed in cells from parkin-knockout mice [21] is reminiscent of the lack of Lewy bodies in parkin-associated human PD cases [13,14], suggesting that Lys[63]-linked polyubiquitination by parkin may be directly involved in the formation of Lewy bodies and that the inability to form these protective inclusion bodies may underlie the rapid disease onset and progression observed in patients with mutations in parkin.

Accumulation of Lys^{63}-linked polyubiquitinated proteins was recently detected in brains of human HD (Huntington's disease) patients [37], further supporting a connection between Lys^{63}-linked polyubiquitination and the formation of pathological inclusion bodies.

Mutations in the Ub-binding domain of p62, an adaptor that binds Lys^{63}-linked polyubiquitin chains and promotes autophagic clearance of protein aggregates [28–30], cause Paget disease [38]. Although Paget disease is primarily a bone disorder, knockout studies in mice revealed age-dependent accumulation of Lys^{63}-polyubiquitinated protein aggregates and neurodegeneration in $p62^{-/-}$ brains [39]. Additional support for the involvement of the aggresome–autophagy pathway dysfunction in neurodegeneration comes from the following: (i) the identification of mutations in dynactin subunit $p150^{Glued}$, a component of the dynein/dynactin motor that plays a critical role in aggresome formation as well as autophagy [40,41], as the cause for human motor neuron disease [42]; and (ii) animal model studies showing that disruption of the dynein/dynactin motor function leads to motor neuron degeneration [43,44] and enhanced toxicity of aggregation-prone proteins [41]. Dysfunction of the aggresome–autophagy pathway has also been implicated in the pathogenesis of two human neurodegenerative diseases, frontotemporal dementia [45] and ALS (amyotrophic lateral sclerosis) [46], by the findings that the disease-causing mutations in the ESCRT (endosomal sorting complexes required for transport)-III subunit CHMP2B (charged multivesicular body protein 2B) cause impairments in autolysosome formation and autophagic clearance, leading to accumulation of Ub-positive protein aggregates and neuronal cell death [47,48].

Previously, it was reported that overexpression of HDAC6, a key regulator of the aggresome–autophagy pathway [10,25], is able to suppress neurodegeneration in *Drosophila* induced by proteasome impairment or by expression of the spinobulbar muscular atrophy-associated mutant protein [49]. Furthermore, pharmacological activation of autophagy with mTOR inhibitors has been shown to reduce neurotoxicity of misfolded and aggregated proteins in cell and animal models of neurodegenerative diseases [8,9,50]. Together, these findings point to a critical role for the aggresome–autophagy pathway in the protection against misfolded protein accumulation and neurodegeneration, and they suggest that targeting this pathway may have therapeutic benefits for treating neurodegenerative disorders.

Conclusions and perspectives

While the role of the Ub–proteasome system in misfolded protein degradation has long been appreciated, the involvement of the aggresome–autophagy pathway in cellular defence against cytotoxic accumulation of misfolded proteins has only recently been recognized. Lys^{63}-linked polyubiquitination by parkin has emerged as a signal for targeting misfolded proteins into aggresomes and facilitating their selective clearance by autophagy [21,26]. Increasing evidence suggests that the Ub-binding proteins HDAC6 and p62 may function as cargo receptors for recognizing Lys^{63}-linked polyubiquitinated misfolded proteins and facilitating their processing through the aggresome–autophagy pathway (Figure 1). Given the recently reported role of parkin in mitophagy [20], it is tempting to speculate that parkin-mediated Lys^{63}-linked polyubiquitination of mitochondria-localized misfolded proteins may act as a signal for targeting damaged mitochondria for mitophagy. Further studies of the molecular mechanisms by which parkin promotes clearance of misfolded proteins and damaged mitochondria should facilitate the development of novel therapies for treating PD as well as other neurodegenerative diseases.

Acknowledgments

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Abbreviations used

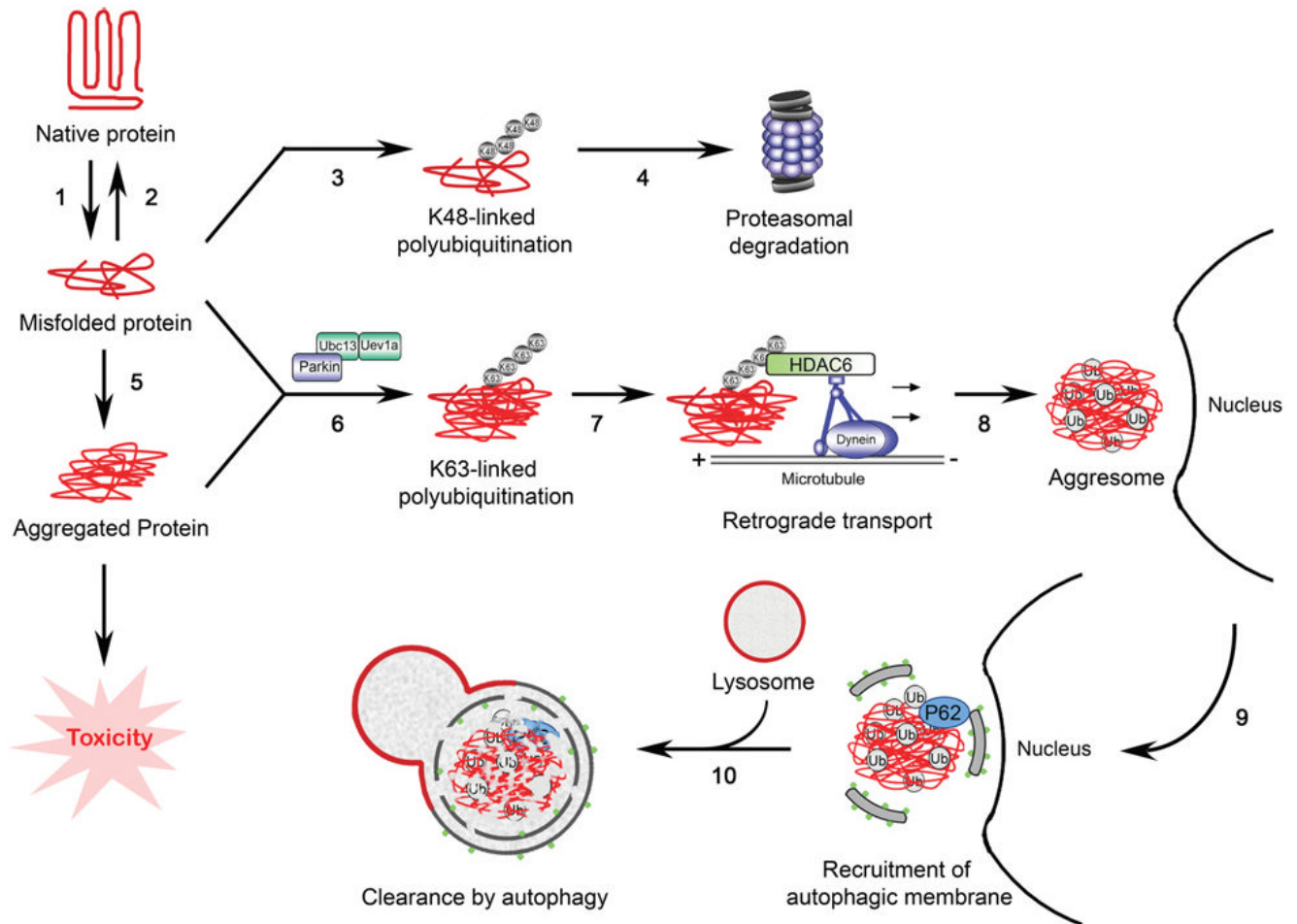| AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
CHMP2B	charged multivesicular body protein 2B
ESCRT	endosomal sorting complexes required for transport
HDAC6	histone deacetylase 6
LIR	LC3-interacting region
MTOC	microtubule-organizing centre
mTOR	mammalian target of rapamycin
PD	Parkinson's disease
Ub	ubiquitin

Figure 1. The aggresome–autophagy pathway and its regulation by parkin-mediated Lys^{63}-linked polyubiquitination

Protein misfolding can occur as a result of genetic mutations or oxidative damage (1). Once formed, misfolded proteins may be refolded by chaperones (2) or tagged with Lys^{48}-linked polyubiquitin chains (3) for degradation by the proteasome (4). When the chaperone and proteasome systems fail or are overwhelmed, misfolded proteins form oligomers and aggregates (5) that can cause cytotoxicity. Recent evidence indicates that, under conditions of proteasomal impairment, PD-linked E3 ligase parkin co-operates with the E2 enzyme Ubc13/Uev1a to mediate Lys^{63}-linked polyubiquitination of misfolded proteins (6). The Lys^{63}-linked polyubiquitin chains promote binding to HDAC6 (7) and thereby link the misfolded proteins to the dynein motor complex for retrograde transport towards the MTOC to form the aggresome (8). Lys^{63}-linked polyubiquitination may also promote binding to p62 and thereby facilitate the recruitment of autophagic membrane to the aggresome for the formation of an autophagosome (9). Subsequent fusion of the autophagosome with the lysosome allows the degradation of misfolded and aggregated proteins by lysosomal hydrolases (10).

Table 1

The aggresome–autophagy pathway components and neurodegenerative diseases

Component	Protein function	Disease	Reference(s)
Parkin	E3 Ub–protein ligase	PD	[12,13]
p62	Ub-binding, LC3-binding	Paget disease	[38]
p150[Glued]	Dynactin subunit	Motor neuron disease	[42]
CHMP2B	ESCRT-III subunit	Frontotemporal dementia, ALS	[45,46]