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Review

Mechanisms of selective autophagy and mitophagy: Implications for neurodegenerative diseases

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A B S T R A C T

Over the past 20 years, the concept of mammalian autophagy as a nonselective degradation system has been repudiated, due in part to important discoveries in neurodegenerative diseases, which opened the field of selective autophagy. Protein aggregates and damaged mitochondria represent key pathological hallmarks shared by most neurodegenerative diseases. The landmark discovery in 2007 of p62/SQSTM1 as the first mammalian selective autophagy receptor defined a new family of autophagy-related proteins that serve to target protein aggregates, mitochondria, intracellular pathogens and other cargoes to the core autophagy machinery *via* an LC3-interacting region (LIR)-motif. Notably, mutations in the LIR-motif proteins p62 (*SQSTM1*) and optineurin (*OPTN*) contribute to familial forms of frontotemporal dementia and amyotrophic lateral sclerosis. Moreover, a subset of LIR-motif proteins is involved in selective mitochondrial degradation initiated by two recessive familial Parkinson's disease genes. PTEN-induced kinase 1 (*PINK1*) activates the E3 ubiquitin ligase Parkin (*PARK2*) to mark depolarized mitochondria for degradation. An extensive body of literature delineates key mechanisms in this pathway, based mostly on work in transformed cell lines. However, the potential role of PINK1-triggered mitophagy in neurodegeneration remains a conundrum, particularly in light of recent *in vivo* mitophagy studies. There are at least three major mechanisms by which mitochondria are targeted for mitophagy: transmembrane receptor-mediated, ubiquitin-mediated and cardiolipin-mediated. This review summarizes key features of the major cargo recognition pathways for selective autophagy and mitophagy, highlighting their potential impact in the pathogenesis or amelioration of neurodegenerative diseases.

1. Cellular quality control and neurodegeneration

Protein aggregates and damaged mitochondria represent key pathological hallmarks shared by most neurodegenerative diseases. From the α-synuclein-containing Lewy bodies and Lewy neurites in Parkinson's disease (PD) to beta-amyloid plaques or neurofibrillary tangles of tau in Alzheimer's disease (AD), these pathological aggregates are highlighted by immunohistochemistry for ubiquitin (Chu et al., 2000). As polyubiquitination represents a classic signal for protein degradation by the ubiquitin-proteasome system, the accumulation of ubiquitinated aggregates was thought to reflect proteasomal failure in neurodegeneration (Moore et al., 2003; Ross and Pickart 2004). The proteasomal system classically degrades soluble, short-lived proteins that can be unfolded to pass inside the cylindrical proteasome structure, which may limit its ability to handle aggregated proteins. The autophagy-lysosomal system represents another major quality control system, which is capable of degrading both protein aggregates and organelles such as mitochondria (Ashford and Porter 1962; Elmore et al., 2001; Fortun et al., 2003; Ravikumar et al., 2002; Rideout et al., 2004). Whereas chaperone-mediated autophagy is also restricted to proteins that can be unfolded (Cuervo, 2004), macroautophagy (hereafter referred to as autophagy) involves the formation of *de novo* organelles called autophagosomes (Fig. 1), which surround and sequester intracellular cargoes of various sizes and compositions. In the last decade, it has become clear that ubiquitin not only regulates proteasomal degradation, but also plays a key role in selective autophagy (Kim et al., 2008; Pankiv et al., 2007; Tan et al., 2007). Accumulation of ubiquitinated protein aggregates may thus reflect dysregulation of either proteasomal and/or autophagic quality control systems.

Impairment in one cellular degradation pathway often results in compensatory upregulation of other pathways, complicating cause and effect relationships (Ding et al., 2003; Xilouri et al., 2009). Nevertheless, there is now extensive evidence from both post-mortem analysis of patient brain tissues (Table 1) and in experimental models of disease (Table 2) that support a key role for autophagy dysregulation in multiple neurodegenerative diseases. These range from proposed deficits in cargo targeting or impaired completion of autophagy (autophagic stress) to excessive degradation of mitochondria, neuronal atrophy and possibly autophagic cell death [Reviewed in (Boland and Nixon 2006; Cherra III and Chu, 2008; Chu 2006; Harris and Rubinsztein 2012; Liu and Levine 2015)]. It is evident that autophagy can be dysregulated at multiple steps and the effects of inducing autophagy may be beneficial or detrimental depending on cellular context and downstream factors (Table 2). Importantly from a therapeutic

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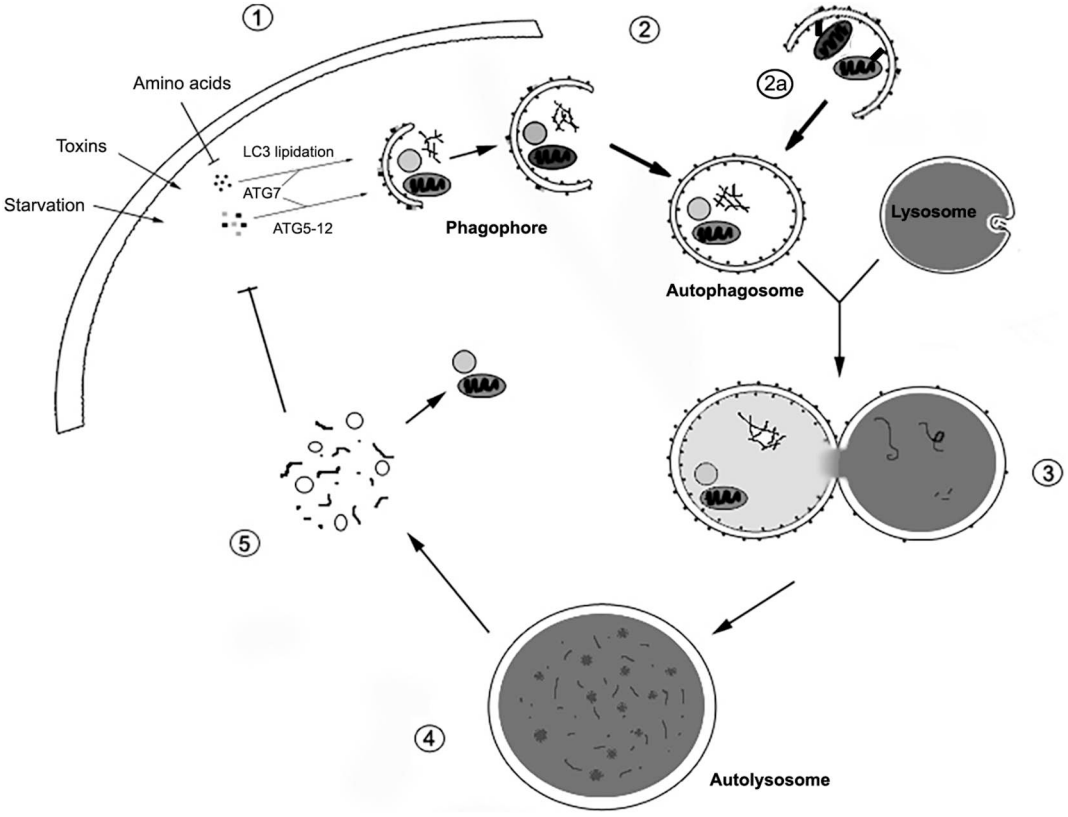

Fig. 1. Major steps in macroautophagy.
Cells integrate a variety of extracellular and intracellular signals that act to inhibit or promote autophagy (1). Autophagy initiation and membrane nucleation mediated by Atg1 and Beclin 1 complexes, respectively, is followed by phagophore membrane extension (2), a process regulated by covalent lipidation of LC3 and other Atg8 family members, to form an autophagosome. Selective autophagy is mediated by molecular interactions that enrich certain cargoes within the phagophore (2a). Maturation of the autophagosome to form an autolysosme proceeds through vesicular fusion with late endosomes and lysosomes (3). Within the acidic lysosomal lumen, enzymatic hydrolysis completes the degradation process (4). Hydrolysis products serve to downregulate autophagy initiation while providing substrates for energy or biosynthetic pathways (5).

Table 1
Altered autophagy and mitophagy in human post-mortem patient brain tissues.

Altered autophagy		
Alzheimer's disease	Granulovacuolar degeneration reflects accumulation of a type of autophagosome by EM	(Okamoto et al., 1991)
	Dystrophic neurites contain numerous autophagic structures by EM	(Nixon et al., 2005)
	Granulovacuolar degeneration colocalizes weakly with LC3 and p62, and strongly with lysosomal associated membrane protein 1	(Funk et al., 2011)
Parkinson's disease	Apoptosis and autophagic degeneration detected in substantia nigra neurons by EM	(Anglade et al., 1997)
Lewy body dementia	LC3-II and beclin 1 increased by WB	(Yu et al., 2009)
Aging	Decreased beclin 1 protein expression with aging by WB	(Shibata et al., 2006)
Altered mitophagy		
Alzheimer's disease	Localization of lipoic acid to lipofuscin by immunogold EM	(Moreira et al., 2007)
	Elevated Parkin and LC3-II protein in mitochondrial fractions by WB; decreased Parkin at high Braak stages	(Ye et al., 2015)
Parkinson's disease and Lewy body dementia	Increased autophagosomes containing abnormal mitochondria and activated ERK1/2 by IF and immunogold EM	(Zhu et al., 2003)
Aging	Phospho-ubiquitin IHC/IF in a 93-year old control and two aged PD patients	(Fiesel et al., 2015)

EM – electron microscopy; IF – immunofluorescence; IHC – immunohistochemistry; WB – Western blot.

standpoint, neurons may be involved in a double bind, whereby upregulation of autophagic flux elicits beneficial clearance of aggregates and damaged mitochondria (Dagda et al., 2009; Ravikumar et al., 2004), but may also contribute to pathological synaptic remodeling, excess mitochondrial depletion and axo-dendritic shrinkage (Cherra III et al., 2013; Gusdon and Chu 2011; Hernandez et al., 2012; Yang et al., 2007). Due to crosstalk among components of the proteostasis network, which includes biosynthetic, chaperone and degradative components, the outcome of autophagy or mitophagy stimulation in a particular pathological context may be difficult to predict and should be experimentally tested.

Although electron microscopy and biochemistry played key roles in the original description and early studies of autophagy, a series of seminal yeast nitrogen starvation studies have contributed to a molecular rennaissance in the study of autophagy. New tools to experimentally manipulate autophagy were made possible by the

Table 2
Altered autophagy and mitophagy in neurodegenerative disease models. Selected studies that illustrate new concepts or mechanisms by which alterations in autophagy may contribute to pathogenesis or neuroprotection, in chronological order by disease.

Disease	Process	Changes observed	Proposed mechanism/impact	References
HD	Autophagy	HD-expanded Htt exon 1 accumulates in early and late autophagic structures in striatal neurons	Kegel - Proposed autophagic cell death; Petersen - susceptibility to DA toxicity	(Kegel et al., 2000); (Petersen et al., 2001)
	Autophagy	Inhibition of mTOR alleviates toxicity in fly and mouse HD models	First paper to show therapeutic potential of inducing autophagy to clear aggregates *in vivo*	(Ravikumar et al., 2004)
	Autophagy	Mutant Htt sequesters beclin 1 and inhibits beclin 1-mediated protein degradation	Impaired beclin-dependent autophagy with aging may predispose to HD	(Shibata et al., 2006)
	Autophagy	Small molecule that upregulates mTOR-independent autophagy in neurons is neuroprotective	mTOR-independent autophagy inducer clears aggregates in primary neurons	(Tsvetkov et al., 2010)
	Autophagy	Mutant Htt interfers with the function of PGC-1alpha, which regulates mitochondrial biogenesis, oxidative stress and TFEB	Upregulation of lysosomal biogenesis by TFEB rescues Htt aggregation and neurotoxicity	(Tsunemi et al., 2012)
	Autophagy	Increased rates of autophagosome marker flux, but decreased cargo proteolysis with empty appearance ultrastructurally	Mutant Htt interferes with cargo recognition for selective autophagy	(Martinez-Vicente et al., 2010)
AD	Autophagy	Autophagic vacuoles generate Abeta	Autophagic structures may promote pathogenesis by serving as reservoirs for Abeta generation	(Yu et al., 2005)
	Autophagy	Beclin 1 deficiency in AD brains; beclin haploinsufficiency exacerbates pathology in APP transgenic mice	Impaired beclin-dependent autophagy induction may predispose to AD	(Pickford et al., 2008)
	Autophagy	Normal neurons have high rates of autophagic flux; inhibiting lysosomal proteolysis causes accumulation of autophagic structures similar in appearance to those observed in AD neurons	Autophagic pathology in AD arises from impaired clearance rather than strong induction alone	(Boland et al., 2008)
	Mitophagy	Increased parkin recruitment in mutant hAPP neurons; depletion of parkin with higher Braak stages	Diminished mitochondrial cargo targeting may occur in later stages of AD	(Ye et al., 2015)
	Autophagy	mTOR activity correlates with tau pathology and behavioral deficits *in vivo*	Suppression of mTOR signaling may be beneficial for tauopathies	(Caccamo et al., 2013)
PD	Autophagy	Mutant SNCA blocks chaperone-mediated uptake of proteins into the lysosome *via* LAMP-2A	CMA blockage contributes to activation of autophagy by mutant SNCA	(Cuervo et al., 2004)
	Autophagy	Increased autophagy in the MPP+ model	First report of beclin-independent autophagy, which promotes cell death	(Zhu et al., 2007)
	Autophagy	Plowey, Cherra- mutant LRRK2 elicits increased autophagy; inhibiting autophagy induction rescues neurite retraction. Alegre-Abarrategui - WT LRRK2 knockdown increases LC3-II flux in HEK293 cells. Schapansky - WT LRRK2 activation induces autophagy in monocytes.	LRRK2 has cell type or compartment-specific effects on autophagy. Autophagy contributes to shrinkage of neuronal processes.	(Alegre-Abarrategui et al., 2009; Cherra III et al., 2010; Plowey et al., 2008; Schapansky et al., 2014)
	Mitophagy	First report of parkin-mediated mitophagy that triggered major advances defining the ubiquitin pathway of mitophagy	Diminished mitochondrial cargo targeting may contribute to recessive PD	(Matsuda et al., 2010; Narendra et al., 2008) and papers too numerous to list
	Mitophagy	Increased mitophagy is observed in PINK1 deficient neuronal cells, in which it plays a neuroprotective role	PINK1 deficiency triggers mitochondrial damage, which elicits mitophagy	(Dagda et al., 2009)
	Autophagy	Beclin 1 gene transfer ameliorates synaptic and dendritic pathology in a lentiviral SNCA overexpression model	Therapeutic potential for beclin 1-dependent autophagy in synucleinopathies	(Spencer et al., 2009)
	Autophagy	Rapamycin effective for clearing acute rotenone induced SNCA aggregates, but not during chronic rotenone exposure	Mitochondrial dysfunction may limit efficacy of autophagy induction	(Yu et al., 2009)
	Autophagy	Lysosomal depletion contributes to accumulation of autophagosomes in the MPTP mouse model	Inducing lysosomal biogenesis alleviates autophagic stress	(Dehay et al., 2010)
	Mitophagy	Selective mitophagy induced by chronic MPP+ treatment in conjunction with impaired mitochondrial biogenesis	Impaired mitochondrial biogenesis determines outcome of mitophagy	(Zhu et al., 2012)
	Mitophagy	Loss of ATP13A2 decreases autophagic flux, resulting in accumulation of ROS-generating mitochondria	Impaired mitochondrial quality control due to lysosomal mechanisms	(Gusdon et al., 2012)
	Mitophagy	SNCA-induced mitophagy and autophagy shortens lifespan	Excess mitophagy may be harmful with aging	(Sampaio-Marques et al., 2012)
	Autophagy	The small molecule ambroxol not only increases glucocerebrocidase activity, but also favors exocytosis while suppressing autophagic flux	Reduction of SNCA levels by ambroxol or TFEB may be due to exocytosis rather than autophagy	(Magalhaes et al., 2018)
	Mitophagy	Cherra, Verma - LRRK2 promotes calcium dysregulation to elicit mitophagy-dependent dendrite retraction in neurons. Zhu - mutant LRRK2 interacts with ULK1 to induce autophagy and mitophagy.	Autophagic neurite retraction may be a side effect of mitochondrial clearance	(Cherra III et al., 2013; Verma et al., 2017; Zhu et al., 2013)

(*continued on next page*)

Table 2 (*continued*)

Disease	Process	Changes observed	Proposed mechanism/impact	References
ALS	Autophagy	Increased autophagy and decreased p-mTOR in SOD1-G93Amice	Descriptive, role of autophagy not explicitly investigated	(Morimoto et al., 2007)
	Mitophagy	Optineurin, which is mutated in ALS, is a mitophagy receptor in parkin-mediated mitophagy	Diminished mitochondrial cargo targeting may contribute to ALS	(Wong and Holzbaur 2014)
	Autophagy	Reducing beclin expression increased lifespan of mutant SOD1 transgenic mice	Autophagy may play a pathogenic rather than a protective role in familial ALS	(Nassif et al., 2014)
FTD	Autophagy	Mutant CHMP2B interferes with autophagosome maturation in cortical neurons and flies; inhibiting autophagy is protective	Autophagy induction may be harmful by increasing autophagic stress	(Lee et al., 2007; Lee and Gao 2009)
	Autophagy	Valosin-containing protein is necessary for autophagosome maturation and this function is disrupted by an IBMPFD mutation	Impaired autophagosome maturation as a pathogenic factor	(Ju et al., 2009)
	Autophagy	Rapamycin-induced autophagy aggravates pathology and weakness in a mouse model of VCP-associated myopathy	Autophagy induction may be harmful by increasing autophagic stress	(Ching and Weihl 2013)
	Autophagy	A152T tau allele causes neurodegeneration that can be ameliorated in a zebrafish model by autophagy induction	Autophagy upregulation may be protective in FTLD-tau	(Lopez et al., 2017)

APP – amyloid precursor protein; CHMP2B – charged multivesicular body protein 2B; Htt – huntingtin protein; IBMPFD - Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia; LRRK2 – leucine-rich repeat kinase 2; MPP + - the complex I inhibitor 1-methyl-4-phenylpyridinium; mTOR – mammalian target of rapamycin; ROS – reactive oxygen species; SNCA – alpha synuclein; SOD1 – superoxide dismutase 1; ULK1 - Unc-51 like autophagy activating kinase 1; VCP – valosin-containing protein; WT – wild type.

identification of evolutionarily conserved core components of the autophagy machinery (Scott et al., 1996; Thumm et al., 1994; Tsukada and Ohsumi 1993). Moreover, the discovery of novel ubiquitin-like conjugation systems that resulted in covalent attachment of yeast Atg8 to the autophagic membrane (Ichimura et al., 2000) enabled the development of new molecular strategies for detangling cause, effect and correlation. With rare exceptions, however, autophagy was still thought to represent predominantly a non-selective bulk degradation system in the first decade of the new millennium. This relatively narrow perspective has been markedly expanded by important discoveries in neurodegenerative disease research, which have moved the field of selective autophagy into the limelight.

2. Selective autophagy in neurons: a brief history

Although research in selective macroautophagy mechanisms has rapidly expanded over the last decade, the ability of cells to selectively degrade specific cargoes had been noted in early ultrastructural studies that predate the term autophagy itself. These pathological studies, referring to autophagosomes as “foci of physiologic autolysis” (Ashford and Porter 1962) or “focal cytoplasmic degradation” noted that the content of autophagic inclusions was dependent upon the type of injury or stimulus (Hruban et al., 1963). Nevertheless, at the dawn of the molecular age of autophagy research following the discovery of *Atg* genes in the 1990’s (Harding et al., 1995; Thumm et al., 1994; Tsukada and Ohsumi 1993), autophagy was still primarily conceptualized, with rare exceptions in yeast, as a bulk degradation response with little mechanism for specificity. A resurgence of interest in autophagy in pathological contexts following the discovery of the Atg8 lipidation reaction (Ichimura et al., 2000) began to shift the emphasis from starvation-induced autophagy to injury or damage-induced autophagy.

In brief, both nonselective and selective macroautophagy proceed through a set of basic steps (Fig. 1). These include: (1) integration of stimulatory and inhibitory signals, (2) extension of phagophore membranes and their fusion to form a spherical autophagosome that encapsulates degradative cargoes, (3) maturation of the autophagosome through additional fusion and transport steps that result in lumenal acidification and delivery of lysosomal enzymes, and (4) enzymatic cargo hydrolysis and release of the products. Recycling of certain autophagy proteins and the biosynthetic usage of degradation products (5) complete the cycle. Additional molecular mechanisms that enrich for specific cargoes within the growing phagophore confer selectivity to this cellular degradation and recycling process (Fig. 1, step 2a).

Research interest in selective mammalian mitophagy emerged from studies of apoptotic pathways in hepatocytes and neurons. Early in the course of apoptosis, live fluorescence imaging studies reveal that a small number of hepatic mitochondria lose their membrane potentials and enter acidic lysosomal compartments (Lemasters et al., 1998). Furthermore, NGF-deprived neurons are able to completely and selectively remove their entire cohort of mitochondria if apoptosis is inhibited (Xue et al., 2001). While this has been proposed to confer tolerance to an anaerobic environment in the short term, these primary cells are nevertheless committed to cell death. In 2002, a marked increase in small aggregates of phosphorylated ERK1/2 were noted in the substantia nigra neurons of patients with Parkinson's disease (PD) and dementia with Lewy bodies (DLB) (Zhu et al., 2002): These were ultrastructurally shown to represent abnormal mitochondria engulfed in autophagosomes (Zhu et al., 2003). These early studies anticipated two essential features of a heavily studied mitophagy pathway described in 2008 (Narendra et al., 2008) – namely, the ability of depolarizing ionophores to trigger complete mitochondrial removal and the potential importance of mitophagy in PD pathogenesis.

Meanwhile, studies focusing on the role of autophagy in clearing protein aggregates in neurological diseases resulted in delineation of a new class of intracellular mammalian selective autophagy cargo receptors. Aggregate-prone proteins such as exon 1 of huntingtin, α-

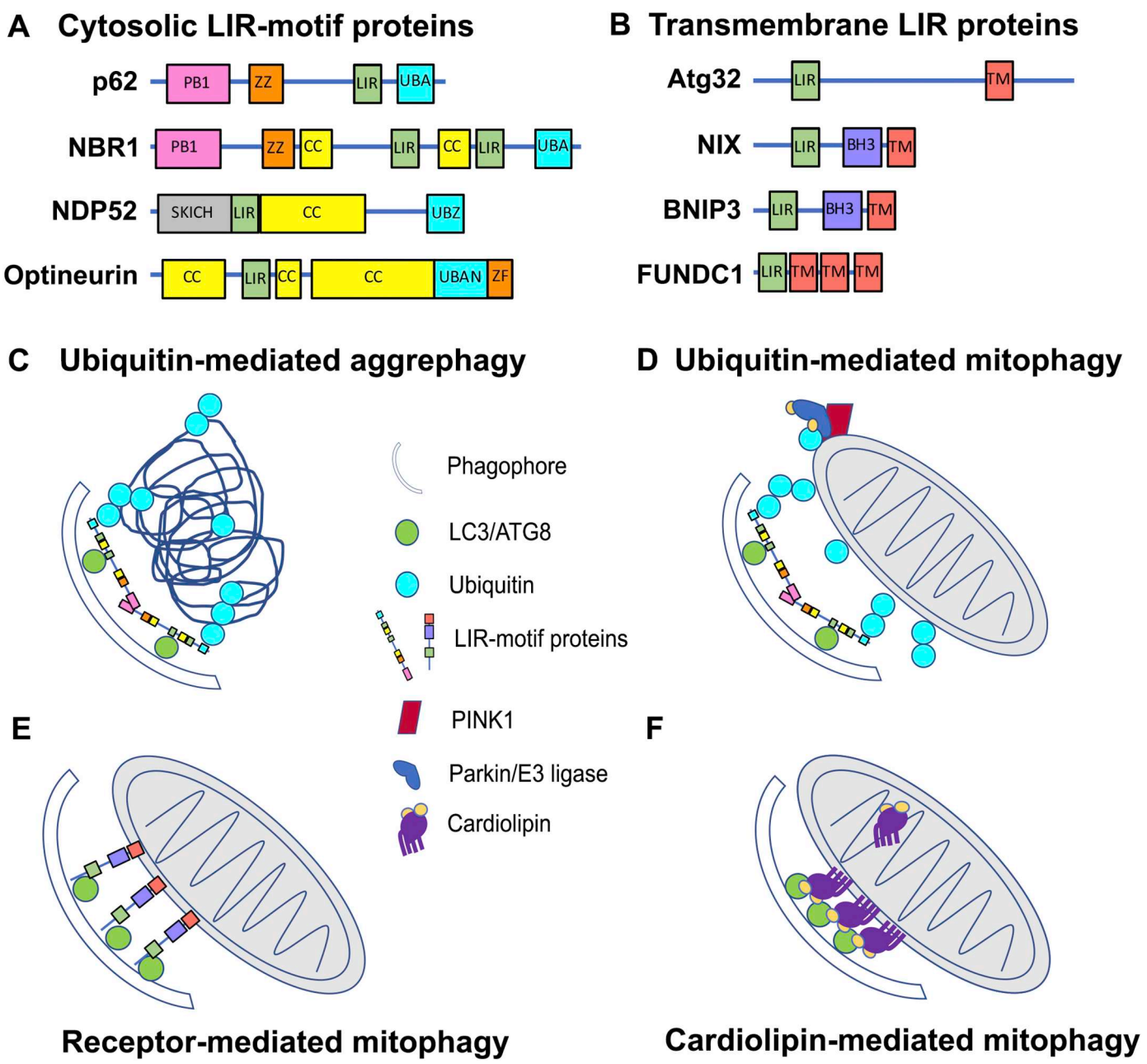

Fig. 2. Cargo receptors and pathways of selective mitophagy.
A subset of proteins that contain LIR-motifs (green) and ubiquitin binding domains (teal) function as selective autophagy receptors for ubiquitinated cargoes (A). LIR-motif proteins that exhibit membrane-spanning sequences (red) function in selective mitophagy (B). Ubiquitin tags on protein aggregates recruit cytosolic LIR-motif proteins, which target them to phagophore-bound LC3 (C). Many of the same LIR proteins also recognize mitochondria that are ubiquitinated following the recruitment and phosphorylations (yellow circle) of Parkin and ubiquitin by PINK1 (D). A group of transmembrane LIR-receptors expressed on the outer mitochondrial membrane function in developmental or hypoxia-induced mitophagy, regulated by transcriptional upregulation or dephosphorylation (E). The inner mitochondrial membrane phospholipid cardiolipin undergoes translocation to the outer membrane, where it can directly interact with LC3 to mediate mitophagy in neurons (F).

synuclein implicated in PD, or aggresomes containing misfolded peripheral myelin protein 22 in hereditary neuropathies could all be degraded by autophagy (Fortun et al., 2003; Ravikumar et al., 2002; Webb et al., 2003). Moreover, pharmacological induction of autophagy proved beneficial in several models of Huntington's disease (HD) (Ravikumar et al., 2004), and the p62/SQSTM1 protein was implicated in this process (Bjorkoy et al., 2005). In 2007, the molecular mechanism by which p62 promotes autophagic aggregate clearance was elucidated (Fig. 2C), due to its ability to simultaneously bind ubiquitinated cargo and the mammalian Atg8 homolog known as microtubule-associated proteins 1A/1B light chain 3 (LC3), a key component of the core autophagy machinery (Pankiv et al., 2007). Indeed, the ability to interact with Atg8 proteins, including members of both the mammalian LC3 subfamily and the gamma-aminobutyric acid receptor-associated protein (GABARAP) subfamily, forms a common theme as additional selective autophagy mechanisms emerge (Stolz et al., 2014). These cargo recognition signals involve not only protein-protein interactions, but also protein-lipid interactions (Chu et al., 2013).

3. LIR-motif proteins – targeting cargo to phagophores

Originally referred to as cargo adapters, because they link autophagic cargoes to LC3 during selective autophagy, these proteins have more recently been referred to as autophagy receptors because they

bind and/or are degraded along with the cargo. The structural motif that ties this family of proteins together is the LC3 interacting region (LIR), which consists of 2–3 acidic residues followed by a 4 residue motif [W/F/Y]-X_1-X_2-[L/I/V] (Birgisdottir et al., 2013). Subsequent to the identification of p62 as a selective autophagy receptor for ubiquitinated protein aggregates, it has become clear that there are both cytosolic and transmembrane proteins that bear the LIR motif (Fig. 2A–B). While most studies have focused upon the LC3 branch of the Atg8 family, which includes three human protein isoforms LC3A, LC3B and LC3C, members of the GABARAP subfamily, which include GABARAP, GABARAPL1 and GABARAPL2/Golgi-associated ATPase enhancer of 16 kDa (GATE-16), are also implicated in cargo targeting (Novak et al., 2010). Notably, genes for LIR-motif proteins such as p62 and optineurin are mutated in rare familial forms of the amyotrophic lateral sclerosis-frontotemporal dementia spectrum (ALS-FTD), implicating deficient cargo-recognition as a possible pathological mechanism. Mutant forms of the HD protein huntingtin may also act to interfere with autophagic cargo sequestration, resulting in decreased protein degradation despite increased turnover of autophagosome markers (Martinez-Vicente et al., 2010).

3.1. Cytosolic LIR-motif proteins

In general, these proteins contain both LIR-motifs and ubiquitin binding domains. They serve to connect cargoes tagged by ubiquitin to Atg8 family members (Fig. 2C). The presence of additional protein-protein interaction domains, such as PB1 or CC1 domains, regulates cargo binding avidity and the ability to cooperate with other cargo receptors or adapters (Nakamura et al., 2010). In addition, some LIR-motif proteins show greater affinity for certain Atg8 family members. For example, NDP52 has a noncanonical LIR lacking an aromatic residue, which accounts a 10-fold greater affinity for LC3C relative to LC3A (von Muhlinen et al., 2012). Given the surface charge differences between the LC3 and the GABARAP subfamilies (Sugawara et al., 2004), it is likely that additional proteins will be discovered that link specific cargoes to the less studied human Atg8 homologs.

In addition to ubiquitinated protein aggregates, p62 mediates degradation of ubiquitinated cytosolic bacteria (Zheng et al., 2009). However, its roles in selective mitophagy (Geisler et al., 2010) and pexophagy have been controversial, and may relate to cargo clustering and enhancement of other cargo targeting mechanisms (Narendra et al., 2010b; Yamashita et al., 2014). Differences observed in different experimental systems may also relate to post-translational regulation of p62, as phosphorylation at S403 appears to be necessary for p62-mediated mitophagy (Matsumoto et al., 2015). NDP52 is another LIR-motif protein implicated in selective autophagy of intracellular bacteria and depolarized mitochondria (Lazarou et al., 2015; von Muhlinen et al., 2012). Multimerization of cargo receptors enhances the avidity of binding to surface-bound LC3B, as well as conferring selectivity for certain types of ubiquitin chains (Wurzer et al., 2015; Xie et al., 2015). Similarly, dimerization of NBR1, which is necessary and sufficient for pexophagy (Deosaran et al., 2013), confers a slight preference for K63-linked ubiquitin over K48-linked ubiquitin (Walinda et al., 2014). Given that both p62 and NBR1 can be found in the same disease-associated protein aggregates (D'Agostino et al., 2011; Mori et al., 2012; Odagiri et al., 2012), it is likely that they cooperate to recognize slightly different cargo modifications, and that multiple cargo targeting mechanisms act together to enhance the overall avidity of interactions with the autophagic machinery.

Optineurin was recently shown to mediate mitophagy in neuronal cells (Wong and Holzbaur 2014). It is also involved in elimination of ubiquitinated bacteria. Phosphorylation of OPTN enhances its ability to bind LC3 and to restrict *Salmonella* growth (Wild et al., 2011). Phosphorylation and the post-translational regulation of autophagy receptors/adapters (Deng et al., 2012) and of Atg8/LC3 family members themselves (Cherra III et al., 2010; Wilkinson et al., 2015), comprise new areas of research emphasis. Given potential redundancies in function among members of this family, understanding how post-translational modifications regulate degradation of specific substrates and how disease mechanisms may hijack or intefere with this process will be important for understanding why mutation of a single LIR-motif protein contributes to neurodegeneration.

3.2. Transmembrane LIR-motif proteins

Another group of LIR-motif containing proteins exhibit a transmembrane domain and are expressed on the outer mitochondrial membrane or endoplasmic reticulum. These include the yeast Atg32 protein (Kanki et al., 2009) and mammalian proteins implicated in developmental or stress-induced mitophagy. These receptors will be discussed in greater detail below. Another member of this group of receptors is the cell cycle progression protein 1 (CCPG1). CCPG1 is upregulated with ER stress and mediates selective reticulophagy (or ER-phagy). It has a canonical LIR motif WTVI, which also functions as a GABARAP-interaction motif with the addition of V/I in the X_1 position (Rogov et al., 2017). In addition, CCPG1 interacts with FIP200, a component of the ULK/Atg1 autophagy initiating complex (Smith et al., 2018). Conceivably, this interaction could provide a mechanism linking cargo recognition to induction of autophagosome formation.

3.3. Beyond cargo recognition

In addition to mediating interactions between Atg8 family members and cargoes for selective autophagy, LIR-motif proteins play other important roles in autophagy regulation. For example, pleckstrin homology and RUN domain containing M1 (PLEKHM1), which is essential for bone resorption, acts to regulate the interaction between the autophagy machinery and endosomal sorting complexes (McEwan et al., 2015). In neurons, the autophagic and endocytic pathways intersect at multiple levels (Plowey and Chu 2011), and genes involved in endosome, retromer and autophagy regulation have been implicated in several neurodegenerative diseases (Lane et al., 2012; Nixon 2013). In addition, dysregulation of autophagic maturation and flux is a prominent feature of several neurodegenerative diseases (Chu 2006). The SNARE protein syntaxin 17 is essential for the fusion of autophagosomes with lysosomes (Itakura et al., 2012). Recently it has been shown that interactions between syntaxin 17 and LC3 is mediated by a LIR-motif (Kumar et al., 2018). The involvement of Atg8 family members in both core autophagosome formation/maturation events and in cargo targeting likely explain early observations that LC3 is covalently deposited on both the inside and outside curvatures of the phagophore as it extends to form an autophagosome (Kabeya et al., 2000).

4. Other cargo recognition mechanisms

4.1. Protein-protein interactions

Among the cargoes that have been shown to undergo selective autophagy is the iron-binding ferritin complex (Dowdle et al., 2014). Recently, it was discovered that the androgen receptor-binding nuclear receptor coactivator 4 (NCOA4) serves as a cargo receptor for ferritinophagy. Interestingly, NCOA4 does not have a classic LIR-motif (Mancias et al., 2014), and the amino acid residues responsible for its assumed interaction with LC3 remain undefined by mapping studies. One possibility is that its association with LC3 puncta is indirect, as GST pull-down was strongest for GABARAPL2 and other cargo receptors were also present in the isolated autophagosomes.

The ubiquilins represent a family of ubiquitin-binding proteins involved in several degradative pathways. Ubiquilin 1, whose polymorphisms modulate risk for Alzheimer's disease (Kamboh et al., 2006), is degraded by both macroautophagy and chaperone mediated autophagy (Rothenberg et al., 2010). Besides protein aggregates, ubiquilin 1

interacts with intracellular *Mycobacterium tuberculosis* and is important in xenophagy (Sakowski et al., 2015), although it does not directly bind LC3 (Rothenberg et al., 2010). Conversely, ubiquilin 4 does bind LC3 through a novel interaction requiring the N-terminal STI1 repeats (Lee et al., 2013). As ubiquilin 4 also binds to ubiquilin 1, it may function as a selective autophagy adapter for ubiquilin 1-labeled cargoes.

Alfy (autophagy-linked FYVE domain containg protein, or WDF3Y), is a large scaffold protein that facilitates formation of autophagosomes around protein aggregates. It is capable of binding directly to p62 as well as to Atg5 (Filimonenko 2010). Moreover, its FYVE domain enables it to interact with PI3P, enriched on autophagic precursor membranes by the activity of the Beclin 1-type 3 phosphatidylinositol 3-kinase (PIK3C3/Vps34) complex. As with CCPG1, this could be a mechanism to coordinate general autophagy processes such as nucleation with cargo recognition. It is likely that additional adapter proteins will be identified that link cargoes not only to Atg8 family members, but also to other components of the core autophagy machinery.

4.2. Protein-lipid and protein-carbohydrate interactions

The ability of non-protein macromolecules to bind to Atg8 family members and mediate cargo recognition for selective autophagy is likely to reflect a growing area given the macromolecular heterogeneity of potential autophagic cargoes. As discussed below, mitochondrial cardiolipin is redistributed to the surface of damaged mitochondria, where it recruits LC3 to mediate the formation of autophagosomes centered on mitochondria (mitophagosomes) (Chu et al., 2013). While it remains unknown whether or not cardiolipin, which is found in both mitochondria and bacteria, participates in xenophagy, the ability of NDP52 to target glycoconjugates through interaction with galectin 8 plays an important role in host defense against bacterial invasion (Thurston et al., 2012). Indeed, galectin 8-mediated recruitment of cargo receptors to bacteria-damaged intracellular membranes occurs earlier and may be essential for the subsequent, more heavily studied phase of ubiquitin-dependent recruitment. It is reasonable to assume that protein-lipid interactions may play an important role in the selective autophagy of lipid droplets (Singh et al., 2009) and other organelles.

5. Mitophagy in neurons

The structural and functional properties of neurons necessitate metabolic dependence on mitochondria (Kann and Kovacs 2007). As such, cellular quality control mechanisms to recognize and recycle mitochondria damaged with usage, aging or disease are critically regulated in neurons (Dagda et al., 2009; Tatsuta and Langer 2008). Mitochondrial quality control may be mediated by antioxidant and chaperone expression, localized remodeling/proteolysis and autophagic degradation (Chu 2010). In addition, mitochondrial proteins may be delivered to the lysosome through mitochondria-derived vesicles (Soubannier et al., 2012). Nevertheless, macroautophagy is currently believed to represent the major mechanism for mitochondrial recycling. Basal mitophagy is an ongoing process, thought to be required for maintaining neuronal health. In addition, mitophagy can be upregulated in neurons by a variety of pathological stimuli.

Alterations in mitophagy have been observed in nearly all genetic or toxic-environmental models of PD pathogenesis (Cherra III et al., 2013; Chinta et al., 2010; Chu et al., 2013; Dagda et al., 2009; Dagda et al., 2008; Osellame and Duchen 2013; Zhu et al., 2007). While many of these studies did not address selectivity, selective degradation of mitochondria is induced in response to low dose treatment with the complex I inhibitor MPP+ (Zhu et al., 2012), commonly used to model PD pathogenesis. The dopaminergic midbrain neurons that degenerate in PD have been shown to experience elevated basal mitophagy *in vivo* compared to other midbrain neurons (Guzman et al., 2018). Excitatory calcium dysregulation in cortical neurons triggered by mutations in the dominant PD gene *LRRK2* (Plowey et al., 2014) also elicits increased mitophagy accompanied by mitochondrial depletion and autophagic dendrite retraction (Cherra III et al., 2013; Verma et al., 2017). Moreover, susceptible neurons in post-mortem studies of patients with PD or DLB exhibit increased numbers of autophagocytosed mitochondria, which accounts for a distinctive punctate staining pattern for activated extracellular-signal regulated protein kinases (ERK1/2) (Zhu et al., 2003). Additional evidence supporting a possible role for mitophagy dysregulation in neurodegeneration include observations that two recessive PD genes (Kawajiri et al., 2010; Matsuda et al., 2010; Narendra et al., 2010a; Vives-Bauza et al., 2010) and two LIR-motif proteins linked to ALS-FTD (Majcher et al., 2015) are involved in the ubiquitin-mediated pathway of mitophagy.

6. Mechanisms of cargo recognition for selective mitophagy

There are many stimuli that result in mitophagosome formation and mitochondrial clearance. These include developmental, environmental and genetic modulation of physiological and pathological cues. Given that a given mitophagy stimulus may trigger more than one pathway, I have classified the pathways with a focus on the specific cargo-associated signals that allow recognition by the autophagy machinery (Fig. 2D–F). Some of these tags are transcriptionally upregulated in response to developmental or hypoxic-ischemic cues, while others are post-translationally modified or exposed to stimulate mitophagy.

6.1. Ubiquitin-mediated mitophagy

In addition to protein aggregates and intracellular bacterial pathogens, mitochondria can be targeted for selective autophagic degradation by virtue of ubiqutin tags on their outer surfaces (Fig. 2D). Details of this process, commonly referred to as the PINK1-Parkin pathway of mitophagy, have been extensively reviewed (Hamacher-Brady and Brady 2016; McWilliams and Muqit 2017; Yin and Ding 2013). In brief, loss of mitochondrial membrane potential causes a disruption in the normal import of mitochondrial proteins. As a result, the PINK1 protein accumulates on the outer mitochondrial membrane (Kawajiri et al., 2010; Matsuda et al., 2010; Narendra et al., 2010a), to recruit and activate Parkin (Shiba-Fukushima et al., 2012) through phosphorylation of ubiquitin (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014). Ubiquitination of outer mitochondrial membrane proteins then leads to their degradation *via* the proteasomal or lysosomal systems (Chan et al., 2011; Yoshii et al., 2011). With relation to Parkin-independent mitophagy mechanisms discussed below, it is interesting to note that other ubiquitin ligases have also been implicated in mitophagy (Szargel et al., 2016).

Optineurin and NDP52 represent the most commonly studied mitophagy adapters, although phosphorylated p62 also contributes to mitophagic clearance (Geisler et al., 2010; Lazarou et al., 2015; Matsumoto et al., 2015; Wong and Holzbaur 2014). Increased staining for phospho-ubiquitin has been observed in degenerating substantia nigra neurons (Fiesel et al., 2015), with a similar staining pattern to phospho-ERK1/2-labeled mitophagosomes in PD/DLB patients (Zhu et al., 2003). Taken together, these studies indicate activation of ubiquitin-mediated mitophagy in PD and related dementias.

PINK1-Parkin-regulated mitophagy represents the most heavily studied pathway of cargo specification. Despite this, the relative importance of the PINK1-Parkin mitophagy pathway in neurons remains controversial (Cummins and Gotz 2017; Grenier et al., 2013; Pogson et al., 2014; Rakovic et al., 2013). Caveats include the frequent requirement of Parkin overexpression, use of chemicals or photoirradiation that elicit severe depolarization and/or cessation of electron transport, and potential differences in mitophagy regulation between tumor cells and neurons (Van Laar et al., 2011). Studies focusing on primary neurons or neurons differentiated from induced pluripotent stem cells have yielded conflicting interpretations concerning

activation of the PINK1-Parkin pathway, ranging from no significant increase over baseline in cortical, striatal or midbrain neurons to involvement of < 20% of Killer red-depolarized mitochondria in hippocampal neurons (Ashrafi et al., 2014; Cai et al., 2012; Rakovic et al., 2013; Van Laar et al., 2011). Moreover, recent *in vivo* studies suggest that PINK1 and Parkin are not required for basal mitophagy in a range of tissues including the brain (Lee et al., 2018; McWilliams et al., 2018). It is thus pertinent to consider alternative pathways of cargo targeting for mitophagy.

6.2. Transmembrane receptor-mediated mitophagy

In yeast, the transmembrane LIR-motif protein Atg32 is responsible for targeting mitochondria to the yeast cargo adaptor Atg11 during mitophagy triggered in media containing lactate as the sole carbon source (Kanki et al., 2009). Although there are no structural homologs of Atg32 or Atg11 in mammals, outer mitochondrial membrane proteins with LIR domains are involved in regulating developmental mitophagy in erthryocytes and lens cells (Fig. 2E). The BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (BNIP3L), which is also referred to as Nix, is transcriptionally upregulated during erythrocyte differentiation. Nix interacts with LC3B or GATE-16 *via* a classic LIR to mediate mitophagic sequestration (Novak et al., 2010). Transcriptional upregulation is also involved in mitophagy induced by hypoxia, during which Nix expression is markedly induced along with the related BNIP3 (Liu et al., 2014). Another mitophagy receptor of the outer mitochondrial membrane is FUN14 Domain Containing 1 (FUNDC1), which is dephosphorylated during hypoxic stress to activate mitophagy (Liu et al., 2012; Lv et al., 2017). Given that Nix overexpression rescues impaired CCCP-induced mitophagy in fibroblasts from patients with mutations in *PINK1* or *PARK2* (Koentjoro et al., 2017), these PD genes are not required for receptor-mediated mitophagy. Other outer membrane proteins with LIR domains such as FK506 Binding Protein 8 (FKBP8) (Bhujabal et al., 2017) have also been reported to recruit LC3A for Parkin-independent mitophagy.

It remains unknown the extent to which Nix and BNIP3 may participate in mitophagy in neurons, although both proteins have been reported to be upregulated in neurons in response to stress (Prabhakaran et al., 2007; Yeh et al., 2011; Zhang et al., 2007). In these contexts, however, these proteins play a pro-death role, and the potential involvement of Nix- or BNIP-mediated mitophagy were not examined.

6.3. Cardiolipin-mediated mitophagy

Recently an alternative mitophagy pathway was discovered in cortical neurons and neuroblastoma cells treated with sublethal doses of two PD toxins, rotenone and 6-hydroxydopamine (Chu et al., 2013). In contrast to protein-protein interactions, this pathway involves the interaction of the phospholipid cardiolipin with LC3 (Fig. 2F). This unusual phospholipid is found in bacteria and the mitochondria of eukaryotic cells, where it is concentrated in the inner mitochondrial membrane. Exposure of cardiolipin to the surface of injured mitochondria requires three enzymatic translocations mediated by the mitochondrial phospholipid scramblase-3 (Chu et al., 2013) and the inner- and outer- membrane spanning hexameric complex of mitochondrial nucleoside diphosphate kinase-D (Kagan et al., 2016). The cardiolipin-mediated mitophagy cargo targeting mechanism does not require PINK1 accumulation or Parkin recruitment to the mitochondria (Chu et al., 2013). Besides neurons, it can be observed in pulmonary cells treated with staurosporine or CCCP, acting upstream to or in parallel with the PINK1-Parkin pathway. Cardiolipin interacts with multiple Atg8 family members *in vitro*, but LC3 appears to be the main binding partner for selective mitophagy in glioma cells (Anton et al., 2016).

In addition to cardiolipin synthase, whose expression is regulated by PINK1 (Chen et al., 2014), tafazzin regulates cardiolipin by remodeling its fatty acyl chains. Interestingly, tafazzin deficiency causes defective mitophagosome biogenesis in fibroblasts (Hsu et al., 2015), implicating a possible differential role of different cardiolipin species in mitophagy. While oxidation of cardiolipin is associated with apoptosis (Shidoji et al., 1999), there is no evidence of cardiolipin oxidation during mitophagy (Chu et al., 2013). Interestingly, brain cardiolipin is composed of fewer unsaturated fatty acids, rendering it more resistant to peroxidation (Bradley et al., 2016). These differences may be predicted to favor cardiolipin-mediated mitophagy over cell death in the brain.

In addition to cardiolipin, ceramide may also act to target mitochondria for autophagy (Sentelle et al., 2012) to elicit a form of autotic cell death (Liu and Levine 2015). In addition, genome-wide screens implicate components of steroid and lipogenesis pathways in a regulatory role for mitophagy (Ivatt et al., 2014).

6.4. Other mitophagy stimuli with unclear cargo targeting mechanims

Several other stimuli elicit PINK1- and/or Parkin-independent mitophagy. These include the application of iron chelators (Allen et al., 2013). While the cargo targeting mechanism remains unknown, it is also independent of BNIP3. Cardiomyocyte mitophagy *in vivo* appears to proceed through two parallel pathways, one that is Parkin-independent, but requiring the mitochondrial fission protein dynamin related protein 1 (Drp1) and one mediated by Parkin (Kageyama et al., 2014). Mitochondrial fission can act to promote degradation of unhealthy segments of mitochondria or to spare healthy segments from degradation (Burman et al., 2017). Fission is also important for PINK1-independent mitophagy elicited in PINK1-deficient neuronal cells (Dagda et al., 2009). Constitutively active ERK1/2 spontaneously localizes to mitochondria, where it is capable of driving mitophagy in the absence of other injurious stimuli (Dagda et al., 2008). ERK1/2 is involved in stimulating a non-canonical form of Beclin-1-independent mitophagy in the MPP+ model (Chu et al., 2007; Zhu et al., 2007), but the mechanism of cargo specification remains elusive. Finally, the activating molecule in Beclin-1-regulated autophagy 1 (AMBRA1) is a LIR-motif protein that has been implicated in both Parkin-dependent (Van Humbeeck et al., 2011) and Parkin-independent mitophagy (Strappazzon et al., 2015).

7. Mitophagy *in vivo*

While there is clearly evidence of mitophagy pathway activation in human brain tissues from neurodegenerative disease patients (Fiesel et al., 2015; Ye et al., 2015; Zhu et al., 2003), whether or not this reflects increased or decreased flux has been difficult to address *in vivo*. In *Drosophila*, PINK1 and Parkin act to regulate mitochondrial protein turnover (Vincow et al., 2013). However, the pattern of protein buildup with PINK1 deficiency is not a subset of the pattern elicited by autophagy (Atg7) deficiency, suggesting that PINK1 regulates respiratory complex proteins through Atg7-independent mechanisms. It remains to be determined whether this may relate to mitochondria derived vesicles (McLelland et al., 2014), or the destabilizing effects of PINK1 deficiency on cristae structure (Dagda et al., 2009) that may be due to its interaction with mitofilin (Tsai et al., 2018). Recently a set of transgenic animals that express sensors for mitochondrial delivery into the acidic compartment of the lysosome have been used to address the potential *in vivo* role of PINK1 and Parkin in basal mitophagy.

The first is a mouse (Sun et al., 2015) transgenically expressing a pH sensitive matrix-targeted Keima protein, which is excited predominantly by 458-nm light in the slightly basic pH of the normal mitochondrial matrix (Katayama et al., 2011). However, the peak excitation shifts to 561-nm in the acidic environment of a normal lysosome. The second mouse, called mito-QC (McWilliams et al., 2016), is based on the differential fluorescence sensitivity of GFP *versus* mCherry to acidic environments (Allen et al., 2013). The tandem reporter is

targeted to the outer mitochondrial membrane, emitting both red and green fluorescence in the neutral cytosol, but only red in acidic lysosomes. An advantage of mito-QC is that the tissues can be fixed, although the mechanism by which the intra-lysosomal GFP fluorescence continues to be suppressed after fixation is unclear.

Although commonly referred to as flux reporters, it is important to keep in mind that the sensors measure autophagosome maturation to autolysosomes rather than actual flux. Changes in the biosynthesis or mitochondrial import rates of the sensor may affect the ratios. Also, the sensors detect pH rather than enzymatic activity, which could be affected by impaired delivery or function of lysosomal enzymes with aging or disease states. These possibilities can be experimentally addressed through other methods. Another consideration is that the sensors on their own are not designed to differentiate selective mitophagy from passive entrapment of mitochondria during bulk autophagy.

Nevertheless, a set of recent papers using these sensors confirm that mitochondria are actively delivered into lysosomes *in vivo*, and that this rate varies in different tissues (McWilliams et al., 2016; Sun et al., 2015). Moreover, the mito-QC mouse has been crossed to *Pink1*−/− mice to reveal that basal mitophagy occurs through a PINK1-independent mechanism (McWilliams et al., 2018). In the pancreatic islet, the rates of mitophagy appeared to be increased in *Pink1*-deficient mice. Increased rates of mitophagy have also been observed in neuroblastoma cells with chronic PINK1 shRNA knockdown (Dagda et al., 2009) and in primary neurons from *Pink1*-knockout mice (Dagda et al., 2011). Interestingly, PINK1 message is downregulated in skeletal muscle by inactivity or type 2 diabetes (Scheele et al., 2007), suggesting that altered mitophagy in beta cells may play a role in metabolic syndrome. *Drosophila* expressing either mt-Keima or mito-QC have been constructed, revealing widespread basal mitophagy in multiple tissues as well (Lee et al., 2018). However, there were no effects of null mutations of either *Pink1* or *Parkin* on rates of mitochondrial delivery to lysosomes.

These studies of basal mitochondrial turnover are consistent with observations that there are multiple distinct mechanisms for targeting mitochondria to the autophagy machinery. It is possible that transmembrane LIR proteins, cardiolipin, or other as yet undefined mechanisms play a key role in basal mitophagy. Alternatively, given the limitations mentioned above, it is also possible that basal mitochondrial turnover results from stochastic rather than selective inclusion of mitochondria in autophagosomes, as neurons exhibit a high rate of housekeeping autophagic flux (Boland et al., 2008). On the other hand, the correlation between dendritic calcium pacemaking and elevated basal mitophagy in dopaminergic substantia nigra neurons (Guzman et al., 2018) suggests that calcium-mediated mitochondrial injury plays a role in elevating basal mitophagy in susceptible neuron types. Indeed, mutations in *LRRK2* result in enhanced excitatory neurotransmission and mitochondrial calcium dysregulation (Cherra III et al., 2013; Plowey et al., 2014; Verma et al., 2017), which leads to depletion of mitochondria from dendrites. Given that PINK1-Parkin, cardiolipin and FUNDC1 pathways are each activated by distinct as well as overlapping stimuli in cultured cells, their potential involvement in response to specific disease-relevant stressors that upregulate mitophagy *in vivo* remains to be determined.

8. Role(s) of PINK1 or Parkin in recessive PD?

While it is clear that loss of PINK1 or Parkin cause recessively inherited parkinsonian neurodegeneration, central questions remain unanswered. What are the possible roles of PINK1 and Parkin in neuroprotection, and which function(s) are lost that cannot be compensated? Deficiency in the function of either protein show striking effects on mitochondrial structure and function in culture systems, with often less prominent effects in mouse models. For example, primary mouse *Pink1*−/− neurons exhibit reduced membrane potential, mitochondrial fragmentation, increased mitophagy and lysosomal expansion (Dagda et al., 2011). Neurons differentiated from induced pluripotent stem cells of PARK2- or PINK1-mutated patients also exhibit mitochondrial dysfunction (Chung et al., 2016). Just as with PINK1-triggered ubiquitin-mediated mitophagy, which is most robust in transformed cells in culture, many of these highly reproducible culture phenotypes are not as striking in *Pink1*−/− mice, and deciphering whether or not these changes play a pathogenic role in human neurodegeneration has not been straighforward.

Other functions of PINK1 that could impact mitochondrial quality involve direct or indirect effects on phosphorylation of complex I subunits, mitochondrial fission or transport proteins, mitochondrial calcium transporters, chaperones and proteins important for cardiolipin synthesis and cristae structure (Chen et al., 2014; Kostic et al., 2015; Morais et al., 2014; Pridgeon et al., 2007; Sandebring et al., 2009; Shlevkov et al., 2016; Tsai et al., 2018; Zhang et al., 2013). Cytosolic functions of PINK1 and Parkin include the regulation of PGC-1α mediated mitochondrial biogenesis (Lee et al., 2017; Shin et al., 2011; Siddiqui et al., 2015), suppression of toxin-induced autophagy (Dagda et al., 2009; Fedorowicz et al., 2014) and activation of mTORC2 (Murata et al., 2011). With regard to neurons, PINK1 acts to promote neuronal differentiation and mitochondrial transport into dendrites (Dagda et al., 2014; Das Banerjee et al., 2017). These alternative roles of PINK1 or Parkin have been more extensively reviewed elsewhere (Arena and Valente 2017; Chu 2010; Scarffe et al., 2014; Steer et al., 2015).

9. Self-eating in neurodegeneration: future perspectives

Advances in basic autophagy research have given rise over the past 25 years to new molecular tools enabling researchers to demonstrate that changes in autophagy or mitophagy play important roles in the pathogenesis of neurodegenerative diseases. In turn, neurodegenerative disease studies focused on protein aggregates and mitochondrial injury have led to major basic science discoveries that firmly establish the existence of specific macromolecular interactions responsible for autophagic cargo recognition and selective autophagy. Research in HD not only revealed a new family of LIR-motif autophagy cargo receptors that recognize protein aggregates (Pankiv et al., 2007), but also provided the first *in vivo* evidence supporting autophagy modulation as a potential disease therapy (Ravikumar et al., 2004). Mitochondrial studies in the PD field led to the discovery of two distinct pathways for selective mitophagy, one involving genes mutated in PD (Kawajiri et al., 2010; Narendra et al., 2010a) and ALS-FTD (Wong and Holzbaur 2014) and the other triggered in neurons by complex I inhibition (Chu et al., 2013) as a model of sporadic PD.

In part as a result of this expansion from starvation-focused to disease-focused autophagy research, it has become evident that the molecular regulation of selective autophagy involves multiple pathways of recognition involving direct and indirect interactions of the core autophagy machinery with cargo receptors, phospholipids and glycoproteins. Multiplication of Atg8 genes and cargo receptor genes during evolution confer at least partial redundancy, and this increased complexity compared to unicellular organisms attests to the importance of autophagic quality control in mammalian neurons. Selective autophagy of just about every intracellular constituent has now been described, giving rise to a new viewpoint that selective autophagy under conditions of adequate nutrition may be the norm rather than the exception.

Several lines of evidence implicate dysregulation not only of autophagy in general, but also of aggrephagy, mitophagy and other forms of selective autophagy in neurodegeneration. Mutations in neurodegenerative disease genes discussed above either directly or indirectly trigger or regulate selective autophagy. Cargo receptors and their ligands, kinases and other regulatory proteins are observed in pathological aggregates or in association with abnormal mitochondria in diseased human tissues (Fiesel et al., 2015; King et al., 2011; Kurosawa et al., 2016; Nogalska et al., 2009; Zhu et al., 2003). Moreover, it is

reasonable to postulate that defective cargo recognition may result from saturation of the autophagy machinery by aggregate-prone proteins that are not properly handled by chaperone and proteasomal systems.

Yet, key questions remain. Given that there are several distinct pathways of cargo selection for mitophagy, to what extent does loss of one pathway contribute to neurodegeneration? Clearly, this answer may depend upon the major pathway(s) operating in a given cell type or tissue under a particular disease-related stress. The new animal tools discussed above will yield an unprecedented opportunity to determine what types of *in vivo* stressors may trigger activation of ubiquitin-mediated, transmembrane receptor-mediated or cardiolipin-mediated pathways of mitophagy.

Likewise, the kinetics and fate of protein aggregates that develop *in vivo*, or in different compartments of primary neurons are areas of research that may be approached using an analogous strategy to follow delivery of aggregate-protein proteins, or of specific cargo receptor proteins into lysosomes in response to different injuries. Whereas different pathways of cargo recognition may be primarily triggered by different stimuli, a multiplicity of direct molecular interactions would enhance avidity for cargo targeting. It is thus conceivable that therapeutic strategies may be designed to upregulate alternative pathways to compensate for disease-related impairments in a given selective autophagy pathway.

Finally, it has become clear from studies in AD, PD, ALS and FTD that upregulated induction of autophagy or mitophagy may have either beneficial or detrimental effects in a particular context (Table 2). An improved understanding of regulatory mechanisms for selective autophagy is necessary to facilitate the development of new strategies to promote degradation of selected cargoes while minimizing excessive turnover of other cellular constituents.

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