

# PINK1 and Parkin

McWilliams, Thomas G.; Muqit, Miratul M. K.

*Published in:*
Current Opinion in Cell Biology

*DOI:*
10.1016/j.ceb.2017.03.013

*Publication date:*
2017

*Document Version*
Final published version

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

# PINK1 and Parkin: emerging themes in mitochondrial homeostasis

Thomas G McWilliams[1] and Miratul MK Muqit[1,2]

The Parkinson's disease (PD)-associated protein kinase, PTEN-induced putative kinase1 (PINK1), and ubiquitin E3 ligase, Parkin function in a common signalling pathway known to regulate mitochondrial network homeostasis and quality control, including mitophagy. The multistep activation of this pathway, as well as an unexpected convergence between the post-translational modifications of ubiquitylation and phosphorylation, has added breadth to our understanding of cellular damage responses during human disease. In concert with these new insights in signal transduction, unique modalities and signatures of vertebrate mitophagy have been unravelled *in vivo* for the very first time. The cell biology of mammalian mitophagy, and the roles of PINK1-Parkin signalling *in vivo* have emerged to be more complex than previously thought.

**Addresses**
[1] MRC Protein Phosphorylation and Ubiquitylation Unit, The Sir James Black Centre, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK
[2] School of Medicine, University of Dundee, Dundee DD1 9SY, UK

Corresponding author: Muqit, Miratul MK (m.muqit@dundee.ac.uk)

**Current Opinion in Cell Biology** 2017, **45**:83–91

This review comes from a themed issue on **Cell regulation**

Edited by **Davide Rugero** and **Reuben Shaw**

http://dx.doi.org/10.1016/j.ceb.2017.03.013



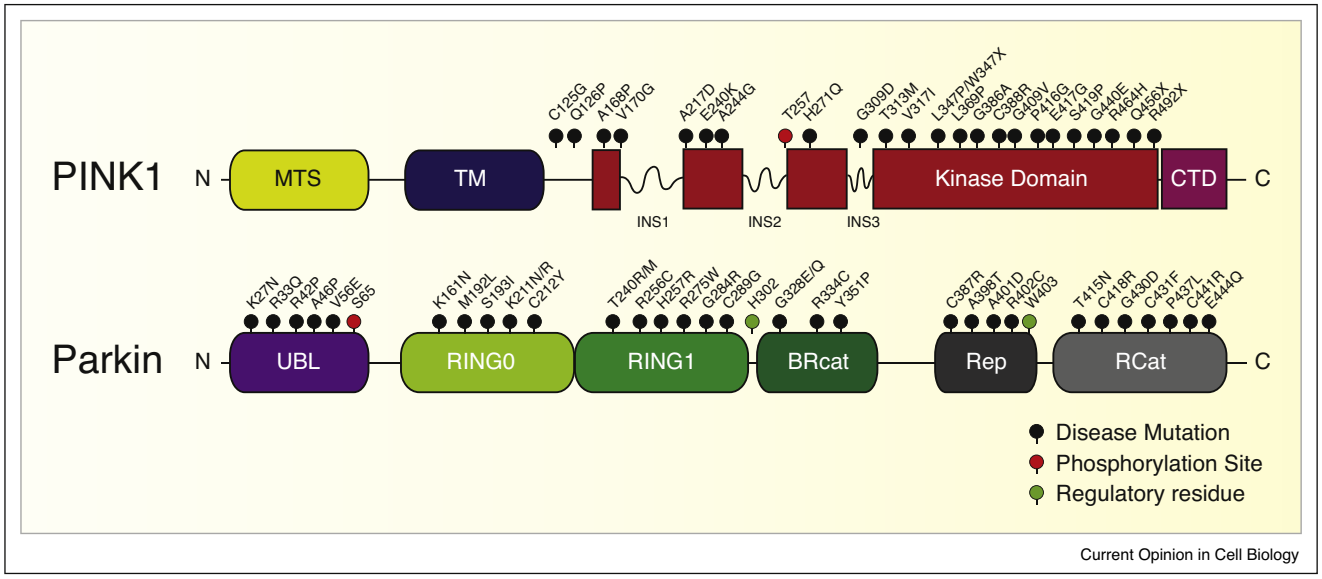
## Introduction: an evolving view of mitochondrial homeostasis

Whilst mitochondria may have granted eukaryotes the ultimate survival advantage during evolution, the dysfunction of these dynamic and functionally pleiotropic organelles confers a potentially devastating vulnerability to mammalian cells. This is particularly relevant in the context of terminally differentiated or long-lived cells such as neurons, cardiomyocytes and renal tubular cells. Complex pathways have evolved to sense, label and sequester damaged or dysfunctional mitochondria to neutralise their cytotoxic potential. One such mechanism is the delivery of compromised mitochondria or associated mitochondrial components to the lysosome for elimination, *via* the autophagy pathway [1]. Mitochondrial autophagy or 'mitophagy' is receiving increased attention as a major pathway that sustains mitochondrial network homeostasis, by regulating mitochondrial number and protecting cells from the deleterious effects of 'mitotoxicity'. Mutations in human PTEN-induced kinase 1 (PINK1) and the RING-IBR-RING (RBR) E3-ubiquitin ligase Parkin (encoded by *PARK6* and *PARK2* genes, respectively) result in early-onset Parkinson's disease (PD) (Figure 1) [2]. Initial discoveries in *Drosophila melanogaster* revealed both of these proteins function within a common, evolutionarily conserved mitochondrial homeostasis pathway. Landmark cell-based studies demonstrated that PINK1-Parkin signalling regulates stimulus-induced mitochondrial clearance *in vitro*, and research on the molecular regulation of this and related responses has proliferated dramatically over the past decade. Whilst the 'PINK1-Parkin axis' has received the great majority of attention, it is important to note that PINK1-Parkin-independent mitophagy is emerging as a burgeoning area of research. Reciprocally, mitophagy-independent functions of PINK1/Parkin are also widely observed. Recent breakthroughs have redefined our view of vertebrate mitophagy *in vivo*, and provoke a reassessment in our understanding of how mitochondrial quality control is regulated in a variety of contexts.

## PINK1, phospho-ubiquitin (p-Ub), Parkin and the regulation of stimulus-induced mitophagy

Groundbreaking work by the Youle laboratory initially linked Parkin to mitophagy, and subsequent contributions from many other laboratories have defined a central role for PINK1 in regulating Parkin following mitochondrial damage. Under steady state conditions, PINK1 is constitutively imported into mitochondria, cleaved and degraded *via* the N-end rule pathway. The proteolysis of PINK1 is mediated by the inner mitochondrial membrane associated PARL protease, and regulated by the recently described SPY complex [3]. Upon the loss of mitochondrial membrane potential that can be induced artificially by mitochondrial uncouplers (*e.g.* carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP)), PINK1 becomes stabilised and activated on the outer mitochondrial membrane (OMM) by mechanisms that are not yet fully elaborated [4,5]. *Drosophila* studies suggest PINK1 levels on 'healthy mitochondria' are regulated by the LON protease, however evidence for this in mammalian cells remains unclear [6]. Cytosolic Parkin is recruited to damaged mitochondria where it becomes activated [5,7•]. Both Parkin translocation and its E3-ligase activity are

**Figure 1**



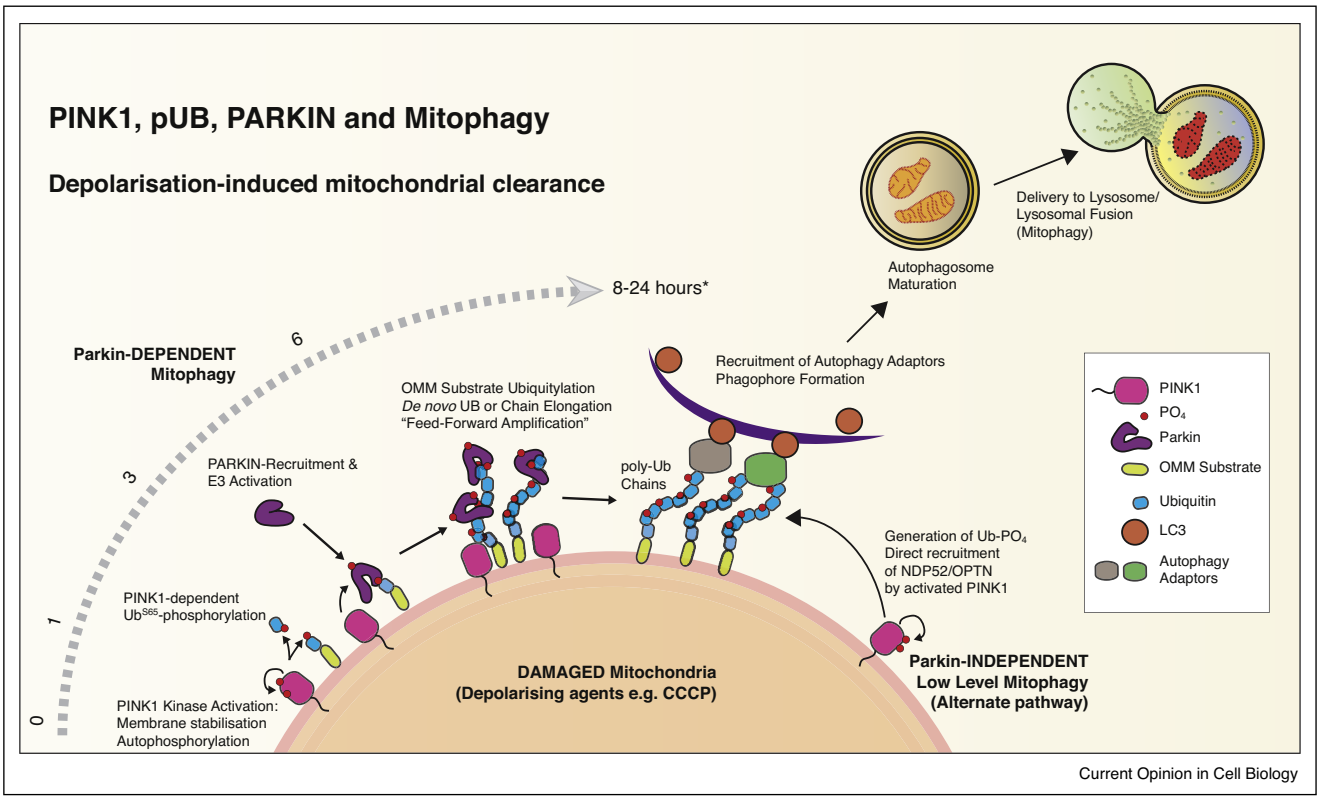

Domain architecture of PINK1 and Parkin.
Schematic depicting the major regions of the mitochondrial-associated kinase PINK1, and the RBR-E3 Ubiquitin Ligase Parkin. Selected human disease mutations are shown in black, key phospho-sites in red and regulatory residues in green. MTS: mitochondrial targeting sequence, TM: transmembrane domain, INS: insertion, CTD: C-terminal domain, UBL: ubiquitin-like domain, RING: really interesting new gene domain, BRcat: benign-catalytic domain, Rep: repressor element, Rcat: required for catalysis domain.

dependent upon the enzymatic activity of PINK1. Over the past two years, the multistep mechanistic regulation of Parkin activation has been elaborated in great detail (Figure 2) [5].

Parkin exists in a native autoinhibited conformation [8]. Upon mitochondrial depolarisation, activated PINK1 phosphorylates both Ubiquitin and Parkin at their respective Ser65 residues [7•,9••,10••,11••,12,]. Detailed structural and biophysical characterisation by independent laboratories demonstrated that phospho-ubiquitin (p-Ub) binds with high affinity to phosphorylated Parkin to allosterically induce conformational changes that promote recruitment of its cognate charged E2, and stimulation of Parkin activity [13–17]. Active Parkin is reported to ubiquitylate myriad putative substrates that reside in the OMM, by elongating pre-existing ubiquitin chains attached to OMM proteins or ubiquitylating substrates *de novo*. Whilst the role of p-Ub as an allosteric activator is evident, whether p-Ub may play other physiological roles is unclear. It has been reported that poly-p-Ub chains are more resistant to proteolysis by several deubiquitinases, suggesting that p-Ub may act as an important signal to maintain Parkin-directed ubiquitylation [12]. Given the pleiotropy of substrates, it is also possible that p-Ub may function as a semaphore to direct or guide Parkin to selected substrates. In this regard, it will prove exciting to determine if other proteins contain similar p-Ub binding pockets akin to that found on Parkin [5,18]. A leading hypothesis suggests particular combinations of poly-p-Ub chain topologies may represent a distinct molecular code required for the selective recruitment of autophagy machinery. Quantitative proteomics revealed damaged mitochondria become decorated with a diverse array of polyubiquitin linkages (Lys 6, 11, 48, 63) [7•]. These OMM-polyubiquitin chains serve as substrates for PINK1 phosphorylation and adaptors for Parkin, resulting in a 'feed-forward' amplification loop that drives the clearance of the damaged organelle and ultimately, the completion of mitophagy (Figure 2) [7•,19,20].

The recruitment of ubiquitin-binding autophagy receptor proteins is necessary for local phagophore formation at damaged mitochondria and, to date six such receptors have been identified: NBR1, NDP52, OPTN, p62/SQSTM1, TAX1BP1 and TOLLIP [5]. Converging studies using clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) genome editing suggest NDP52/OPTN are essential for PINK1-Parkin-dependent mitophagy, and TAX1BP1 to a lesser extent [21•,22•]. Furthermore, TBK1 phosphorylation of these receptors enhances interactions between LC3 and ubiquitin [22•,23]. Interestingly, PINK1 may also generate p-Ub and recruit NDP52/OPTN independently of Parkin to drive low-level mitophagy, implying the involvement of additional ubiquitin E3 ligases [21•]. Until recently, it was widely assumed that the ATG-conjugation machinery is crucial for

**Figure 2**




PINK1, p-Ub, Parkin and mitophagy.
The molecular regulation of depolarisation-induced mitochondrial clearance is well established. PINK1-Parkin-dependent signalling triggers cycles of ubiquitylation on the OMM, which result in the recruitment of the autophagy machinery, autophagosome formation and eventual clearance of the damaged organelle. Times are approximate and refer to observations from *in vitro* studies. PINK1 can also drive low-level mitophagy, independently of Parkin.

autophagosome biogenesis and cargo incorporation. A new study has cast doubt on this, suggesting that while LC3 is essential for autophagosome-endolysosome fusion, it is dispensable for the autophagic encapsulation of damaged mitochondria [24]. Consistent with a role outside of autophagosome biogenesis, other recent observations indicate LC3 conjugation is instead required to orchestrate the degradation of the inner autolysosomal membrane following autophagosome-lysosome fusion [25].

It has recently been reported that the mitochondrial ubiquitin ligase 1 (MUL1; also known as MULAN and MAPL) functions with Parkin to regulate degradation of paternal mitochondria from sperm, but the mechanism by which MUL1 induces mitophagy is unknown [26]. Rab GTPase proteins have also been implicated in mitophagy, and two RAB-GAPs (TBC1D15 and TBC1D17) modulate the activity of Rab7 to regulate encapsulation of damaged mitochondria by the autophagosome [27]. Recently published phosphoproteomic analyses demonstrated that Rab8A, 8B and 13 are indirect substrates of PINK1 [28•]. The temporal dynamics of Rab phosphorylation following mitochondrial insult is consistent with their potential contribution to the later stages of mitophagy [28•]. The links between PINK1, TBK1 and Optineurin signalling are intriguing, given previous reports that Optineurin is an effector protein of Rab8A, suggesting a potential nexus. Given the function of NDP52/OPTN/TBK1/Rab7 in xenophagy and the ancient origins of mitochondria from $\alpha$-proteobacteria, interesting parallels have also been drawn between mitophagy and xenophagy [5,29].

The idea that dysregulated PINK1-Parkin signalling leads to impaired mitophagy is an attractive hypothesis, however there has been no clear validation of this mechanism in the aetiology of PD. Although PINK1-Parkin mediated mitophagy is highly reproducible *in vitro*, it is difficult to reconcile the actual contribution of PINK1-Parkin signalling to mitochondrial homeostasis *in vivo* due to some potentially confounding factors. (1) The majority of studies utilise proliferating cell lines and although such systems are unrivalled for the tractable

molecular dissection of signalling pathways *in vitro*, they relate poorly to the post-mitotic and complex A9 mesencephalic dopaminergic (mesDA) nigrostriatal projection neurons that selectively degenerate in PD. (2) High levels of PINK1/Parkin expression are usually required to detect a robust induction of mitophagy (4). It is difficult to relate the cytotoxic induction stimuli (*e.g.* CCCP) to an analogous physiological correlate that evokes a similar level of mitochondrial clearance *in vivo*. Additionally, genetic ablation of germ-line *Pink1* or *Parkin* in mice does not recapitulate the overt neuropathology or motor dysfunction that manifests in PD.

However, a recent proof-of-concept study has highlighted the importance of endogenous *Parkin* in mediating neural mitochondrial homeostasis *in vivo* [30•]. To simulate conditions of mitochondrial stress in the absence of endogenous Parkin and investigate the effect of this on nigrostriatal pathway integrity, the authors crossed *Parkin*-deficient mice with mitochondrial *Polg*$^{D257A}$ 'mutator' mice. The well-characterised mutator model accumulates mitochondrial DNA mutations because of defective mitochondrial proofreading by polymerase γ. Although individual mutants (*Parkin*-deficient or *Polg*$^{D257A}$ mice) alone did not exhibit gross dopamine/dopaminergic (DA) neuropathology or behavioural abnormalities, double-mutant animals with loss of *Parkin* under conditions of constitutive mitochondrial stress *in vivo* exhibited age-dependent DA neuron loss and levodopa-responsive motor dysfunction [30•]. Whilst this important study demonstrated the requirement of Parkin for DA neuronal homeostasis under conditions of mitochondrial stress *in vivo*, it remains unclear if this pathology is a consequence of dysregulated mitophagy in double-mutant mice. Nevertheless, this important *in vivo* finding demonstrates the contribution of endogenous Parkin to mesDA neural integrity under conditions of stress *in vivo*. Interestingly and unlike *Pink1* knockout (KO) mice, a recently generated *Pink1* KO rat model exhibits locomotor dysfunction and age-related DA cell loss, in addition to altered neural metabolomics [31,32]. In contrast, *Parkin* KO rats do not exhibit significant PD-related pathology due to probable redundancy of E3 ubiquitin ligases, for example MUL1 [26].

Fiesel and Springer provided intriguing evidence for the physiological relevance of canonical PINK1-Parkin signalling in humans by demonstrating the age-dependent accumulation of p-Ub in post-mortem brains, and its reciprocal absence in SNpc sections from a patient with a compound heterozygous *PARK6* mutation [33]. Although studies in cultured cells and post-mortem human brain suggest PINK1 to be the ubiquitin kinase for Ser65-Ub, this does not preclude a role for other protein kinases that may generate p-Ub *in vivo*, as ubiquitin can be phosphorylated at several other sites. Recent advances in cell reprogramming enabled Chung *et al.* to recapitulate the molecular pathophysiology of PD *in vitro* using induced pluripotent stem cell (iPSC)-derived DA neurons from PINK1/Parkin-patients [34]. Reprogrammed patient neurons exhibited aberrant mitochondrial morphology as well as a propensity to accumulate α-synuclein [34]. Importantly, this study also demonstrated the influence of culture conditions on modelling molecular pathophysiology *in vitro*, however the contribution of impaired mitochondrial turnover to the observed pathology remains unclear.

## Alternate signals: PINK1 and PARKIN-independent mitophagy

Because of their striking roles in mediating stimulus-induced mitochondrial clearance *in vitro*, PINK1 and Parkin tend to reap the limelight in most mitophagy-related discussions. However, it should be noted that PINK1-p-Ub-Parkin-independent mechanisms of mitophagy exist and these constitute an equally exciting and emerging area of research. These could also be viewed as ubiquitin-dependent and ubiquitin-independent mechanisms of selective autophagy, reviewed in Ref. [35]. The first such mechanism described was NIX-dependent mitophagy (originally reported by the Ney laboratory and shown to be an ATG8 receptor by Dikic and colleagues) [1]. NIX and the closely related BNIP3 are induced by hypoxia, trigger autophagy and have apoptotic-related functions. They both localise to the OMM and contain LC3-interation motifs (LIR; WXXLL), thus acting as autophagy receptors. Upon activation, BNIP3 and NIX prompt opening of the mitochondrial permeability transition pore (mPTP), depolarisation and recruitment of LC3/GABARAPs for autophagosome formation. NIX has been shown to be important for mitochondrial elimination during developmental erythrocyte maturation. Cross talk between NIX/BNIP3 and PINK1-Parkin mitophagy pathways is unclear, although NIX has been implicated in the mitochondrial translocation of Parkin.

The OMM-protein FUNDC1 (Fun14 Domain Containing 1) has also been implicated as a receptor in hypoxia-induced mitophagy. The affinity of FUNDC1 with LC3 is controlled by Src-mediated phosphorylation of Tyr$^{18}$ within its N-terminal LIR. Under hypoxia, de-phosphorylation of Tyr$^{18}$ enhances the FUNDC1-LC3 interaction and evokes mitophagy. The Ser-Thr protein kinase ULK1 has been reported to phosphorylate FUNDC1 to regulate mitophagy [1].

Iron chelation *via* deferiprone has also been shown to induce mitophagy in a PINK1-Parkin-independent manner. This is particularly intriguing, as dysregulated iron metabolism has been linked to neurodegenerative disease and clinical trials with deferiprone and PD are currently underway. It should be noted that the signalling pathway regulating deferiprone-induced mitophagy remains to be elucidated and it will be interesting to test

the contribution of other ubiquitin E3 ligases, such as the recently described MUL1 E3 ligase [26]. Similarly ubiquitin can be phosphorylated at several other sites and their relevance to mitophagy and the identity of the upstream kinases regulating these residues remains unknown [36].

## Orchestrating mitochondrial quality control *in vivo*

A major challenge in the field has been to accurately and faithfully measure mitophagy *in vivo*. The inability to measure this process in mammalian tissues has provoked great suspense surrounding the *bona fide* contribution of PINK1-pUB-Parkin signalling to mitochondrial turnover *in vivo*.

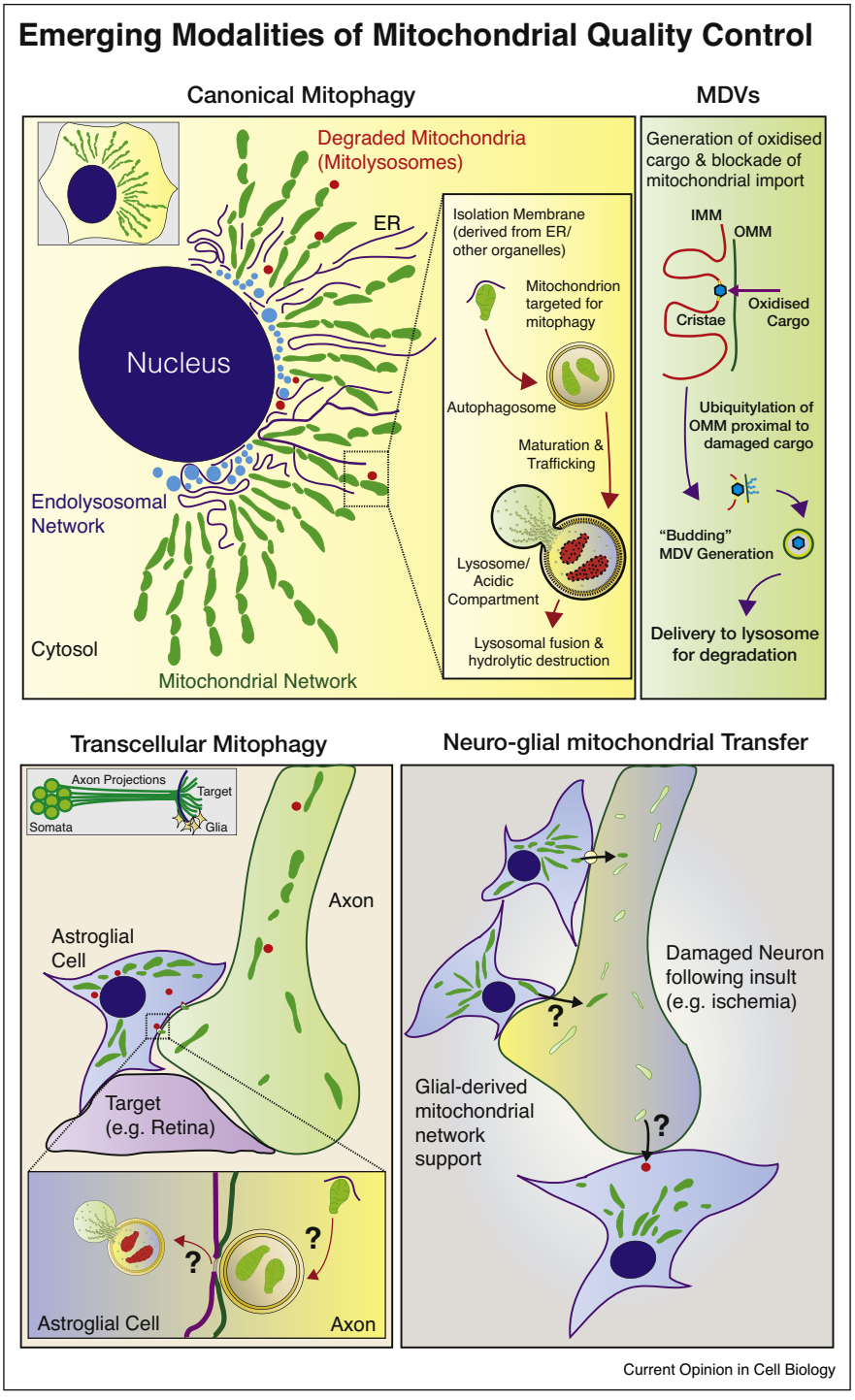
Powerful insights into eukaryotic mitophagy have been obtained from budding yeast, from the initial *in vivo* studies by the Lemasters laboratory to more recent work on p-Ub signalling. Intriguingly, although there is no known PINK1 orthologue in yeast, Swaney *et al.* observed the presence of Ser65-p-Ub in *S. cerevisiae* upon exposure to oxidative stress [37]. This raises the exciting possibility that an alternate, PINK1-like ubiquitin kinase may exist. Invertebrate *in vivo* mitophagy has also been investigated in novel reporter assays using *C. elegans*, where *Pink1* and *Parkin* were reported to orchestrate ageing-associated mitophagy [38]. However, these studies relied upon induction stimuli, as the basal rate of turnover in nematodes appears low. Fly genetics has also provided important insights into the regulation of mitochondrial homeostasis by PINK1 and Parkin [39].

In terms of mammalian mitophagy *in vivo*, tool development has been integral to the field and a combination of mouse genetics with fluorescent-reporter proteins has culminated in the recent rise of mitophagy reporter mouse models [40]. The recently reported *mito*-QC and mt-Keima mouse models generated by the Ganley and Finkel laboratories have unearthed the dramatic, striking and heterogeneous nature of basal mitophagy *in vivo* [41••,42••]. Although these models operate on a similar pH-based principle, the Keima protein is incompatible with tissue fixation and users are limited to making regional inferences about mitophagy *in vivo*. Conversely, the end-point *mito*-QC model facilitates the facile resolution of both mitophagy and mitochondrial network architecture within labelled subsets of cells *in vivo* at subcellular resolution. Collectively, these observations have profound implications for our view of mitochondrial turnover in mammals. The vast majority of our molecular knowledge on mitophagy is derived from studies of stimulus-induced mitophagy *in vitro*. The heterogeneity of basal turnover between tissues and even between cell types within the same organ is striking, for example in the kidney—where mitophagy between distal and proximal tubules is dramatically different. Furthermore, it will be essential to determine the contribution of developmental mitophagy to tissue function. From these findings and others, it is likely that multiple pathways regulate mitophagy in a context-dependent and cell-specific manner.

Ultimately, it will be essential to define the contribution of PINK1, p-Ub and Parkin to the regulation of basal mitophagy in vertebrates by exploiting these recently described approaches. Accurately identifying basal mitophagy within precise cellular subsets will be essential to understand if dysfunctional mitophagy underpins the selective vulnerability of particular neuronal subsets in complex degenerative disorders such as PD. Given the postulated contribution of dysregulated mitophagy to the aetiology of PD, how can we reconcile such selective pathophysiology in humans with the widespread, basal nature of mitophagy *in vivo*? If PINK1/Parkin are the master regulators of basal mitophagy, might we not expect patients to exhibit a greater degree of extra-nigral mitochondrial-associated pathology? These findings raise the interesting idea that PINK1/Parkin may be required to protect against a distinct type of stress-evoked mitophagy *in vivo*, as observed in Ref. [30•]. Collectively, these observations should stimulate an interesting debate on the cell subtype-specific functions of PINK1/Parkin *in vivo*.

Destructive and protective modalities of mitochondrial transfer have recently been reported in neurons (Figure 3). Interestingly, depolarisation-induced axonal mitophagy was described *in vitro* in neurons with overexpressed Parkin [43]. The exciting phenomenon of *trans*-cellular mitochondrial degradation (or axonal *trans*-mitophagy) has also recently been described *in vivo* within the optic nerve head [44•]. Remarkably, axonal mitochondria destined for destruction are extruded from terminal processes, engulfed by neighbouring astrocytes and eliminated within their lysosomes. Given the inordinate complexity of the mature nervous system with $\sim 10^{15}$ connections, this topography may account for how a spatial economy of destruction is achieved in mammalian neurons when faced with the challenge of distance [45]. An equally intriguing discovery with implications for mitochondrial quality control is neuro-glial mitochondrial transfer, recently described *in vitro* following simulated ischemic insult [46]. Might the acquisition of healthy mitochondria serve to equilibrate network homeostasis, and how is this orchestrated with basal or induced mitophagy during ageing? Furthermore, do these new mitophagy modalities rely on PINK1-Parkin signalling, and could particular permutations of poly-p-Ub chains specify these alternate delivery routes to the lysosome? Although further work is required to substantiate the frequency of such events in a variety of contexts, both discoveries have profound implications for our understanding of nervous system architecture at a subcellular level.

**Figure 3**




Emerging modalities of mitochondrial quality control.
Our knowledge of mitochondrial homeostasis has expanded, and new routes to the lysosome have been uncovered. Canonical mitophagy refers to PINK1-Parkin-dependent or independent mitophagy that has been classically studied in cultured cells. The mitochondrial-derived vesicle (MDV) pathway is emerging as a key regulator of mitochondrial quality control. Damaged mitochondria can also be extruded from cells and degraded by lysosomes in neighbouring cells (Transcellular mitophagy), and damaged neurons may acquire healthy mitochondria from glial cells (Neuro-glial mitochondrial transfer). It is unclear if these latter modes of delivery are dependent on PINK1-Parkin signalling.

## Emerging themes from cell biology to pathology

Alternate pathways have emerged where damaged mitochondrial components can be excised and delivered to acidic endolysosomal compartments for degradation. In response to oxidative stress, the McBride and Fon laboratories have shown that PINK1 and Parkin mediate the cargo-dependent export of excised mitochondrial regions as membrane derived vesicles (MDVs) for delivery to lysosomes [47•,48]. The Qa-SNARE Syntaxin-17 was recently implicated in this process [49]. Furthermore, a physiological role for PINK1-Parkin signalling-dependent generation of MDVs has been found in mitochondrial antigen presentation (MitAP) [50]. In response to cellular stress, a novel PINK1-Parkin-mediated MDV pathway exports mitochondrial-derived self-antigens for presentation at the cell surface, triggering an immune response [50]. This unexpected discovery of MitAP provides an interesting link between autoimmunity and PD [51]. The complexity of signalling pathways regulating the delivery of damaged mitochondria to the lysosome also needs to be evaluated in light of emerging findings in organelle crosstalk [52]. A variety of mitochondria-organelle contact sites are emerging as key regulators of lipid transport, mitochondrial transport and distribution [52]. Such crosstalk is also exemplified by the recent and exciting revelation of the dual mitochondrial-ER origins of peroxisomes [53]. The development of next-generation chemical probes for monitoring Parkin-activation should also prove valuable in resolving the contribution of PINK1-Parkin signalling to these new and exciting aspects of mitochondrial cell biology [54]. Intriguing links between mitophagy and metabolic regulation are also emerging, especially with respect to cardiovascular development. The Dorn laboratory demonstrated a role for Parkin during cardiac perinatal development, by showing that cardiomyocyte-specific ablation of *Parkin* at this stage provokes a lethal cardiomyopathy caused by the retention of foetal mitochondria. A wave of mitophagy was observed in late embryonic *mito*-QC mice, suggesting that a defined window of developmental cardiomitophagy *in utero* may orchestrate an essential phase of metabolic adaption during early life [33].

## Concluding remarks

Our view of mitophagy, signal transduction and its contribution to the pathophysiology of PD should be evaluated in the light of mesDA neurobiology. It is estimated that a single SN DA neuron can innervate 2.7% of the total striatal volume, synapsing with up to 75,000 medium spiny neurons [55,56]. Furthermore, our understanding of DA neuron function itself is continually evolving with the advent of more sensitive tools [57,58]. The selective nature of mitophagy, as distinct from non-selective general macro-autophagy cannot be understated. Although general macroautophagy is a catabolic process that evolved to sustain cells during instances of nutrient deprivation, basal mitophagy proceeds in cells that have ample access to nutrients, and represents a unique quality control mechanism required to sustain the integrity of the mitochondrial network. Moreover, major differences have been unearthed with respect to the regulation of this process *in vivo*. Despite major research efforts in this field, several important questions remain unanswered (Box 1). Ultimately, it will be imperative to combine state of the art signalling studies with cutting edge *in vivo* cell biology to clarify the precise contribution of mitophagy pathways to mammalian neural integrity.

**Box 1 Outstanding questions in the field.**

- What is the precise contribution of PINK1-Parkin-mediated mitophagy to basal mitophagy *in vivo*?
- Is a chronic 'mitotoxic' stress required to induce PINK1-Parkin mediated mitophagy *in vivo*?
- Given the prevalence of basal mitophagy in tissues, if PINK1-Parkin mediates mitophagy *in vivo*—how can this be reconciled with the highly selective degeneration observed in PD?
- What are the upstream activation stimuli of PINK1-Parkin signalling *in vivo*?
- Do PINK1, p-Ub or PARKIN play a role in trans-cellular mitochondrial trafficking, that is during axonal *trans*-mitophagy or neuroglial transfer?
- Do specific ubiquitin chain topologies or associated patterns of these act as mitophagy-associated molecular patterns for phagophore formation?
- What is the function of PINK1-Rab signalling in mitophagy?
- How are mitochondrial-organelle membrane contacts orchestrated under conditions of acute or chronic mitochondrial stress?
- Do other ubiquitin kinases/ligases regulate mitophagy in mammals?

## Funding

The research of the authors is supported by the Wellcome Trust (101022/Z/13/Z), Medical Research Council; Parkinson's UK; Michael J. Fox Foundation for Parkinson's disease research, J Macdonald Menzies Charitable Trust, Biotechnology and Biological Sciences Research Council and the EMBO YIP programme.

## Acknowledgement

Due to space constraints, we were unable to provide a comprehensive citation of all the relevant primary literature. We apologise to those whom we have omitted. We thank Ian Ganley for critical reading of the manuscript.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Rogov V, Dotsch V, Johansen T, Kirkin V: **Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy**. *Mol. Cell* 2014, **53**:167-178.

2. Bras J, Guerreiro R, Hardy J: **SnapShot: genetics of Parkinson's disease**. *Cell* 2015, **160** 570–570 e571.

3. Wai T, Saita S, Nolte H, Muller S, Konig T, Richter-Dennerlein R, Sprenger HG, Madrenas J, Muhlmeister M, Brandt U *et al.*: **The membrane scaffold SLP2 anchors a proteolytic hub in mitochondria containing PARL and the i-AAA protease YME1L**. *EMBO Rep.* 2016, **17**:1844-1856.

4. Pickrell AM, Youle RJ: **The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease**. *Neuron* 2015, **85**:257-273.

5. Yamano K, Matsuda N, Tanaka K: **The ubiquitin signal and autophagy: an orchestrated dance leading to mitochondrial degradation**. *EMBO Rep.* 2016, **17**:300-316.

6. Thomas RE, Andrews LA, Burman JL, Lin WY, Pallanck LJ: **PINK1-Parkin pathway activity is regulated by degradation of PINK1 in the mitochondrial matrix**. *PLoS Genet.* 2014, **10**:e1004279.

7. • Ordureau A, Sarraf SA, Duda DM, Heo JM, Jedrychowski MP, Sviderskiy VO, Olszewski JL, Koerber JT, Xie T, Beausoleil SA *et al.*: **Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis**. *Mol. Cell* 2014, **56**:360-375.

By quantitative ubiquitin proteomics, this paper demonstrates the contribution of feed-forward and feed-back amplification cycles of Parkin activation by PINK1 and Phospho-ubiquitin following mitochondrial depolarisation.

8. Chaugule VK, Burchell L, Barber KR, Sidhu A, Leslie SJ, Shaw GS, Walden H: **Autoregulation of Parkin activity through its ubiquitin-like domain**. *EMBO J.* 2011, **30**:2853-2867.

9. •• Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, Banerjee S, Youle RJ: **PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity**. *J. Cell Biol.* 2014, **205**:143-153.

10. •• Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, Hofmann K, Alessi DR, Knebel A, Trost M, Muqit MM: **Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65**. *Biochem. J.* 2014, **460**:127-139.

11. •• Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T *et al.*: **Ubiquitin is phosphorylated by PINK1 to activate parkin**. *Nature* 2014, **510**:162-166.

These collective contributions describe the phosphorylation of ubiquitin at Serine 65 by PINK1. Phospho-ubiquitin is essential for the full activation of Parkin E3 ligase activity and induction of mitophagy. These papers highlight a previously unrecognised interplay between the post-translational modifications of phosphorylation and ubiquitin.

12. Wauer T, Swatek KN, Wagstaff JL, Gladkova C, Pruneda JN, Michel MA, Gersch M, Johnson CM, Freund SM, Komander D: **Ubiquitin Ser65 phosphorylation affects ubiquitin structure, chain assembly and hydrolysis**. *EMBO J.* 2015, **34**:307-325.

13. Kazlauskaite A, Martinez-Torres RJ, Wilkie S, Kumar A, Peltier J, Gonzalez A, Johnson C, Zhang J, Hope AG, Peggie M *et al.*: **Binding to serine 65-phosphorylated ubiquitin primes Parkin for optimal PINK1-dependent phosphorylation and activation**. *EMBO Rep.* 2015, **16**:939-954.

14. Kumar A, Aguirre JD, Condos TE, Martinez-Torres RJ, Chaugule VK, Toth R, Sundaramoorthy R, Mercier P, Knebel A, Spratt DE *et al.*: **Disruption of the autoinhibited state primes the E3 ligase parkin for activation and catalysis**. *EMBO J.* 2015, **34**:2506-2521.

15. Wauer T, Simicek M, Schubert A, Komander D: **Mechanism of phospho-ubiquitin-induced PARKIN activation**. *Nature* 2015, **524**:370-374.

16. Sauve V, Lilov A, Seirafi M, Vranas M, Rasool S, Kozlov G, Sprules T, Wang J, Trempe JF, Gehring K: **A Ubl/ubiquitin switch in the activation of Parkin**. *EMBO J.* 2015, **34**:2492-2505.

17. Yamano K, Queliconi BB, Koyano F, Saeki Y, Hirokawa T, Tanaka K, Matsuda N: **Site-specific interaction mapping of phosphorylated ubiquitin to uncover Parkin activation**. *J. Biol. Chem.* 2015, **290**:25199-25211.

18. Durcan TM, Fon EA: **The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications**. *Genes Dev.* 2015, **29**:989-999.

19. Ordureau A, Heo JM, Duda DM, Paulo JA, Olszewski JL, Yanishevski D, Rinehart J, Schulman BA, Harper JW: **Defining roles of PARKIN and ubiquitin phosphorylation by PINK1 in mitochondrial quality control using a ubiquitin replacement strategy**. *Proc. Natl. Acad. Sci. U. S. A.* 2015, **112**:6637-6642.

20. Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, Matsuda N: **Phosphorylated ubiquitin chain is the genuine Parkin receptor**. *J. Cell Biol.* 2015, **209**:111-128.

21. • Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI, Youle RJ: **The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy**. *Nature* 2015, **524**:309-314.

22. • Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW: **The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy**. *Mol. Cell* 2015, **60**:7-20.

These papers describe the key autophagy adaptor proteins involved in the downstream steps of mitophagy. Lazarou *et al*., also provide evidence for PINK1-mediated Parkin-independent mitophagy.

23. Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P, Zaffagnini G, Wild P, Martens S, Wagner SA *et al.*: **Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria**. *Proc. Natl. Acad. Sci. U. S. A.* 2016, **113**:4039-4044.

24. Nguyen TN, Padman BS, Usher J, Oorschot V, Ramm G, Lazarou M: **Atg8 family LC3/GABARAP proteins are crucial for autophagosome-lysosome fusion but not autophagosome formation during PINK1/Parkin mitophagy and starvation**. *J. Cell Biol.* 2016, **215**:857-874.

25. Tsuboyama K, Koyama-Honda I, Sakamaki Y, Koike M, Morishita H, Mizushima N: **The ATG conjugation systems are important for degradation of the inner autophagosomal membrane**. *Science* 2016, **354**:1036-1041.

26. Rojansky R, Cha MY, Chan DC: **Elimination of paternal mitochondria in mouse embryos occurs through autophagic degradation dependent on PARKIN and MUL1**. *eLife* 2016, **5**.

27. Yamano K, Fogel AI, Wang C, van der Bliek AM, Youle RJ: **Mitochondrial Rab GAPs govern autophagosome biogenesis during mitophagy**. *eLife* 2014, **3**:e01612.

28. • Lai YC, Kondapalli C, Lehneck R, Procter JB, Dill BD, Woodroof HI, Gourlay R, Peggie M, Macartney TJ, Corti O *et al.*: **Phosphoproteomic screening identifies Rab GTPases as novel downstream targets of PINK1**. *EMBO J.* 2015, **34**:2840-2861.

Combining phosphoproteomic screening and biochemical analyses, this study implicates several Rab proteins including Rab8A as downstream targets of PINK1 following mitochondrial damage.

29. Randow F, Youle RJ: **Self and nonself: how autophagy targets mitochondria and bacteria**. *Cell Host Microbe* 2014, **15**:403-411.

30. • Pickrell AM, Huang CH, Kennedy SR, Ordureau A, Sideris DP, Hoekstra JG, Harper JW, Youle RJ: **Endogenous Parkin preserves dopaminergic substantia nigral neurons following mitochondrial DNA mutagenic stress**. *Neuron* 2015, **87**:371-381.

This important proof-of-concept study demonstrates that genetic ablation of Parkin results in age-dependent neurodegeneration under conditions of constitutive mitochondrial stress by crossing Parkin-null mice with the PolG mitochondrial mutator mouse model.

31. Dave KD, De Silva S, Sheth NP, Ramboz S, Beck MJ, Quang C, Switzer RC 3rd, Ahmad SO, Sunkin SM, Walker D *et al.*: **Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease**. *Neurobiol. Dis.* 2014, **70**:190-203.

32. Villeneuve LM, Purnell PR, Boska MD, Fox HS: **Early expression of Parkinson's disease-related mitochondrial abnormalities in PINK1 knockout rats**. *Mol. Neurobiol.* 2016, **53**:171-186.

33. Fiesel FC, Ando M, Hudec R, Hill AR, Castanedes-Casey M, Caulfield TR, Moussaud-Lamodiere EL, Stankowski JN, Bauer PO, Lorenzo-Betancor O: **(Patho-)physiological relevance of PINK1-dependent ubiquitin phosphorylation**. *EMBO. Rep.* 2015, **16**:1114-1130.

34. Chung SY, Kishinevsky S, Mazzulli JR, Graziotto J, Mrejeru A, Mosharov EV, Puspita L, Valiulahi P, Sulzer D, Milner TA *et al.*: **Parkin and PINK1 patient iPSC-derived midbrain dopamine neurons exhibit mitochondrial dysfunction and alpha-synuclein accumulation**. *Stem Cell Rep.* 2016, **7**:664-677.

35. Khaminets A, Behl C, Dikic I: **Ubiquitin-dependent and independent signals in selective autophagy**. *Trends Cell Biol.* 2016, **26**:6-16.

36. Herhaus L, Dikic I: **Expanding the ubiquitin code through post-translational modification**. *EMBO Rep.* 2015, **16**:1071-1083.

37. Swaney DL, Rodriguez-Mias RA, Villen J: **Phosphorylation of ubiquitin at Ser65 affects its polymerization, targets, and proteome-wide turnover**. *EMBO Rep.* 2015, **16**:1131-1144.

38. Palikaras K, Lionaki E, Tavernarakis N: **Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans***. *Nature* 2015, **521**:525-528.

39. Vincow ES, Merrihew G, Thomas RE, Shulman NJ, Beyer RP, MacCoss MJ, Pallanck LJ: **The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo**. *Proc. Natl. Acad. Sci. U. S. A.* 2013, **110**:6400-6405.

40. McWilliams TG, Ganley IG: **Life in lights: tracking mitochondrial delivery to lysosomes in vivo**. *Autophagy* 2016, **12**:2506-2507.

41. •• McWilliams TG, Prescott AR, Allen GF, Tamjar J, Munson MJ, Thomson C, Muqit MM, Ganley IG: **mito-QC illuminates mitophagy and mitochondrial architecture in vivo**. *J. Cell Biol.* 2016, **214**:333-345.

42. •• Sun N, Yun J, Liu J, Malide D, Liu C, Rovira II, Holmstrom KM, Fergusson MM, Yoo YH, Combs CA *et al.*: **Measuring in vivo mitophagy**. *Mol. Cell* 2015, **60**:685-696.

These two papers employ novel reporter mouse models to describe the unique basal nature of mammalian mitophagy for the first time *in vivo*. These technological advances herald powerful tools to uncover the physiological roles of mitophagy.

43. 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**:655-670.

44. • Davis CH, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T, Kinebuchi M, Phan S, Zhou Y, Bihlmeyer NA *et al.*: **Transcellular degradation of axonal mitochondria**. *Proc. Natl. Acad. Sci. U. S. A.* 2014, **111**:9633-9638.

Classically, mitochondrial delivery to the lysosome has been studied in the context of intracellular degradation. This paper is the first to describe axonal *trans*-mitophagy *in vivo* and provokes a reassessment of how mitophagy proceeds in complex systems.

45. Davis CH, Marsh-Armstrong N: **Discovery and implications of transcellular mitophagy**. *Autophagy* 2014, **10**:2383-2384.

46. Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH: **Transfer of mitochondria from astrocytes to neurons after stroke**. *Nature* 2016, **535**:551-555.

47. • 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**:282-295.

This study implicates PINK1-Parkin signalling in the regulation of mitochondrial-derived vesicles (MDVs) following mitochondrial damage. MDVs were noted to contain selective damaged mitochondrial cargos that are targeted to the lysosome for destruction.

48. Sugiura A, McLelland GL, Fon EA, McBride HM: **A new pathway for mitochondrial quality control: mitochondrial-derived vesicles**. *EMBO J.* 2014, **33**:2142-2156.

49. McLelland GL, Lee SA, McBride HM, Fon EA: **Syntaxin-17 delivers PINK1/parkin-dependent mitochondrial vesicles to the endolysosomal system**. *J. Cell Biol.* 2016, **214**:275-291.

50. Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, Fazel A, Bergeron JJ, Trudeau LE, Burelle Y *et al.*: **Parkinson's disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation**. *Cell* 2016, **166**:314-327.

51. Roberts RF, Fon EA: **Presenting mitochondrial antigens: PINK1, Parkin and MDVs steal the show**. *Cell Res.* 2016, **26**:1180-1181.

52. Murley A, Nunnari J: **The emerging network of mitochondria-organelle contacts**. *Mol. Cell* 2016, **61**:648-653.

53. Sugiura A, Mattie S, Prudent J, McBride HM: **Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes**. *Nature* 2017, **542**:251-254.

54. Pao KC, Stanley M, Han C, Lai YC, Murphy P, Balk K, Wood NT, Corti O, Corvol JC, Muqit MM *et al.*: **Probes of ubiquitin E3 ligases enable systematic dissection of parkin activation**. *Nat. Chem. Biol.* 2016, **12**:324-331.

55. Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, Kaneko T: **Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum**. *J. Neurosci.* 2009, **29**:444-453.

56. Bolam JP, Pissadaki EK: **Living on the edge with too many mouths to feed: why dopamine neurons die**. *Mov. Disord.* 2012, **27**:1478-1483.

57. Crittenden JR, Tillberg PW, Riad MH, Shima Y, Gerfen CR, Curry J, Housman DE, Nelson SB, Boyden ES, Graybiel AM: **Striosome-dendron bouquets highlight a unique striatonigral circuit targeting dopamine-containing neurons**. *Proc. Natl. Acad. Sci. U. S. A.* 2016, **113**:11318-11323.

58. Howe MW, Dombeck DA: **Rapid signalling in distinct dopaminergic axons during locomotion and reward**. *Nature* 2016, **535**:505-510.