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Guardian of mitochondrial function: an expanded role of Parkin in skeletal muscle

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Parkin is a cytosolic E3 ubiquitin ligase encoded in mammals by the *Park2* gene. Upon mitochondrial damage and loss of mitochondrial membrane potential, phosphatase and tensin homologue (PTEN)-induced putative kinase 1 (PINK1) accumulates on the outer mitochondrial membrane and via phosphorylation of Parkin at serine 65 it activates Parkin ligase activity and initiates its translocation to the mitochondria. Subsequently, Parkin conjugates ubiquitin chains on proteins located on the outer mitochondrial membrane causing signal amplification and recruitment of the autophagosome to initiate mitophagy. Despite an increased understanding of how Parkin activation initiates mitophagy at a cellular level and the importance of the PINK1/Parkin pathway for the maintenance of a healthy mitochondrial pool, literature regarding the role of Parkin in the regulation of mitochondrial content and function in skeletal muscle is scarce.

A recent study published in *The Journal of Physiology* by Gouspillou *et al.* (2018) advances our understanding of the role of Parkin in the regulation of skeletal muscle mitochondrial content and respiratory function. Using *Park2*$^{-/-}$ mice, Gouspillou *et al.* (2018) report that ablation of Parkin causes a decrease in mitochondrial respiratory function, as well as reduced activity of certain respiratory chain complexes, and increased susceptibility to the opening of permeability transition pores. In addition, ablation of Parkin decreased the expression of genes regulating mitochondrial biogenesis and the content of the mitochondrial fusion protein mitofusin 2 (MFN2), whilst increasing mitochondrial fission protein dynamin-related protein 1 (DRP1). Despite the decrease in the expression of key genes regulating mitochondrial biogenesis, mitochondrial content was similar between the *Park2*$^{-/-}$ and wild-type animals. Although confirmation by transmission electron microscopy is required, this suggests that Parkin ablation leads to decreased mitochondrial connectivity and increased mitochondrial fragmentation, without a change in mitochondrial content. Furthermore, Parkin ablation possibly leads to an accumulation of suboptimal respiratory complex subunits that are unable to be replaced, potentially due to a decreased turnover, resulting in decreased mitochondrial respiratory function.

Mitochondrial respiration in permeabilised fibres is considered the gold-standard technique to assess mitochondrial respiratory function in skeletal muscle, as it directly measures oxygen consumed by the mitochondria to generate ATP and it allows the assessment of the contribution of individual respiratory complexes to mitochondrial respiration. While no difference was seen in maximal ADP-stimulated mitochondrial respiration through complex I+II ($CI+II_P$), Gouspillou *et al.* (2018) observed that maximal ADP-stimulated mitochondrial respiration through complex I (CI_P) and complex IV (CIV_P) was 48% and 34% lower, respectively, in permeabilised gastrocnemius muscle fibres of *Park2*$^{-/-}$ mice. Similarly, a 52% reduction in the acceptor control ratio was observed, and enzymatic activity of complexes I and IV was also lower, in *Park2*$^{-/-}$ mice. Previous studies have shown that Parkin plays a multi-faceted role in mitochondrial quality control, as it not only initiates mitophagy but is also important for the turnover of specific subunits in all the respiratory complexes (I–V); complex I is the most susceptible as it is the largest and most intricate complex (Vincow *et al.* 2013). The study of Gouspillou *et al.* (2018) adds to the repertoire of functions of Parkin by indicating its role in the maintenance of mitochondrial respiratory function in skeletal muscle, and possibly also the quality control of complexes containing a larger number of mtDNA-encoded subunits (complex I and complex IV contain 7 and 3 mtDNA-encoded subunits, respectively). How Parkin carries out the selective turnover of particular respiratory complex subunits, whether it only affects mtDNA-encoded subunits, or if it specifically affects certain respiratory complexes (i.e. complex I and IV) in skeletal muscle are questions of interest that remain unanswered.

Despite the long-standing idea that mitochondrial content and mitochondrial respiratory function change simultaneously, results from the present study (Gouspillou *et al.* 2018) confirm recent findings that this is not always the case (Granata *et al.* 2018). Gouspillou *et al.* (2018) report that despite a decrease in mitochondrial respiratory function in *Park2*$^{-/-}$ mice, there was no change in mitochondrial content (indirectly assessed using citrate synthase activity, which has previously been reported to correlate with mitochondrial volume density). There was also a dissociation between the effects of Parkin on changes in mRNA and protein. Although Parkin ablation resulted in a significant decrease in the expression of key genes regulating mitochondrial biogenesis (e.g. peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and mitochondrial transcription factor A (TFAM)), the content of proteins encoded by these genes remained unchanged. Parkin ablation did also result in the reduction of MFN2 protein content and an increase in DRP1 protein content, which could lead to a less interconnected and more fragmented mitochondrial reticulum that would be more susceptible to subsequent stresses. Collectively, the results of this study (Gouspillou *et al.* 2018) show how the different components of mitochondrial dynamics are influenced by the ablation of Parkin and demonstrate how different proteins involved in the normal function of processes involved in mitochondrial dynamics (biogenesis, fusion, fission, mitophagy) interact and affect each other. This is clearly a complex area requiring further research.

Despite the well-known role of PINK1 and Parkin in mitochondrial degradation, other pathways are also known to regulate the breakdown of mitochondrial proteins. Gouspillou *et al.* (2018) demonstrate that the expression of genes encoding

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autophagy proteins (e.g. LC3, GABARAPL1 and BNIP3) and autophagic flux are both significantly increased in the skeletal muscle of *Park2*$^{-/-}$ mice. This may indicate a compensatory upregulation of general autophagy to compensate for the reduced mitochondria-specific degradation when Parkin is absent. However, whether other degradation pathways, such as proteasomal degradation and mitochondria-derived vesicles, are also increased in *Park2*$^{-/-}$ mice, as an additional compensatory mechanism, remains a question of interest. Increased DRP1 and decreased MFN2 protein abundance could also be a compensatory mechanism of Parkin ablation, selectively dividing mitochondria to facilitate their clearance through autophagy.

Despite the importance of Parkin in skeletal muscle (Gouspillou *et al.* 2018), it has been reported that PINK1 is not needed for basal mitophagy to occur in metabolically demanding tissues such as skeletal muscle; this suggests that the PINK1–Parkin pathway is not the only pathway responsible for degradation of mitochondrial proteins. This is supported by findings from research conducted in rodent skeletal muscle, which indicates that the PINK1/Parkin pathway may not be important for *in vivo* basal mitophagy but is needed for an adequate mitochondrial respiratory function, a reduction in oxidative damage (Gouspillou *et al.* 2018), and a decrease in reactive oxygen species production following a long-term physiological stressor such as exercise (Chen *et al.* 2018). Surprisingly, Parkin ablation has been studied in predominantly glycolytic muscles, such as the tibialis anterior and gastrocnemius (Chen *et al.* 2018; Gouspillou *et al.* 2018), and it is possible that the effects of Parkin ablation may be aggravated in oxidative muscles, where autophagy flux is higher. Future research should investigate the role of Parkin on mitochondrial quality control *in vivo*, and whether this regulation differs between glycolytic and oxidative muscles.

PINK1 and Parkin are emerging as key mediators of mitophagy in health and disease due to their role in preventing the accumulation of damaged mitochondria, and both have been proposed to be involved in the pathogenesis of age-related diseases such as Parkinson's disease (Vincow *et al.* 2013). Mounting evidence observing mitophagy as a key process to maintain contractile and mitochondrial respiratory function supports the importance of the PINK1–Parkin pathway for mitochondrial quality control (Chen *et al.* 2018; Gouspillou *et al.* 2018). Despite the increase in research within the field, studies conducted in humans are limited to the assessment of proteins through immunoblotting techniques and there is a need to use more sensitive techniques. A recent report suggests that only 0.1% of the mitochondrial pool was concurrently identified as engulfed by LC3 in rodents (Laker *et al.* 2017). This physiological level of degradation cannot be expected to be measured with traditional techniques such as immunoblotting (Laker *et al.* 2017); these techniques, therefore, need to be used in combination with microscopy techniques to identify mitophagic events (i.e. mitochondria engulfed by autophagosomes, or colocalised with autophagosomes or lysosomes). Once such techniques are further employed in human studies, we will be able to better understand the potential role of mitophagy in human health and disease.

While animal studies can benefit from models of gain or loss of function to investigate the role of specific proteins on cellular function, this is not possible in humans. However, exercise (as a stressor) may provide a good example of how mitophagy can be studied in human skeletal muscle, as mitophagy has previously been suggested to be activated by exercise (Laker *et al.* 2017) and increases in mitochondrial respiratory function and mitochondrial content are a well-known adaptation to endurance exercise (Granata *et al.* 2018). Unveiling how mitochondrial quality control occurs following exercise may provide important insights into how healthy mitochondria are regulated in humans.

The study published in *The Journal of Physiology* by Gouspillou *et al.* (2018) provides an important contribution to the field of Parkin and mitophagy. It advances the understanding of the role of Parkin in skeletal muscle, and its importance for mitochondrial turnover and the maintenance of mitochondrial respiratory function and dynamics. However, many questions still remain. What is the importance of Parkin in skeletal muscle for *in vivo* mitophagy? What is the role of Parkin-mediated mitochondrial quality control in health and disease? What are the mechanisms by which Parkin alters the mitochondrial respiratory function and respiratory complexes turnover? Answering these questions will be important in shaping the direction of future research.

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Additional information

Competing interests

None declared.

Author contributions

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