

## Life in lights: Tracking mitochondrial delivery to lysosomes in vivo

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### ABSTRACT

The past decade has seen an intensive and concerted research effort into the molecular regulation of mitophagy, the selective autophagy of mitochondria. Cell-based studies have implicated mitophagy in the pathology of diverse conditions ranging from cancer to neurodegeneration. However, a definitive link between mitophagy and the etiology of human disease remains to be demonstrated. Moreover, we do not know how pervasive mammalian mitophagy is in vivo and fundamental questions remain unanswered. For example, is mitophagy common to all tissues under basal conditions or does it only occur in highly oxidative tissues under stress? This paucity of knowledge is largely due to a lack of experimentally tractable tools that can measure and monitor mitophagy in tissues. Our recent work describes the development of *mito-QC*, a mouse model to study mitophagy at single cell resolution in vivo.

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Measuring mitophagy is not trivial, and descriptions of mitophagy in the literature have relied on the use of colocalization of overexpressed proteins, dyes and/or surrogate biochemical assays. However, these methods are not applicable to monitoring mitophagy in tissues. A welcome contribution to the field was made recently by the laboratory of Toren Finkel, in the form of the *mt-Keima* mouse. However, due to *Keima*'s technical limitations and its incompatibility with fixation, it was still not possible to visualize mitophagy in selectively labeled populations of cells in vivo. To overcome this limitation, we exploited our previously published assay that involved mitochondrial outer membrane labeling with a tandem mCherry-GFP fusion protein coupled to the mitochondrial targeting sequence of FIS1. This approach enabled the visualization of cellular, “steady-state” mitochondrial networks in yellow. However, when mitochondria are delivered to lysosomes, GFP fluorescence becomes quenched by the acidic microenvironment, whereas mCherry fluorescence remains stable. These mCherry-only positive mitolysosomes provide a binary, end-point readout of mitophagy. The success of this extensively validated approach in cultured cells led to the generation of a transgenic mouse based on the same principle. Thus, to reflect its utility in illuminating mitochondrial quality control (QC) in vivo, we named our mouse model “*mito-QC*.”

*mito-QC* mice are healthy and, aside from fluorescence, their mitochondrial function and morphology appear indistinguishable from wild type. Importantly, we determined that *mito-QC* is compatible with fixation. This means it is possible to use immunohistochemical methods to label cellular subtypes

in fixed tissue samples from our mouse. TOMM20 and LAMP1 immunostaining verified the mitochondrial and lysosomal origins of mCherry-GFP and mCherry-only signals respectively, and this was further confirmed by electron microscopy.

*mito-QC* initially revealed the striking and distinct organization of mitochondrial networks within different tissues. This was even evident in related tissue types such as muscle. The characteristic chain-like arrangement of individual mitochondria is clearly evident in cardiomyocytes and we were able to resolve the recently described mitochondrial reticulum in skeletal muscle. Particularly intriguing were the mitochondrial networks in muscle fibers of the adult tongue, whose studies have largely been confined to classical EM work in the 1950s.

With respect to mitophagy, *mito-QC* immediately demonstrated its nature as a constitutive process. Mitophagy is evident in all tissues examined; however, there is both inter- and intra-tissue variation. The latter is most striking within the renal tubules of the kidney. Using the distal convoluted tubule marker SLC12A3/NCC, we demonstrated that the highly energetic proximal convoluted tubules (PCTs) are a major site of mitophagy in the mammal. Although we observed turnover in nearly all cells of the kidney, mitophagy in PCTs is particularly impressive. Whole organ imaging using immunolabeling-enabled 3-dimensional imaging of solvent-cleared organs (iDISCO) and cross sections of renal tubules revealed a polarized organization of mitochondrial destruction. Whereas renal tubules are densely populated with mitochondria, we observed a coronal border of mitolysosomes, concentric to the PCT lumen. The regulation and physiological significance of such positioning in these cells merits further investigation. As distal

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convoluted tubules contain more mitochondria than PCTs, our findings also reveal that the degree of mitophagy within a given anatomical structure is not proportional to its mitochondrial content.

Due to its postulated links with neurodegenerative disease, neural mitophagy is a burgeoning topic of considerable interest. Given the cellular heterogeneity and anisotropic nature of the nervous system, the precise identification of individual cell types is possible only through the use of specific markers. We labeled *mito*-QC cerebellar sections with anti-CALB/calbindin, a marker of Purkinje neurons, and can resolve mitochondria in neuronal processes and somata. We were also able to visualize neuronal mitophagy, with the great majority of mCherry-only mitolysosomes present in somata, suggesting the cell body is a major region of neuronal mitochondrial turnover in vivo, at least in this neural population.

What next for mitophagy? Our work reveals the constitutive and spatiotemporal nature of vertebrate mitophagy in vivo. Indeed, the high levels of mitophagy we observed under steady-state conditions suggest it is critical to mitochondrial homeostasis. Pioneering work from Richard Youle and others has established fundamental roles for the PINK1-PARK2 signaling pathway in

regulating depolarization-induced mitophagy. It will be critical to determine the precise contribution of PINK1-PARK2 signaling and other pathways to basal mitophagy in vivo, and delineate the contribution of mitophagy to the pathophysiology of Parkinson disease. Additionally, several other questions remain. What are the precise intrinsic and extrinsic signals that trigger mitochondrial elimination in vivo? What underpins the cell and tissue heterogeneity of basal mitophagy? Is a particular threshold of mitophagy required to sustain mitochondrial network integrity? What is the physiological significance of mitophagy during mammalian development?

We think that *mito*-QC represents the latest advance in the recent rise of reporter mouse models to study dynamic cell biology in vivo. We anticipate that our model will provide researchers with a tractable way to measure the physiological end-point of mitophagy. Not only will *mito*-QC serve to validate numerous factors that have already been suggested to play a role in mitophagy, we predict that it will also be a powerful tool for new discovery in studies of development and disease. Ultimately, we hope our experimental findings will provoke a renewed appreciation for mitophagy as a constitutive process in vivo.