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Issue: *Mitochondrial Research in Translational Medicine***Optogenetic probing of mitochondrial damage responses**Wei Yuan Yang<sup>1,2</sup><sup>1</sup>Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan. <sup>2</sup>Institute of Biochemical Sciences, College of Life Sciences, National Taiwan University, Taipei, Taiwan

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It is now possible to functionally **impair mitochondria through light illumination with high specificity**. These optogenetic tools permit precise control on the timing, location, and extent of mitochondrial damage within a cell population with subcellular resolution, allowing quantitative probing of the various types of mitochondrial damage responses within cells. This approach can generally be extended toward the probing of other organelle damage responses.

**Keywords:** Parkin; mitochondria; autophagy; Parkinson disease; mitophagy; optogenetics; photochemical control; mitophagy; photosensitizer; quality control

**Introduction**

Cells need to cope with mitochondrial injuries to remain viable. Improper mitochondrial damage responses can affect cellular function, accumulate unwanted stress, and could be detrimental. For example, it has been shown that a lack of mitophagy against damaged mitochondria contributes to Parkinson disease.<sup>1,2</sup> A wide range of mitochondrial quality-control mechanisms have been described. Chaperones and proteases present within the mitochondrial matrix are thought to selectively eliminate oxidized and misfolded proteins, thereby cleansing the matrix space to lessen mitochondrial stress.<sup>3,4</sup> Small vesicles, termed *mitochondria-derived vesicles*, can bud off from the mitochondrial outer membrane, carrying portions of defective mitochondria with them into the cytoplasm for further processing and turnover.<sup>5</sup> A cell can even exocytose vesicles containing defective mitochondria into the extracellular space for other cells to process, a pathway termed mitoptosis.<sup>6</sup> One of the most studied and well-characterized pathways for mitochondrial quality control is mitophagy, in which cells manage to selectively wrap up dysfunctional mitochondria for degradation within the lysosomal lumen.<sup>2,7</sup> Aside from the repair and removal of dysfunctional

mitochondria within the cytoplasm, one may also expect many other types of pathways to become activated. For instance, cells may accelerate mitochondrial biogenesis to replenish their functional mitochondrial pool in response to damage.

One way to experimentally investigate mitochondrial damage responses is to artificially elicit mitochondrial dysfunction through optogenetic schemes. By using light to specifically impair mitochondria, researchers have the opportunity to precisely define the number of mitochondria that are dysfunctional at one time (e.g., from every mitochondrion within the entire cell population to only one or a few within a single cell), as well as when and where within a cell this damage occurs (and the responses can then be analyzed using any method of choice). All of these parameters can affect a cell's decision on how it wants to respond. For example, through these schemes the differential effects of damage within axons, dendrites, the cell body, and the distal regions in a neuron can be compared.<sup>8</sup>

**Two commonly used means for initiating mitochondrial dysfunction through light are direct laser ablation and chromophore-assisted light inactivation (CALI) through the use of mitochondria-targeting photosensitizers.**

## Direct laser ablation

Exposure to strong laser light can disrupt cellular integrity, and this strategy has been extensively used in neuroscience where individual neurons within neuronal networks are ablated for functional characterization.<sup>9</sup> Similarly, focused illumination of a subcellular location will locally disrupt cellular compartments and materials near the site of light activation and can be applied toward probing organelle functions.<sup>10,11</sup> One of the earlier demonstrations of this strategy of analyzing the fate of damaged mitochondria was in the 1970s,<sup>12</sup> when it was found that both blue and green laser light can elicit damage, with the illuminated mitochondria undergoing paling and coagulation. A key discovery made using this damage-inducing scheme was later reported by Kim *et al.*, where the selective removal of photodamaged mitochondria by the cellular autophagy pathway was observed.<sup>13</sup> Using 488 nm illumination with intensities that were 10,000- to 40,000-fold greater than the typical laser power used in confocal fluorescence microscopy, the authors reported light dose-dependent loss of mitochondrial membrane potential in primary mouse hepatocytes, indicating laser-mediated mitochondrial ablation. Interestingly, the same phenomenon was not observed with 543 nm illumination, suggesting that mitochondria are more sensitive to 488 nm than to 543 nm light. Live-cell imaging revealed that photodamaged mitochondria (by 488 nm light) gradually recruited individual EGFP-LC3 puncta to their periphery; EGFP-LC3 puncta eventually connected into a full ring and enclosed damaged mitochondria for autophagic clearance. This led to the proposal that cells can selectively recognize photo-damaged mitochondria and target them specifically for autophagic clearance. The observed mitophagy is distinct from other reported forms of selective autophagy (e.g., Parkin-mediated mitophagy), where it is thought that LC3-coated membranes extend into cup-shaped membranes to engulf substrates.<sup>14</sup>

## CALI mediated by fluorescent proteins

One of the key hypotheses on why mitochondria are prime targets for damage is that they harbor the electron transport chain (ETC): a leak of electrons from the ETC can result in the generation of reactive oxygen species (ROS), thereby

oxidizing/damaging biomolecules within mitochondria. This damaging effect can be mimicked through the use of CALI.<sup>15,16</sup> **With mitochondria-targeted chromophores that are active in ROS generation upon excitation, light illumination will result in ROS-mediated mitochondrial impairment.** Highly specific mitochondrial labeling afforded by the chromophores will ensure selective impairment without the generation of unwanted side effects.

**Genetically encoded fluorophores, such as KillerRed (and its monomeric variant SuperNova) and miniSOG (and the enhanced Q102L mutant<sup>17</sup>), have been specifically developed to mediate light-activated ROS production.**<sup>18–20</sup> These proteins can be efficiently brought into the mitochondrial matrix through mitochondrial targeting sequences, allowing researchers to specifically elicit ROS production within the mitochondrial lumen. With mitochondria-targeted KillerRed, it is possible to activate Parkin-mediated mitophagy and has allowed clear demonstration that Parkin selectively targets illuminated mitochondria.<sup>21–23</sup> The main advantage of this scheme over direct laser ablation lies in (1) the enhanced damage specificity provided by the mitochondria-specific chromophores and (2) the low light intensities required (tens of microwatts; only  $\sim 10\times$  of the normally used laser power for long-term live-cell fluorescent imaging; this again avoids unwanted side effects). Genetically encoded chromophores will also permit the investigation of mitochondrial damage responses within subtypes of cells in an organism.

## CALI mediated by mitochondrial dyes

Small-molecule dyes can offer superior spectral properties as well as ROS-generating capabilities when compared to genetically encoded photosensitizers. A wide range of mitochondrial dyes have been developed for tracking mitochondrial function and dynamics, and can be directly utilized for CALI. These small molecules are commercially available and can be directly supplied to cells to achieve mitochondrial localization, making them accessible to most laboratories. For example, the mitochondrial membrane potential indicator tetramethylrhodamine has been successfully used in place of KillerRed to trigger Parkin-mediated mitophagy with spatiotemporal precision.<sup>22</sup>

A particularly useful aspect of the small-molecule mitochondrial markers is that some fluoresce well

outside of the visible spectrum. These dyes are spectrally separated from commonly used fluorescent indicators in cell biology, and therefore facilitate their combined use with live-cell imaging. Commercially available far-red fluorescent markers, such as Mitotracker DeepRed, MitoView 633, and Rhodamine 800, have all been shown to successfully elicit mitochondrial damage responses through the use of 635 nm laser illumination.<sup>24</sup>

## Advantages and applications

Optogenetic schemes allow spatiotemporally precise induction of mitochondrial damage responses. Among these schemes, CALI is particularly simple and robust, as it requires only low light intensities to achieve mitochondrial dysfunction. These operations leave the overall structure of mitochondria intact: many mitochondrial matrix contents remain in place even after light-activated impairment.<sup>24</sup>

The spatiotemporal precision afforded by optogenetic schemes allows investigation of the temporal ordering of mitochondrial damage responses, as well as how the responses differ within distinct subcellular regions of a cell. For example, the scheme has been utilized to investigate mitochondrial quality control within the axonal regions of a neuron. Selective impairment of only a few axonal mitochondria minimized cellular toxicity and allowed validation of the role of Parkin-mediated mitophagy in neuronal axons.<sup>8</sup> One exciting direction will be to further achieve all-optical control over mitochondrial positioning, movement, and function to precisely define the interplay between different damage response programs. This can be done by combining CALI-based mitochondrial impairment schemes with light-activated motor protein recruitment strategies for controlling organelle transport.<sup>25</sup> Aside from operations on cultured cells, it will be desirable to investigate mitochondrial damage responses directly within intact organisms. Combining the use of tissue-specific promoters for targeted expression of genetically encoded photosensitizers within mitochondria and two- or three-photon excitation will allow mitochondrial injuries to be elicited within defined subregions of a tissue.<sup>26</sup>

It is important to quantitatively define the input–output relationships for mitochondrial damage responses; namely, to understand how cells differentially react to varying numbers of injured mitochondria as well as different extents of the mitochondrial

injuries within their cytoplasm. Different extents of mitochondrial injuries may differentially affect the energy status and stress level of a cell, and elicit distinct sets of damage responses. Cells may also want to tune the strengths of their repair/degradation and biogenesis programs to precisely accommodate for the amount of damaged mitochondria present. These issues can now be probed through optogenetic schemes through changing light illumination patterns, durations, and intensities. For example, in Parkin-mediated mitophagy, it has been found that the number autophagosome initiation sites correlate with the amount of Parkin-labeled mitochondria present within the cytoplasm, indicating precise cellular sensing of mitophagy substrates in a cell.<sup>24</sup>

One less obvious advantage offered by optogenetic damage-inducing schemes is that, under many situations, they can be used to improve image-based analysis (by selectively impairing only one or a few mitochondria within the cell cytoplasm during live-cell imaging). Having large numbers of defective mitochondria simultaneously present within the cell cytoplasm complicates tracking and image analysis. This becomes even more evident during Parkin-mediated mitophagy when defective mitochondria are collated toward the nucleus to become mito-aggregates,<sup>1,27</sup> and the formation of these large clusters (with large numbers of defective mitochondria) can make structural and morphological analysis by light microscopy difficult. On the contrary, when only a small fraction of mitochondria are impaired (through the use of the optogenetic approach), it becomes experimentally feasible to directly resolve, under live-cell imaging, how cells disassemble Parkin-labeled mitochondria into small fragments for piecemeal autophagic engulfment and turnover during mitochondrial dysfunction.<sup>28</sup>

The CALI-mediated mitochondrial impairment scheme can easily be extended to probe damage responses for other organelles. While different organelles have distinct molecular identities, their chemical compositions are similar: all are cellular structures assembled from common biomolecules (e.g., proteins and phospholipids). Most organelles will therefore be similarly susceptible to ROS-mediated damage. The rampant development of organelle-specific dyes and the identification of organelle-targeting sequences in cell biology allow one to easily find chromophores suitable for CALI-mediated organelle impairment. With the light

illumination conditions for functional impairment already worked out for mitochondria, only minimal fine tuning of the experimental conditions will be required to establish specific organelle-damaging schemes. Finally, as organelles are membrane-bound compartments, these membrane barriers will help restrict light-activated ROS from affecting cytoplasmic materials (with the use of chromophores that reside in the lumen), thereby minimizing unwanted side effects. Together, these points suggest CALI-based organelle impairment schemes to be an attractive, general experimental strategy for probing organelle damage responses. Indeed, this approach was recently used to demonstrate that dysfunctional lysosomes can be selectively targeted by the cellular autophagy machinery for removal.<sup>29</sup>

### Conflicts of interest

The author declares no conflicts of interest.

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