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OA treatment and Quantification of Parkin recruitment to mitochondria

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We use this protocol and it's working

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Abstract

Defective mitochondrial quality control in response to loss of mitochondrial membrane polarization is implicated in Parkinson's disease by mutations in PINK1 and PRKN. Application of in situ cryo-electron tomography (cryo-ET) made it possible to visualize the consequences of mitochondrial depolarization at higher resolution than heretofore attainable. Parkin-expressing U2OS cells were treated with the depolarizing agents oligomycin and antimycin A (OA), subjected to cryo-FIB milling, and mitochondrial structure was characterized by in situ cryo-ET. Phagophores were visualized in association with mitochondrial fragments. Bridge-like lipid transporter (BLTP) densities potentially corresponding to ATG2A were seen connected to mitophagic phagophores. Mitochondria in OA-treated cells were fragmented and devoid of matrix calcium phosphate crystals. The intermembrane gap of cristae was narrowed and the intermembrane volume reduced, and some fragments were devoid of cristae. A subpopulation of ATP synthases re-localized from cristae to the inner boundary membrane (IBM) apposed to the outer membrane (OMM). The structure of the dome-shaped prohibitin complex, a dodecamer of PHB1-PHB2 dimers, was determined in situ by sub-tomogram averaging in untreated and treated cells and found to exist in open and closed conformations, with the closed conformation is enriched by OA treatment. These findings provide a set of native snapshots of the manifold nano-structural consequences of mitochondrial depolarization and provide a baseline for future in situ dissection of Parkin-dependent mitophagy.

Cell culture and OA treatment

1 U2OS cell lines stably expressing mCherry-Parkin and BFP-mito were established ahead of experiments.

2 On the day before the experiment, split cells into 8 chamber slides at a density of 20-30,000 cells per well and let them recover overnight.

3 On the following day, incubate cells with fresh media for 30minutes. After incubation, replace media with fresh media again, or media containing 3uM Oligomycin, 3uM Antimycin A, or 3uM Oligomycin and Antimycin A. Incubate cells with new media for 3hrs.

4 After final incubation, brings cells to the microscope for imaging.

Imaging and Analysis

5 At the microscope, capture at least 3 indepedent fields of cells (minimum 10 cells per field). For this experment we must capture the mCherry and BFP channels to investigate Parkin recruitment to mitochondria under various conditions

6 Open one individual field with 2 channels in FIJI. First use Process > Subtract background and process each image with a 50 pixel rolling ball radius to remove background signal.

7 Binarize each background subtracted image using Process > Binary > Make binary using default settings.

8 To determine a Pearson coefficient for each treatment, use the JACoP plugin with auto-thresholding for each channel and record the resultant Pearson coefficient for each channel.

9 Repeat this for all 3 independent fields and determine an average value.

10 Repeat the experiment at least 2 additional times, capturing 3 more fields each time. Record the average value from each experiment like previously.

11 To determine significance, apply a paired t-test between pairs of conditions using the triplicate average obtained from 3 replicates (9 fields of cells).