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EM grid seeding for cryo-FIB milling

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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.

EM grid preparation and cell seeding

1 Glow discharge carbon coated gold quantifoil R2-2 200 mesh EM grids at 15mA for 30 seconds

2 In a laminar flow hood, float EM grids carbon side down on 30 uL drops of 0.01% poly-L-lysine solution. Simultaneously, add 100 uL of 0.01% poly-L-lysine solution to each well of an 8-chamber plate with removable wells. Incubate both under UV for 30min at room temperature.

3 After incubation, split cells using 0.25% trypsin and resuspend to 100,000 cells/mL in complete media

4 Before seeding cells, remove poly-L-lysine solution from the 8 chamber plate and replace with 200uL complete media. Remove grids from atop the drops of poly-L-lysine and rinse them 3 times in complete media before placing into the bottoms of each of the wells of the 8 chamber plate.

5 Add 200 uL of cell solution to each well such that 20,000 cells per EM grid are seeded. Let cells recover overnight before treating and freezing.