New live imaging combined 3D-CLEM revealed a quick response of mitochondrial transformation from tubular to a globular form after loss of membrane potential

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Mitochondria are known to have a distinctive tubular shape, but the molecular mechanism to maintain their shape has not been elucidated. The fission and fusion mechanism has been well studied and the balance of which are considered to be closely linked in their morphology and function. When the balance shift toward fission, fragmentation of mitochondria would be observed within the cell which display a huge number of globular mitochondria. Such fragmentation of mitochondria is generally accepted as a typical reaction of cells for oxidative stresses, and the process has been reported to be caused by differential modulation of mitochondrial fission-fusion proteins. Here, uncoupler administration has been frequently used for the induction of an experimental mitochondrial fragmentation. Uncoupler such as carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) induces loss of mitochondrial membrane potential and triggers drastic structural changes in mitochondria from a tubular to a globular shape, which had been considered as a result of frequent mitochondrial fission. At the time, resultant globular mitochondria were known to show unusual ring/doughnut shape under light microscopy. However, the detail transformation process has not been estimated.

In order to evaluate the early processes of structural changes after uncoupler administration, we have reported an original correlative microscopic method combined with fluorescence microscopic live imaging and FIB-SEM tomography (Fig1) [1]. Live-imaging under confocal microscopy showed that most mitochondria in mouse embryonic fibroblasts (MEFs) changed their shape from a tubular shape to a globular shape without any fission and showed typical ring shapes within 10 min after CCCP. However, our correlative observation showed most ring mitochondria under light microscopy did not have a true through hole; rather, they had various indents, and 47% showed stomatocyte shapes with vase-shaped cavities (Fig 2), which is the most stable physical structure without any structural support if the long tubular shape shortens into a sphere with redundant membrane [1].

In this study, we further examined another type of ionophore to evaluate whether the unique transformation process associate with loss of membrane potential or not. Valinomycin is known to work as uncoupler by the effect of its potassium ionophore. On the other hand, calcimycin as a calcium ionophore is known to affect cellular calcium concentration but not affect mitochondria membrane potential. Our CLEM observation showed that Varinomycin induce mitochondrial transformation almost same as the case of CCCP. On the contrary, calcimycin administration showed drastic fission of mitochondrion and resulted small true sphere mitochondria. Therefore, vase-shaped mitochondria formation was probably a specific event after the loss of membrane potential.

These results suggested that uncoupling would trigger the collapse of prospective mitochondrial structural support mechanisms.

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[1] Miyazono Y et al., Sci Rep. 2018;**8(1)**:350. (2018)

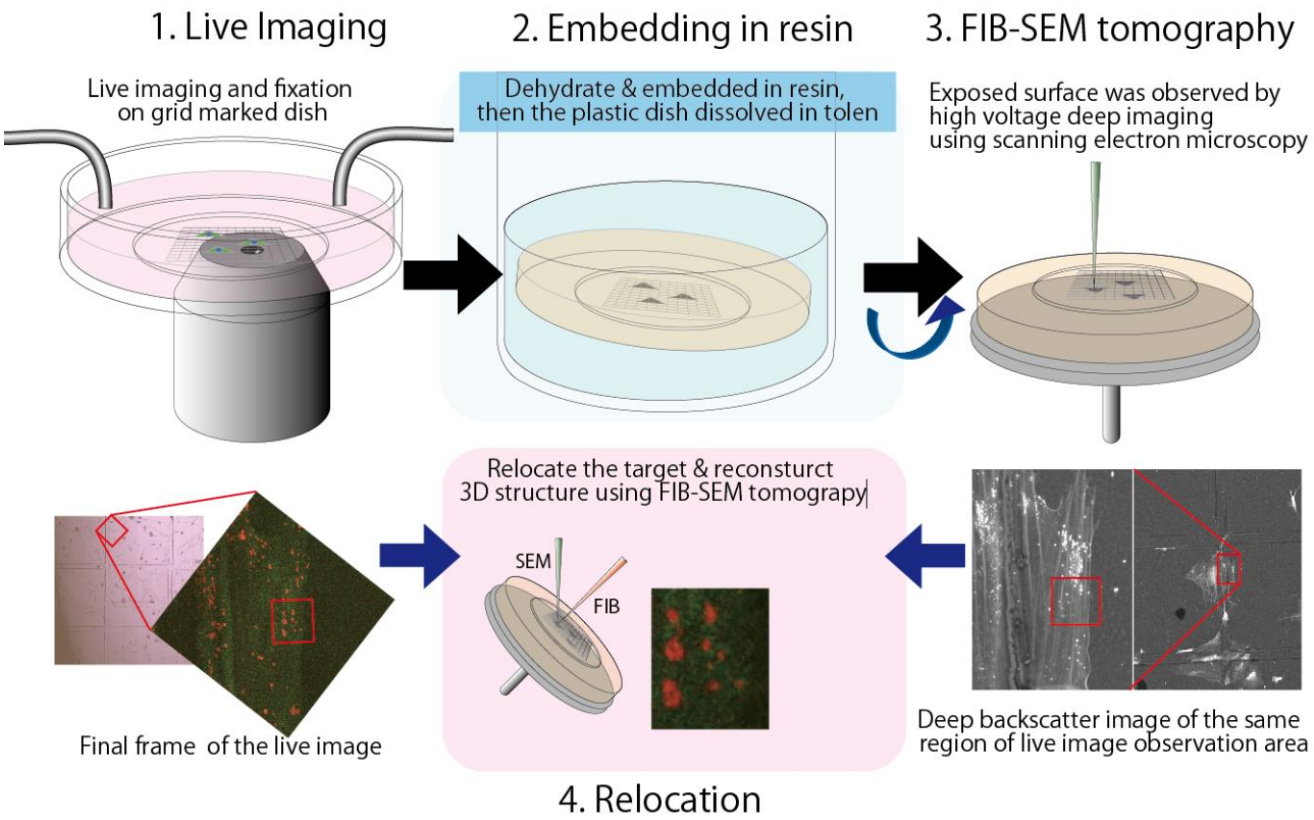

Fig. 1 Work flow of live imaging combined 3D-CLEM

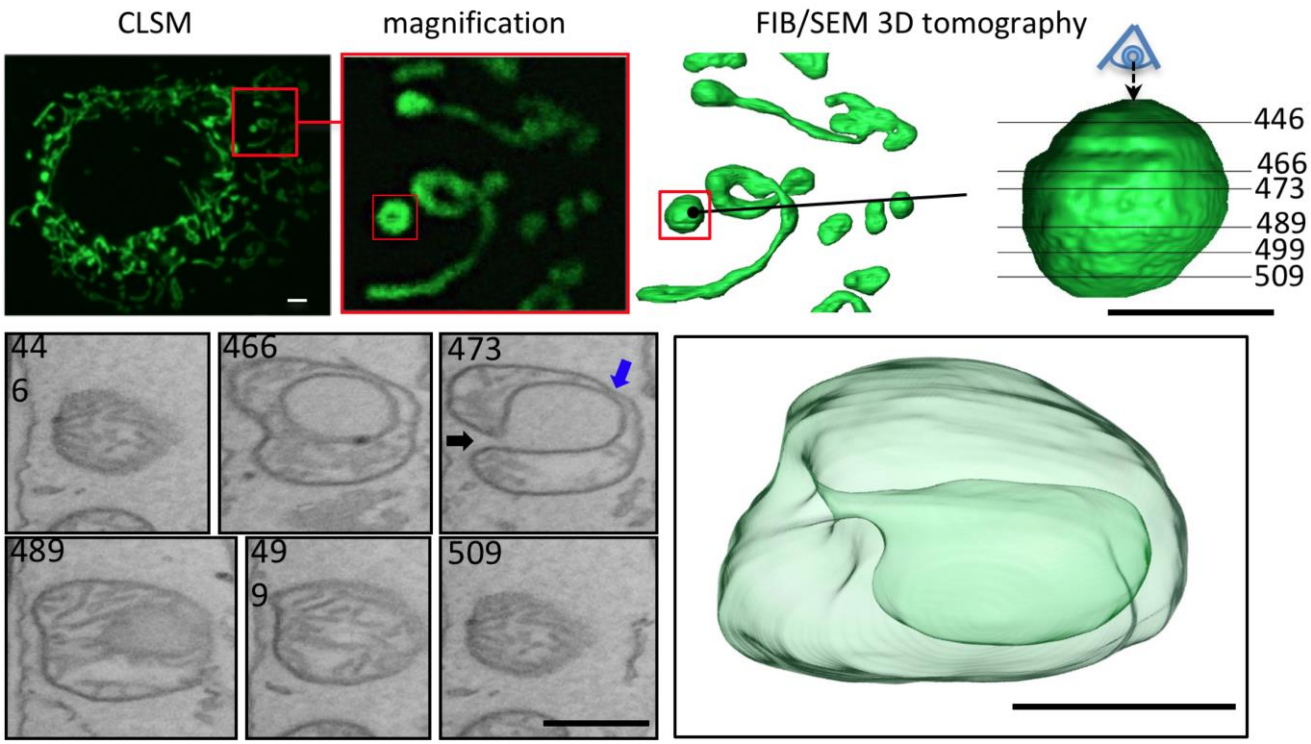

Fig. 2 Correlative observation of CCCP administrated ring-shape mitochondria between light microscope and FIB-SEM tomography.