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Visualization of Mitochondrial Membrane Dynamics in Primary Cultured Neurons by Cryo−electron Tomography*

Dear Editor,

Mitochondria acts as a cellular organelle that produces ATP and buffers Ca^{2+} , and plays an important role in neuronal growth, survival and function[1]. Loss of mitochondria will make the ATP supply insufficient, resulting in synaptic transmission dysfunction[2]. Further, presynaptic mitochondrial dysfunctions are often associated with severe neurological diseases[3]. Abnormal mitochondrial function can also lead to neurodegenerative diseases because of the degeneration of synapse transmission, such as Alzheimer's disease[4] and Parkinson's disease[5]. In neurons, mitochondria have a wide range of size, shape and number, which can be constantly changed through fission and fusion events to form a highly dynamic and organized network[6] and also a way of mitochondrial quality control.

In recent years, cryo-electron microscopy/tomography (Cryo-EM/ET) has become a powerful tool for high resolution structural information in a native state. Studies in yeast with cryo-ET have shown that endoplasmic reticulum (ER) determines mitochondrial fission sites[7]. In HeLa cells, cryo-ET studies found that small mitochondria budded out of mother mitochondria *in situ*[8]. However, intact mammalian cells are too thick for cryo-ET to obtain good resolution and image contrast. Neurons which can extend to form a network are thin enough to be observed under cryo-ET and are also good model with abundant mitochondria for studying mitochondrial membrane dynamics. Here we cultured primary hippocampal neurons and observed mitochondrial membrane dynamics by cryo-ET and 3D reconstruction. We found that mitochondrial central and peripheral fission both occurred at ER contact sites and some mitochondria with "matrix-free" region. This may ensure the original mitochondria are always in good quality and normal function at all times.

Mitochondrial fission begins with the constriction of outer mitochondrial membrane (OMM), and then inner mitochondrial membrane (IMM) split apart. Central fission usually produces two daughter mitochondria of similar size, mass and shape, whereas peripheral fission may aim to remove the damaged mitochondrial components transported to lysosome for degradation. We observed the spontaneous mitochondrial fission in cultured neurons. In central fission, we can clearly see ER wrap round the mitochondrial fission sites at different stages (Figure 1). At early stage, the OMM has begun to constrict, but the IMM was still connected (Figure 1a−d and PIBB 20210302_Movie_S1. avi in **Supplementary**). In another central fission at a later stage, the IMM and matrix have been completely divided into two separate parts (Figure 1e−h and 20210302_Movie_S2. avi in **Supplementary**). As long as the OMM is separated, the mother mitochondria will thoroughly split into two independent daughter mitochondria. Because these mitochondrial fissions occurred in the neuronal axons, it was not surprising to see microtubules accompanying mitochondria. However, microtubules, which act as axon extension driver, were not involved in this mitochondrial fission event. Instead, the long-distance movements of mitochondria from soma to presynaptic and back are mainly carried out through the microtubules network[9].

In the peripheral fission, mitochondria bud selectively damaged IMM, OMM and matrix proteins to form a small mitochondrion, also called mitochondria-derived compartments (MDCs) [10]. MDCs required fission machinery for release and served as a way of mitochondrial quality control. Previous live-cell imaging and conventional EM studies reported that ER and actin have no

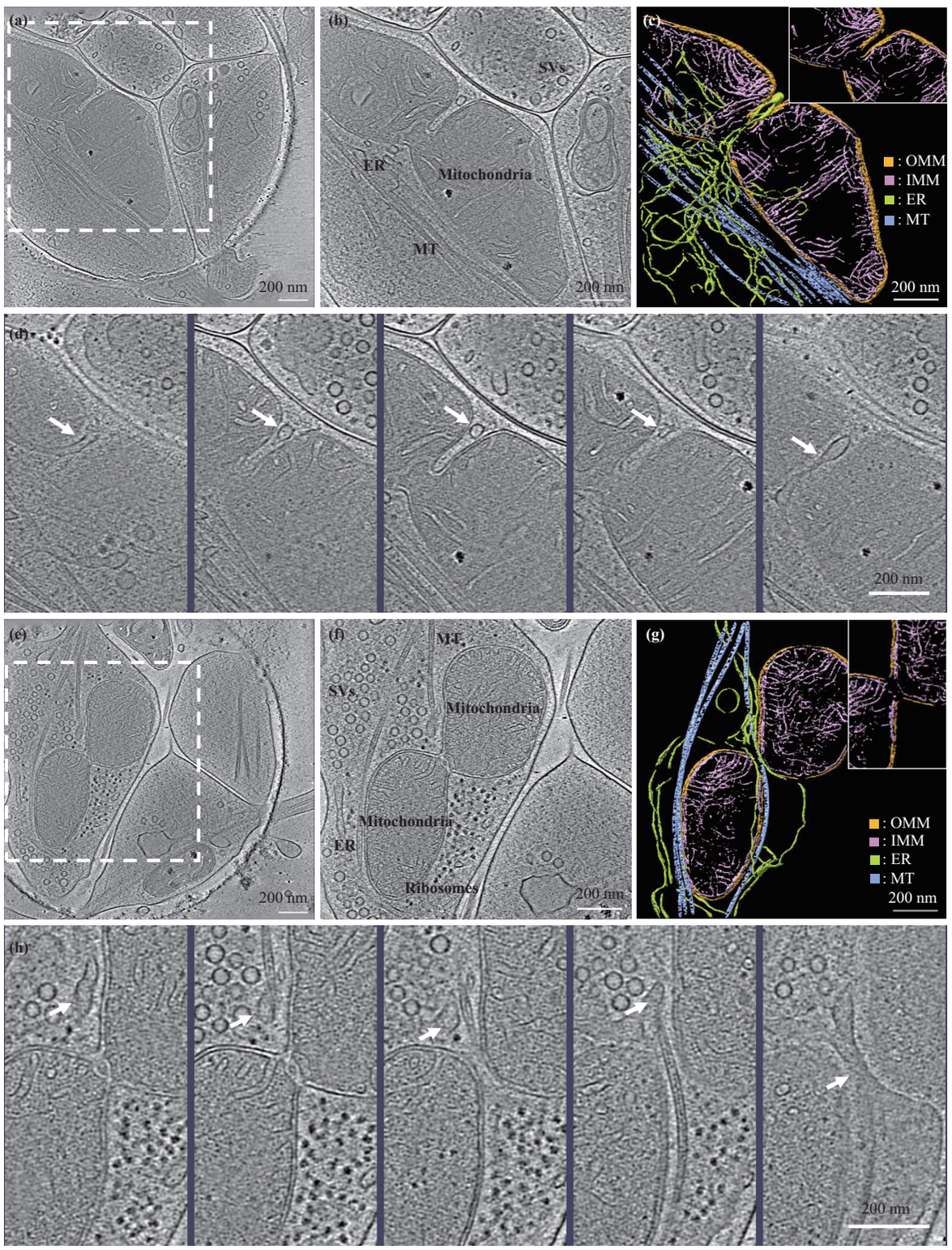

Fig. 1　High−resolution structure of two typical mitochondrial central fission occurred at ER contact sites in primary neurons

(a) A tomographic slice showing a mitochondrial central fission at early stage. (b) Enlarged image of white boxed area in (a). (c) 3D segmentation of the tomogram in (b) with color key. (d) Slices of mitochondrial division tomogram at different *z* values, showing that IMM was still connected. White arrows indicate the ER wrap around the mitochondria. (e−h) Another mitochondrial central fission events at a later stage. Note that the IMM was separated in this tomogram. Synaptic vesicles (SVs), microtubules (MT), endoplasmic reticulum (ER).

contribution to the initial constriction of mitochondria peripheral fission[11]. However, in frozen-hydrated neuron close to native state, our results showed that ER also wrapped around the fission site in peripheral fission mitochondria. ER can be seen above and below the fission site, and the MDCs thrown out also contained OMM, IMM and matrix, with no significant difference from the mother mitochondria morphologically (Figure 2a and 20210302_Movie_S3.avi in **Supplementary**). Mitochondrial central and peripheral fission both occurs at ER contact sites.

In addition, we also observed some distinct morphological characteristics of mitochondrial membrane dynamics. The OMM extended outward and separated from IMM, leaving a "matrix-free" region with no inner membranes and matrix components. At the junction of varicosity/axon, it often appeared that as cell membrane space contracted, the OMM became longer and narrower, and there was no IMM and matrix in the narrowed and extended part of OMM (Figure 2c and 20210302_Movie_S5.avi in **Supplementary**). The dramatic mitochondrial membrane morphology may be determined by narrow space of axons. Moreover, such "matrix-free" mitochondrial membrane morphology can also be seen in traditional axonal varicosity (Figure 2b and 20210302_Movie_S4.avi in **Supplementary**). Morphologically, the OMM showed a constricted shape similar to the initial action of mitochondrial fission. It was worth noting that ER neighbored mitochondria, but it didn't wrap around the mitochondria. It was a mystery whether ER was involved in the morphology of IMM and OMM separation because it was so abundant and widespread in cells. Therefore, OMM and IMM, the most important structure of mitochondria, were not always spatially together. These two mitochondrial membranes may separate, forming a "matrix-free" area. Whether the appearance of this mitochondrial morphology is related to specific biological process is

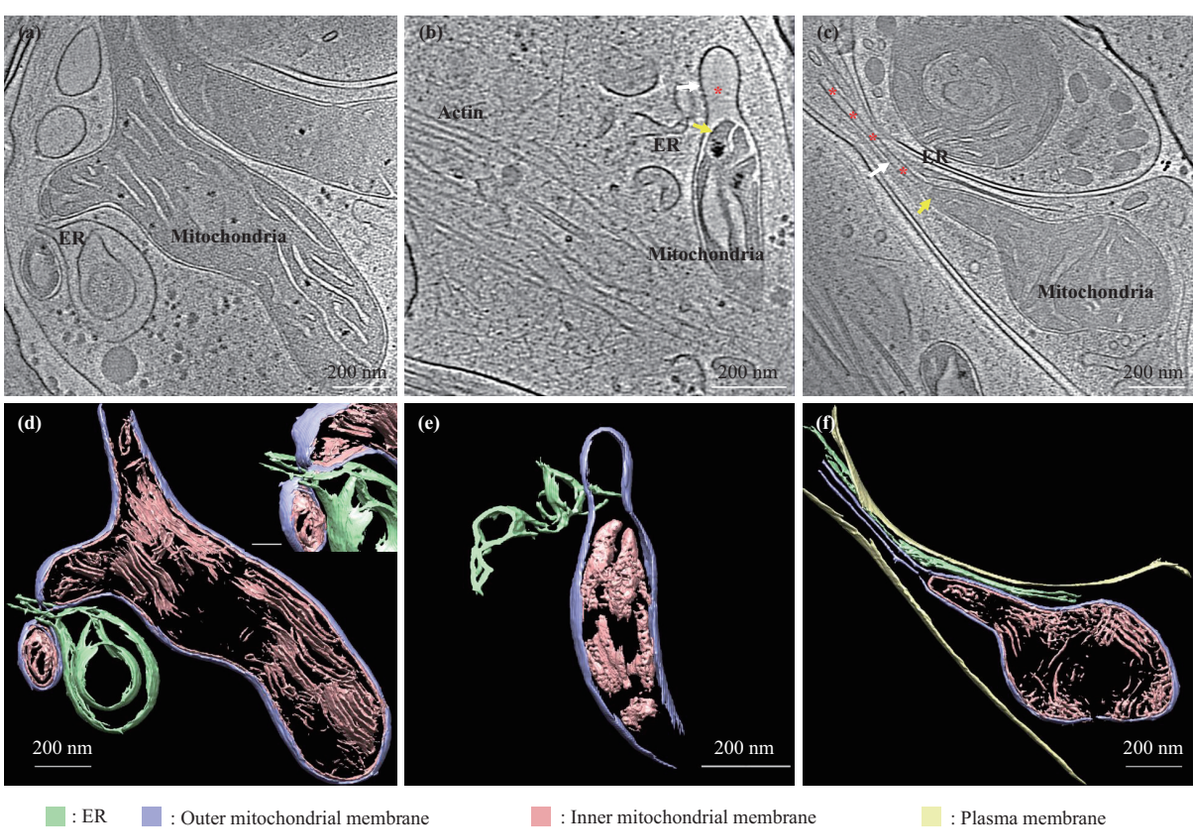

Fig. 2　Distinct morphological features of mitochondrial membrane dynamics

(a) Slice through a tomogram showing a mitochondrion of peripheral fission, with ER wrap around the fission site. 3D segmentation of the tomogram shown in (d). (b) Slice through a tomogram showing the OMM separated from IMM, remaining a "matrix-free" area. White arrow indicates the extended OMM, yellow arrow represents the IMM, and red asterisk displays the "matrix-free" area. 3D segmentation of the tomogram shown in (e). (c) Slice through a tomogram showing a mitochondrion with "matrix free" region at the junction of varicosity/axon. The meaning of each symbol is consistent with that in (b). 3D segmentation of the tomogram shown in (f).

still unknown.

In summary, from cultured primary hippocampal neurons in native state by cryo-ET and 3D reconstruction, we observed that both the central and peripheral mitochondrial fission have some spatially contact with ER, which is wrapped round the fission sites. In particular, some mitochondria would form a "matrix-free" region, and the cause and mechanism of this phenomenon need to be studied in the future.

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Supplementary PIBB_20210302_Doc_S1. pdf and PIBB_20210302_Movie_S1−S5. avi are available online (http://www.pibb.ac.cn or http://www.cnki.net).

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