Electron tomography and serial block face scanning electron microscopy complement each other in 3D morphological characterization of cell organelles

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Keywords: 3D EM, electron tomography, serial block face scanning electron microscopy

The current 3D electron microscopy (EM) techniques enable morphological analysis of cell organelles and even cells and tissues at high resolution. The new developments in light microscopy (LM) technology allow resolution below diffraction limit i.e., 200 nm but these techniques can never resolve cellular context at the resolution of EM. The advantage of LM that cannot be obtained by EM is observation of living cells; thereby, both LM and EM techniques are needed to obtain an all-embracing understanding about the functions and morphological alteration of many cellular events.

The current best methods providing 3D information of cell structures at EM level are electron tomography (ET) and serial block face scanning electron microscopy (SBF-SEM) or focused ion beam SEM. In addition of using two different types of EM, these methods also differ from each other in respect of achievable volume and resolution. The ultrastructures of biological specimens are best preserved by using high pressure freezing (HPF) after which the specimens are substituted at low temperatures (FS). HPF/FS can be combined with ET; however the requirement of high contrast (extra staining) for SBF-SEM, hampers the use of rapid freezing in SBF-SEM specimen preparation.

To fully appreciate the complex structure of the endoplasmic reticulum (ER), the 3D EM techniques have to be applied [1, 2]. Volumes reconstructed by ET are moderate, typically up to few tens of cubic microns, whereas by using SBF-SEM it is easy to collect data from volumes up to several thousand cubic microns. Large and multiform organelle such as the ER is a perfect target for SBF-SEM because the resolution achieved by it is sufficient to define the different structures of ER i.e., tubules, sheets and fenestrations that are not resolved by conventional 3D LM techniques (Figure 1). On the other hand, the resolving power of SBF-SEM is not adequate to resolve smaller structures like membrane connections, intermediate filaments and ribosomes which are more suitable targets to be studied by ET (Figure 2A). Since ET reveals the 3D structures it enables quantification of membrane bound ribosomes in different structure types of the ER (Figure 1C). We have previously shown that the morphology of the ER is not altered by conventional chemical fixation compared to HPF/FS. Chemical fixation allows cytochemical staining to be applied which greatly facilitates the segmentation and modelling of complex ER structures.

The localization of target protein using pre-embedding immunolabelling is challenging technique for 3D EM methods due to the use of electron dense metal ions: In ET the electron dense material creates artefacts in the reconstruction covering up the structures and in SBF-SEM it conveys the contrast from the biological material. However, if the amount of a specific, silver enhanced gold is low, it is possible to inspect the localization by ET. Also, by optimizing the amount of label it might be possible to extent the pre-emmedded immunoEM technique to SBF-SEM.

The studies of autophagosomes using ET revealed small connections between the phagophore/autophagosome membrane and the closely located ER membrane, especially with the ER located inside the autophagosome [3] (Figure 2B). These tiny connections are not resolvable by SBF-SEM; however SBF-SEM is definitive tool to study the occurrence of these structures in different stages of autophagosome formation in large number of cells. In similar manner SBF-SEM can be used to quantify lipid droplets in several cells, and analyze the amount of ER membranes in vicinity of lipid droplets in 3D. ET can be used to characterize the membrane connections, which are under the resolution limit of SBF-SEM; however, by applying only ET the possibility for statistical analysis is limited.

Since SBF-SEM technique provides analysis of large volumes, it is possible to use it as a screening tool to find the phenotype in large number of specimens. After discovering the target...
phonotype, it can be further characterized by ET which provides higher resolution. Tiny objects are difficult to catch in thin EM sections and are not resolved by LM or SBF-SEM. Thus, the most powerful tool to study cellular organelles is compounded with combination of different imaging methods [4].

References

[2] M Puhka et al., Progressive sheet- to- tubule transformation is a general mechanism for endoplasmic reticulum partitioning in mammalian cells, accepted for publication in Mol. Biol. Cell
[4] The authors gratefully acknowledge funding from the Academy of Finland (project no. 131650) and Biocenter Finland. The project concerning the morphology of lipid droplets is done in collaboration with Dr. Yuki Ohsaki and Prof. Elina Ikonen. Mervi Lindman is thanked for excellent technical assistance with ET and SBF-SEM specimens.

Figure 1. By using SBF-SEM whole HuH-7 cells can be imaged with e.g. pixel size of 5x5x25 nm (A). Modeled ER in HuH-7 cell shows rough ER depicted in yellow and smooth ER in green, volume 4x2x1 µm (B).

Figure 2. Tomographic slices reveal smooth/rough ER near the ER exit site in HuH-7 cell (A) and connections between phagophore/autophagosome membrane and the ER in NRK-52E cell (arrow heads, B). Ribosomal density on different types of the ER can be quantified from modeled tomogram (C, ER depicted in yellow, ribosomes in red).