Contacts between the Endoplasmic Reticulum and Other Membranes in Neurons

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Abstract

The close apposition between Endoplasmic Reticulum (ER) and subcellular organelles serve key functions in cell physiology. Such functions include synthesis of membrane and membrane lipids, Ca^{2+} storage, and metabolic processing. In this research project, we aimed to systematically analyze the structure and distribution of ER together with other inter organelles as well as their interaction points, especially focusing on mitochondria-associated membrane (MAM) contacts. Images generated from serial-block face scanning electron microscopy (SBEM) and electron tomography (ET) with the aid of automatic segmentation were used to reconstruct organelles three-dimensionally. Samples obtained from Purkinje and granule cells within a neuron and spine were analyzed. ER-mitochondrial contacts were abundant and extended into all cell compartments even to the most distant regions, with ER showing a strong tendency to form a network embracing mitochondria. Here, our study supplies anatomical and structural data on ER and other organelle networks, providing an unique reference for understanding the function and significance of ER and inter-organelle interactions.

Introduction

The endoplasmic reticulum is a network of interconnected tubules and cisternae, which forms a wide network continuous with the outer nuclear membrane. The specialized subdomains of ER are ribosome-rich areas (rough ER) and ribosome-free areas (smooth ER). Focusing on the subcellular ER network in neurons, we evaluated two types of cells- Purkinje and Granule. Purkinje cell, one of the largest neurons in human brain, is a class of GABAergic neurons located in the cerebellum and are characterized by its large number of dendrites. The inhibitory functions of Purkinje cell regulate motor activity and learning. In comparison, granule cells are one of the smallest and abundant neurons in the brain. Granule cells form numerous synaptic connections with the Purkinje cell by exerting excitatory effects through parallel fibers.

Methods

Serial Block-face scanning Electron Microscopy (SBEM) SBEM is a method to obtain high resolution 3D images from a sample. SBEM consists of an ultramicrotome positioned inside a vacuum chamber of scanning electron microscope (SEM). To obtain the images, samples are first fixed and stained, then embedded in an epoxy resin. With a diamond knife cutting a thin section from the face of the block, surfaces are imaged by detection of electrons. After a section is imaged, the block is elevated back to focal plane and the process is repeated throughout the entire sample. The obtained images are stacked for segmentation and quantification.

Electron Tomography (ET) ET is a tomography technique used to generate detailed 3D images of the target sample. An electron beam is passed at incremental degrees of rotation around the center of a sample, resulting in a tilt series to generate a model in a process similar to that of SBEM.

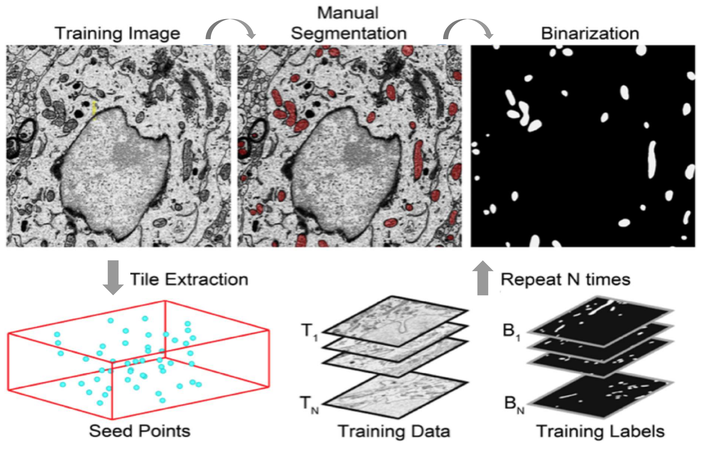

Fig. 1. Automatic Segmentation of Organelles in EM Image Stacks All images obtained from SBEM are first converted and appended to an 8-bit MRC stack using IMOD. The sequential images are then aligned in the XY plane. By matching the histograms of the image stack to the first image, the consistency of the stack is maintained (Coltucetal.,2006). After selecting an organelle of interest, a set of organelle-specific images are generated to train a CHM pixel classifier. Such training tiles are extracted from a set of seed points throughout the image stack. When all training images are collected, CHM pixel classifier is trained through numerous bottom-up and top-down steps. The collected data are then combined into a single classifier adjusted to natural resolution. Then, a probability map is generated from previously obtained test set and goes through binarization. The binarized outputs are converted to MRC format and the contours are meshed together to generate a 3D model.

Results

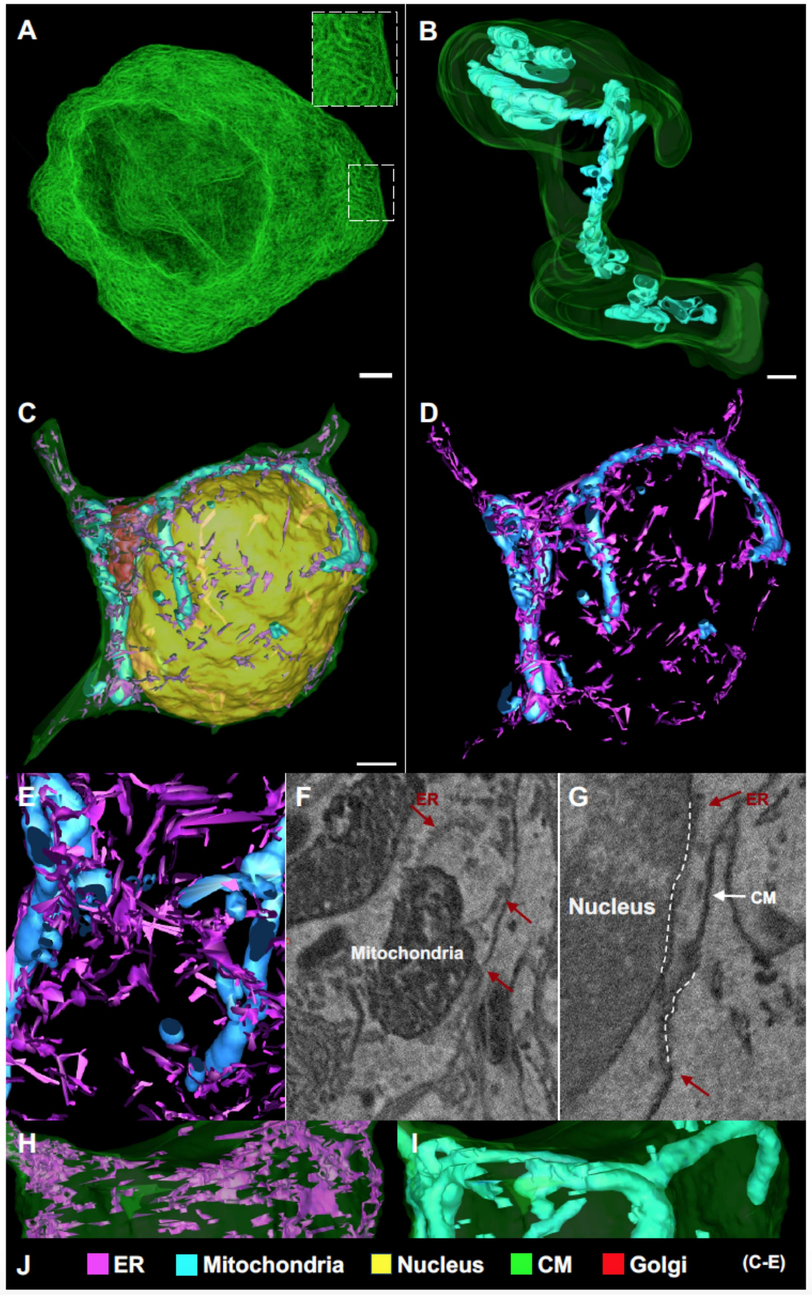

Fig. 2. Three-dimensional reconstruction of cell organelles and ER contact points from image stack of a neuronal cell body. (*A*) Complete model of ER network from Purkinje cell. (*B*) Three-dimensional model of the spine showing ER network from electron tomography image stacks. (*C*) All membranous organelles were reconstructed in a granule cell, color coded as shown in (*H*). (*D* and *E*) A portion of the ER and mitochondria from image (*C*). Note the contacts between the organelles and ER's tendency to form a network around mitochondria. (*F* and *G*) Single EM image showing a cross-section of the granule cell, demonstrating ER contacts with mitochondria in the plane of the image (*F*) and with nucleus and membrane(white dotted line, *G*). (*H* and *I*) Sections of 3D model of another granule cell sample. Structural similarity between the network of ER and mitochondria is displayed. (*J*) Color code for images (*C-I*). (Scale bars: 500 nm in *A*,*C*,*D*; 400 nm in B, Pixel size: 4.764nm x 4.764nm x 70nm)

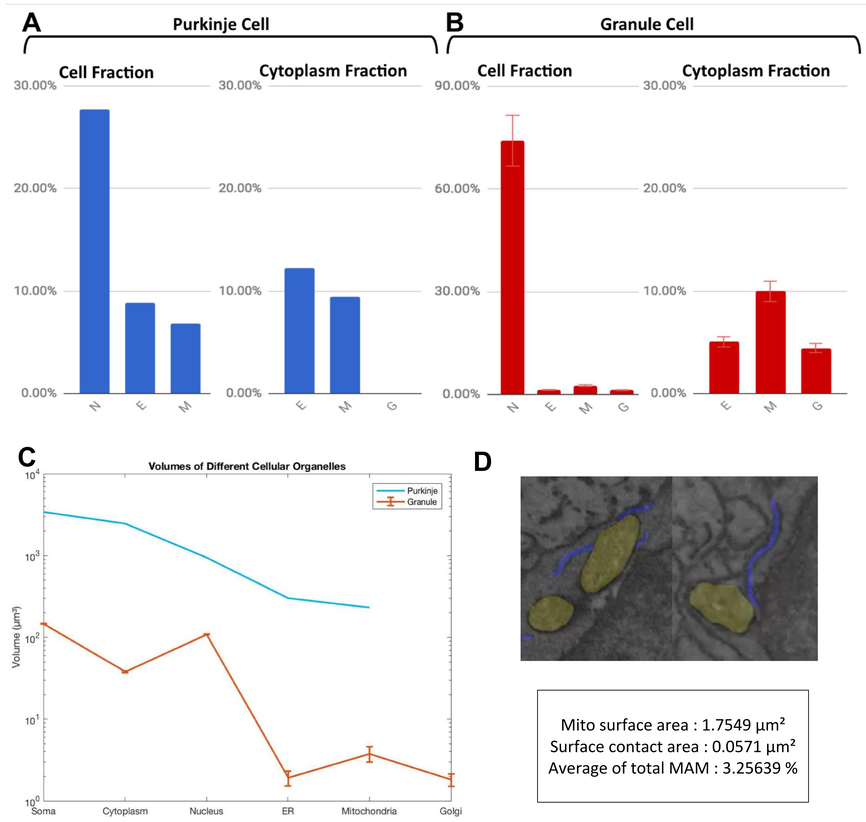

Fig. 3. Analysis of the volume and cell/soma fraction of Purkinje and granule cells (*A*) Cell and cytoplasm fraction of a single Purkinje cell. Note that the percentage for cytoplasm fraction of nucleus does not exist. Data for golgi apparatus were not collected. (*B*) Data was collected from two different granule cell samples. Note the added value for soma fraction of golgi and the different scale of cell fraction. (*C*) Volumes of each subcellular organelles of Purkinje and granule cell. All data scaled in μm. (*D*) MAM contacts analyzed

Conclusion

Through intensive analysis of SBEM and ET data, we were able to track and reconstruct subcellular organelles within neurons, especially focused on interactions between ER and mitochondria. Although a great difference in ER and mitochondrial fraction were found from the cell fraction data between Purkinje and granule cells, cytoplasmic fraction data are more feasible in comparing the organelle fraction due to the small size of the granule cell. Interestingly, the mitochondrial cytoplasmic fractions of both cell types were very similar while the Purkinje cell had much higher ER fraction than the granule cell. While both ER and mitochondria take charge of Ca^{2+} homeostasis, protein folding specifically occurs along the ER membrane, which provides further insights into the differences in cellular functions between Purkinje cells and granule cells. Collectively, our results on the substantial amount of ER presence and networks in a neuron advances our knowledge on the structural interpretation of organization and interactions of ER in neurons and its significance in cell physiology.

References

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