3D Structure Studies of the Pancreatic Beta Cell by High Resolution Electron Microscope (EM) Tomography

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The beta cells of the endocrine pancreas are the sole source of insulin in humans. Death of the beta cells, or their failure to make/release adequate amounts of insulin, results in the disease known as Diabetes. We are using large volume, dual-axis EM tomography to study the mechanisms involved in insulin biosynthesis, processing and trafficking in 3D at approximately 5 nm resolution in beta cells preserved in situ in intact pancreatic “islets of Langerhans” isolated from mice and humans. 300-400 nm-thick sections cut from high-pressure frozen, freeze-substituted and plastic-embedded beta cells/islets are imaged in a 300keV Technai F30 intermediate voltage EM (FEI), using motorized rotation holders capable of high tilt (GATAN). “Tilt series” image data are acquired automatically to a large format (4k), ultra-high sensitivity phosphor CCD camera (GATAN) as the sections are serially tilted through 0.75° or 1° increments over a range of 130-140°, about two orthogonal axes using the microscope control program SerialEM [1]. Semi-automated methods for CCD data acquisition, image montaging and image alignment by cross-correlation controlled by SerialEM help to minimize electron dose to the specimen. Tilt series images are brought into register with one another by cross-correlation, and then more accurately aligned by tracking the positions of small (5 or 10 nm) gold fiducial markers on the surface(s) of the sections using a modification of the TILTALIGN program that uses subsets of fiducials to solve more accurately for local distortions/alignments [2, 3]. Tomograms calculated by R-weighted back-projection from each set of aligned tilts are then matched to each other in 3D and combined to produce a single, high resolution 3D reconstruction [4]. 3D cellular reconstructions generated in this way and analyzed using the IMOD software package [5] have demonstrated the complexity of structural relationships among organelles of the insulin biosynthetic pathway [3, 6] and revealed novel connections between compartments that are normally spatially/functionally distinct [7]. To complement these insights into insulin trafficking in the beta cell to whole cell structure-function relationships, we have now undertaken to reconstruct an entire beta cell in 3D at ≤5nm resolution. This reconstruction, which will be approximately 100-fold larger than any produced so far at comparable resolution [Marsh et al. (2001) PNAS 98:2399-2406], will by necessity require the parallel development of complex 3D algorithms for extracting useful structural/biological information from the data in a semi-automated manner [7, 8]. The use of automated data mining tools, combined with the development of robotics instrumentation for loading/unloading specimens into the microscopes together with automated EM data collection routines [9-11], will mean that subsequent reconstructions of whole cells will be carried out exponentially faster in the future. Such a “Visible Cell” atlas will provide a unique structural framework which will serve as a major informatics/3D visualization/educational resource for the molecular cell biology, Diabetes and computational simulation communities.
References

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