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Maturation Times of Pancreatic Beta Cell Secretory Granules Estimated from Serial Block-Face Electron Microscopy

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We have used serial block-face electron microscopy (SBEM) [1] to study the maturation of secretory granules in mouse pancreatic islets of Langerhans [2], which are micro-organs 100–200 micrometers in diameter containing ~1,000 cells, mainly consisting of insulin-secreting β-cells and glucagon-secreting α-cells, whose purpose is to control blood glucose. Here, we explore the possibility of deriving information about the maturation times for β-cell secretory granules by analyzing the 3D ultrastructure of whole cells at snapshots in time. It is assumed that the β-cells are in homeostasis, and that the morphology of immature granules containing proinsulin can be readily distinguished from the morphology of mature insulin granules. For islets in homeostasis, the rate of loss of proinsulin from immature β-cell granules is equal to the rate of formation of insulin packaged in mature granules, and the rate of insulin loss from mature granules is equal to the rate of insulin secretion from the cell, which can be determined from pulsed ^{35}S-radiolabeling of sulfur-containing amino acids [3].

A complete analysis of β-cell 3D volumes reveals a subpopulation of secretory organelles that are characterized by amorphous cores and an absence of electron-lucent halos. These organelles, which comprise ~2% of the total secretory granule population, are situated in the region of the trans-Golgi network; these are identified as immature secretory granules containing the prohormone, proinsulin. Another subpopulation of secretory granules that display very narrow electron-lucent halos comprises an additional ~2% of the total granule population; these are attributed to immature granules with cores that are beginning to transform to insulin through the action of proenzyme convertases, which cleave off the C-peptide from proinsulin. The remaining ~ 96% of the secretory granules display wide electron-lucent halos, which are characteristic of a fully mature state.

To estimate the lifetimes of the newly formed proinsulin granules, it is insufficient simply to count the numbers of secretory granules in different morphological states, since it is possible that granules fuse with each other or split apart during the maturation process. Instead, the relative numbers of moles of proinsulin and insulin are estimated from the volumes of the granule cores using a previously demonstrated stereological approach [4], together with the known molecular masses of proinsulin and insulin, and knowledge of the protein density. 2D stereological measurements of the mature and immature granule core areas, as well as the cell slice areas were made on orthoslices spaced by 500 nm perpendicular to the block face throughout the 3D β-cell volumes. By analyzing the ultrastructure, it was found that the newly formed proinsulin secretory granules remain in a morphologically defined immature state for ~100 minutes, which is consistent with indirect biochemical measurements [5]. From similar measurements on the transforming immature granules, which display narrow halos, it was found that those granules remained in a morphologically defined state for an additional 2 hours. Our results suggest that some types of dynamic information about cellular behavior can be extracted from a detailed 3D morphological analysis of cells fixed at a snapshot in time, if those systems are in a state of homeostasis [6].

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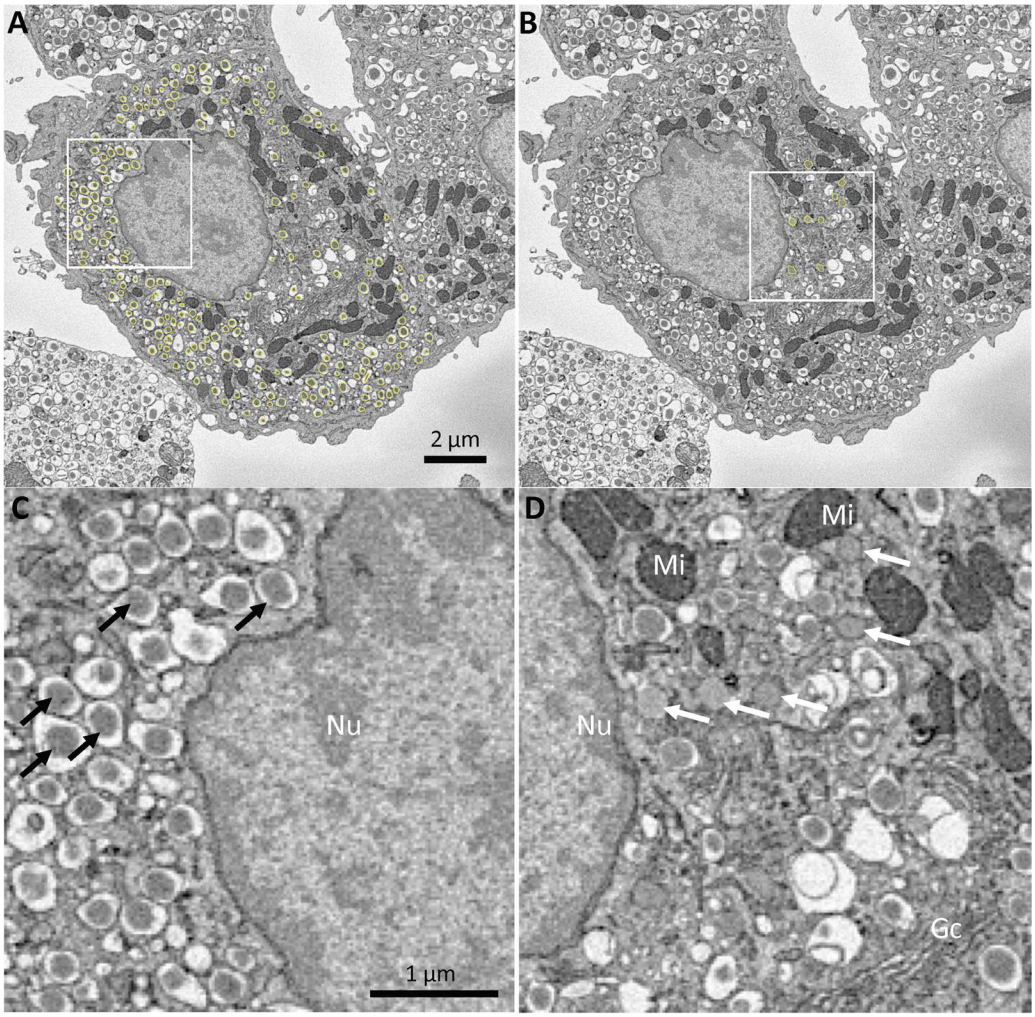

Figure 1. Orthoslice through 3D volume of pancreatic β-cell, obtained using a Carl Zeiss Microscopy SIGMA VP SEM with a Gatan 3View serial block face system. Stereological area measurements were made on: (A) cores of mature secretory granules; and (B) cores of immature secretory granules, located in the region of the trans-Golgi network; granule cores are outlined in yellow, and measurements were made using the NIH ImageJ program. Ultrastructural views at higher magnification from the square regions in A and B show the mature insulin granules (C) with dense cores and electron-lucent halos (black arrows); and (D) immature proinsulin granules with less staining and without electron-lucent halos (white arrows). Nu, nucleus; Gc, Golgi complex; Mi, mitochondria.

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