Estimation of Maturation Time for Secretory Granules in Mouse Pancreatic Islet Beta Cells by Serial Block Face Scanning Electron Microscopy

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We have used serial block-face scanning electron microscopy (SBF-SEM) [1,2] to study the maturation of secretory granules in beta cells of mouse pancreatic islets of Langerhans, which are micro-organs 100 - 200 micrometers in diameter containing ~1,000 cells, mainly consisting of insulin-secreting beta cells and glucagon-secreting alpha cells, whose purpose is to control blood glucose (Figures 1 and 2). A better quantitative understanding of the relationship between structure and function of secretory processes in beta cells is important since their malfunction results in diabetes. Here, we explore the possibility of deriving information about the maturation times for beta cell secretory granules by analyzing the 3D ultrastructure of whole cells at snapshots in time.

It is assumed that the beta cells are in homeostasis, and that the morphology of the immature granules containing proinsulin (with lower density cores and narrow halos) can be readily distinguished from mature insulin-containing granules (with dense crystalline cores and large halos). For islets in homeostasis, the rate of loss of proinsulin from immature beta 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 beta cell. The number of mature secretory granules per cell was determined from stereological measurements on random slices through the 3D data, which gave 10,000±1,500 per beta cell; and the number of immature granules per beta cell was determined by manual counting to be 107±4 (± s.e.m.). The time taken for the beta cells to release 50% of their insulin was determined to be 96±12 hours by pulse chasing the islets with [^{35}S]-cysteine and [^{35}S]-methionine and detecting [^{35}S]-insulin [3]. We could then estimate the average maturation time for immature proinsulin granules as 105±15 minutes. This result is consistent with earlier data on pulse chasing pancreatic islets with [^{3}H]-leucine and measuring [^{3}H]-proinsulin, which gave a time of ~60 minutes to convert 35% of proinsulin to insulin [4].

Our experiments demonstrate that it is possible to deduce dynamic information about secretory cells from SBF-SEM analyses of 3D cellular ultrastructure, coupled with physiological and biochemical data [5].

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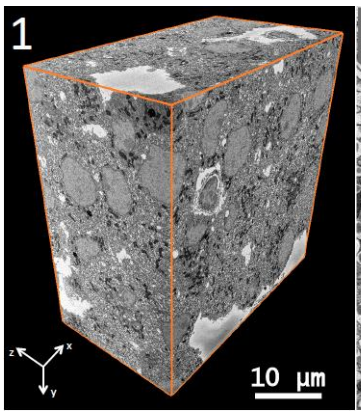

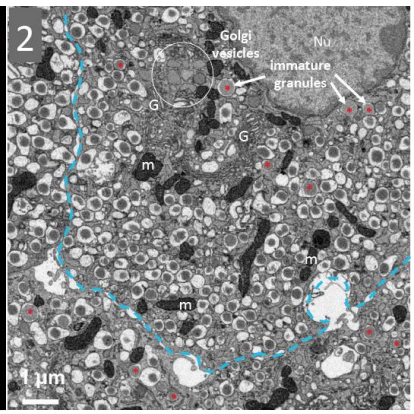

Fig. 1. Volume of mouse pancreatic islet of Langerhans that was analyzed by SBF-SEM.
Fig. 2. Plane through beta cell outlined by blue dashed curve, showing nucleus (Nu), Golgi membranes (G), and mitochondria (m); cluster of Golgi vesicles indicated by circle, and immature granules (red asterisks) characterized by thin halos; most granules are in mature form with dense crystalline cores and large halos.