


SPOTLIGHT

# Granular detail of $\beta$ cell structures for insulin secretion

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**Pancreatic  $\beta$  cells secrete insulin in response to increased glucose concentrations. Müller et al. (2021. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202010039>) use 3D FIB-SEM to study the architecture of these cells and to elucidate how glucose stimulation remodels microtubules to control insulin secretory granule exocytosis.**

The pancreatic islet  $\beta$  cell is a prototypical model for regulated protein secretion, which has been studied extensively because of its importance for diabetes in humans. Upon stimulation by increased glucose concentrations,  $\beta$  cells mobilize insulin-containing secretory vesicles to the cell surface. These “insulin secretory granules” fuse at the plasma membrane to release insulin into the circulation. Insulin then acts on the liver to inhibit glucose production and on muscle and fat cells to stimulate glucose uptake, thus returning blood glucose concentrations to a narrow physiological range. During the development of diabetes, this negative feedback system fails. In type 1 diabetes,  $\beta$  cells are destroyed by an autoimmune process; in type 2 diabetes, attenuated insulin action (“insulin resistance”) occurs together with impaired  $\beta$  cell function. In both cases, blood glucose concentrations rise above the normal range. Overall, glucose homeostasis requires a delicate balance between glucose-stimulated insulin secretion from  $\beta$  cells and insulin-stimulated effects on glucose metabolism in liver, muscle, fat, and other cell types.

Each  $\beta$  cell contains 5,000 to  $\geq 10,000$  insulin secretory granules, and acute glucose stimulation causes exocytosis of only 1–2% of this pool (1, 2). Both readily releasable and reserve pools of granules contribute to insulin secretion. Classically, the readily releasable pool has been considered

as vesicles that are docked at the plasma membrane; the reserve pool, which is larger and more important, resides deeper within the cell and relies on microtubule-based transport to reach the cell surface. Yet, other data show that newly synthesized insulin is preferentially released, and aged insulin granules are targeted for degradation in lysosomes, implying that microtubules play a more complicated role in granule trafficking (3). In the setting of insulin resistance, the flux of insulin through the secretory pathway is increased. Demands are placed upon the machinery for folding of the insulin precursor, proinsulin, for its proteolytic conversion to produce insulin, and for insulin secretory granule maturation. As well, in type 2 diabetes, lipids and other metabolites act directly on  $\beta$  cells to impair glucose-stimulated insulin secretion. What steps are affected by this critical pathophysiologic insult is not well understood, in part because basic mechanisms by which glucose stimulation remodels microtubules to promote insulin release remain undefined.

To better understand these processes, Müller et al. used focused ion beam scanning electron microscopy (FIB-SEM) to image microtubules, insulin secretory granules, and other organelles in whole primary mouse  $\beta$  cells and to study the effects of glucose stimulation on these structures (4). Together with advances in sample preparation, image

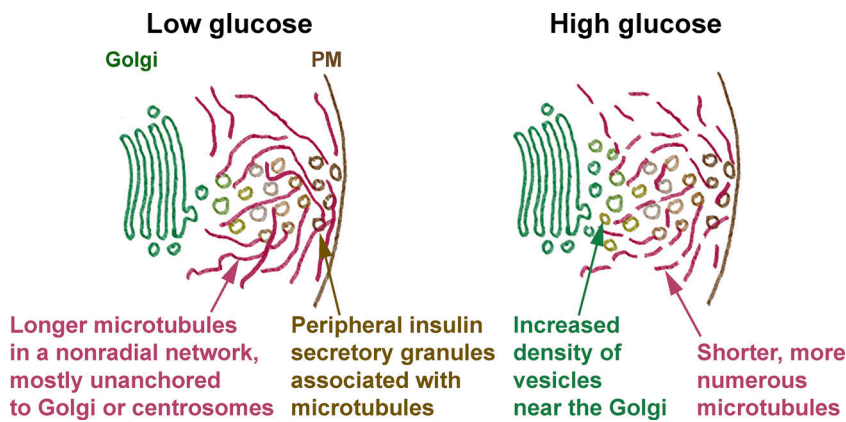
segmentation, and analysis, FIB-SEM is uniquely suited to this task. The resulting 3D images have a voxel size of  $4 \times 4 \times 4$  nm and encompass volumes of 20–30  $\mu\text{m}$  in x, y, and z dimensions. This is sufficient to image microtubules, which have an outer diameter of ~25 nm, in whole  $\beta$  cells, which are 10–20  $\mu\text{m}$  in diameter. Moreover, the imaging was performed on intact islets, rather than on dissociated cells, which may better preserve insulin secretory granule dynamics. The images captured seven  $\beta$  cells in all, three in a low-glucose (unstimulated) condition and four in the glucose-stimulated state. Finally, the images were quantified in a way that controlled for the overall geometry of the cells.

The data show that the  $\beta$  cell microtubule network is nonradial, dense, tortuous, and mostly not connected to either centrioles or the Golgi complex, so that microtubules appear to be freely positioned in the cytosol (Fig. 1). This is similar to other differentiated cells (5) but in contrast to previous data suggesting that in  $\beta$  cells most microtubules originate at the Golgi (6). The microtubule cytoskeleton negatively regulates insulin granule exocytosis in unstimulated cells (7). Yet, the FIB-SEM data suggest that the effect of glucose is not simply to disinhibit this effect. Glucose stimulated an approximately threefold increase in the number of microtubules and an approximately threefold decrease in the

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**Figure 1. Regulation of  $\beta$  cell architecture by glucose.** In low-glucose conditions (left panel), microtubules form a dense, nonradial network. The tubules are mostly not anchored to the Golgi complex or to centrosomes but are associated with insulin secretory granules near the plasma membrane (PM). After stimulation with high-glucose concentrations (right panel), the microtubules are shorter and more numerous, and an increased density of vesicles near the Golgi was observed.

average length of each microtubule, so that total tubulin polymer density remained unchanged (4). The images also show that microtubule ends and insulin secretory granules are enriched near the plasma membrane and indicate an important role for microtubules in positioning the granules for exocytosis. Intriguingly, glucose stimulation did not cause any marked change in the association of secretory granules with microtubules, suggesting that it may act by other mechanisms to increase microtubule-based transport (3). The data also demonstrate an increase in the number of secretory granules near the Golgi in glucose-stimulated cells, raising the possibility that glucose may promote budding of nascent secretory vesicles at the trans-Golgi network. If so, it is not yet clear whether this is a direct effect or if it is secondary to increased flux through the secretory pathway.

The observation that glucose stimulation results in shorter, more numerous microtubules, without changing tubulin polymer density, suggests a possible role for severing enzymes such as katanin, spastin, or fidgetin (8). Whether and how such enzymes

may be stimulated by glucose is not known. Glucose stimulates insulin secretion by increasing the ATP/ADP ratio, in part due to local ATP generation by pyruvate kinase, as well as by oxidative phosphorylation (9). Although these microtubule-severing enzymes are AAA ATPases, it is not clear that ATP availability acts physiologically to regulate their activities. Other data show that in  $\beta$  cells, glucose-responsive kinases phosphorylate tau, causing it to dissociate from microtubule plus ends to destabilize microtubules and to promote remodeling (10). Could such kinases regulate severing enzymes as well?

The technological achievements of Müller et al. are impressive, and the work will serve as a model for the analysis of cellular architecture in other cell types. In  $\beta$  cells, the results may be extended by using FIB-SEM to study effects of various genetic manipulations, by using EM-visible tags, and by examining diabetes models. It may be informative to image granules of different ages or to use drugs to manipulate microtubules or membrane lipids, or that act on  $\beta$  cells to enhance insulin secretion (3). In this study,

Müller and colleagues used a 1-h high-glucose stimulation, but it may be interesting to test other time points to determine the effects of more acute versus chronic glucose exposure. Although FIB-SEM can image only fixed, static cells, the work will complement other studies using super-resolution imaging and live cell microscopy. Aside from these future directions, it is worthwhile to pause and celebrate the present work, which is the first to reconstruct the entire 3D architecture of the microtubule network in a primary mammalian cell during interphase. The movies in the supplement are gorgeous. The work bodes well for the future of FIB-SEM and will stimulate new directions to understand both diabetes physiology and regulated protein secretion.

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