

# Heterogeneity in Pancreatic $\beta$ -Cell Population

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**All pancreatic  $\beta$ -cells are identified by specific morphological characteristics. Similarity in microscopic features is not necessarily associated with identity in functional properties. In vitro studies on isolated rat  $\beta$ -cells have indicated intercellular differences in the threshold for glucose-induced shifts in metabolic redox state. The cellular heterogeneity in glucose sensitivity results in a dose-dependent recruitment of glucose-exposed  $\beta$ -cells into biosynthetic and secretory activities. The molecular basis of this diversity is not known. Indirect evidence supports the concept that the in situ pancreatic  $\beta$ -cell population is also composed of functionally diverse subpopulations. The heterogeneity in glucose responsiveness is expected to create subpopulations of  $\beta$ -cells with either constant, fluctuating, or occasional glucose-dependent functions; whether any subpopulation is preferentially responsive to other regulatory factors and/or committed to other activities is unknown. Morphological markers may help identify  $\beta$ -cell subpopulations in situ and quantify their size in conditions known to affect total  $\beta$ -cell mass or function. The concept of a functionally heterogeneous  $\beta$ -cell population influences views on the role of pancreatic  $\beta$ -cells in health and disease. *Diabetes* 41:777-81, 1992**

**T**he pancreatic  $\beta$ -cell has been defined on the basis of its microscopic characteristics after fixation. The  $\beta$ -cell type can be identified by aldehyde-fuchsin staining of its cystein-rich vesicles, by ultrastructural recognition of its typical secretory

granules, by immunolabeling of its hormonal products, or by in situ hybridization of cell-specific mRNA. The number of  $\beta$ -cells per pancreas is unknown but probably varies with the species and its stage of development. The functional characterization of the cells has been derived primarily from in vitro experiments on perfused pancreas or isolated islet preparations from adult rodents. The results were usually interpreted with the implicit assumption that the pancreatic  $\beta$ -cell population is composed of functionally identical cells. This assumption has likely emerged from the notion that all  $\beta$ -cells appear morphologically similar. However, analogy for morphological features does not imply identity in functional properties. Observations in purified islet cell preparations led to the proposal that the endocrine pancreas is composed of functionally diverse  $\beta$ -cell subpopulations (1). Experiments with single  $\beta$ -cells indicated the existence of intercellular differences in glucose responsiveness (1-5). This perspective reviews the experimental support for the concept of functional heterogeneity within the pancreatic  $\beta$ -cell population. The model will be discussed for its possible implications on the role of pancreatic  $\beta$ -cells in health and disease.

## INTERCELLULAR DIFFERENCES IN GLUCOSE RESPONSIVENESS IN VITRO

Direct evidence for a functional heterogeneity among  $\beta$ -cells came from autoradiographs of isolated rat islet  $\beta$ -cells in which newly synthesized proteins had been labeled at different glucose concentrations (2). In none of the conditions was a homogeneous cell population detected. At glucose levels as low as 1 mM, 5% of the  $\beta$ -cells were in active biosynthesis, whereas the remaining 95% were inactive. A rise in glucose dose dependently increased the number of biosynthetically active cells (Fig. 1). At  $\geq 10$  mM glucose, 70% of the  $\beta$ -cells participated in the biosynthetic response, but 30% remained inactive. A dose-dependent recruitment of active  $\beta$ -cells was also observed in intact islet tissue (2),

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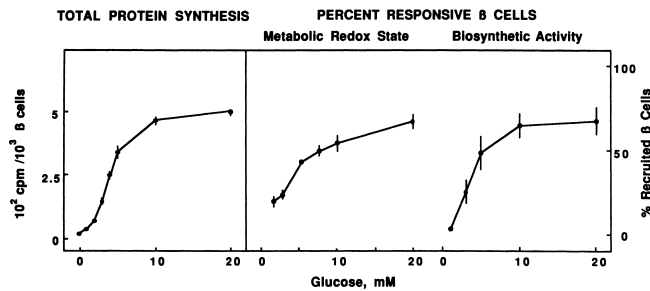


FIG. 1. Parallellism between glucose-induced protein synthesis by the rat pancreatic  $\beta$ -cell population (A) and the dose-dependent recruitment of  $\beta$ -cells into metabolic (B) and biosynthetic (C) activity. Data taken from refs. 2 and 4. ©Proc Natl Acad Sci USA and Am Soc Clin Invest.

generating comparable rates of insulin synthesis as in single-cell preparations (2). The dose-response curves obtained from total  $\beta$ -cell populations thus represent an integration of heterogeneous responses from functionally differing  $\beta$ -cells rather than a summation of progressively increasing activities in identical cells (Fig. 1).

The presence of glucose-unresponsive  $\beta$ -cells was also noticed during analysis of metabolic signals in glucose-exposed  $\beta$ -cells (3,4). With cellular NAD(P)H autofluorescence intensity as parameter for the metabolic state of the cells, it was demonstrated that individual  $\beta$ -cells differ markedly in their sensitivity for glucose (3,4; Fig. 1). Cells undergoing a redox shift at low glucose exhibited a low threshold for glucose-induced protein synthesis (4). A similar parallellism was found for cells responding only to intermediate or high glucose concentrations. Subpopulations of  $\beta$ -cells can be isolated on the basis of their metabolic responsiveness to a particular glucose concentration (3,4). Cells with lower threshold for glucose-induced metabolic changes were also characterized by a lower threshold for glucose-elicited insulin release (5). These experiments on purified rat  $\beta$ -cells indicate that in vitro-studied  $\beta$ -cell populations are functionally heterogeneous because of intercellular differences in glucose sensitivity (Fig. 2). Fluctuations in glucose concentration vary the number of  $\beta$ -cells that participate in the processes of insulin synthesis and release (Fig. 3).

In vitro studies on islet tissue seem to evaluate only those subpopulations of  $\beta$ -cells that respond to the prevailing glucose levels. Differences in the number of responsive  $\beta$ -cells can therefore be considered as one cause for interlaboratory and interassay differences in the rates of insulin release or biosynthesis. At any glucose concentration, the glucose-responsive subpopulation differs from the unresponsive one by its ability to produce and release newly formed hormone. Although the released amounts of newly synthesized and of preformed insulin will only depend on the activities of the recruited cells, the amounts contained in the islet tissue correspond to hormone reserves in both responsive and unresponsive subpopulations (Fig. 3). Coexistence of responsive and unresponsive  $\beta$ -cells can thus lead to higher ratios of newly formed over preformed insulin in

## HETEROGENEITY IN GLUCOSE SENSITIVITY

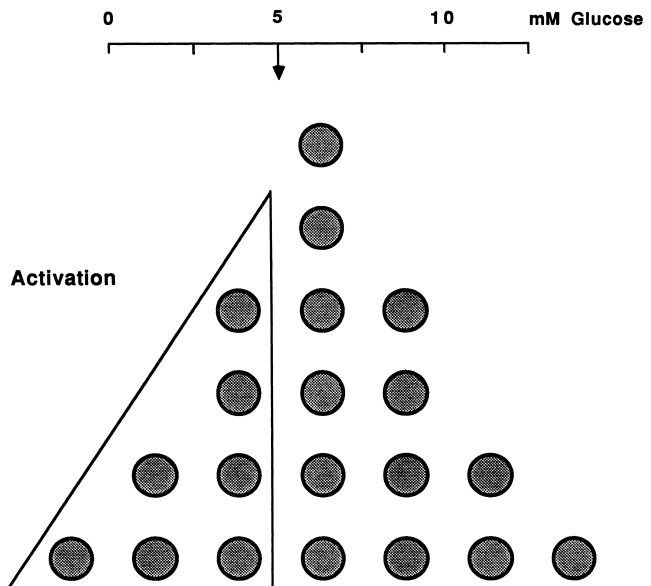


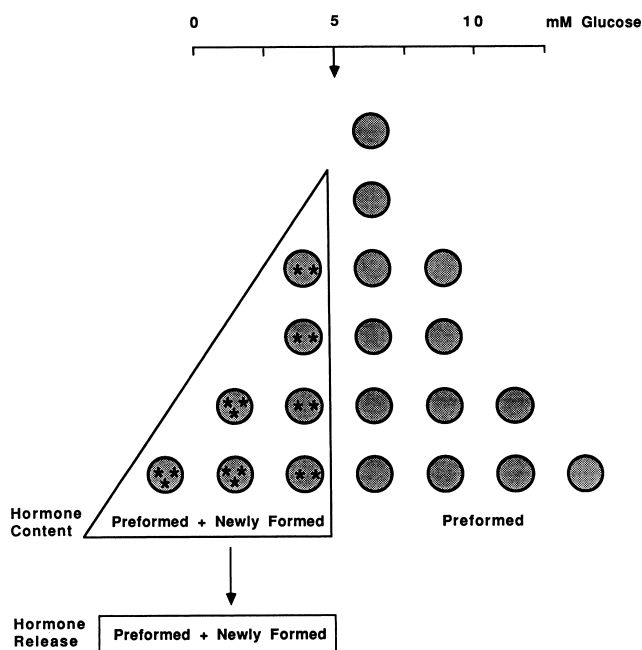
FIG. 2. Model of pancreatic  $\beta$ -cell population composed of subpopulations with different sensitivities to glucose. Most cells can be activated by glucose, but the percentage of activated cells varies with glucose concentration. The figure illustrates the activated subpopulation at 5 mM glucose, comprising the cells already active in the absence of glucose plus those recruited between 0 and 5 mM glucose.

the medium than in the tissue (Fig. 3). Such higher ratios have indeed been measured in experiments with intact islets, suggesting preferential release of newly synthesized insulin (6–11). However, these measurements are not necessarily indicative for an alternative route of secretion along which newly formed granules shortcut the regulated release of preformed hormone within the same cell. According to our model, detection of preferential release of newly synthesized insulin may as well result from unequal contribution of functionally different cells to the secretory response (Fig. 3).

Studies in unpurified islet cell preparations support the concept of heterogeneity in glucose-inducible  $\beta$ -cell functions. With a hemolytic plaque assay to detect secretory responses of individual cells, Salomon and Meda (12) noticed that not all  $\beta$ -cells responded to a maximal glucose stimulus. For most cells, this state of glucose responsiveness or unresponsiveness remained for several hours (13). When  $\beta$ -cells were exposed to lower glucose concentrations, the percentage of actively secreting cells decreased dose dependently (14). By combining the hemolytic plaque assay with autoradiography, Bosco et al. (15) found that most actively secreting  $\beta$ -cells were also activated in their biosynthetic function and in their release of newly synthesized hormone. Possible influences from other cell types or from structurally coupled cells (1,16) cannot be neglected in these unpurified cell preparations.

The molecular basis for the intercellular differences in glucose sensitivity has not been clarified. Measurements

## HETEROGENEITY IN GLUCOSE RESPONSIVENESS



**FIG. 3. Model of glucose responsiveness in the pancreatic  $\beta$ -cell population. At 5 mM glucose, the subpopulation of activated cells is stimulated for hormone synthesis (\*) and release. Newly formed insulin is only secreted by the activated subpopulation. The ratio of newly formed to preformed insulin in the medium can thus be higher than that in the total  $\beta$ -cell population because the cellular hormone content also comprises the preformed reserves of the nonactivated subpopulation.**

in isolated islets indicated the existence of intercellular differences in glucose-induced electrical activities (17). With dispersed islet cells, Misher et al. (18) noticed different thresholds in the glucose-dependent gating of ATP-sensitive  $K^+$  channels (18), which is considered an important step in the activation of insulin release (19). This heterogeneity may result from differences in cellular rates of glucose utilization and oxidation, because  $\beta$ -cell subpopulations with a lower threshold for glucose-induced functions metabolized glucose at a higher rate (20). The site that is responsible for this metabolic variability has not been identified. Potential key regulators, e.g., glucose transporter (21), glucokinase (22,23), and other glucose-induced signals (24,25), can be considered. In a recent study, Jetton and Magnuson (26) described a heterogeneous expression of glucokinase in the pancreatic  $\beta$ -cell population, suggesting that variations in this enzyme may confer a graded glucose responsiveness to different  $\beta$ -cells.

#### FUNCTIONAL DIVERSITY IN SITU

It is technically difficult to compare individual  $\beta$ -cell functions in intact organs. Indirect support can nevertheless be found in favor of a functional diversity in situ. The variety of  $\beta$ -cell locations is suggestive for heterogeneity in functions. Occurrence as isolated single cells or as units in aggregates of variable size and composition may

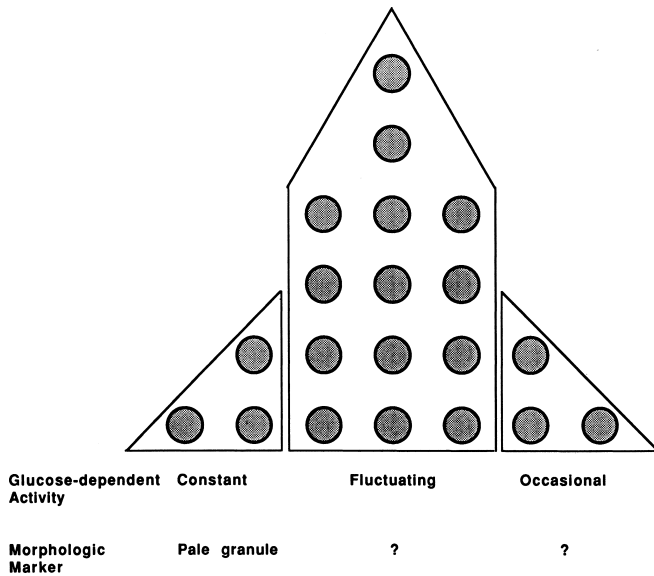
be related to different states of activity (1,27). A localization in the dorsal part of the pancreas appears associated with stronger secretory responses than one in the ventral part (28). At the level of the islets, peripherally located  $\beta$ -cells can be functionally differentiated from centrally located ones (29–31), whereas proximity to neural endings should predispose to an altered environmental regulation.

Insofar as  $\beta$ -cell functions have been compared in situ at the cellular level, signs of diversity have been noticed. In the adult rat, only a small fraction of  $\beta$ -cells is involved in mitosis (32), probably having little participation in the insulin secretory response. On the other hand, most  $\beta$ -cells appeared biosynthetically active in pancreases that were perfused with  $^3H$ -leucine and 10 mM glucose (unpublished observations); at lower glucose levels, the percentage of activated  $\beta$ -cells varied dose dependently, as previously described for purified single-cell preparations and in intact islets (2). The glucose-induced recruitment of activated  $\beta$ -cells was recognized as a major determinant of the steep dose-response curves that characterize  $\beta$ -cell functions in the physiological range (2). The pancreatic capacity to discharge variable quantities of insulin for relatively minor fluctuations of glucose may thus highly depend on a dose-dependent recruitment of  $\beta$ -cell subpopulations with differing sensitivities to glucose.

Morphological observations support the view that  $\beta$ -cells in situ differ in their individual sensitivity for glucose. It has been demonstrated recently that  $\beta$ -cells with sensitivity to low glucose levels contain a higher percentage of pale secretory granules than cells that are only responsive to high glucose levels (4). The pale granule subtype is considered an immature form because of its content in unprocessed hormone (33). It is not only recognized in isolated  $\beta$ -cells but also in  $\beta$ -cells of the intact pancreas (34). Instead of assuming that these proinsulin-rich granules are homogeneously distributed over all pancreatic  $\beta$ -cells, we propose a heterogeneous distribution with the highest density in cells that are sensitive to low glucose levels (Fig. 4). A non-uniform distribution of hormonal products is consistent with immunocytochemical observations. Orci et al. (33) identified  $\beta$ -cells that are rich in proinsulin and poor in insulin. Furthermore, the staining intensity for insulin exhibits marked intercellular differences. After prolonged glucose stimulation, centrally located  $\beta$ -cells are much more degranulated than peripheral cells (35), possibly as a result of intercellular differences in glucose responsiveness and/or in the initial state of granulation. Heterogeneity in cellular insulin content has also been observed in vitro (1). It was noticed that  $\beta$ -cells that are structurally coupled to somatostatin-containing  $\delta$ -cells are more densely granulated than those attached to  $\beta$ -cells (1).

#### POSSIBLE PHYSIOLOGICAL IMPACT OF HETEROGENEITY IN GLUCOSE RESPONSIVENESS

Differences in glucose sensitivity result in a dose-dependent recruitment of  $\beta$ -cells into glucose-inducible functions. The subpopulation of  $\beta$ -cells that are already activated at basal glucose levels is expected to maintain



**FIG. 4. Possible physiological relevance of cellular heterogeneity in glucose sensitivity and responsiveness. The pancreatic  $\beta$ -cell population is expected to consist of subpopulations with constant, fluctuating, or occasional glucose-dependent activities. Morphological markers may help identifying these subpopulations in situ. A high ratio of pale to dark granules could qualify as marker for the subpopulation with constant activity.**

a continuous activity, filling its hormone reserves and meeting hormone needs during and between meals (Fig. 4). Fluctuations in glycemia, such as during meals, are thought to cause on-and-off responses in a subpopulation of  $\beta$ -cells that are activated by physiological glucose concentrations above basal (Fig. 4). Supraphysiological glucose levels may amplify the secretory response of these two subpopulations without increasing further their rates of hormone production (2,36); alternatively, they may liberate hormone reserves from a subpopulation with occasional glucose-dependent activities, possibly regulated by other secretagogues. The physiological role of the glucose-unresponsive subpopulation is unclear; it is unknown whether these cells are at rest, responsive to other regulators or intercellular signals, or committed to other functions such as mitosis.

There is no need to postulate that  $\beta$ -cells remain firmly associated, throughout their life, to one of these subpopulations. Transitions from one functional state to another may occur as a result of cell aging, prior activities, changing environmental factors, or intercellular signals. The number of  $\beta$ -cells in each subgroup may vary accordingly. Morphological markers that can distinguish functionally differing  $\beta$ -cells would allow assessment of the concept of functional heterogeneity in vivo; they would be particularly useful for determining the relative sizes of each subpopulation (Fig. 4). Pale granules may serve such marker function because they have been associated with the subpopulation of  $\beta$ -cells that are sensitive to low or intermediate glucose concentration. In our view, a high proportion of these granules may be characteristic for  $\beta$ -cells that are already activated at basal glucose levels (Fig. 4). When counted over the

entire  $\beta$ -cell population, an increased proportion of pale-over-dark mature granules may indicate a rise in the percentage of  $\beta$ -cells that produce and release insulin in the basal state. Such an increased ratio has been noticed in laboratory animals under cortisone treatment (37) or under influence of a growth hormone-producing tumor (38), in obese (39) and pregnant (40) rats; it was also found in insulinoma cells (41). Each of these conditions is characterized by increased basal insulin levels, which is in support of our hypothesis. They are also associated with growth in the total  $\beta$ -cell mass, suggesting that growth factors influence the size of a particular  $\beta$ -cell subpopulation. It is, in this context, interesting to note that pale secretory granules were also much more abundant in fetal and neonatal than in adult rabbit  $\beta$ -cells (34).

**HETEROGENEITY IN  $\beta$ -CELL PATHOLOGY**

A decline in mass and/or function of the pancreatic  $\beta$ -cell population can cause diabetes. The disease is clinically detected after most cells are destroyed or functionally deficient. It is conceivable that not all  $\beta$ -cells are equally susceptible to these pathological processes, or that not all are simultaneously affected. Lack of information on the sequence of events at the level of the pancreatic  $\beta$ -cells keeps this view largely speculative. However, the available observations make it attractive for further testing.

Pancreases of insulin-dependent diabetic patients contain <10% of the normal  $\beta$ -cell mass (42); in several cases, regions have been identified with multiple groups of surviving insulin-containing  $\beta$ -cells that appear unaffected by the autoimmune reactivity (43). That not all  $\beta$ -cells simultaneously undergo the same pathological process is also evident from the frequent remissions that are noticed after clinical onset of the disease; in many cases, endogenous insulin production continues for years after diagnosis of insulin-dependent diabetes. It is unclear which cellular properties or which environmental conditions can protect human  $\beta$ -cells from the diabetogenic process or make them more vulnerable. Experiments with rat cells have indicated that intercellular differences in oxidative state can explain differences in the sensitivity of  $\beta$ -cells to cytotoxic agents and in their defense reactions (44,45). Surviving  $\beta$ -cells may not permanently resist an autoimmune or any other cytotoxic reactivity. However, their presence at a late stage of the pathological process raises the possibility that they are remnants of larger subpopulations that resisted successive cytotoxic attacks.

In non-insulin-dependent diabetes, the pancreatic  $\beta$ -cell mass may be decreased but certainly not depleted of insulin-containing cells and insulin reserves (46). According to in vivo studies, the islet  $\beta$ -cells fail to exhibit appropriate secretory responses to glucose (47). In glucose-exposed rat  $\beta$ -cells, normal dose-response curves are generated as a result of intercellular differences in metabolic responsiveness (2). Loss of the normal responsiveness to physiological glucose concentrations may thus indicate that the heterogeneity in glucose recognition has been altered, at least in the subpopulation of cells that are responsive to fluctuating glucose levels (Fig. 4). It is of course not surprising that

loss of heterogeneity in the pancreatic  $\beta$ -cell population results in a pathological condition if the functional diversity among  $\beta$ -cells indeed plays the physiological role that we would like to postulate. A molecular analysis of the cellular heterogeneity is needed to further assess the validity of this model.

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