Impact of islet architecture on β-cell heterogeneity, plasticity and function

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Abstract | Although β-cell heterogeneity was discovered more than 50 years ago, the underlying principles have been explored only during the past decade. Islet-cell heterogeneity arises during pancreatic development and might reflect the existence of distinct populations of progenitor cells and the developmental pathways of endocrine cells. Heterogeneity can also be acquired in the postnatal period owing to β-cell plasticity or changes in islet architecture. Furthermore, β-cell neogenesis, replication and dedifferentiation represent alternative sources of β-cell heterogeneity. In addition to a physiological role, β-cell heterogeneity influences the development of diabetes mellitus and its response to treatment. Identifying phenotypic and functional markers to discriminate distinct β-cell subpopulations and the mechanisms underpinning their regulation is warranted to advance current knowledge of β-cell function and to design novel regenerative strategies that target subpopulations of β cells. In this context, the Wnt/planar cell polarity (PCP) effector molecule Flattop can distinguish two unique β-cell subpopulations with specific transcriptional signatures, functional properties and differential responses to environmental stimuli. *In vivo* targeting of these β-cell subpopulations might, therefore, represent an alternative strategy for the future treatment of diabetes mellitus.

All β cells can sense glucose concentration and respond to metabolic demand by both producing and secreting insulin to regulate blood glucose levels; however, remarkable phenotypic and functional β-cell heterogeneity exists between islets or even within the same islet. The first evidence of phenotypic β-cell heterogeneity was provided in 1960 when differences in nuclear size were observed with regard to the specific regional location of β cells in the islet of Langerhans[1] (TABLE 1). In 1968, differences were reported in β-cell membrane potentials between central and peripheral β cells located in the same islet[2], proving the concomitant existence of functional β-cell heterogeneity (TABLE 2). The findings of these early studies have since been confirmed and expanded using advanced technologies (TABLES 1–3).

Variations are apparent in the size, granularity and insulin levels of β cells[3–5]. Freeze–fracture of isolated rat pancreatic islets followed by electron microscopy enabled the internal organization of β-cell membranes to be studied and permitted visualization of the heterogeneous surface structure of β cells and their components, such as gap junctions[6]. In the 1980s, autofluorescence-activated cell sorting was first used to analyse and purify β cells according to their responsiveness to glucose, based on the specific variations in reduction–oxidation state and subsequent changes in the endogenous fluorescence of adenine dinucleotides[7]. Such techniques provided evidence of intercellular differences in glucose sensitivity of individual β cells[7], which in turn lead to cellular diversity in biosynthetic and secretory activities[8]. Radioactive labelling of nucleotides and amino acids aided discovery and/or confirmation of the differences in β-cell proliferation[9], metabolic coupling[10] and glucose-dependent stimulation of insulin synthesis and secretion[11]. Glucose infusion techniques were used to explore β-cell functionality *in vivo*[12]. Static and dynamic incubation of pancreatic islets and/or β cells with insulin secretagogues revealed how β cells are heterogeneous in terms of insulin release[12,13]. The use of laser confocal scanning microscopy, together with 3D imaging software, enabled the role of the islet cytoarchitecture in β-cell heterogeneity to be explored in both animals and humans[14]. Importantly, human β cells also display marked variation in terms of insulin production and secretion[15,16].

The development of β-cell-specific transgenic reporter mice further widened understanding of β-cell heterogeneity by allowing clear discrimination of distinct subpopulations of β cells based not only on insulin expression but also on the expression of non-β-cell hormones (glucagon, somatostatin and pancreatic polypeptide)[17]. In the past 10 years, sensitive cellular and molecular biological techniques and single-cell analysis

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Key points

- Phenotypic and functional β-cell heterogeneity arise during β-cell development *in utero*; however, such heterogeneity is also acquired during the postnatal period owing to cell maturation, ageing and plasticity
- Islet composition, cell polarity, cell-to-cell adhesion and cell-to-matrix adhesion all contribute to β-cell heterogeneity, as does the interaction of endocrine cells with vessels, nerves and the microenvironment
- Plasticity allows islet cells to adapt to physiological changes, such as pregnancy; this process might also be triggered by pathological insults, such as prolonged exposure to hyperglycaemia
- Heterogeneous subpopulations of β cells can be recognized by the expression of specific markers, including insulin, PDX1, E-Cadherin, Flattop, PSA-NCAM, DKK3, ST8SIA1 and CD9
- Flattop is a novel Wnt/PCP effector that can discriminate between proliferative and mature β cells
- Targeting specific β-cell subpopulations could advance efforts to induce therapeutic regeneration of endogenous islet cells among individuals with diabetes mellitus

have led to the discovery of biomarkers of β-cell heterogeneity (TABLE 3). A deep understanding of inter-islet and intra-islet β-cell heterogeneity is of great biological importance. Furthermore, such knowledge holds clinical relevance for diseases such as diabetes mellitus, which are characterized by β-cell loss or dysfunction and consequent deregulation of glucose metabolism.

This Review aims to provide a comprehensive overview of the different levels of β-cell heterogeneity, with particular emphasis on the role of islet architecture in defining phenotypic and functional plasticity among β cells. Furthermore, findings by both our research group and other investigators highlight the mechanisms that underpin this heterogeneity and provide novel tools to effectively discriminate between β-cell subpopulations owing to their distinct functional properties. This Review offers the basis for future investigations that aim to exploit β-cell heterogeneity and plasticity for diagnostic and therapeutic purposes in the context of regenerative therapies for diabetes mellitus.

Establishment of β-cell heterogeneity

Although it is clear that different subpopulations of β cells exist, questions remain regarding when such heterogeneity is established; how it is regulated; the physiological importance of cellular diversity; and its role in the development of diabetes mellitus.

Heterogeneity arises during development of the pancreas. In addition, this process is subject to postnatal regulatory mechanisms owing to the ability of β cells to modify their gene and protein expression profiles in response to specific endogenous and exogenous cues (a phenomenon known as β-cell plasticity). Heterogeneity stems from the fact that not all β cells are exposed to the same environment during the various stages of their lifespan. During fetal development, the pancreas originates from the ventral and dorsal buds, each of which receives distinct signals from the surrounding tissues[18]. Differences in ontogeny between ventrally and dorsally derived islets (and β cells) are responsible for differences in innervation, blood supply and endocrine composition of the islets, all of which influence islet-cell composition and heterogeneity in adulthood[19].

Furthermore, β cells can originate from different progenitor cells — even within the same pancreatic region or islet — and so display some degree of intrinsic heterogeneity. A study using mouse chimaeras (experimental animals characterized by a mixture of cells from two different embryos with different genotypes) detected the formation of heterogeneous islets that comprised cells originating from the progenitors of both embryos, indicating the polyclonal origin of pancreatic islets[20].

Impact of 3D islet architecture

Islet compaction is a pivotal process in determining β-cell function. Freshly isolated β cells maintained in suspension culture release markedly less insulin than dispersed β cells cultured for 4 days as a monolayer attached to a substratum[21] or β cells that are aggregated or re-aggregated in islets[22]. This finding is indicative of functional cooperation between islet cells[23] and stresses the importance of a multidimensional structure in the regulation of β-cell function[21]. The compaction of both β cells and non-β cells within the islet occurs non-randomly, such that each islet has a specific 3D architecture and cellular composition. The islet niche comprises different cell types (endocrine, neuronal, endothelial, mesenchymal and blood cells) that are interconnected by extracellular matrix (ECM), cell-to-cell adhesion molecules, cell-to-matrix adhesion molecules and gap junctions, all of which actively support endocrine cell processes[24]. Islet composition, cell polarity, homotypic cell-to-cell contacts, heterotypic cell-to-cell contacts and interactions with the surrounding tissues and environmental cues provide regional differences in glucose responsiveness and insulin secretion, indicating that the 3D islet architecture has a pivotal role in β-cell heterogeneity[24].

Islet composition and cell-to-cell contacts. In rodents, β cells are the most abundant endocrine cell type within the pancreas. They are mainly located in the core of the islets, surrounded by α, δ, ε and pancreatic polypeptide cells, which secrete glucagon, somatostatin, ghrelin and pancreatic polypeptide, respectively. By contrast, islet organization in the human pancreas remains a matter of debate. Some studies show that all endocrine cell types in human islets are distributed throughout this structure and that β cells are intermingled with the non-β cells[14,16]. Conversely, other studies have reported that human islet structure is similar to the mantle-core organization observed in rodents[25,26]. Accordingly, islets might be considered a composite of singular mantle–core units, whereby small clusters of β cells are surrounded by a mantle of non-β cells[25]. Despite divergence among studies, the findings of several independent investigations agree that, when compared with rodents, human islet composition is much more heterogeneous[14] and dependent on both age and size[24–26]. The relative contribution to rodent islets is 80% for β cells, 14% for α cells and 6% for δ cells; by contrast,

Table 1 | **Evidence of phenotypic β-cell heterogeneity**

Heterogeneous feature	Consequence or observation	Species	Reference
Nuclear size	Nuclear DNA content varies among β cells with the occurrence of diploid, tetraploid and octaploid cells	Human	Ehrie & Swartz[126]
	The smallest nuclei are located centrally in large islets	Rat	Hellerstrom *et al.*[1]
Granularity	Subpopulations of β cells with distinct granularity display different levels of insulin expression	Mouse	Katsuta *et al.*[82]
	β cells structurally coupled to somatostatin-containing cells are densely granulated	Rat	Pipeleers[3]
Gap junctions	• Gap junctions are most frequent among β cells and are located at the periphery of the islet • Such heterogeneity is preserved during stimulation of insulin secretion	Rat	Meda *et al.*[38]
Homotypic and heterotypic cell contacts	Heterotypic contacts are most abundant at the cell periphery, whereas homotypic β-cell contacts are abundant in the islet core	Rat	Meda *et al.*[38]
Mitochondria	• Mitochondria within the β cell are metabolically heterogeneous • BAD (implicated in mitochondrial recruitment of glucokinase) influences the level of heterogeneity	Mouse	Wikstrom *et al.*[122]
Polarity	β cells positioned around the capillaries all exhibit the same orientation of granular accumulation	Rat	Bonner-Weir[55]
Hormone content	β cells heterogeneously co-express multiple genes that encode hormones	Mouse	Katsuta *et al.*[82]; Guz *et al.*[89]

BAD, Bcl2-associated agonist of cell death.

the human β-cell population varies from 28% to 75%, the α-cell population from 10% to 65% and the δ-cell population from 1.2% to 22.0%[14].

Islet composition is determined during embryonic development[26] and is crucial for normal adult islet cell coupling and functioning. Rat islets derived from the ventral bud (which gives rise to the inferior part of the head and the uncinate process of the pancreas) contain more pancreatic polypeptide cells and fewer α and β cells than islets formed from the dorsal bud (which gives rise to the superior part of the head, as well as the body and tail of the pancreas)[27–29]. Similar differences were found in human pancreata[30]. Both the location of the islet within the pancreas and the location of the β cell within an islet determine functional heterogeneity of the cells in adulthood[26,28]. Variation in islet composition between the pancreas head and tail could promote regional differences in insulin secretion among rodents[28]. Indeed, one study found that islets located in the pancreas tail of rats have higher rates of stimulated insulin biosynthesis and secretion than islets located in the pancreas head[28]. By contrast, another study failed to identify regional differences in glucose-stimulated insulin secretion among isolated human islets[31].

Cellular composition is crucial to establish between-islet heterogeneity but it also contributes to within-islet heterogeneity by determining the amount of homotypic and heterotypic cell-to-cell contacts that occur. The cellular organization of islets in rodents clearly favours homotypic cell-to-cell interactions in the islet core (where the β cells reside), whereas heterotypic cell-to-cell contacts are more frequently observed in the periphery because the α, δ, ε and pancreatic polypeptide cells are intermingled at this site[32].

Most information on the influence of cell-to-cell contacts on insulin secretion comes from reaggregation studies, whereby dissociated β cells are allowed to recompact with either other β cells or non-β cells. The establishment of either homotypic or heterotypic contacts was associated with large differences in insulin secretion[33]. In particular, homologous contacts between β cells potentiated maximal insulin secretion in both animals and humans[15,33]. Contacts between α cells and β cells also increase insulin secretion compared to single β cells, whereas decreased insulin levels were observed in aggregates of β cells and δ cells[15,23]. Furthermore, in rodents, centrally located β cells became degranulated faster than peripheral β cells upon acute glucose challenge[12], indicating that homotypic cell contacts could be responsible for the acute insulin response under conditions of metabolic need[15,23].

Although the effect of locally released hormones and peptides on insulin secretion might partially explain the observed functional differences among homotypic and heterotypic cell-to-cell contacts[34], a role for cell adhesion molecules (CAMs) and/or cell junctions has also been suggested owing to the capacity of these cell surface components to maintain islet integrity and function. In particular, β cells communicate via gap junctions, which are believed to regulate the coordinated functioning of these cells via synchronization of their activities[35]. The finding that human β cells have decreased numbers of homotypic cell-to-cell contacts[15,26] and lack synchronization of calcium oscillations[16] suggested a crucial role for gap junctions in mediating functional homotypic β-cell contacts in rodents[16,36,37]. Nevertheless, this hypothesis contrasts with the finding in rats that gap junctions are most abundant among peripheral β cells where heterotypic cell-to-cell contacts are frequent[38]. Given that β cells and non-β cells are also coupled by gap junctions and further interlinked by CAMs, the expression and abundance of specific cell-surface molecules might determine the formation of specific homotypic or heterotypic islet-cell aggregates. For example,

Table 2 | **Evidence of functional β-cell heterogeneity**

Heterogeneous feature	Consequence or observation	Species	Reference
Electrical activity and/or membrane potential	Cells with large membrane potentials are located near the islet surface	Mouse	Dean & Matthews[2]
	Different thresholds for glucose-induced electrical activity observed among β cells	Mouse	Beigelman *et al.*[127]
	Some, but not all, β cells are electrically coupled by low-conductance junctional channels	Rat and mouse	Meda *et al.*[36,128]
Glucose responsiveness	Differences observed in reduction–oxidation state and adenine dinucleotide fluorescence during autofluorescence-activated cell sorting	Rat	Van De Winkel & Pipeleers[7]
	Different glucose utilization and oxidation patterns attributed to intercellular differences in the levels of glucose phosphorylation	Rat	Heimberg *et al.*[129]
	Different thresholds in glucose-mediated gating of ATP-sensitive potassium channels, electrical activity and calcium rise were observed in dispersed β cells	Mouse and rat	Beigelman *et al.*[127]; Herchuelz *et al.*[130]; Jonkers & Henquin[131]
	• At low glucose concentrations (1 mM), only 5% of isolated β cells were responsive • At high glucose concentrations (>10 mM), 70% of the β cells were responsive	Rat	Pipeleers[98]
	At 1 mM, 5 mM and 20 mM concentrations of glucose, 18%, 43% and 70% of the β cells shifted from basal to increased reduction–oxidation state, respectively	Rat	Kiekens *et al.*[132]
	Heterogeneous glucose-stimulated expression of the insulin gene exhibited by β cells	Rat	de Vargas *et al.*[133]
	Centrally located β cells become degranulated faster than peripheral β cells after prolonged exposure to glucose	Rat	Stefan *et al.*[12]
Insulin secretion	Large islets secrete more insulin than small islets	Rat	Kitahara & Adelman[83]
	Dissociated β cells display substantial heterogeneity in insulin release	Rat	Salomon & Meda[13]
	• The β cells non-responsive to glucose secrete quantitatively lower amounts of insulin than glucose-responsive β cells, despite the presence of comparable insulin stores	Rat	Van Schravendijk *et al.*[8]
	• A small minority of the β-cell population is responsible for the majority of insulin secretion • Pairs of β cells secrete more insulin than single β cells	Human	Wojtusciszyn *et al.*[15]
	• 75% of β cells showed constant secretory patterns under conditions of repeated glucose stimulation • 25% of β cells shifted from a secretory to a non-secretory state	Rat	Giordano *et al.*[134]
	Intraislet and interislet variation in insulin secretion observed among isolated β cells	Rat	Hiriart & Ramirez-Medeles[135]
	• Subpopulations of β cells *in vivo* respond differently to physiological and pharmacological levels of insulin secretagogues upon stimulation with either glucose or glibenclamide (an insulin secretagogue) • Degranulation of β cells occurs at different rates, depending both on their location within the islets and the location of the islets within the pancreas	Rat	Stefan *et al.*[12]
Metabolic coupling	Nucleotide exchange was observed in 50% of the recipient β cells contacting donor β cells in monolayer culture	Rat	Meda *et al.*[10]
Dye coupling	Dye exchange among β cells is restricted to a limited number of neighbouring β cells owing to heterogeneity in the extent of coupling between β cells located close together	Rat	Meda *et al.*[42]
Calcium oscillations	The rise in intracellular calcium levels observed in response to glucose is heterogeneous within the islets	Mouse	Jonkers & Henquin[131]
	The pattern of intracellular calcium increase and sensitivity to different secretagogues were highly heterogeneous among β cells	Rat	Herchuelz *et al.*[130]
Biosynthetic activity	Large and highly glucose-responsive β cells exhibit high rates of insulin synthesis after incubation in the presence of glucose	Rat	Kiekens *et al.*[132]
	Labelling β cells with radioactive leucine uncovered a heterogeneous response in insulin synthesis	Rat	Schuit *et al.*[11]; Olsson & Carlsson[50]
Proliferative activity	Only 3% of β cells in the adult islet exhibit proliferative capacity	Mouse	Hellerström & Swenne[9]
Adaptation to physiological and pathological stressors	Compared with β cells in the gastric and duodenal regions of the pancreas, β cells in the splenic region increased proliferation and glucose-induced insulin release in response to a high-fat diet	Mouse	Ellenbroek *et al.*[27]
	Most, but not all, β cells within an islet increased proliferation during pregnancy	Rat	Parsons *et al.*[80]

Table 2 (cont.) | **Evidence of functional β-cell heterogeneity**

Heterogeneous feature	Consequence or observation	Species	Reference
Response to treatment	Tolbutamide induced heterogeneous increase in intracellular calcium levels in β cells	Rat	Herchuelz *et al.*[130]
	The β-cell subpopulation markedly degranulated by glibenclamide treatment displayed more biosynthetic activity than the β-cell subpopulation that was less sensitive to glibenclamide treatment	Rat	Ling *et al.*[123]
	Subpopulations of β cells respond differently to glibenclamide	Rat	Stefan *et al.*[12]
Susceptibility to damage	The subpopulation of glucose-unresponsive β cells was the most susceptible to oxidative damage	Rat	Van De Winkel & Pipeleers[7]
	Intercellular differences in oxidative state might explain differences in the sensitivity of β cells to cytotoxic agents	Rat	Pipeleers[3]
	Glucose-responsive β cells were more predisposed to cell damage and suppression of insulin release in response to IL-1β than the glucose-unresponsive β cells	Rat	Ling *et al.*[97]
	Loss of β cells was more pronounced in the head region than the tail region of the pancreas among patients with type 2 diabetes mellitus	Human	Wang *et al.*[31]
Blood supply	Most, but not all, β cells are aligned along capillaries	Rat	Bonner-Weir[55]
	Most β cells are in close proximity to vascular cells and are aligned along blood vessels in a random order	Human	Cabrera *et al.*[16]
Innervation	Only a small proportion of β cells receive an axon terminal	Mouse and human	Woods & Porte[63]; Rodriguez-Diaz *et al.*[65]

the expression of CAMs on the surface of islet cells was postulated to have a role in the ordered distribution of cells within islets[39,40]. In particular, CAMs have been implicated in the budding of endocrine cells from ducts and seem to affect sorting of islet cells during cluster formation[40]. Neuronal CAM (NCAM) knock-out mice are associated with alterations in islet organization, with α cells infiltrating the islet core[40].

Junctional structures (for example, connexins) and cell-to-cell and cell-to-matrix adhesion molecules (such as integrins, cadherins and CAMs) also positively influence intracellular communications[34] and insulin secretion among β cells[35,41]. Moreover, the heterogeneous and dynamic distribution of gap junctions on β cells is associated with regional differences in β-cell coupling in animals[6,37,42]. The extent to which differences in gap junction abundance correlate with β-cell variability in terms of insulin secretion *in vivo* remains to be elucidated. Conversely, a role for adhesion molecules in functional heterogeneity *in vivo* is supported by reports that show how distinct expression patterns for E-cadherin and the sialylated form of the NCAM (PSA-NCAM) on β cells correlate with different insulin secretory responses of β cells[43,44].

The islet microenvironment. The islet microenvironment comprises a network of ECM, mesenchymal cells, nerves and blood vessels that signal via neighbouring tissue interactions, as well as neuronal and circulatory routes, to β cells and thereby affect their function. Ontogenic differences between the pancreas head and tail sections are primarily responsible for differences in innervation and blood supply among the islets. The ventral pancreas is supplied with blood via the mesenteric artery and receives sympathetic innervation from the superior mesenteric ganglion, whereas the dorsally derived pancreas is supplied with blood by the coeliac artery and is innervated by the coeliac ganglion[19]. Within-islet heterogeneity is also dependent on vascularization and innervation. Central and peripheral β cells are exposed to different extracellular environments and differentially interact with the microvasculature and autonomic innervation. As components of blood (including oxygen) and neurotransmitters participate directly in the regulation of insulin secretion[45,46], these regional differences could increase functional heterogeneity between the peripheral and central β cells.

Distinct patterns of blood perfusion and vascular density among dorsally derived and ventrally derived islets are associated with heterogeneity in β-cell function and proliferation[27]. Moreover, the use of static reconstruction techniques[47,48] and live *in vivo* imaging of islet blood flow within the pancreas[49] suggest that the vascular anatomy of the islets varies depending on their size[47] and location relative to the large vessels within the pancreas[48]. Small islets usually have two to three vascular penetration points, whereas large islets have more than three of these sites[48]. Islets might receive capillary branches if they are located in the periphery of the pancreas or branches directly from large vessels if they are located centrally[48]. Therefore, it seems legitimate to assume that some β cells are bathed directly by the systemic circulation, whereas other β cells receive blood that has already passed through other pancreatic or islet regions. This position implies that some β cells could be functionally active owing to the high amount of oxygen and blood components that they receive from the circulation, whereas other β cells are dormant and so constitute a reserve pool to meet metabolic demands.

Similarly, two distinct pools of high-blood perfused islets (with high oxygen consumption) and low-blood perfused islets (with low oxygen consumption) have

been described[45,50,51]. These islets (and their β cells) display unique functional features, with highly oxygenated islets having higher glucose-stimulated insulin release, higher β-cell proliferation and higher susceptibility to cellular stress than the low oxygenated islets[50,51]. This finding suggests that blood perfusion and oxygen consumption are good indicators of functional heterogeneity among pancreatic islets and β cells. A method has been devised to monitor oxygen tension and islet function *in vitro* and *in vivo* by using pimonidazole, a marker of the low oxygen tension[50]. Among awake wild-type rats, pimonidazole accumulated in 20–25% of the islets, which were less vascularized and characterized by lower insulin biosynthetic activity than the islets which

Table 3 | **Markers of β-cell heterogeneity**

Heterogeneous feature	Consequence or observation	Species	Reference
Cell surface markers			
PSA-NCAM	• PSA-NCAM^{high} β cells are more responsive to insulin secretagogues than PSA-NCAM^{low} β cells • The ratio of PSA-NCAM^{high} to PSA-NCAM^{low} cells varies with the metabolic conditions • The two populations of β cells have distinct gene expression profiles	Rat	Karaca *et al.*[44]
E-Cadherin	Expression correlates with insulin secretion	Rat	Bosco *et al.*[43]
ST8SIA1 and CD9	Co-staining of human islets with antibodies against these markers revealed clear heterogeneity and distinguished four β-cell subpopulations with distinct gene expression profiles and different insulin-secretion kinetics	Human	Dorrell *et al.*[111]
Intracellular markers			
Insulin	Cellular fluorescence intensity after insulin immunostaining varies within the same islet	Rat	Jorns *et al.*[5]
	• Heterogeneous immunoreactivity to insulin exhibited by β cells, which disappears under conditions of starvation • Centrally located β cells express less insulin than peripheral β cells	Rat	Jorns *et al.*[5]
	The β cells structurally coupled to δ cells express more insulin than those coupled to β cells	Rat	Pipeleers[3]
	• Transgenic mice expressing GFP linked to the insulin promoter exhibit three distinct subpopulations of β cells on the basis of GFP expression • These subpopulations display distinct insulin secretory activity upon glucose stimulation	Mouse	Katsuta *et al.*[82]
	Heterogeneous insulin staining found among dispersed β cells	Human	Szabat *et al.*[100]; Johnson *et al.*[136]
Pale secretory granules	High-glucose responsive β cells have more of these granules than low-glucose responsive β cells	Rat	Kiekens *et al.*[132]
Flattop (Fltp)	Distinguishes a mature subpopulation (Fltp^{+}) and a proliferative subpopulation (Fltp^{-}) of β cells with distinct genetic profiles and differential responses to metabolic stressors	Mouse	Bader *et al.*[77]
Glucokinase	Heterogeneous immunoreactivity among β cells	Rat	Jetton & Magnuson[104]; Heimberg *et al.*[129]
	Heterogeneous immunoreactivity among β cells, which disappears under conditions of starvation	Rat	Jorns *et al.*[5]
GLUT2	Low expression levels identify a rare β-cell subpopulation with stem-cell properties	Human and mouse	Smukler *et al.*[95]
	Identifies a pool of precursor β cells during islet regeneration	Mouse	Guz *et al.*[89]; Wang *et al.*[103]
	In a model of dedifferentiation, Glut2^{low} cells might represent a resident population capable of forming new functional β cells	Mouse	Beamish *et al.*[102]
DKK3	Found in a subset of β cells	Human	Hermann *et al.*[112]
VMAT2	• Only 10% of all β cells were negative for expression of this marker in the pancreata of healthy individuals and patients with type 1 or type 2 diabetes mellitus • By contrast, 70% of β cells scattered in the exocrine tissue were negative for this marker	Human	Saisho *et al.*[115]
Nuclear markers			
PDX1	Heterogeneous staining found among dispersed β cells	Human	Szabat *et al.*[100]; Johnson *et al.*[136]
	In combination with insulin, this marker identifies distinct maturation states of β cells	Mouse	Szabat *et al.*[100]

DKK3, Dickkopf 3; GFP, green fluorescent protein; GLUT2, glucose transporter 2; PDX1, pancreatic/duodenal homeobox protein 1; PSA-NCAM, sialylated form of the neural cell adhesion molecule; VMAT2, synaptic vesicular amine transporter.

were negative for pimonidazole accumulation[50]. After removing 60% of the pancreas mass, the remaining islets became well-perfused and displayed a marked reduction in pimonidazole accumulation, indicating that dormant islets can be induced to maintain normoglycaemia upon metabolic need. Islet capillaries can also enhance insulin secretion and β-cell survival by providing soluble factors and ECM proteins[52–54]. Hence, proximity to capillaries might be an additional cause of β-cell heterogeneity and plasticity.

Most endocrine cells within an islet, including the β cells, are in close proximity to endothelial cells and smooth muscle cells[16,25]. The organization of β cells adjacent to blood vessels closely resembles the rosette-like structure previously reported in rodents[25,55]. The intraislet microcirculation imposes a specific directional flow of blood through the islets, which is crucial for both intercellular communication and communication between islet endocrine cells. Vascular perfusion within the islet is ordered among both rodents and humans, with the β cells perfused first, followed by the α and δ cells[56,57]. Hence, insulin released in the microcirculation affects glucagon and somatostatin secretion within the islet, whereas glucagon affects only somatostatin secretion[57]. Somatostatin probably acts directly on the exocrine tissue via the islet–acinar portal system[58].

Within each islet, the endocrine cells also communicate with each other extracellularly through the release of their secretory products into the interstitial fluid[34]. This paracrine effect explains the beneficial role of α–β cell aggregates and the detrimental effects of δ–β cell aggregates owing to the insulin-stimulating effect of glucagon and the insulin-inhibitory effect of somatostatin, respectively[34]. Furthermore, α cells are a major source of GLP-1 within the islets, which could also affect the function of adjacent β cells[59].

The autonomic nervous system is responsible for islet innervation[60] and controls islet development, maturation, mass and function[61,62]. By employing genetic and chemical ablation of sympathetic neurons in mice, sympathetic innervation was found to be necessary for establishing pancreatic islet shape and cytoarchitecture during development, which in turn is critical for regulation of glucose metabolism[61]. Only a small proportion of β cells receive axon terminals[63], and studies in mice suggest that sympathetic islet innervation is largely concentrated at the islet periphery[64]. Human β cells are even more sparsely innervated than those of mice[65]. Few parasympathetic cholinergic axons penetrate the human islet; however, neuron fibres preferentially innervate smooth muscle of blood vessels within the islet, possibly regulating highly vascularized islet regions[65]. Although these findings can lead to speculations on regional functional heterogeneity within islets, clear evidence is still lacking. The observed differences in NCAM among endocrine cells[39] might support a role for innervation in β-cell heterogeneity.

Collectively, the organization of each islet and its particular relationship with the vasculature and the nervous system offers a tight spatial compartmentalization of β-cell responses and so might subdivide β cells in distinct functional subpopulations.

β-Cell polarity. Establishment of polarity during fetal development is crucial for terminal differentiation and spatial localization of cells within a tissue. Pancreatic β cells derive from epithelial tissue but they do not usually exhibit the classic columnar epithelial organization, whereby the apical domain is opposite to the basal domain. Nonetheless, cuboidal or columnar epithelial patterns of organization were observed in some histological sections[55].

The available evidence suggests that β cells predominantly resemble the polar structure of hepatocytes, with apical regions along the lateral surfaces[66]. In 1985, polarization of plasma membrane domains in pancreatic endocrine cells was first demonstrated by viral budding[67]. The β-cell-specific glucose transporter 2 (GLUT2) was later found to localize at the lateral side, directly adjacent to a neighbouring β cell[68]. Furthermore, rat β cells are typically organized in rosette-like structures surrounding blood vessels[55]. The β cells within these structures segregate the nucleus to the basal side and the insulin granules to the apical side facing the central capillary[55], supporting the concept that insulin secretion into the bloodstream occurs apically. By contrast, an alternative theory suggests that exocytosis of insulin-containing granules from β cells occurs mainly on the lateral side into the interstitial space from where the granules reach the systemic circulation[52,69].

Nevertheless, it is clear that β cells are polarized with respect to the vasculature. By using a pancreatic slice preparation, which maintains the native structural organization of the islets, and different 3D imaging methods, three distinct polar domains can be identified in β cells[70]. These polar domains include an apical region where cilia are projected into the extracellular lumen; a large lateral domain characterized by expression of GLUT2; and a basal region where β cells are in contact with the vasculature. Importantly, the same polar organization was found in humans[70]. Another study found that adjacent β cells (both human and mouse) meet at sharp angles or edges that form specialized surface microdomains where signalling molecules are segregated[71]. Thus, polarization not only has a role in β-cell positioning within the islets, but also probably helps to define specific signal and process compartmentalization within individual β cells.

The first insight into the regulation of β-cell polarity on a molecular level was provided by a functional analysis of the serine–threonine liver kinase B1 (LKB1)[66,72]. Deletion of LKB1 in adult β cells resulted in translocation of the β-cell cilia to the basal side and of the nucleus to the apical side. Precise nuclear and ciliary positioning, therefore, depend on intracellular polarity, strongly pointing to LKB1 as a regulator of this process in β cells. The fact that LKB1 also regulated β-cell function and size further suggests a role of polarity acquisition in β-cell morphology and homeostasis.

Planar cell polarity (PCP) — defined as the polarity perpendicular to the apical–basal polarity — regulates the orientation of cells within the plane of an epithelium, intracellular organelle positioning and ciliary positioning through expression of a conserved set of core PCP genes.

These genes encode both membrane-bound and cytoplasmic proteins localized symmetrically and asymmetrically, which are crucial for the establishment and maintenance of PCP[73]. Activators of PCP signalling include the secreted non-canonical Wnt proteins (for example, WNT4, WNT5a and WNT11) that bind to Frizzled receptors and transduce the signal into the cell. Although the role of non-canonical Wnt signalling in pancreas development is well established[74], little is known about the role of PCP during islet neogenesis and β-cell maturation. Expression and asymmetric localization of core PCP proteins were reported in the embryonic pancreas[75]. Genetically modified mice lacking genes encoding two core PCP components (*Celsr2* and *Celsr3*) had reduced numbers of endocrine cells at embryonic day 14.5, which was associated with diminished glucose clearance capacity[75]. CELSR3 probably acts on β-cell differentiation by activating PCP signalling.

The actin cytoskeletal regulator RhoA/ROCK is a downstream effector of the Wnt/PCP signal transduction pathway. RhoA/ROCK has a role in adult β-cell function, including insulin secretion[76], indicating that β-cell polarity can directly affect β-cell function.

Finally, we have demonstrated the involvement of Wnt/PCP in islet formation and maturation, as well as the existence of functional β-cell heterogeneity in active PCP signalling, as indicated by the expression of the novel marker Flattop (encoded by *Fltp*)[77]. *In vitro* activation of Wnt/PCP signalling triggered expression of maturation markers, including the transcription factors NKX6.1 and MAFA and urocortin-3 (a neuropeptide), and improved glucose-stimulated insulin secretion. We also have evidence to suggest that Wnt/PCP has a role in the postnatal maturation of β cells[77]. Collectively, the studies described throughout this section highlight the importance of polarity and intracellular signal positioning for β-cell heterogeneity, maturation and function.

β-Cell plasticity

During the life course, physiological or pathological conditions can alter islet composition and 3D architecture and further affect β-cell heterogeneity, both between and within the islets. This process is the result of the highly plastic behaviour of islet endocrine cells, in particular β cells, which adapt their physiology, morphology and function upon exposure to specific endogenous and exogenous cues.

Physiological plasticity. The proliferative activity of β cells decreases rapidly after birth. Only 3% of β cells in the adult mouse islet exhibit proliferative capacity[9], and proliferation is almost negligible among human β cells[78].

During pregnancy, β cells respond to lactogenic hormones by undergoing extensive hyperplasia to meet increased metabolic demand[79]. Considerable heterogeneity exists in islet responsiveness to cell proliferation during mid-to-late pregnancy in the rat[80], suggesting that variability in hormonal adaptation might be responsible for the development of gestational diabetes mellitus. The observed boost in β-cell proliferation could cause changes in islet composition and affect the intraislet localization of β cells, as well as their function. Indeed, β-cell hyperplasia was accompanied by a substantial decrease in the threshold for glucose-stimulated insulin secretion[79,80].

During ageing, replication of pre-existing β cells and neogenesis both contribute to maintain an adequate β-cell mass[81]. Different cellular origin might account for changes in islet composition, potentially giving rise to unique β-cell subpopulations. Studies in rodents suggest that ageing regulates the size and granularity of some β cells, thereby influencing insulin secretion[82,83]. Substantial changes in the proportion of β cells co-expressing multiple hormones have been reported between neonatal and adult mice[17].

Pathological plasticity. A change in islet composition is found among diabetic animals and humans[84,85], which is accompanied by displacement of the endocrine cells from their characteristic location within the islet[84]. Although it is unclear whether these changes are a cause or a consequence of disease, this imbalance probably affects the net hormonal output of the endocrine pancreas and contributes to dysregulation of blood glucose levels.

Experimental models of β-cell dysfunction indicate that distinct pools of β cells can originate from other cell types under pathological conditions, including endocrine or exocrine cells[86]. Accordingly, single-gene manipulation or β-cell depletion is sufficient to drive transdifferentiation of α cells or δ cells towards the β-cell lineage[87,88]. Newly formed β cells might display distinct features, such as co-expression of multiple hormones, as shown in a model of islet regeneration[89].

De novo generation of β cells from exocrine progenitors derived from the pancreatic duct or acinar also occurs after mild (but prolonged) hyperglycaemia among healthy and diabetic rodents[90,91]. This finding indicates that progenitor, immature and mature β cells might coexist and so contribute to β-cell heterogeneity under diabetic conditions. This hypothesis is supported by the fact that chronic exposure to pathological stressors or artificial gene deletion induces dedifferentiation of β cells into an immature state, with the appearance of subpopulations of β cells that express progenitor markers (for example neurogenin-3; encoded by *Ngn3*) but lack expression of maturation markers (for example, insulin, pancreatic/duodenal homeobox protein 1 (PDX1), NKX6.1 and MAFA)[92,93]. Dedifferentiated endocrine cells could be reverted into mature hormone-expressing cells upon insulin therapy[93], suggesting a potential window of therapeutic opportunity. According to the 'five stages' theory of diabetes progression, the continual demise of β cells is accompanied by sequential changes in β-cell mass, phenotype and function[94]. Hence, both basic research and therapeutic efforts would benefit greatly from the identification of specific markers that distinguish discrete β-cell populations during the progressive deterioration that leads to diabetes mellitus.

Biological and clinical relevance

Physiology. The existence of heterogeneous populations of β cells provides pancreatic islets with high functional flexibility in terms of insulin secretion and enables them to correctly sense and adapt to physiological changes in the environment.

Central and peripheral β cells respond to glucose with different kinetics, suggesting that one population could be responsible for the acute-challenge response whereas the other population could contribute to insulin output after a sustained challenge[12,30]. Other studies have noted the existence of specialized pancreatic stem-cell populations or distinct pools of β-cell precursors, both of which are expected to display marked phenotypic and functional differences from the mature adult β cells[95]. Although this hypothesis remains debated[96], islets are believed to contain progenitor-like cells characterized by the capacity to undergo self-renewal and a precursor gene expression profile[95]. Indeed, the existence of a low oxygenated and functionally dormant subpopulation of islets has been described, which could be of functional importance as a cellular reserve during situations of increased metabolic need[50].

Disease. Attention has also focused on the role of β-cell heterogeneity in the pathogenesis of disease. Regional heterogeneity occurs in the adaptation of β cells to pathological stressors[27]. In this regard, β cells from the splenic region were more likely to increase proliferation and glucose-stimulated insulin secretion in response to 6 weeks of a high-fat diet (HFD) than β cells from the duodenal and gastric regions[7]. Heterogeneity among β cells might also determine susceptibility to pathogenic stressors. For example, β cells with low glucose responsiveness were markedly more susceptible to oxidative damage than β cells with high glucose responsiveness[3,7]. A pool of highly functional, highly blood-perfused islets in the intact pancreas displayed increased susceptibility to cellular stress by cytokines and hypoxia[51]. Furthermore, glucose-responsive β cells were predisposed to damage by IL-1β and suppression of insulin release[97]. Among patients with type 2 diabetes mellitus, β-cell loss was more pronounced in the head region than the tail region of the pancreas[31,96].

Cellular and molecular characterization of these metabolically variant subpopulations of β cells is warranted to identify markers and understand the molecular principles of heterogeneity. Genomic, proteomic and metabolic analysis of functionally distinct populations of β cells with regard to proliferation, glucose response, insulin secretion, pathogenic susceptibility and response to antidiabetic treatment might aid identification of a specific signature of markers that could be used to monitor changes in islet heterogeneity in pathophysiological conditions. Increased understanding of the endogenous and exogenous modulation of such markers could potentially lead to new therapeutic strategies aimed to recapitulate a specific β-cell function that is either lost or dysfunctional in diabetes mellitus.

Markers of β-cell heterogeneity

Identification and characterization of markers of β-cell heterogeneity remains the ultimate goal to enable targeting of specific subpopulations for regenerative therapy in diabetes mellitus. Pancreatic insulin-producing β cells express a unique and complex profile of molecules, comprising transcription factors, membrane transporters, metabolic enzymes, G-protein coupled receptors (GPCRs) and CAMs, which mirror their phenotypic and functional status (TABLE 3).

Insulin

Insulin is obviously the best described β-cell marker; furthermore, expression levels of this hormone are heterogeneous. Morphological analysis of insulin expression revealed that β cells in the centre of the islet express less insulin than β cells located at the periphery[5]. Transgenic mice expressing green fluorescent protein (GFP) under the control of the mouse insulin promoter (MIP; MIP–GFP) allowed distinction of three subpopulations of β cells characterized by distinct levels of GFP intensity[82]. This heterogeneity was detected throughout the animals' lifespan and correlated with β-cell function. Variation in islet composition between the pancreas head and tail can also contribute to heterogeneity of insulin expression[28]. Indeed, islets located in the pancreas tail display higher insulin biosynthesis rates than islets located in the pancreas head[28]. A similar result was observed in a mouse model of obesity and diabetes mellitus[27]. Furthermore, β cells structurally coupled to δ cells contain more insulin than those coupled to other β cells[3].

Insulin heterogeneity might also reflect different maturation states of β-cell subpopulations. Single-gene expression analysis of MIP-GFP cells revealed that a small portion of β cells still co-express multiple hormones, a phenotype that usually characterizes immature β cells[17]. The co-expression of islet hormones that occurs in endocrine progenitors during development and in adult mice might reflect the residual progenitor potential of a reserve pool of a β cells during conditions of metabolic demand. Finally, the density of pale granules that contain unprocessed (immature) insulin seems to associate with the sensitivity of β cells to glucose; therefore, granule density could represent a marker of highly responsive cells[98].

Pancreatic/duodenal homeobox protein 1

The transcription factor PDX1 is a master regulator of β-cell development and function[99]. Heterogeneous expression of this protein has been detected in both human and mouse pancreas, possibly reflecting the distinct maturation status of β-cell subpopulations[100]. Expression analysis indicated that 25% of human and mouse β cells were PDX1^{+}/insulin^{low}. This subpopulation displayed a gene expression profile that resembled that of immature developing β cells, with high levels of the transcription factor MAFB and low levels of glucokinase and GLUT2, accompanied by increased proliferative capacity and diminished insulin secretory capacity. A portion of these cells differentiated into mature β cells in culture without undergoing cell division[100]. Furthermore, chronic

mild glucose infusion among rats triggered the formation of small common duct epithelium clusters composed of PDX1$^+$/insulin$^-$ cells and PDX1$^+$/insulin$^+$ cells, suggesting a role for these molecules as markers of β-cell neogenesis and heterogeneity[90]. The appearance of precursor PDX1$^+$ cells (and somatostatin$^+$ cells) was also noted in a model of regeneration following β-cell ablation[89].

GLUT2

Uptake and sensing of glucose by β cells is required to initiate appropriate insulin secretion. GLUT2 is the major glucose transporter isoform present in mouse β cells; its expression starts early during development and increases during β-cell maturation and acquisition of metabolic competence[101]. Low expression levels of GLUT2 characterize a rare subpopulation of β cells that express low levels of insulin and retain the properties of stem and/or progenitor cells[95]. An *in vivo* dedifferentiation model identified a population of insulin$^+$/GLUT2^{low} cells that represented 3.5% of all insulin-expressing cells[102]. The majority of these cells were localized within clusters of less than five β cells; they exhibited a higher proliferation rate than insulin$^+$/GLUT2$^+$ β cells isolated from young mice at postnatal day 7 and high plasticity towards endocrine, ductal and neural lineages. These cells might represent a resident population capable of generating new functional β cells *in vivo*[102]. Furthermore, a pool of precursor GLUT2$^+$ cells was reported to appear during β-cell regeneration following targeted β-cell ablation[89,103], making GLUT2 a promising tool to explore plasticity among subpopulations of β cells.

Glucokinase

Glucokinase is a pivotal glucose-sensing enzyme among β cells and its expression correlates with the abundance of insulin granules and GLUT2 expression[104]. Heterogeneous polarized localization patterns of glucokinase immunostaining were detected among β cells in contact with intraislet capillaries, with the highest density in the cytoplasmic region close to the pericapillary space[5]. The expression of glucokinase becomes heterogeneous with changes in metabolic status and precedes insulin heterogeneity[5]. Glucokinase might, therefore, be of use to discriminate discrete subpopulations of β cells that retain differential metabolic activity.

E-Cadherin

A variety of cadherins are expressed by β cells. These calcium-dependent transmembrane proteins are involved in cell-to-cell adhesion, islet compaction and formation[105], and β-cell activity[106]. E-Cadherin regulates proliferation and glucose responsiveness of β cells[107] and its expression is reduced in transgenic animal models of type 2 diabetes mellitus[108]. Heterogeneous expression of E-cadherin was reported in adult rodent β cells[43], which was regulated by insulin secretagogues. High levels of E-cadherin expression in β cells correlated with increased expression of insulin and greater secretory capacity compared to β cells expressing low levels of E-cadherin, indicating the importance of tight cell-to-cell contacts for physiological β-cell function[43].

PSA-NCAM

Heterogeneous expression of PSA-NCAM has been reported in β cells[44]. The advantage of PSA-CAM as a marker for β-cell heterogeneity in the pancreas is its restricted expression to this cell type and the regulation of its surface expression by cellular activity, such as insulin exocytosis[109].

PSA-NCAM has been used to sort β-cell subpopulations with distinct glucose responsiveness in healthy rats[110]. Rat β cells were divided into PSA-NCAM^{high} and PSA-NCAM^{low} subpopulations based on the level of expression; these subpopulations were characterized by distinct mRNA profiles, metabolic status, cellular complexity and insulin secretion patterns[44]. The PSA-NCAM^{low} β cells exhibited a small increase in calcium concentration after glucose stimulation, reduced ATP levels and impaired expression of genes involved in the generation of metabolic signals, such as *Glut2* and *Gck* (encoding glucokinase). The PSA-NCAM^{low} subpopulation also showed deficiency in cAMP-dependent pathways and a marked degree of dedifferentiation. The PSA-NCAM^{high} and PSA-NCAM^{low} cells further differed in the distribution of F-actin at the apical plasma membrane, and in expression of proteins, pumps and ion channels required for insulin exocytosis. The proportion of PSA-NCAM^{high} and PSA-NCAM^{low} β cells varied according to the insulin demand in two separate animal models of diabetes mellitus and acute hyperglycaemia[44]. The observed differences correlated with disease progression, highlighting the pathophysiological relevance of PSA-NCAM and its potential use in monitoring functional β-cell mass *in vivo*. Nonetheless, the validity of this marker in humans remains to be investigated.

ST8SIA1 and CD9

The cell surface markers ST8SIA1 and CD9 distinguish four distinct subpopulations of β cells in adult human islets[111]. These subpopulations were named from most abundant to least abundant as follows: β1 (ST8SIA1$^-$ and CD9$^-$), β2 (ST8SIA1$^-$ and CD9$^+$), β3 (ST8SIA1$^+$ and CD9$^-$) and β4 (ST8SIA1$^+$ and CD9$^+$). They display clear differences in both basal and stimulated insulin secretion, suggesting variation in insulin-release kinetics. The ST8SIA1$^-$ population (β1 and β2) was enriched for gene ontology (GO) terms related to protein secretion, whereas the ST8SIA1$^+$ population (β3 and β4) was enriched for GO terms related to neurogenesis[110]. Indeed, the ST8SIA1$^-$ populations secreted more insulin than the ST8SIA1$^+$ populations, despite similar insulin content. Moreover, although the four subpopulations expressed common β-cell markers, such as PDX1, MAFA and NKX6.1, a subset of genes consistently displayed differential expression levels. The frequencies of these β-cell subpopulations were altered among individuals with type 2 diabetes mellitus, suggesting that they might react differently to metabolic stress and pathogenic cues, thereby highlighting the potential clinical relevance of these findings[111].

Dickkopf 3

Gene expression profiling of isolated human islets revealed high levels of *DKK3* mRNA (encoding Dickkopf 3) among β cells; furthermore, immunohistochemical analysis revealed heterogeneous expression of the protein Dickkopf 3 in these cells[112]. Dickkopf 3 is a member of a family of secreted Wnt antagonists; however, its function in regulating the canonical or non-canonical Wnt signalling pathway remains to be clarified. Studies in zebrafish reported a specific expression pattern of the *Dkk3b* gene (homologous to human *DKK3*) in the islet during pancreas development and its possible involvement in endocrine differentiation[113]. Surprisingly, however, *Dkk3*-null mice did not display any abnormalities during either embryonic development or in adulthood[114], suggesting that its function could be redundant.

Synaptic vesicular amine transporter

Approximately 80% of the β cells in the pancreas of diabetic and nondiabetic individuals express synaptic vesicular amine transporter (VMAT2; also known as monoamine transporter and vesicular amine transporter 2)[115]. The overall pattern of VMAT2 expression was similar among patients with type 1 or type 2 diabetes mellitus. The proportion of VMAT2-negative cells increased to 70% among the β cells scattered in the exocrine tissue at a distance from the islets[115]. However, the role of VMAT2 in β cells has not yet been determined.

β-Cell heterogeneity–3D architecture link

Efforts are still ongoing to identify novel markers of β-cell heterogeneity. One such marker is the Wnt/PCP effector Flattop (also known as Fltp)[77,116].

This molecule was originally discovered in a screen to identify target genes of the forkhead box transcription factor FOXA2 that regulates polarization and epithelialization in the endoderm germ layer[117,118]. Indeed, *Fltp* is expressed in definitive endoderm-derived organs, such as the pancreas, lungs and gastrointestinal tract, particularly in regions where Wnt/PCP signalling components are expressed and the pathway is active[119,120]. *Fltp* represents a surrogate marker for Wnt/PCP activity that could be used to study the effects of specific PCP signals on β-cell homeostasis and function. The PCP pathway orientates cells and organelles within the plane of tissues and so is a key player in organizing islet architecture and function. Both FOXA2 (REF. 121) and Wnt/PCP[75] are crucial for pancreas and islet development and planar polarization. Given that tissue organization and cell-to-cell interaction are critical for β-cell heterogeneity and function, we investigated the expression of Fltp in the pancreatic islets and its potential role in β-cell pathophysiology.

To study the establishment of tissue polarity at the molecular level, we employed a genetically modified mouse model that enabled the expression and function of *Fltp* to be assessed in all endocrine cells of the islets of Langerhans[119,120]. At embryonic day 18.5, Fltp venus reporter (FVR) activity was detectable only in β cells of compacted islets and the number of FVR$^+$ β cells increased in islets after birth (~50% of β cells at postnatal day 1) and throughout the β-cell maturation process (80% of mature β cells in adult islets; FIG. 1). This finding suggests that β-cell heterogeneity could be driven by Wnt/PCP-mediated planar polarization of these cells during postnatal maturation. Interestingly, the FVR$^+$ cells were often found in rosette-like structures (FIG. 1), suggesting that Wnt/PCP signalling might be important for the polarity and functional tuning of mature insulin-producing β cells. By contrast, we observed a higher proliferative capacity among FVR$^-$ β cells during pregnancy and postnatal β-cell expansion compared to the FVR$^+$ population (FIG. 1).

Molecular and transcriptional analysis of FVR$^+$ and FVR$^-$ endocrine cells revealed distinct gene expression, structural and physiological features among the two subpopulations[77]. The FVR$^+$ cells were enriched in mRNAs encoding β-cell maturation markers, receptors, signalling components, mitochondrial proteins and molecules involved in metabolism. By contrast, the FVR$^-$ cells were associated with GO terms, such as mitogen-activated protein kinases (MAPKs) and GPCRs; they also expressed low levels of GLUT2 (REF. 77), a finding previously reported in insulin-expressing multipotent progenitor cells from mouse and human islets[95]. Additionally, glucose-stimulated insulin secretion was decreased in reaggregated FVR$^-$ β cells compared to FVR$^+$ β cells, suggesting differences in metabolic status among these subpopulations.

Using a Cre recombinase/*loxP*-mediated genetic lineage tracing approach[120], we found that Fltp-lineage$^-$ and Fltp-lineage$^+$ endocrine subpopulations reacted differently under physiological and pathological conditions known to affect glucose metabolism[77]. Fltp-lineage$^-$ β cells underwent cell expansion during pregnancy, whereas Fltp-lineage$^+$ β cells underwent β-cell compensatory growth and/or hypertrophy after 8 weeks of a HFD. When we transplanted islets into the anterior chamber of the mouse eye to perform longitudinal analysis of the two Fltp subpopulations, we observed that the Fltp-lineage$^+$ population initially decreased in number, whereas the Fltp-lineage$^-$ population underwent expansion, during the revascularization period. However, 4 weeks after transplantation, the Fltp-lineage$^+$ subpopulation recovered, possibly owing to the conversion of the Fltp-lineage$^-$ cells into Fltp-lineage$^+$ cells. This finding indicates that Fltp-lineage$^-$ β cells might function as a reservoir of cells that can compensate for metabolic demand, whereas the Fltp-lineage$^+$ β cells are the mature cells with high metabolic activity. Furthermore, longitudinal analysis of Fltp-lineage$^-$ and Fltp-lineage$^+$ endocrine cells during 8 weeks of a HFD revealed a marked increase in the cross-sectional area of the Fltp-lineage$^+$ endocrine-cell population over time, suggesting that this β-cell subpopulation accounts for the pathological islet-cell response. In humans, we genotyped seven single-nucleotide polymorphisms (SNPs) tagging all common variation in the *FLTP* orthologue (CFAP[122]) and investigated their distribution and association with metabolic traits and body fat content in a cohort of more than 2,000 individuals diagnosed with prediabetes[77].

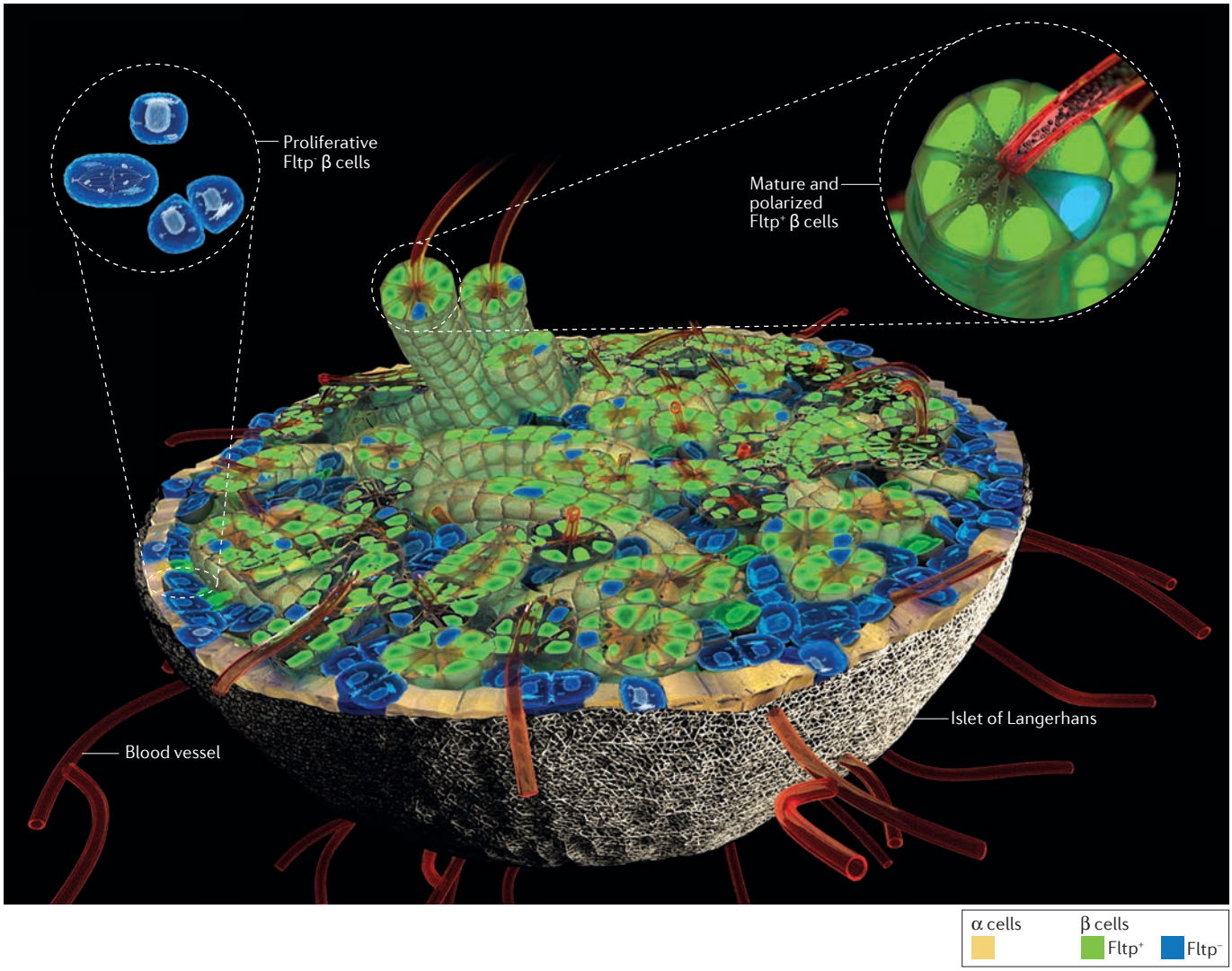

Figure 1 | **A 3D model of functional β-cell heterogeneity.** The schematic shows a mouse pancreatic islet. The β cells — located within the core (blue and green) — are the most abundant cell type (85%). Non-β cells are located at the periphery. Of these, α cells (yellow) comprise 10% of the endocrine-cell population, with the final 5% comprising δ cells, pancreatic polypeptide cells and ε cells (not shown). Blood vessels (red) penetrate the islet at different levels and heterogeneously interact with the endocrine cells. The novel Wnt/PCP effector Flattop (Fltp) distinguishes two β-cell subpopulations with distinct molecular, physiological and ultrastructural features. In all, 80% of β cells express Fltp (green). These cells are terminally differentiated, highly polarized and secrete insulin, probably owing to active Wnt/PCP signalling that enables preferential localization around the blood vessels in rosette-like structures to fine-tune glucose sensing and insulin secretion. The β cells lacking Fltp (blue) comprise 20% of the total β-cell mass. These cells are less polarized and have less secretory activity than Fltp⁺ β cells but are more responsive to environmental cues. The Fltp⁻ β cells exhibit high proliferative capacity and can mature into Fltp⁺ β cells, suggesting that they constitute a reserve pool of immature cells that respond to external signals.

We found that one SNP (*rs75715534*) was associated with decreased insulin secretion among obese individuals. Additionally, expression levels of *FLTP*, *PDX1* and *GLUT2* were downregulated in islets isolated from individuals with progressive diabetes mellitus, suggesting a novel role for non-canonical Wnt signalling in the maturation and degeneration of β cells[77].

Fltp seems to represent a unique tool to study how β-cell heterogeneity is established, as well as the mechanisms that regulate this process under both physiological and pathological conditions. We have described a role for Wnt/PCP signalling in islet compaction, as shown by increased mRNA expression of *Fltp* at the time when islets start to form and acquire their unique 3D structure[77]. Our results support the concept that active Wnt/PCP signalling regulates β-cell polarization, maturation and function both during development and in the adult. By stimulating 2D and 3D *in vitro* cultures of mouse and human β cells with Wnt/PCP ligands, we were able to trigger expression of β-cell maturation markers and to increase glucose-stimulated insulin secretion[77].

The mechanisms underpinning these effects probably reflect Wnt/PCP-mediated (and Fltp-mediated) regulation of actin and microtubules in the cytoskeleton and changes in gene expression; however, this hypothesis remains to be tested. Other unanswered questions include the relationship between Wnt/PCP and Fltp within the islet niche. Heterogeneity in Fltp expression was observed both within and between islets when we compared head and tail regions of the pancreas and islets of different size[77]. In addition, the mechanisms that regulate conversion of immature Fltp$^-$ β cells to mature Fltp$^+$ β cells must be elucidated in detail.

In summary, Fltp can discriminate between proliferative and metabolically active β cells[77]. Hence, exogenous manipulation of Wnt/PCP signalling or endogenous changes in Fltp expression might enable investigation of new dimensions of β-cell heterogeneity under conditions that affect functionality of this cell type. The distinct profile of receptors and signal transduction pathways that characterize the Fltp$^+$ and Fltp$^-$ subpopulations might offer a targeted approach for *in vivo* β-cell regeneration. One study has already demonstrated that therapeutic targeting of different subpopulations of β cells is possible[123]. Chronic treatment with an insulin secretagogue induced heterogeneous degranulation of β cells; the subpopulations that were most degranulated exhibited increased biosynthesis of insulin, even in the absence of glucose[123].

Using FLTP as a marker might also facilitate *in vitro* maturation of human stem cells to β cells for replacement strategies or to create functional islets for tissue-engineering approaches. Furthermore, expression of *Fltp* is not restricted to β cells. This molecule is also expressed in other endocrine cells[77], potentially providing a means to investigate intraislet transdifferentiation, plasticity and heterogeneity among α cells or δ cells, which could also ultimately affect β-cell function and regeneration.

Conclusions

Technological advances have confirmed previously described principles of β-cell heterogeneity and marked a new era in scientific breakthroughs. Such heterogeneity arises during development and could reflect the existence of more than one developmental pathway for β cells[17,101]. Indeed, β cells exposed to the same environment display some degree of intrinsic heterogeneity in their rates of insulin secretion[13]. Dissociated β cells exhibit a clear self-organizing ability to form aggregates with appropriate 3D architecture and insulin-release patterns comparable to intact islets[124]. The islet cytoarchitecture and surrounding microenvironment support the heterogeneous yet specific functions of β cells during the postnatal period.

Heterogeneity can also arise from β-cell plasticity or from different routes of β-cell neogenesis under physiological or pathological conditions. Visualization and discrimination of heterogeneous β-cell populations with specific markers, and increased understanding of the mechanisms that regulate β-cell function, is required to fully understand β-cell physiology and pathophysiology. Novel markers, such as Fltp, could provide data on the activity of signalling pathways of pivotal importance in islet compaction, maturation and function (for example, Wnt/PCP). Such studies could potentially enhance knowledge of β-cell heterogeneity and guide new regenerative treatments for diabetes mellitus and other metabolic diseases.

An important question is the extent to which β-cell heterogeneity occurs *in vivo* among humans and how it contributes to the development of metabolic disease. Most studies conducted to date have used animal models to identify the principles and mechanisms of β-cell heterogeneity. Although the composition of human islets might resemble that of rodents[25], it is unclear whether functional between-species differences exist[16]. Variation in the expression of gap junction or adhesion molecules has been reported among mammalian species. For example, mouse islets lacking connexin 36 display similar calcium oscillations to human islets[125]. Consequently, caution must be exercised when extrapolating results from rodents to human.

Nonetheless, the available data clearly support the concept that β-cell heterogeneity is present in human islets and could contribute to the pathogenesis of diabetes mellitus[31,111]. Understanding how distinct subpopulations of β cells are dynamically regulated upon diabetogenic cues (for example, a HFD) will aid design of appropriate strategies to recapitulate physiological heterogeneity and regenerate functional β-cell mass. Technical limitations still need to be overcome and it is possible that isolation of islets and β cells from the *in vivo* environment could alter their features. For these reasons, research and therapy would definitely benefit from the validation of β-cell-specific markers localized on the cell surface or the development of advanced technologies, such as targeted *in vivo* labelling, to monitor the extent and modulation of β-cell heterogeneity *in situ*, as well as the response to pharmacotherapy. This approach could open the door to a new generation of therapeutic interventions in diabetes mellitus, whereby endogenous cellular function could be monitored, reprogrammed and appropriately reinstated.

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Competing interests statement
The authors declare no competing interests.

Review criteria
The PubMed database was searched for English language full-text papers published from 1960 (when β-cell heterogeneity was first discovered) to March 2016, using the following search terms: "β-cell heterogeneity", "β-cell plasticity", "islet architecture" and "β-cell regeneration". The identified references were then used to find further leads, which were also included in this Review.