Metabolic and functional heterogeneity in pancreatic β cells

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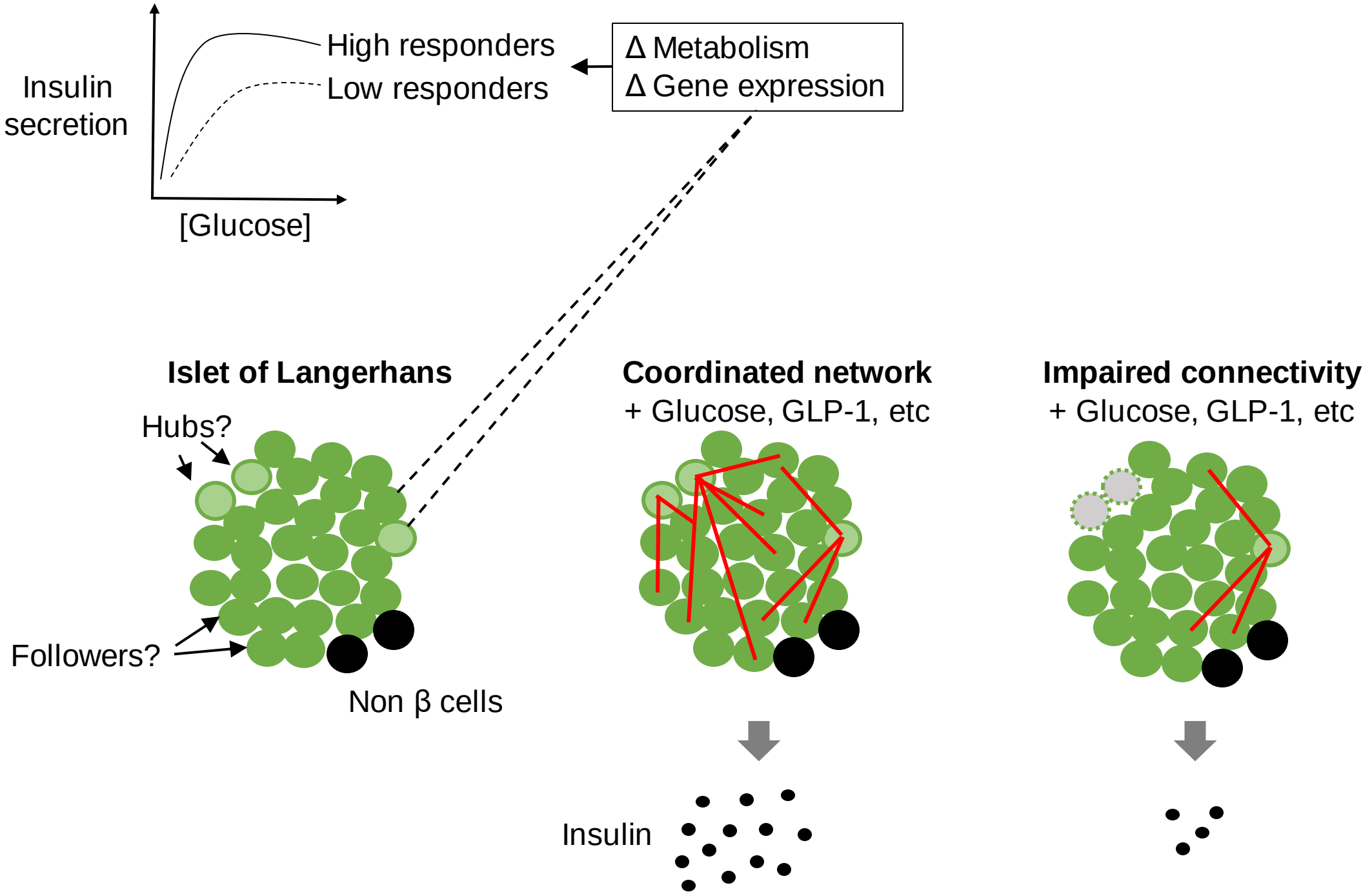
Insulin secretion
[Glucose]
High responders
Low responders
Δ Metabolism
Δ Gene expression
Islet of Langerhans
Hubs?
Followers?
Non β cells
Coordinated network
+ Glucose, GLP-1, etc
Impaired connectivity
+ Glucose, GLP-1, etc
Insulin

Metabolic and functional heterogeneity in pancreatic β cells.

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Highlights:

- Pancreatic β cells exhibit metabolic heterogeneity
- Metabolic heterogeneity is critical for the regulation of insulin secretion
- Hub β cells coordinate the action of other β cells within the islet
- β cell sub-populations can be characterised by their specific gene expression profiles

Abstract

Metabolic and secretory heterogeneity are fundamental properties of pancreatic islet β cells. Emerging data suggest that stable differences in the transcriptome and proteome of individual cells may create cellular hierarchies which, in turn, establish coordinated functional networks. These networks appear to govern the secretory activity of the whole islet and be affected in some forms of diabetes mellitus. Functional imaging, e.g. of intracellular calcium dynamics, has led to the demonstration of "small worlds" behaviour, and the identification of highly connected "hub" (or "leader") cells, and of follower populations subservient to them. Subsequent inactivation of members of either population, for example using optogenetic approaches or photoablation, has confirmed the importance of hub cells as possible pacemakers. Hub cells appear to be enriched for the glucose phosphorylating enzyme glucokinase, and for genes encoding other enzymes involved in glucose metabolism, compared to follower cells. Recent findings have shown the relevance of cellular hierarchy in islets from multiple species including human, mouse and fish, and shown that it is preserved *in vivo* in the context of the fully vascularised and innervated islet. Importantly, connectivity is impaired by insults which mimic the diabetic milieu, including high glucose and/or fatty levels, and by the ablation of genes associated with type 2 diabetes risk in genome-wide association studies. We discuss here the evidence for the existence of these networks and their failure in disease settings. We also briefly survey the challenges in understanding their properties.

Introduction:

Loss of functional pancreatic β cell mass underpins the development of both Type 1 and Type 2 diabetes (T2D) [1-4]. Environmental and genetic factors contribute to the loss of functional β cell mass in T2D (reviewed in [5, 6]). The β cell is the only source of circulating insulin and the loss of regulated insulin secretion leads to dysregulated energy homeostasis.

The notion that not all β cells are the same has received much recent interest, yet is far from new. Thus, a number of studies since the 1960s have reported that β cells exhibit, for example, different sensitivities to glucose, and distribution and numbers within any given islet, and that heterogeneity is linked to differences in ability to secrete insulin [7-22]. There are also published data [23-32] to indicate that there is heterogeneity in the distribution of islets themselves between different parts of the pancreas, [33, 34], though recent data question this possibility in healthy human pancreata [35]. It should be emphasized that functional heterogeneity is not unique to β cells. For example, pituitary cells have been shown to exhibit heterogeneous response to hormonal stimulation [36-38]. Importantly, in recent years the repurposing by us and others of technologies developed to map the heterogeneity in the pituitary response for the study of the pancreatic islet has led to breakthroughs in our understanding of β cell heterogeneity within the context of an islet [39, 40]. In this review, we will look at how β cell heterogeneity contributes to islet function and systemic glucose homeostasis, and how the loss of this heterogeneity may lead to disease. We will focus on the heterogeneous metabolic responses exhibited by β cells and discuss how this affects islet function and glucose homeostasis. We also discuss the implications of heterogeneity for the treatment of diabetes, including β cell replacement therapy.

β cell metabolism is central to secretory function

Islets of Langerhans consists of α, β, δ, polypeptide P (PP) and ε cells ([41] and references therein). The rodent islet typically consists of a core of β cells with a mantle of the other cell types [42], whilst human islets exhibit more intermingling of the different cell types [43]. β cells are the predominant cell type in mammalian islets of Langerhans typically comprising ~70% of cells in rodent and ~ 50% in human islets [44]. Islets themselves make up about 0.7% of the volume of the pancreas and are the only source of circulating insulin [45]. β cell function - the ability to release insulin in response to changes in the concentration blood glucose or other stimuli such as incretin hormones - is the sum of cell autonomous function for each β cell within the islet in response to external stimuli and the interaction of the cells within the islet [46].

β cells secrete insulin in response to changes in blood glucose concentration (see Fig. 1; reviewed in [47]). Insulin secretion is tightly regulated through the metabolism of glucose in these cells. The "canonical" pathway for glucose-stimulated insulin secretion is summarised in Figure 1 and is as follows. Glucose is transported in to β cells by the low affinity glucose transporter (GLUT2/SLC2A2): glucose concentrations equilibrate across the plasma membrane as the capacity for transport exceeds that for phosphorylation by the low affinity hexokinase, glucokinase [48]. Glucokinase is not inhibited by its own product, exhibits positive cooperativity at 4-10 mM glucose (i.e. within the normal physiological range) and has no/low specificities for other hexoses at physiologically relevant concentrations [49]. Gluconeogenesis is low in β cells due to limited phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase expression (www.biogps.org). Thus, glucose transport [50] and glucose phosphorylation [51] are important control points which contribute to the overall characteristics of insulin release observed *in vivo*., with glucokinase serving as the key "flux generating" [52-55] step in glycolytic metabolism.

ATP (or, more precisely, ATP/ADP ratio) is a central intracellular regulator of insulin release.

Moreover, mitochondrial metabolism of glycolytically-derived pyruvate and NADH are central to glucose sensing by pancreatic β cells [56-59]. Additionally, pyruvate carboxylase favours flux of pyruvate-derived carbons in to the tricarboxylic cycle, providing "anaplerotic" input to maintain adequate levels of citrate cycle intermediates [60, 61]. Uniquely in β cells, glycolytic flux is closely coupled to oxidative phosphorylation by suppression of lactate dehydrogenase (LDHA) [53, 62, 63] and monocarboxylate transporter (MCT-1/SLC16A1) [64-66] (two amongst a host of β cell "disallowed" genes, so called as they are expressed at high levels in other cell types; [67]; see below) expression, alongside high levels of glycerol phosphate dehydrogenase expression [53, 68-70].

Stimulation of intramitochondrial dehydrogenases, and increased mitochondrial membrane potential (occurring downstream of increases in cytosolic calcium concentration), enhance oxidative metabolism in response to high glucose [71-73]. Thus, under normal physiological conditions, β cells are poised to favour glycolytic and oxidative metabolism of glucose [70, 74] over alternative pathways [75], resulting in enhanced ATP synthesis. ATP leads to the closure of ATP-sensitive potassium channels [76, 77], leading to membrane depolarisation and the opening of voltage-gated calcium channels [78, 79]. Calcium entry via voltage-gated Ca^{2+} channels then leads to activation of secretory granule-associated small N-ethylmaleimide-sensitive factor receptor proteins [80], granule fusion with the plasma membrane [81], and insulin release. Aside from the above "K_{ATP} channel dependent" pathway, insulin secretion is amplified by K_{ATP}-channel independent, glucose metabolism-dependent pathways [59, 82]. Of particular recent interest is a pathway dependent on efflux of mitochondrial citrate and the generation of NAD(P)H in the cytosol [83] Several other oxidisable metabolites such as leucine, ketoisocaproate and glutamine are able to stimulate insulin release via the same metabolic ATP-dependent signalling pathways downstream of glucose entry [56, 57, 84] (Fig. 1).

Exposure to hyperglycaemia leads to alteration of the expression of the above and other "disallowed" genes involved in β cell metabolism, contributing to the disruption of β cell function [85]. Thus, islets from rats subjected to 90 % pancreatectomy (resulting in exposure to chronic hyperglycaemia) exhibit increased expression of genes involved in gluconeogenesis (glucose-6-phosphatase, fructose-1,6-bisphosphatase), regulation of pyruvate flux (lactate dehydrogenase, monocarboxylate transporters), and mitochondrial function (uncoupling protein 2), and decreased expression of GLUT2, representing loss of the metabolic programme that is characteristic of β cells (Fig. 1).

In summary, the β cell is an glucose sensor *par excellence* due its ability to enhance ATP production through the metabolism of glucose almost exclusively via glycolysis and oxidative phosphorylation [70, 74, 86, 87]. The ability of the β cell to release insulin in response to glucose is intrinsically linked to the unique metabolism exhibited by this cell type.

β cells display heterogeneous metabolic profiles

Individual β cells have different sensitivities to glucose.

It has been known since the 1980s that individual rat islet β cells display different sensitivities to glucose and this had an impact on their ability to synthesise and secrete insulin [7-15]. Rodent β cells increase the synthesis and secretion of insulin in response to glucose by recruiting β cells in a dose-dependent manner [8, 11, 12]. It was found that β cells from dispersed rat islets could be broadly separated in to two groups: high (which secreted insulin avidly) or low (which secreted less insulin) responsive populations [10, 13, 88]. These highly responsive β cells released insulin at lower stimulatory glucose concentrations, and released more insulin in response to glucose in a dose-dependent manner, than the low responsive β cells. High responsive β cells also displayed increased insulin synthesis [7, 10, 13] and preferential release of the newly synthesised insulin [10, 13], but accounted for only a small

proportion of the total β cell population. For example, in rats, only 18% of β cells were highly glucose responsive [7], but this population expands following sustained exposure to high glucose concentrations [88], possibly as an adaptive mechanism. The increased insulin biosynthesis observed in these highly responsive β cells was not associated with altered electrophysiological characteristics of the membrane [14] but was associated with increased glucose-induced metabolism in the highly responsive β cells [7], suggesting that metabolic heterogeneity may account for the functional diversity between the two β cell populations. The above heterogeneity in response to glucose may be beneficial in that it allows fine-tuning of insulin release through the concerted action of β cells with different sensitivities to glucose, and may be a mechanism to avoid hypoglycaemia through excessive insulin release. This phenomenon is also observed in human islets. Antigenically-distinct populations of FACS-purified β cells were shown to have diverse gene expression profiles, and distinct sensitivities to glucose as an insulin secretagogue (as determined by functional assays using pseudo-islet aggregates of purified β cell sub-types) [34]. Some of the differentially expressed genes within these β cell subpopulations in human islets are associated with β cell maturation, glucose metabolism, insulin secretion, and the pathophysiology of type 2 diabetes [34]. These populations of β cells with heterogeneous function are present in normal adult islets and their distribution was found to be altered in type 2 diabetes [34].

The differences in glucose responsiveness are known to be due (at least in part) to differences in the expression of genes encoding for enzymes involved in the regulation of glucose metabolism (discussed in the next section). However, there is evidence that the heterogeneity in metabolic responses may also be due to factors other than the islet cells themselves. For example, two subpopulations of islets which had different abilities to secrete insulin had previously been identified based on the degree of vascularisation occurring in the islets [89]. Thus, islets which were highly vascularised exhibited better β cell function with higher metabolic activity than low-oxygenated islets [89], i.e. metabolic heterogeneity could result

from differential exposure to the cellular environment. It has previously been reported that 20-25 % of the islets in intact rat pancreata were hypoxic; the hypoxic islets had lower metabolic activity and were thought to represent a dormant subpopulation that can be recruited on metabolic demand, which effectively serves as a reserve population that can be activated on demand [90]. Could this population of hypoxic islets be recruited via adaptive alterations in vascularisation induced by changes in the system's metabolic status? This is a question that merits an answer, and can be investigated by using state-of-the-art techniques where islets are grafted in the anterior chamber of the eye, subjected to changes in the milieu, with dynamic imaging of changes in the cells and the surrounding tissue [40, 91-94].

Differences between β cell can be ascribed to altered gene expression.

In the last decade, there has been a renewed interest in β cell heterogeneity, an interest reignited due to the possibility to study this phenomenon using single cell transcriptomics, and other omics, alongside functional analysis of single β cells within the islet. The concept that β cells have a specific gene expression signature is not new, and indeed we have known for over two decades that the expression of certain metabolic and other housekeeping genes are selectively "disallowed" in the β cell (reviewed in [47]). The advent of techniques (e.g. massive parallel sequencing; RNASeq) which allow gene expression screening, in combination with methods for cell type enrichment, gave us a picture of the gene expression profile one could expect from a *single* islet cell. Thus, single cell RNASeq has revealed heterogeneous gene expression between the same type of islet cells (reviewed in [95]), and indicated that our definitions of cell identity may be inadequate. Although limited to the expression of the most abundant transcripts (typically ~ 5,000 transcripts at optimal read depth; this limitation has significant implications for the ability to determine if heterogeneous detection is the same as heterogeneous expression [96]), it has been reported that heterogeneity in the expression of genes is apparent in single β cells and includes genes involved in endoplasmic reticulum stress, β cell maturation and β cell function [97-100]. This approach has led to the identification

of five potentially novel sub-types of β cells [99]. These findings are reminiscent of the four subtypes of human β cells identified based on differences in the abundance of candidate proteins determined using mass cytometry in single human islet cells [101]. Whether increases in heterogeneity can occur as part of the loss of insulin secretory function in disease states including T2D has also been considered [99, 102]. Disruption of expression of some of the genes that have been implicated with increased risk of type 2 diabetes, as identified by genome wide association studies, have also been shown to lead to loss of β cell connectivity [103, 104], further implicating loss of coordinated β cell action as part of the disease mechanism.

Immature β cells display elevated expression of genes that are normally expressed during β cell development, have low insulin content and poor insulin secretion, and gene markers which indicate the potential for proliferation [102, 105, 106], reinforcing the idea that the gene expression programme for proliferation and differentiation are incompatible phenomena [107]. Likewise, β cells that have been exposed to hyperglycaemia dedifferentiate (at least partially) and also display gene expression profiles that are akin to that seen in cells undergoing the β cell developmental programme [85, 102]. Single cell RNASeq of human islets revealed that β cells from type 2 diabetic donors reacquire the gene expression profiles described for developing endocrine cells, suggesting dedifferentiation [100]. A recent study using single cell RNASeq on human islets demonstrated that human islet cells can dedifferentiate and transdifferentiate *ex vivo*, and that gradual cell fate transitions may occur [108] (Fig.2). Thus, Teo and colleagues detected the presence of polyhormonal cells, confirming data from a previous study from the Kaestner group [100]. Another study found overlap in gene expression signatures between major and minor islet cell types [109]. A sub-population of functionally immature β cells that exist at the islet periphery, so called “virgin” (urocortin-3-negative) β cells that represent an intermediate stage in the transdifferentiation of α cells into mature β cells, thought to represent a neogenic niche that serves as a source for β cell

243 replenishment, were recently identified in mice [110]. Thus, the different subgroups of β cells
244 may represent β cells undergoing gradual cell fate transitions *in vivo* (Fig. 2), with adaptation
245 of the metabolic gene expression profile to allow function given a particular metabolic status.
246 Furthermore, a recent elegant study using isotope microscopy using mouse tissues revealed
247 that pancreatic endocrine cells, including β cells, can have widely varying ages i.e. some β
248 cells replicate whilst others do not [111]; this observation was also made in zebrafish, where
249 functional heterogeneity arose due to the presence of older and younger β cells that display
250 different glucose responsiveness [112]. Could some of the older β cells have had a "previous
251 life" as another cell type?
252
253 Alteration of β cell identity may not only pertain to the pathophysiology of type 2 diabetes: Non
254 obese diabetic (NOD) mice were found to harbour a subset of β cells which acquire a
255 senescence-associated secretory phenotype (SASP) which is underpinned by alterations in
256 gene expression [113]. One of the challenges now is to try to amalgamate the information
257 that has been gained through the identification of these various sub-groups to form a coherent
258 road map of β cell function within the environment of an islet. To start with, is there any overlap
259 between the subgroups identified using different markers? Were the different subgroups of
260 β cells generated along defined developmental programmes, or on ones which deviate from
261 the developmental programme that we have currently defined for archetypal β cells? These
262 questions merit answers and pain-staking efforts at single cell transcriptomics may yield these
263 answers. However, these studies reveal that our current definition of what constitutes a β cell
264 is probably inadequate and we likely need a better definition to take the field forward. Of note,
265 lineage tracing approaches (e.g. as used in mouse models [114, 115]) are likely to be required
266 to demonstrate cell fate transitions, as recently demonstrated in human islets [116].
267
268 *β cells within an islet operate as an interconnected, but hierarchical, syncytium: the hub cell*
269 *hypothesis*

One of the possibilities that emerges from the demonstration of β cell heterogeneity is that different subpopulations may adopt discrete roles within a functioning islet syncytium, with individual cells or subgroups performing a controlling or pace-making role through intercellular interactions. We know, for example, that dispersed individual β cells secrete insulin less well than clusters of cells, a gain of function likely due to cell-cell coupling [117] via the presence of cell-to-cell adhesion and/or junctional communication between cells [11, 118-120]. Loss of junctional communication has been shown to lead to altered stimulation threshold and kinetics of insulin release in rodent islets, leading to less efficient insulin secretion in response to nutrient load [118, 119], i.e. the cells in the islets of Langerhans exhibit cooperativity. Moreover, there is heterogeneity in the expression patterns for genes encoding for gap junction proteins in β cells, indicating differences in the connected nature between individual β cells [121]. This phenomenon is found in both rodent and human islets [39, 121, 122], (reviewed in [123]). However, the degree of cooperativity between β cells in human islets was enhanced only in the presence of glucagon-like peptide 1, and was inhibited in the presence of free fatty acids [124]. Human β cells also exhibit heterogeneity, and cooperativity between β cells is a function of this heterogeneity [39].

Importantly, a sub-population of β cells within the islet, which exhibit a less mature phenotype (albeit with some critical exceptions; see below), and have higher mitochondrial membrane potential, appear to be the regulators or "hub" cells which make connections with, and control the activities of, other "follower" β cells [39]. Recently, we have shown that this same hierarchy is apparent in islets engrafted in the anterior chamber of the eye [40], where the islets become revascularised and innervated, as well as in the living fish embryo [40]. In the latter species, photo-ablation of "hub", but not "follower" cells, led to a loss of islet-wide Ca^{2+} dynamics, confirming a role for these cells in islet pace-making in the living animal. Of note, however, it was recently suggested that cell-cell connectivity and synchronicity was not evident in adult zebrafish islets, which do not express a homologue of mammalian connexin 36 (important for

297 β cell connectivity in mammalian islets), although the lack of tight cell-cell coupling of
298 intracellular calcium responses did not impair β cell glucose responsiveness [125]. In contrast,
299 our own findings [126] have demonstrated excellent cell-cell coupling and synchronisation in
300 adult fish islets. The reasons for these differing findings are not clear: differences in islet
301 isolation procedure or culture conditions may be involved. Zebrafish have four Cx36-like
302 proteins which display more than 85% homology to mammalian Cx36 [127]. Thus, whilst
303 zebrafish β cells may not express Cx35b [125], it seems quite likely that other Cx36
304 homologues are expressed and able to form functional gap junctions between zebrafish β
305 cells.
306
307 Whilst hub/follower cell ablation experiments have not, as yet, been performed on islets
308 transplanted into the mouse eye, Granger causality analysis – an approach used in finance to
309 identify institutions whose performance predicts that of others across a sector, e.g. banking
310 [128] – showed the existence of "Granger leaders" i.e. likely hub cells, in islets that have
311 been transplanted at this site [40].
312
313 Examined *ex vivo*, hub cells displayed increased expression of glucokinase relative to
314 followers, indicating that these cells are sensitised to glucose and have a high metabolic rate
315 [39]. Correspondingly, analysis of published RNASeq data allowed imputation, based on the
316 expression of high levels of *GK* and lower levels of *Pdx1* and *Ins1* in the hub versus the
317 follower population, of elevated levels of other genes involved in glucose oxidation, and
318 defined a tentative transcriptome of these cells [40]. Future studies in which each population
319 is identified and then labelled (e.g. with the use of photo-convertible fluorescent proteins), and
320 subjected to RNASeq, will be required to confirm or refute these findings. Of particular interest
321 will be to establish the stability of each population. Do hubs display a particular localisation
322 e.g. proximity to capillaries or other islet cell types? Of note, our recent findings [40] assessing
323 this behaviour *in vivo* in the anterior eye chamber or living fish embryo indicate that hub/leader

cells tend to be more towards the periphery of the islet than was apparent in isolated islets [39], emphasizing the importance of studying the islet in its native context.

Interestingly, enhanced sensitivity to glucose may also be the reason why these cells are more susceptible to diabetic insults [39] and their loss or dysfunction may thus be critical to islet dysfunction in type 2 and, conceivably, type 1 diabetes.

Is the "less mature" phenotype a consequence of this increased sensitivity to diabetic insults, i.e. dedifferentiation in the face of metabolic stress, or is it a characteristic of this sub-type of β cells? To answer this question, techniques that allow us to trace cell fate over time will be required. This is not a trivial task as the "identity" of the sub-types of cells is defined by the cell's gene expression programme and there are few methods which allow us to track changes in a substantial number of gene targets in living cells by lineage tracing (see above). Alternatively, we could consolidate the data that is already available to describe different sub-types of β cells, as identified by different cellular/functional markers, and use this reconciled information to guide how we track the cells over time, in a refinement of the approach we have adopted recently [40].

We note that the influence of overall islet function of any one β cell will be determined by signalling events within the cell itself, and by signalling events of neighbouring cells within a syncytium, with a subset of β cells able to influence the actions of a group of others, thereby allowing coordinated islet activity [118], i.e. the islet is in effect a syncytium of mosaic cells. This raises the intriguing possibility that the ability to identify and modulate the activity of these cells, and/or create and replace such cells, may have therapeutic potential.

It is important to note that the hub cell hypothesis has been challenged by data indicating that the manifestation of cellular heterogeneity, as defined by synchronicity of alterations in

intracellular calcium and NAD(P)H responses, are not apparent in earlier studies in the intact islet [119, 120, 129], where the gradient established by glucose entry was found to be more important in determining the strength of response of individual cells within the islet. Echoing these earlier findings, the calcium signal in β cells within an islet where hub cells have been silenced, is diminished in amplitude [39], but temporal synchronisation was still apparent [39], possibly indicating the existence of other pathways for synchronisation. Additionally, where NAD(P)H autofluorescence was measured as an output to assess metabolic heterogeneity, whilst a large proportion of beta cells did not display heterogeneous responses to glucose (leading to the conclusion that metabolic heterogeneity was not important, and supporting the importance of a glucose gradient), a small proportion of the β cells were different in the pattern of their responses, and these regions of differences can be seen in the periphery (where the calcium signal was initiated in response to glucose) and the core (where the calcium signal spread) of the islet [129]. This is not divergent from the hub cell hypothesis, in as much as only a few hub cells display a different metabolic profile [39, 40]. It must be emphasized that imaging experiments providing data both for and against the existence of hub cells have largely been performed using islet cultures in suspension. It is unclear whether the cellular dynamics, potentially induced by diffusion effects affecting glucose concentrations in the islet in such experimental systems [119], exist *in vivo* where glucose is delivered by the capillary network. Our recent careful assessment of the changes in calcium responses in individual β cells within islets *in vivo* in the living zebrafish before and after ablation of the hub cells, and in engrafted mouse islets in the eye chamber subjected to causality analysis (see above) offer further support for the existence of hub cells [39]. Hub deletion assays in the intact perfused pancreas will provide the acid test for the existence of hub cells *in vivo* but represent a considerable challenge.

Translational perspectives

There has been much interest in the ability to preserve, conserve, and/or enhance β cell function, and in β cell replacement either from regeneration or from a heterologous source, as a treatment for diabetes. β cell heterogeneity is an important consideration for all therapeutic strategies since heterogeneity appears to be a component of normal islet function. For example, studies by Meda and colleagues [15] showed that high and low responsive β cells, which together accounted for 75% of the β cell population, retain their enhanced or diminished ability to secrete insulin after multiple stimulations. However, ca 25 % of β cells were able to shift between the two states, i.e. there is a subpopulation of low responsive β cells which are able to shift to a high responsive state. These low responsive cells may indeed be the immature or dedifferentiated β cells that we discussed in an earlier section of this article, which exhibit flexibility in cell identity. Are these cells related to the hub cells that we described in the previous section? Are they the same cells? What about the "sleeping β cells" which have apparently escaped immune destruction that are found in type 1 diabetes (reviewed in [130])? What are the hallmarks of these β cells and could they share the same immature phenotype, to allow them to escape death? Careful cross reference of single cell transcriptomic data obtained from these "different" sub-populations- identified using different cell markers- followed by "wet" tests (e.g. optogenetics, real-time imaging of cells in a more natural environment, etc) may shed light on these questions.

It may be possible to harness the apparent flexibility in cell identity of regenerative subpopulations of β cells for replacement therapy. It is currently unclear whether these flexible cells are from the same cell population that has been previously identified as bihormonal or polyhormonal [100, 102, 108, 131-137], as observed in the foetal pancreas [138, 139], and are more dedifferentiated. The frequency of bihormonal cells are reported to increase in type 2 diabetes [132], but there is currently no information as to how these cells came about and no functional data from such cells, so it is difficult to ascertain what role they may or may not play in metabolic control. To elucidate this we may need methods to allow us to trace cell fate

over time- do these cells "mature" and become functional β cells or do they serve another function? It may be possible to harness the β cell's ability to shift to a high responsive state (i.e. mature) to enhance functional β cell mass in the diabetic state, an adaptive response which may already exist *in vivo* [88]. It is clear that our current definition of what constitutes a functional β cell is deficient as it is based on the presence of cell maturity markers, the ability to respond to glucose, and the ability to secrete insulin. There are clearly β cells that do not have (all) these properties but do exert important roles in regulating the overall function of the islet [140] and, therefore, glucose homeostasis. In fact, β cells seem to exist on an identity spectrum. Thus, we need to evaluate the bigger picture when we come to assess endocrine pancreas health and function, rather than just focus on the classical parameters we have been using to define cell identity. The fact that not all β cells are the same may also impact on how we interpret functional data that we obtain from our current *in vitro* and *in vivo* genetic manipulation models. Most of these involve a binary on-off switch for the expression of specific genes but in reality the regulation of gene expression in the same cell type within the islet is more nuanced, which asks questions as to how accurately we are replicating the "normal" system outside of the single manipulation that we wanted to effect.

Concluding remarks and future perspectives

Where do the different populations of β cells come from, i.e. can we identify precursor populations for each? If so, do the different sub-populations of β cells follow different developmental programmes? Or could these cells have originated from progenitor cells from different parts of the developing pancreas? Are hub cells permanently tasked to this role, or is there a "duty roster" whereby individual cells are promoted to and then demit from this role? How are signals transmitted from hubs to followers? Our current working model is that most human β cells originate from progenitor cells that follow a particular developmental programme, with β cells dedifferentiating and losing function in the "diseased" state, or

transdifferentiating from other cell types as an adaptive response. Our new insights in to β cell heterogeneity demonstrate that there are certainly still many questions to answer possibly with the advent of new technologies (reviewed in [141]). It is clear that it is insufficient to just have β cells to replicate the precise homeostatic function exerted by the islet *in vivo*. Since the islet operates as an intricate controlled functional unit, in the context of stem cell therapy, is it necessary to recapitulate the whole complement of high and low responsive β cells (and other islet cell types with their heterogeneous counterparts [142]) with correct architecture to recreate an islet that behaves as nature intended? There is still much work to be done.

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Figure legends

Figure 1. Canonical pathway for glucose-stimulated insulin secretion. Pathways normally active in the β cell are in black; "disallowed" pathways are in grey.

Figure 2. Fate transitions *in vivo*? There is evidence in the literature that suggests that cells can change identities, possibly adopting intermediate states in between. Mature β cells have been postulated to arise from immature β cells, which may or may not be the same immature cells that constitute hub cells, which in turn can be derived from other islet cell types, pancreatic ductal cells, islet progenitor cells, etc.

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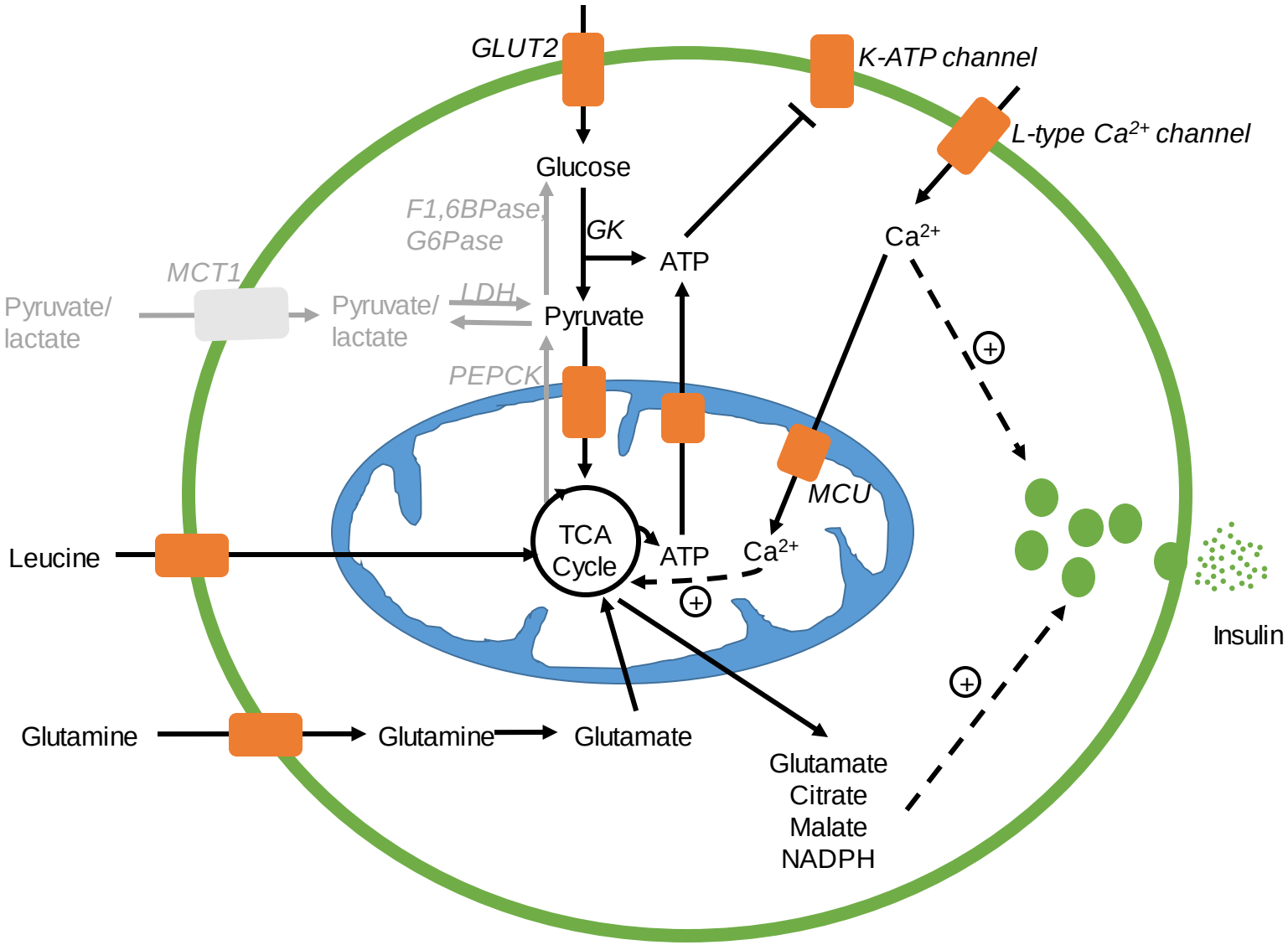
GLUT2
K-ATP channel
L-type Ca2+ channel
Glucose
F1,6BPase,
G6Pase
GK
ATP
Ca2+
MCT1
Pyruvate/
lactate
Pyruvate/
lactate
LDH
Pyruvate
PEPCK
MCU
TCA
Cycle
ATP
Ca2+
Leucine
Insulin
Glutamine
Glutamine
Glutamate
Glutamate
Citrate
Malate
NADPH

Figure 1

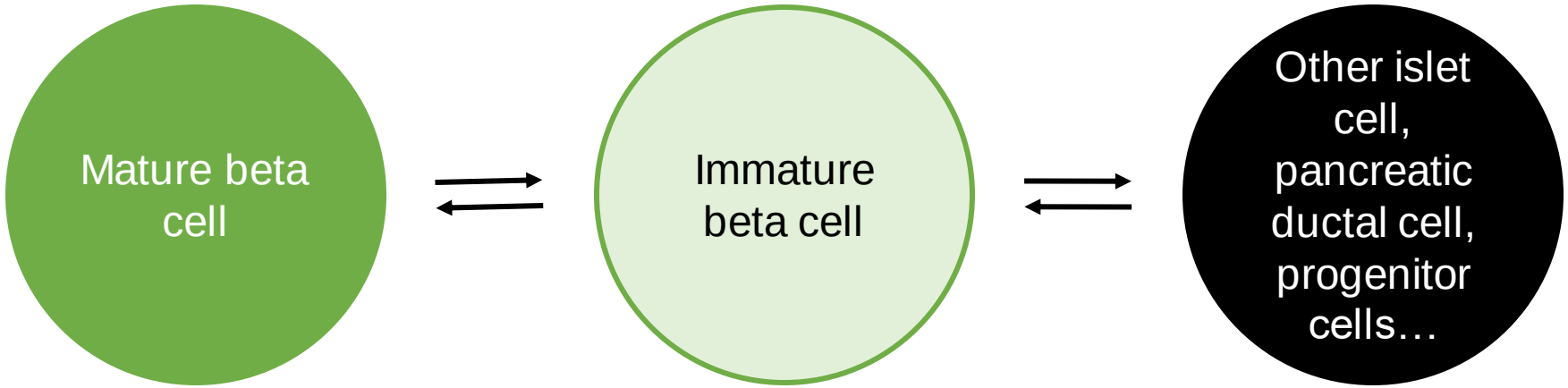
Mature beta cell
Immature beta cell
Other islet cell, pancreatic ductal cell, progenitor cells…

Figure 2