

## Beta cell connectivity in pancreatic islets

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## **REVIEW**

### **Beta cell connectivity in pancreatic islets: a type 2 diabetes target?**

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1 **Summary sentence:** beta cell communication during type 2 diabetes

2

3 **Pubmed was searched using combinations of the following:** “islet”, “diabetes”, “electrical”,  
4 “activity”, “channels”, “calcium”, “sodium”, “potassium”, “incretin”, “GLP-1”, “GIP”,  
5 “coordination”, “synchrony”, “dynamics”, “gap junction”, “connexin 36”, “paracrine”, “architecture”,  
6 “structure”, “autocrine”, “genetics”, “neural”, “tcf7l2”, “adcy5” and “gipr”.

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8

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10

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12

1 **Abstract**

2 Beta cell connectivity describes the phenomenon whereby the islet context improves insulin  
3 secretion by providing a three-dimensional platform for intercellular signaling processes. Thus,  
4 the precise flow of information through homotypically interconnected beta cells leads to the  
5 large-scale organization of hormone-release activities, influencing cell responses to glucose and  
6 other secretagogues. Although a phenomenon whose importance has arguably been under-  
7 appreciated in islet biology until recently, a growing number of studies suggest that such cell-cell  
8 communication is a fundamental property of this micro-organ. Hence, connectivity may plausibly  
9 be targeted by both environmental and genetic factors in type 2 diabetes mellitus (T2DM) to  
10 perturb normal beta cell function and insulin release. Here, we review the mechanisms that  
11 contribute to beta cell connectivity, discuss how these may fail during T2DM, and examine  
12 approaches to restore insulin secretion by boosting cell communication.

13

14

15

16

17 **Abbreviations:** AC, adenylyl cyclase; ACh, acetyl-choline; ADP, adenosine diphosphate; ATP,  
18 adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Cx36, connexin 36; Epac,  
19 exchange protein activated by cAMP; fMCI, functional multicellular calcium imaging; GABA,  
20 gamma aminobutyric acid; GIP, glucose-dependent insulinotropic polypeptide; GJ, gap junction;  
21 GLP-1, glucagon-like peptide-1; GWAS, genome-wide association studies; GPCR, G-protein  
22 coupled receptor;  $K_{ATP}$ , ATP-sensitive  $K^+$  channel; SST, somatostatin; SNP, single nucleotide  
23 polymorphism; T2DM, Type 2 diabetes mellitus; VDCC, voltage-dependent  $Ca^{2+}$ -channel.

## 1 **Introduction**

2 Type 2 diabetes mellitus (T2DM) is a global epidemic that currently consumes ~10% of the  
3 healthcare budget in the developed world [1]. This syndrome has a complex aetiology but can be  
4 summarised as a failure of the beta cell mass to adequately compensate for insulin resistance, or  
5 alternatively a primary beta cell defect that leads to insulin resistance. The resulting glucose  
6 intolerance, coupled with dyslipidemia, drives a range of costly secondary complications  
7 including retinopathy, vasculopathy, renal failure, cancer and cardiovascular disease [2,3].  
8 Consequently, elucidation of the mechanisms underlying the control of insulin secretion from  
9 individual beta cells has been the focus of intense research efforts. Thus, in response to an  
10 elevation of blood glucose, equilibration of the sugar across the plasma membrane occurs rapidly  
11 and is achieved *via* either the low affinity glucose transporter *Glut2/slc2a2* (rodents) or the higher  
12 affinity transporter *Glut1/slc2a1* (man) [4]. The low affinity hexokinase, glucokinase is then  
13 chiefly responsible for determining glycolytic flux towards pyruvate [5]. Conversion of the latter  
14 to acetyl-CoA in the mitochondrial matrix, and its oxidation via the tricarboxylate cycle, then  
15 ensues [6,7]. The resultant increases in the ratio of free ATP to ADP (ATP:ADP) in the cytosol  
16 [8] and sub-plasma membrane domain [9] then leads to closure of ATP-sensitive K<sup>+</sup> channels  
17 (K<sub>ATP</sub>), membrane depolarisation and the influx of calcium (Ca<sup>2+</sup>) through voltage-dependent  
18 Ca<sup>2+</sup>-channels (VDCC) [6,7,10,11]. Together with the activation of a less well-defined  
19 “amplifying” pathway [12,13], localized increases in the intracellular free Ca<sup>2+</sup> concentration  
20 [14], including at the surface of the secretory granule [15], then provoke insulin release through  
21 interactions with the exocytotic machinery [16,17].

22

23 By comparison, the population-level regulation of insulin release is less well understood,  
24 although the idea that it may contribute to T2DM risk has been suggested [18-22]. Providing  
25 evidence that cell-cell interactions are a prerequisite for proper hormone secretion is the  
26 observation that beta cells *incommunicado* (*i.e.* as isolated cells) release less insulin *per capita*  
27 than their properly-connected counterparts within the intact islet [19,23,24]. Indeed, a feature of  
28 the endocrine pancreas is the three-dimensional encapsulation of beta, and other cell types, into  
29 islets of Langerhans, a biological scaffold for cell-cell communications. Since these microorgans  
30 are conserved throughout the mammalian kingdom and beyond [25], albeit with important  
31 differences in the numbers of each cell type and their arrangement within the islet (see below),  
32 the intraislet mechanisms governing insulin secretion may represent an underappreciated target  
33 through which T2DM insults provoke hyperglycemia. Building upon recent findings from our  
34 own [26-28] and others’ [20-22,29-31] laboratories, the aim of the present review is to describe  
35 our current understanding as to how beta cell-beta cell communication (hereafter referred to as

1 “connectivity”) contributes to the normal regulation of insulin secretion in healthy subjects. We  
2 also discuss how changes in this property may contribute to T2DM risk in genetically-susceptible  
3 individuals.

#### 5 **Islets as discrete secretory units**

6 The term “endocrine pancreas” describes the thousands (millions in man) of islets of Langerhans  
7 scattered throughout the exocrine tissue. Each islet can range in size from 20-400  $\mu\text{M}$  and  
8 comprises alpha- (glucagon), beta- (insulin), delta- (somatostatin), epsilon- (ghrelin) and  
9 pancreatic polypeptide (PP) cells. Strikingly, islets are evolutionarily-stable structures and are  
10 present in most mammals studied to date, including the Beluga whale, with a similar range of  
11 sizes reported in each species [25]. With the exception of bats, horses, hyenas, primates and  
12 humans, the arrangement of endocrine cells within islets is similar [25]. Thus, in rodent islets, the  
13 most-studied model, beta cells form a central core, with alpha cells occupying the mantle  
14 [25,32,33]. Suggesting that this may be a consequence of the vasculature, blood flow has been  
15 shown to follow an inner-outer flow pattern, irrigating beta before alpha cells in this species [34],  
16 and the vasculature appears to be instructive for pancreas development [35]. By contrast, beta  
17 cells in human islets are interspersed with alpha cells, in part the consequence of the tertiary  
18 folding of an initial trilaminar alpha-beta-alpha sheet, which promotes heterologous contacts  
19 [33,36-38]. As well as differences in islet architecture, alterations to cell proportion are also  
20 apparent between species. For example, the ratio of beta:alpha cells in rodent islets is  $\sim 4:1$ ,  
21 whereas in humans it is  $\sim 1.25:1$ . Such divergence in islet architecture likely influences cell-cell  
22 communication by altering the extent and nature of cell-cell signaling processes, and may be an  
23 important source of species differences in islet function. Regardless, the islet structure is  
24 permissive for insulin secretion, and beta cells in two dimensions display blunted responses to  
25 input, both in terms of  $\text{Ca}^{2+}$  signaling and magnitude hormone release [27,39-41].

#### 27 **High-speed imaging of beta cell connectivity**

28 Over the last decade, advances in microscopy have allowed cell dynamics to be monitored *in situ*  
29 within the intact tissue setting [42]. Key to this is the use of high-speed imaging, which when  
30 combined with highly-sensitive detectors, allows a large area to be rapidly traversed at cellular  
31 resolution. In terms of endocrine organ function, the physiologically relevant output is hormone  
32 release. However, large-scale imaging of exocytosis in individual cells is only just becoming  
33 possible, although the currently available dyes possess signal-to-noise ratios incompatible with  
34 high-speed acquisition at visible light wavelengths [43-46]. To circumvent these issues,  
35 membrane voltage or intracellular  $\text{Ca}^{2+}$  concentrations can instead be used as a proxy for  $\text{Ca}^{2+}$ -

1 dependent hormone release [47-50]. To this end, functional multicellular  $\text{Ca}^{2+}$  imaging (fMCI),  
2 originally used to map activity in cortical circuits [51-53], has recently been adapted for use in  
3 beta cells [27,28]. By coupling a laser bank to a Nipkow spinning disk, the millisecond  
4 organization of beta cell population  $\text{Ca}^{2+}$ -spiking activity can be captured in near real-time with  
5 reduced phototoxicity and photobleaching. Following acquisition, the datasets are subjected to  
6 non-deterministic Monte Carlo-based models to identify the cells with similar behavioural  
7 profiles, *i.e.* those with correlated activity and which are assumed to contribute to the same  
8 secretory process [42,54]. Statistical significance is determined by shuffling the experimental  
9 dataset and calculating the likelihood of detecting the same correlation pattern due to chance. A  
10 functional connectivity map can then be constructed based on the location of significantly  
11 correlated cells pairs, allowing perturbations to beta cell connectivity to be evaluated (see Figure  
12 1, top panel, for an example). In a refinement of this method, beta cell metabolic interconnectivity  
13 has recently been mapped in intact islets by monitoring intracellular free ATP:ADP dynamics, as  
14 for  $\text{Ca}^{2+}$  [55]. When using these techniques, it is important to note that the territories of  
15 communicating beta cells within intact islets are larger than those that can be recorded, limiting  
16 any physiological inferences that can be drawn.

17

### 18 **Islet wiring patterns**

19 Network science principally relies on the use of graph theory to identify the interactions that  
20 govern behavior in complex systems (see [42] for a review of network science in Endocrinology).  
21 Using these approaches, it has become increasingly clear that network topology tends to be  
22 conserved (*e.g.* scale-free and random) irrespective of the components examined (*e.g.* cells *versus*  
23 people) [42,56,57]. Recent research has shown that graph theory is also applicable to the  
24 description of complex dynamics in the endocrine pancreas. Thus, analysis reveals that beta cells  
25 comprise glucose-responsive scale-free networks in which cells can communicate over long  
26 distances, through presently undefined mechanisms [29]. Such network topologies are defined by  
27 a power-law distributed link probability in which a minority of cells (termed highly-connected  
28 nodes) host the majority of connections and are said to possess small-world properties if there is a  
29 tendency towards formation of cliques (six degrees of separation concept) (Figure 1, bottom  
30 panel). Price was the first to describe scale-free networks, noting that journal citations follow a  
31 power-law distribution, sharing features in-keeping with Pareto's law (the 'rich-get-richer'  
32 hypothesis) [58]. Subsequently, Barabasi and Albert showed that preferential attachment is  
33 responsible for the emergence of scale-free properties [59]. Notably, scale-free distributions are  
34 ubiquitous and have been described in social networks, computer networks, neural networks and  
35 anterior pituitary networks [54,60-63]. An important feature of scale-free networks is robustness

1 at low wiring cost: the chances of a random attack disabling communication are low and the use  
2 of hubs to route information reduces signal transmission length [42]. However, should the highly-  
3 connected nodes be specifically targeted, the network is vulnerable to collapse, since a high  
4 proportion of links will be lost (Figure 1, bottom panel). Therefore, an interesting but untested  
5 possibility is that highly-connected beta cell nodes may represent a subpopulation which is  
6 particularly susceptible to T2DM insults. Conversely, these highly-connected nodes may serve as  
7 a functional reserve to maintain islet function in the face of gross perturbation by allowing the re-  
8 distribution of information, again, a hypothesis that requires experimental validation.

### 9 10 **Mechanisms underlying beta cell-beta cell connectivity**

11 Neural circuits have a clear basis for long-range connectivity, since neurons send out axonal  
12 projections that can form synapses located millimetres apart. By contrast, it is less easy to  
13 conceptualize how beta cells within the islet can communicate over long distances to organize  
14 their activities. Might this involve, for example, “physical connections” (*e.g.* through islet inter-  
15 neurons) between remote cells, or alternatively linearly-connected “trains” of beta or other cells  
16 along which signals are transmitted to a distant cell(s) from a controller (“pacemaker”) at a  
17 coordination hub? In any case, the islet possesses a formidable signaling toolbox (see Figure 2).  
18 This is reviewed in depth elsewhere [20,21,28,64], so here we limit our discussion to the  
19 pathways which may conceivably underlie connectivity between beta cells.

20  
21 **Gap junctions:** The best characterized cell-cell coupling mechanism in the pancreas is provided  
22 by gap junctions (GJ). Beta cells within rodent and human islets are homotypically-connected by  
23 connexin 36 (Cx36 or GJD2) [65,66]. GJs comprised of Cx36 are charge and size-selective  
24 channels that allow the intercellular passage of ions (*e.g.*  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  and  $\text{Zn}^{2+}$ ) and nucleotides  
25 (*e.g.* ATP) [19,20,67]. Providing evidence that Cx36 is critical for coordinating islet activity are  
26 the observations that dispersed beta cells fail to synchronize their responses to glucose, and islets  
27 lacking Cx36 display more stochastic activity patterns due to increases in beta cell functional  
28 heterogeneity [31,68-70]. GJ-linkages are essential for the regulation of normal hormone release,  
29 since mice deleted for Cx36 are glucose intolerant and display impaired pulsatility, as well as  
30 elevated basal insulin secretion [22,68,71]. It is unclear how GJs could account for the long-range  
31 functional connections that project between distant cells, as practically all beta cells express Cx36  
32 protein, meaning that communication should encompass even close neighbors [65,72]. However,  
33 heterogeneity exists in fluorescence recovery after photobleaching (FRAP) within islets [73],  
34 suggesting that connectivity patterns between individual beta cells may at least reflect differences  
35 in functional GJ coupling. As proposed above, this may lead to the formation of linear groups of

1 cells, tightly interconnected in three dimensions between one another, but (relatively) isolated  
2 from neighboring cells outside the train, thus forming a conduit for the passage of ionic ( $\text{Ca}^{2+}$ ) or  
3 other (*e.g.* paracrine, see below) signals.

4  
5 **Neural:** Islets receive rich innervation from the autonomic nervous system, and neural regulation  
6 of insulin secretion is critical for normal glucose homeostasis *in vivo*. The existence of a physical  
7 network of neurons to couple remote beta cells within the islet thus provides a conceptually  
8 straightforward model to explain recent experimental observations [26,27,29]. Indeed, insulin  
9 release is strongly stimulated by postganglionic cholinergic fibres that signal via acetylcholine  
10 (ACh)-mediated activation of muscarinic receptors to phase-set and synchronize beta cell activity  
11 within and, potentially, between islets [74-76]. Such activation underpins the cephalic phase of  
12 insulin secretion in anticipation of food [77]. In addition, other neuropeptides including pituitary  
13 adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) may  
14 contribute to the parasympathetic control of beta cell function [74,78]. By contrast, insulin release  
15 is suppressed by noradrenergic sympathetic neurons that signal via  $\alpha$ 2-adrenoreceptors to open  
16  $\text{K}_{\text{ATP}}$  channels [74,79,80], although a stimulatory effect of noradrenaline has also been observed,  
17 probably through effects on cAMP accumulation and  $\beta$ -adrenoreceptor activation [81,82].  
18 Marked differences exist in the neural regulation of insulin secretion between rodents and man.  
19 Thus, human islets are relatively devoid of parasympathetic nerve fibres [83], and glucose-  
20 sensitization of beta cell activity instead relies upon ACh release from vesicular acetylcholine  
21 transporter-expressing alpha cells [84,85]. This lack of direct innervation may partly explain why  
22 beta cell glucose responses in human islets are largely stochastic, with synchrony detected only  
23 between small cell clusters [27,33,86]. Conversely, the assessment of whether neurons contribute  
24 to long-range connectivity in mouse islets firstly requires confirmation of cholinergic fibre  
25 survival in isolated islets, followed by their specific manipulation (*e.g.* using patch clamp).

26  
27 **Primary cilia:** Cilia can be regarded as cell extensions that act as signaling hubs due to  
28 expression of G-protein coupled receptors, ion channels and transcription factors [87]. Primary  
29 cilia are immotile and are formed from a ring of nine microtubule doublets wrapped in a  
30 membrane sheath [88]. While studies of *Kif3a*, *Lkb1* and *Rfx3* knockout mice have all invoked a  
31 role for cilia in pancreatic development (*i.e.* ductal and endocrine cell specification) [87,89-91],  
32 little is known about their involvement in cell-cell signaling processes within the islet. Given the  
33 role of cilia in signal transmission in in other tissues [92], and potentially in exosome-mediated  
34 intercellular communications [93], we believe this warrants further investigation.



1 ***Paracrine signaling:***

2 Intercellular communication may also be possible *via* the production and secretion of messengers  
3 which act on neighboring cells [20,21,28]. Over 230 secreted factors have been identified in  
4 rodent islets [94], and a number of signalling loops with roles in the regulation of beta cell  
5 function and insulin release are now well characterised (see references [21,28,64]). Despite this, it  
6 is unclear how paracrine factors could contribute to the complex functional islet wiring patterns  
7 described using graph theory [29,30], since all beta cells within the molecule diffusion path  
8 would be expected to be affected. Although it is plausible that active transport mechanisms and  
9 cognate receptor expression levels/patterns may allow more precise communication between beta  
10 cells, this needs further study.

11

12 Despite the plethora of signaling mechanisms available within the islet, we suggest that a  
13 combination of modalities is required for producing the complex activity patterns that underlie  
14 beta cell-beta cell communication and connectivity. Notably, differences in signaling input,  
15 together with alterations to islet architecture, may play an important role in determining species-  
16 specific responses to secretagogues such as glucose and incretins.

17

18 **Glucose and GLP-1-regulated connectivity: metabolic signals**

19 It is generally acknowledged that metabolic activity within individual beta cells is oscillatory, and  
20 that this generates the membrane bursting activity required for  $\text{Ca}^{2+}$  influx and exocytosis [95].  
21 Whether metabolic oscillations are driven by  $\text{Ca}^{2+}$  oscillations, or *vice versa*, is still the source of  
22 debate [95,96], but the islet context seems to be critical, since dispersed beta cells display reduced  
23 periodicity in mitochondrial potential [97]. Moreover, total internal reflection fluorescence  
24 (TIRF) microscopy of mouse islets has shown that near-membrane glucose-induced oscillations  
25 in ATP:ADP are coordinated between small beta cell clusters [98], confirming earlier  
26 observations that employed lower resolution autofluorescence imaging of NAD(P)H [99-101].  
27 The mechanisms underlying the synchronous propagation of energy status between beta cells  
28 remain unknown, but may reflect  $\text{Ca}^{2+}$  feedback and intrinsic metabolic behaviour [96], or  
29 alternatively, metabolic coupling *via* GJs [102,103].

30

31 In addition to glucose, secretory potentiators, including members of the incretin family, are able  
32 to influence beta cell energetics. The incretin, glucagon-like peptide 1 (GLP-1), is released from  
33 the gut in response to bile transit and glucose-dependently augments insulin secretion [104-106].  
34 While its effects on cAMP-Epac2, MAPK and beta-arrestin signaling pathways are well-  
35 characterised [107-109], little is known about whether GLP-1 alters the beta cell metabolic

1 setpoint to influence ATP:ADP. Whereas luciferase-based studies by us have demonstrated a role  
2 for GLP-1 in mitochondrial ATP synthesis in clonal MIN6 beta cells [110], others have observed  
3 no effect of the incretin in rodent islets using biochemical detection methods [111]. Since ATP  
4 dynamics and/or cell heterogeneity may mask actions of incretin on metabolism, the effects of  
5 GLP-1 on intracellular free ATP:ADP were monitored with cellular resolution by expressing the  
6 recombinant probe Perceval throughout the first few layers of rodent and human islets [8,55,112].  
7 Using these methods, we found that GLP-1 engages a metabolically-coupled subnetwork of beta  
8 cells to amplify insulin secretion, an action that is dependent upon  $\text{Ca}^{2+}$  influx and elevations in  
9 cAMP [55]. Of note, in these studies, beta cells within mouse islets responded coordinately to  
10 GLP-1 with synchronous ATP:ADP oscillations, whereas human islets exhibited more random  
11 dynamics. Thus, the regulation of beta cell-beta cell metabolic connectivity may potentially  
12 contribute to the disparate actions of incretin in rodents and man, although confirmation of this  
13 will require simultaneous measures of  $\text{Ca}^{2+}$  and ATP:ADP in islets of both species.

14

#### 15 **Glucose- and GLP-1-regulated connectivity: $\text{Ca}^{2+}$ signals**

16  $\text{Ca}^{2+}$ -imaging of pancreatic islet slices has revealed that glucose likely drives large-scale increases  
17 in population synchrony by coaxing activity in a scale-free and small-world network of beta cells  
18 [29,30,49]. Notably, propagation of  $\text{Ca}^{2+}$  waves *via* GJs is hypothesised to underlie islet dynamics  
19 in response to glucose, since the length of individual correlated links depends on Euclidean  
20 distance, although long-range communications are still evident [29]. Confirming these findings,  
21 we have recently shown that the rapid (ms) oscillations in electrical activity are similarly dictated  
22 by scale-free and small-world beta cell wiring patterns [113]. Thus, under conditions of high  
23 glucose, beta cells work together as defined subpopulations to orchestrate and drive insulin  
24 release from the islet.

25

26 As well as glucose, insulin secretion is also reliant upon the amplifying or potentiating actions of  
27 incretins. Indeed, in humans, almost 70% of the insulin-raising effects of oral glucose challenge  
28 can be attributed to the incretin effect [114]. Notably, the insulinotropic activity of exogenously-  
29 administered GIP and GLP-1 is diminished in T2DM [115,116], suggesting that altered beta cell  
30 incretin responsiveness may contribute to the disease state, although causality is not well defined  
31 [117]. Since the single biggest T2DM risk factor remains obesity, and high BMI individuals  
32 present with reduced GLP-1-stimulated insulin secretion [118,119], excess lipid may target  
33 incretin action to impair beta cell function. To investigate this, we subjected human islets to fMCI  
34 to map population dynamics, and found that both GIP and GLP-1 recruit a highly coordinated  
35 subnetwork of GJ-coupled beta cells to augment insulin secretion [27,28]. This process of

1 incretin-regulated beta cell connectivity may be a target for the insulin-lowering effects of free  
2 fatty acid (FFA), since it could be disrupted in a GJ-dependent manner following exposure to a  
3 lipotoxic milieu, and was inversely correlated with donor BMI [27]. Mechanistically, this may  
4 involve FFA-induced overexpression of inducible cAMP early repressor gamma (ICER- $\gamma$ ), a  
5 protein that binds a cAMP-response element in the Cx36 promoter [120,121]. By contrast, a  
6 similar effect of incretin on beta cell interactivity was not present in mouse islets, but could be  
7 revealed by placing mice on a high fat diet to disrupt normal glucose responses [27,28]. We  
8 therefore speculate that such divergent regulation of the incretin axis, potentially stemming from  
9 structural and functional differences in islet architecture, may represent a novel target for pro-  
10 diabetogenic insults in man.

11

## 12 **Genes and connectivity**

13 Type 2 diabetes has a strong hereditary component [122-124]. Consequently, genome wide  
14 association studies (GWAS) have identified a number of gene variants linked with an increased  
15 odds ratio (OR) of developing elevated fasting glucose and T2DM. Although the effects of these  
16 variants are usually quite small, their very existence indicates that genes in the associated loci are  
17 highly likely to play a role in disease aetiology [125,126]. While gene variants and glucose  
18 homeostasis are well studied in man, relatively less is known about their precise mechanisms of  
19 action at the islet level [125], and in particular upon beta cell connectivity. Several dozen (>90)  
20 risk-associated polymorphisms have been identified to date, and those with the strongest OR for  
21 development of T2DM, or with known effects on beta cell-cell communication, are discussed  
22 below (see Figure 3):

23

24 ***TCF7L2***: *TCF7L2* is a member of the canonical Wnt-signaling pathway and a transcriptional  
25 partner for beta-catenin. Individuals who possess a single nucleotide polymorphism (SNP),  
26 rs7903146, in intron 3 of the *TCFL72* gene on chromosome 10, have an increased risk of  
27 developing T2DM, with an OR of 1.45 for the T allele [127-130]. This is believed largely to be  
28 due to defects in insulin secretion (insulin sensitivity is slightly impaired in T allele carriers), as  
29 well as a markedly (~50%) attenuated incretin effect [127,131,132] (though see [125] for a  
30 discussion of a role for hepatic glucose handling). Although the subject of debate, these results  
31 have subsequently been confirmed in conditional rodent models and human islets. Thus, *TCF7L2*  
32 silencing leads to impaired insulin secretion from isolated mouse and human islets [133,134], and  
33 deletion of *Tcf7l2* throughout the pancreas or selectively in the beta cell causes glucose  
34 intolerance [135,136], particularly after oral glucose administration, with the observed effects  
35 increasing with age or exposure to a high fat diet (HFD). Of note, a further study failed to detect

1 any effects on glycemia of deleting *Tcf7l2* in the adult beta cell, although this report was  
2 restricted to examination of intraperitoneal glucose tolerance in young (<12 wks) animals [137].  
3 GLP-1-stimulated insulin secretion is strongly inhibited by *Tcf7l2* elimination *in vitro* [134,135],  
4 the latter due largely to reduced GLP-1R expression and defects in the exocytotic apparatus  
5 [133,135,138,139]. Interestingly, when investigated in dissociated islets, TCF7L2 knockdown  
6 leads to a slight potentiation of glucose-induced  $Ca^{2+}$  increases [133,140], although only single  
7 (or clusters) of beta cells were studied, precluding analysis of synchrony or coordination. By  
8 contrast, ablation of the *Tcf7l2* gene selectively in the beta cell through *Ins1Cre*-directed  
9 recombination of *flox*'d alleles impairs these increases when assessed in the intact islet setting  
10 [136]. The reasons for these differences remain obscure but suggest that either silencing in non-  
11 beta cells in the former case, or altered beta cell-beta cell interactions in the latter, are at play. Of  
12 note, *Tcf7l2* silencing in INS1 cells lowers the expression of  $Ca^{2+}$  channel subunits [141],  
13 suggesting that TCF7L2 may exert control, either directly or indirectly, over the  $Ca^{2+}$ -signaling  
14 machinery. Of relevance, when studied in islets from mice maintained on a high fat diet (HFD),  
15 glucose-stimulated beta cell connectivity in *Tcf7l2* null animals was significantly reduced *versus*  
16 that of control animals [136] (manuscript submitted). Of note, this alteration was not associated  
17 with any changes in GJ mRNA expression, though may conceivably involve changes in Cx36  
18 protein abundance.

19

20 **ADCY5:** ADCY5 gene products encode isoform V of the adenylate cyclase family, a type III  $Ca^{2+}$ -  
21 inhibited enzyme tasked with generation of cAMP [142,143], a second messenger involved in  
22 glucoregulation as part of the “amplifying” pathway [144]. Whereas other isoforms predominate  
23 in the rodent islet, ADCY5 is amongst the most abundant members of this family in human beta  
24 cells [26,145]. The T2DM-associated SNP rs11708067 on chromosome 3 lies within intron 3 of  
25 the *ADCY5* gene and is associated with increased fasting glucose and 2-hour glucose, but not oral  
26 glucose responses [146], with an OR of 1.23 for the major A-allele [147]. Using lentiviral shRNA  
27 approaches to silence gene and protein expression in human islets, we have recently shown that  
28 ADCY5 is required for the coupling of glucose but not incretin to insulin secretion [148].  
29 Although the former is partly due to impaired insulin processing (*i.e.* proinsulin → insulin  
30 conversion) [149], islets depleted for ADCY5 also displayed impaired glucose- but not GLP-1-  
31 induced increases in cAMP, and consequent impairments in glucose-induced metabolism  
32 (ATP:ADP ratios). Moreover, ADCY5-silenced islets showed more stochastic long-term  
33 evolutions in coordinated beta cell activity following glucose exposure [148]. By contrast, GLP-  
34 1-regulated connectivity was normal, suggesting that ADCY5 is unlikely to link incretin signaling  
35 to cAMP generation and beta cell communication. Thus, ADCY5 preferentially affects glucose-

1 induced human islet dynamics, possibly through cAMP, which has been shown to increase GJ  
2 conductance and trafficking [22,73,150], although this has only been so far demonstrated in  
3 rodent tissues.

4  
5 **ZnT8:** The R325W variant of SLC30A8, the gene encoding zinc transporter 8 (ZnT8), is  
6 associated with reduced insulin secretion. ZnT8 is highly expressed in beta cells where its  
7 activation leads to Zn<sup>2+</sup> accumulation in secretory granules, promoting normal insulin  
8 crystallization, storage and processing [151-154]. While global *ZnT8* deletion results in mild  
9 insulin secretory deficits, which are only observed *in vivo* and are undetectable at the dispersed  
10 islet level [151,152], beta-cell specific deletion of the same gene has been reported either to  
11 inhibit [153] or stimulate [155] insulin release from isolated islets. Indeed, it has been suggested  
12 that defects in glycemia resulting from either global or beta cell specific ZnT8 elimination  
13 [152,153,155] are due to enhanced insulin clearance by the liver [155]. In any case, and  
14 complicating the picture further, rare loss-of-function mutations in *SLC30A8* protect against  
15 T2DM in man [156]. Nonetheless, alterations in ZnT8 expression lead to altered Ca<sup>2+</sup>/Zn<sup>2+</sup>-  
16 handling [133,152,157], and GJ gating is dependent on fine-regulation of both ions in the vicinity  
17 of the plasma membrane [158,159]; whether this also applies to islets is unknown. Thus, while an  
18 effect of *ZnT8* risk alleles on beta cell-beta cell connectivity is not entirely implausible, further  
19 studies are required to assess effects of the gene on coordinated activity and the mechanisms  
20 underlying this (*e.g.* changes in Cx36 expression or GJ function).

21  
22 It should be noted that the studies concerning *ADCY5*, *TCF7L2* and beta cell connectivity were  
23 conducted on models in which expression has essentially been eliminated (through gene silencing  
24 or genomic deletion). It is likely that any phenotype observed *in vivo* in man is a consequence of  
25 more subtle cellular changes coupled with exposure to a permissive environment. It remains to be  
26 seen whether similar effects can be recapitulated in tissue obtained from normoglycemic donors  
27 harboring specific risk alleles. Lastly, even the strongest GWAS hits only marginally contribute  
28 to T2DM risk and effects of gene variants on beta cell coordination should not be overinterpreted  
29 in the absence of defined mechanisms/targets.

### 30 31 **Rescuing beta cell connectivity during T2DM**

32 Since the intraislet regulation of insulin release may be altered by both genes and the environment  
33 to reduce insulin secretion, beta cell connectivity may represent a novel target for the  
34 pharmaceutical restoration of functional beta cell mass. While up-regulated GJ-signaling provides  
35 a logical starting point for the enhancement of beta cell connectivity, investigation of Cx36-

1 modulating compounds has so far been complicated by their off-target effects. Notwithstanding, a  
2 recent study has described a panel of seventeen molecules that increase beta cell-beta cell  
3 communication, and further screening is warranted to validate their activity profiles and  
4 specificity [160]. In addition, atlases of both GPCR and paracrine factor expression/secretion  
5 have been reported for human and rodent islets [94,161], potentially accelerating the elucidation  
6 and development of putative candidates for manipulation of beta cell connectivity. Alternatively,  
7 personalized medicine/deep-phenotyping approaches [162] could be used to identify individuals  
8 where the beneficial effects of GLP-1 and GIP to enhance beta cell connectivity may be exploited  
9 [27,28]. For example, carriers of ADCY5 risk alleles are predicted to respond well to the insulin-  
10 raising actions of the incretins, as this gene preferentially impacts glucose action [148]. By  
11 contrast, obese subjects would potentially benefit more from the pro-communicatory effects of  
12 the sulfonylureas due to altered GLP-1 and GIP signaling inputs [27,163,164]

13

#### 14 **Future perspectives**

15 The network description of beta cells is still in its infancy and more refined methods are required  
16 to better delineate connection topology. Without statistical methods such as Granger causality it  
17 is impossible to say whether coordinated behavior in an individual cell is the origin or  
18 consequence of the connections it shares with its neighbours [42,165]. Likewise, our  
19 understanding of the structural basis for functional connectivity is presently lacking and imaging  
20 approaches are required that allow the large-scale interrogation of any underlying physical cell-  
21 cell linkages. This is particularly applicable to human islets, where differences in architecture  
22 may lead to divergent regulation of insulin secretion and susceptibility to T2DM insults  
23 [28,37,64]. Lastly, it remains unknown how beta cell population dynamics are influenced by  
24 episodes of functional/pathological plasticity in the pancreas, and whether a wiring footprint  
25 persists during T2DM that can be exploited to restore insulin secretion.

26

#### 27 **Summary**

28 The three-dimensional organization of beta cells into islets produces a gain-of-function in insulin  
29 release by fine-tuning beta cell intercommunication. Each islet operates as a self-supported  
30 signaling unit in which the spatiotemporally-precise propagation of information between  
31 neighboring and distant cell ensembles is facilitated by GJ, neural and paracrine communications.  
32 Using imaging approaches together with statistical methods borne from graph theory, the flow of  
33 information throughout the beta cell population can be monitored online and mapped. Pertinently,  
34 coordinated activity in rodent islets appears to be driven and orchestrated by a subpopulation of  
35 beta cells, and wiring density can be increased by both glucose and incretin to stimulate hormone

1 release. We therefore propose that, alongside “cell autonomous” effects, environmental and  
2 genetic insults may target the inraislet regulation of insulin secretion to precipitate beta cell  
3 dysfunction and glucose intolerance, contributing to the risk of developing T2DM.

4

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1 **FIGURE LEGENDS**

2  
3 **Figure 1: Imaging and mapping beta cell network topology.** (Above) Functional multicellular  
4  $\text{Ca}^{2+}$  imaging is used to monitor the large-scale organization of glucose-induced population  
5 dynamics (*above, left*). By subjecting the resulting traces (from ~ 50-100 individual cells per  
6 islet) to correlation analyses, cells with coordinated activity can be identified and a functional  
7 connectivity map plotted based upon position within the imaged field (x-y) (*above, right*). Scale-  
8 free connection distributions are typified by a minority of cells that host the majority of  
9 connections (nodes), while maintaining streamlined information flow due to a short pathlength.  
10 Although robust in the face of random attack, they are prone to collapse following a targeted  
11 attack (*below, left*). By contrast, non-scale free networks (*e.g.* random or lattice) may not  
12 efficiently propagate signals due to a long pathlength, and random attacks significantly reduce  
13 capacity (*below, right*).  
14

15 **Figure 2: Schematic showing single cell and population level beta cell signaling.** At the  
16 molecular level, glucose is transported into the beta cell before undergoing glycolysis to increase  
17 the ratio of free cytosolic ATP:ADP. This closes  $\text{K}_{\text{ATP}}$  channels, leading to opening of VDCC,  
18  $\text{Ca}^{2+}$  influx and  $\text{Ca}^{2+}$ -dependent exocytosis. At the population level, beta cell dynamics are further  
19 dictated by signaling circuits involving paracrine, juxtacrine, autocrine, electrotonic (GJ), neural  
20 and ciliary communications.  
21

22 **Figure 3: Potential mechanisms by which T2D-associated genes may alter beta cell**  
23 **connectivity.** *ZnT8* gene variants disrupt cytosolic  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  handling, and both of these ions  
24 are required for normal GJ activity. *ADCY5* gene variants decrease glucose-stimulated cAMP  
25 rises, a second messenger shown to increase GJ communications between beta cells. By contrast,  
26 *TCF7L2* gene variants may disrupt normal GJ function through effects upon glucose-stimulated  
27  $\text{Ca}^{2+}$  increases, as well as GLP-1-stimulated cAMP generation.  
28  
29