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REVIEW

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

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

Keywords Mouse · Human · Signaling · Insulin · Diabetes · Imaging · Network

Abbreviations

AC	Adenyl cyclase
ACh	Acetylcholine
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
Cx36	Connexin 36
Epac	Exchange protein activated by cAMP
fMCI	Functional multicellular calcium imaging
GABA	Gamma aminobutyric acid
GIP	Glucose-dependent insulinotropic polypeptide
GJ	Gap junction
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association studies
GPCR	G protein-coupled receptor
K_{ATP}	ATP-sensitive K^+ channel
SST	Somatostatin
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
VDCC	Voltage-dependent Ca^{2+}-channel

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Introduction

Type 2 diabetes mellitus (T2DM) is a global epidemic that currently consumes ~10 % of the healthcare budget in the developed world [1]. This syndrome has a complex etiology but can be summarized as a failure of the beta cell mass to adequately compensate for insulin resistance, or alternatively a primary beta cell defect that leads to insulin resistance. The resulting glucose intolerance, coupled with dyslipidemia, drives a range of costly secondary complications including retinopathy, vasculopathy, renal failure, cancer, and cardiovascular disease [2, 3]. Consequently, elucidation of the mechanisms underlying the control of insulin secretion from individual beta cells has been the focus of intense research efforts. Thus, in response to an elevation of blood glucose, equilibration of the sugar across

the plasma membrane occurs rapidly and is achieved via either the low affinity glucose transporter Glut2/*slc2a2* (rodents) or the higher affinity transporter Glut1/*slc2a1* (man) [4]. The low affinity hexokinase, glucokinase is then chiefly responsible for determining glycolytic flux toward pyruvate [5]. Conversion of the latter to acetyl-CoA in the mitochondrial matrix, and its oxidation via the tricarboxylate cycle, then ensues [6, 7]. The resultant increases in the ratio of free adenosine triphosphate (ATP) to adenosine diphosphate (ADP) (ATP:ADP) in the cytosol [8] and subplasma membrane domain [9] then leads to closure of ATP-sensitive K^+ channels (K_{ATP}), membrane depolarization and the influx of calcium (Ca^{2+}) through voltage-dependent Ca^{2+}-channels (VDCC) [6, 7, 10, 11]. Together with the activation of a less well-defined "amplifying" pathway [12, 13], localized increases in the intracellular free Ca^{2+} concentration [14], including at the surface of the secretory granule [15], then provoke insulin release through interactions with the exocytotic machinery [16, 17].

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

Islets as discrete secretory units

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

High-speed imaging of beta cell connectivity

Over the last decade, advances in microscopy have allowed cell dynamics to be monitored in situ within the intact tissue setting [42]. Key to this is the use of high-speed imaging, which when combined with highly sensitive detectors, allows a large area to be rapidly traversed at cellular resolution. In terms of endocrine organ function, the physiologically relevant output is hormone release. However, large-scale imaging of exocytosis in individual cells is only just becoming possible, although the currently available dyes possess signal-to-noise ratios incompatible with high-speed acquisition at visible light wavelengths [43–46]. To circumvent these issues, membrane voltage or intracellular Ca^{2+} concentrations can instead be used as a proxy for Ca^{2+}-dependent hormone release [47–50]. To this end, functional multicellular Ca^{2+} imaging (fMCI), originally used to map activity in cortical circuits [51–53], has recently been adapted for use in beta cells [27, 28]. By coupling a laser bank to a Nipkow spinning disk, the millisecond organization of beta cell population Ca^{2+}-spiking activity can be captured in near real time with reduced phototoxicity and photobleaching. Following acquisition, the 1datasets are subjected to nondeterministic Monte

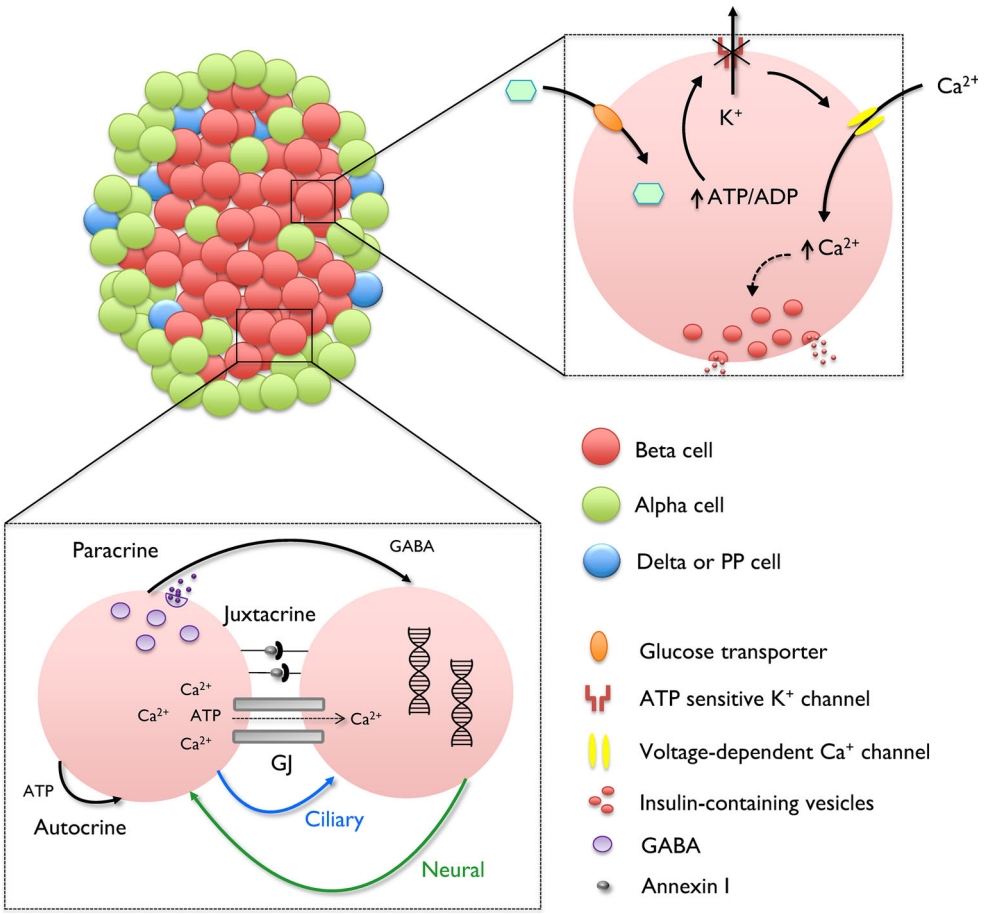

Fig. 1 Imaging and mapping beta cell network topology. (*Above*) Functional multicellular Ca^{2+} imaging is used to monitor the large-scale organization of glucose-induced population dynamics (*above*, *left*). By subjecting the resulting traces (from ~ 50–100 individual cells per islet) to correlation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

Carlo-based models to identify the cells with similar behavioral profiles, i.e., those with correlated activity and which are assumed to contribute to the same secretory process [42, 54]. Statistical significance is determined by shuffling the experimental dataset and calculating the likelihood of detecting the same correlation pattern due to chance. A functional connectivity map can then be constructed based on the location of significantly correlated cell pairs, allowing perturbations to beta cell connectivity to be evaluated (see Fig. 1, top panel, for an example). In a refinement of this method, beta cell metabolic interconnectivity has recently been mapped in intact islets by monitoring intracellular free ATP:ADP dynamics, as for Ca^{2+} [55]. When using these techniques, it is important to note that the territories of communicating beta cells within intact islets are larger than those that can be recorded, limiting the physiological inferences that can be drawn.

Islet wiring patterns

Network science principally relies on the use of graph theory to identify the interactions that govern behavior in complex systems (see [42] for a review of network science in Endocrinology). Using these approaches, it has become increasingly clear that network topology tends to be conserved (e.g., scale-free and random) irrespective of the components examined (e.g., cells vs. people) [42, 56, 57]. Recent research has shown that graph theory is also applicable to the description of complex dynamics in the endocrine pancreas. Thus, analysis reveals that beta cells comprise glucose-responsive scale-free networks in which cells can communicate over long distances, through presently undefined mechanisms [29]. Such network topologies are defined by a power-law distributed link probability in which a minority of cells (termed highly connected nodes) host the majority of connections and are said to possess small-world properties if there is a tendency toward formation of cliques (6° of separation concept) (Fig. 1, bottom panel). Price was the first to describe scale-free networks, noting that journal citations follow a power-law distribution, sharing features in keeping with Pareto's law (the 'rich-get-richer' hypothesis) [58]. Subsequently, Barabasi and Albert showed that preferential attachment is responsible for the emergence of scale-free properties [59]. Notably, scale-free distributions are ubiquitous and have

been described in social networks, computer networks, neural networks, and anterior pituitary networks [54, 60–63]. An important feature of scale-free networks is robustness at low wiring cost: the chances of a random attack disabling communication are low and the use of hubs to route information reduces signal transmission length [42]. However, should the highly connected nodes be specifically targeted, the network is vulnerable to collapse, since a high proportion of links will be lost (Fig. 1, bottom panel). Therefore, an interesting but untested possibility is that highly connected beta cell nodes may represent a subpopulation which is particularly susceptible to T2DM insults. Conversely, these highly connected nodes may serve as a functional reserve to maintain islet function in the face of gross perturbation by allowing the redistribution of information, again, a hypothesis that requires experimental validation.

Mechanisms underlying beta cell–beta cell connectivity

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

Gap junctions

The best characterized cell–cell coupling mechanism in the pancreas is provided by gap junctions (GJs). Beta cells within rodent and human islets are homotypically connected by connexin 36 (Cx36 or GJD2) [65, 66]. GJs comprising Cx36 are charge- and size-selective channels that allow the intercellular passage of ions (e.g., Ca^{2+}, Na^{+}, and Zn^{2+}) and nucleotides (e.g., ATP) [19, 20, 67]. Providing evidence that Cx36 is critical for coordinating islet activity are the observations that dispersed beta cells fail to synchronize their responses to glucose, and islets lacking Cx36 display more stochastic activity patterns due to increases in beta cell functional heterogeneity [31, 68–70]. GJ linkages are essential for the regulation of normal hormone release, since mice deleted for Cx36 are glucose intolerant and display impaired pulsatility, as well as elevated basal insulin secretion [22, 68, 71]. It is unclear how GJs could account for the long-range functional connections that project between distant cells, as practically all beta cells express Cx36 protein, meaning that communication should encompass even close neighbors [65, 72]. However, heterogeneity exists in fluorescence recovery after photobleaching (FRAP) within islets [73], suggesting that connectivity patterns between individual beta cells may at least reflect differences in functional GJ coupling. As proposed above, this may lead to the formation of linear groups of cells, tightly interconnected in three dimensions between one another, but (relatively) isolated from neighboring cells outside the train, thus forming a conduit for the passage of ionic (Ca^{2+}) or other (e.g., paracrine, see below) signals.

Neural

Islets receive rich innervation from the autonomic nervous system, and neural regulation of insulin secretion is critical for normal glucose homeostasis in vivo. The existence of a physical network of neurons to couple remote beta cells within the islet thus provides a conceptually straightforward model to explain recent experimental observations [26, 27, 29]. Indeed, insulin release is strongly stimulated by postganglionic cholinergic fibers that signal via acetylcholine (ACh)-mediated activation of muscarinic receptors to phase set and synchronize beta cell activity within and, potentially, between islets [74–76]. Such activation underpins the cephalic phase of insulin secretion in anticipation of food [77]. In addition, other neuropeptides including pituitary adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) may contribute to the parasympathetic control of beta cell function [74, 78]. By contrast, insulin release is suppressed by noradrenergic sympathetic neurons that signal via α2-adrenoreceptors to open K_{ATP} channels [74, 79, 80], although a stimulatory effect of noradrenaline has also been observed, probably through effects on cyclic adenosine monophosphate (cAMP) accumulation and β-adrenoreceptor activation [81, 82]. Marked differences exist in the neural regulation of insulin secretion between rodents and man. Thus, human islets are relatively devoid of parasympathetic nerve fibers [83], and glucose-sensitization of beta cell activity instead relies upon ACh release from vesicular acetylcholine transporter-expressing alpha cells [84, 85]. This lack of direct innervation may partly explain why beta cell glucose responses in human islets are largely stochastic, with synchrony detected only between small cell clusters [27, 33, 86]. Conversely, the assessment of whether neurons contribute to long-range connectivity in mouse islets firstly requires confirmation of cholinergic

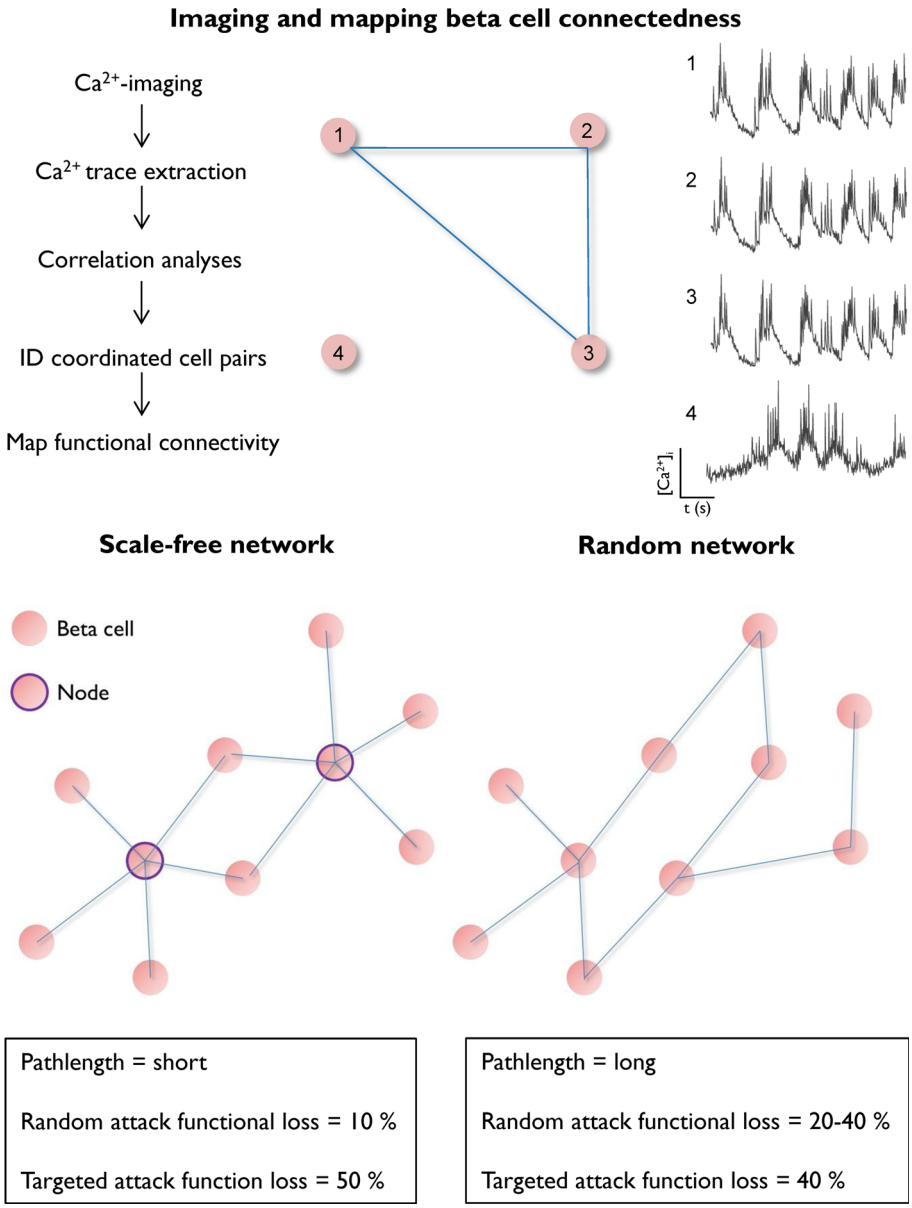

Fig. 2 Schematic showing single cell and population-level beta cell signaling. At the molecular level, glucose is transported into the beta cell before undergoing glycolysis to increase the ratio of free cytosolic ATP:ADP. This closes K_{ATP} channels, leading to opening of VDCC, Ca^{2+} influx, and Ca^{2+}-dependent exocytosis. At the population-level, beta cell dynamics are further dictated by signaling circuits involving paracrine, juxtacrine, autocrine, electrotonic (GJ), neural and ciliary communications

fiber survival in isolated islets, followed by their specific manipulation (e.g., using patch clamp).

Primary cilia

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

Paracrine signaling

Intercellular communication may also be possible via the production and secretion of messengers which act on neighboring cells [20, 21, 28]. Over 230 secreted factors have been identified in rodent islets [94], and a number of signaling loops with roles in the regulation of beta cell function and insulin release are now well characterized (see references [21, 28, 64]). Despite this, it is unclear how paracrine factors could contribute to the complex

functional islet wiring patterns described using graph theory [29, 30], since all beta cells within the molecule diffusion path would be expected to be affected. Although it is plausible that active transport mechanisms and cognate receptor expression levels/patterns may allow more precise communication between beta cells, this needs further study.

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

Glucose and GLP-1-regulated connectivity: metabolic signals

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

In addition to glucose, secretory potentiators, including members of the incretin family, are able to influence beta cell energetics. The incretin, glucagon-like peptide-1 (GLP-1), is released from the gut in response to bile transit and glucose-dependently augments insulin secretion [104–106]. While its effects on cAMP-Epac2, MAPK, and beta-arrestin signaling pathways are well characterized [107–109], little is known about whether GLP-1 alters the beta cell metabolic set point to influence ATP:ADP. Whereas luciferase-based studies by us have demonstrated a role for GLP-1 in mitochondrial ATP synthesis in clonal MIN6 beta cells [110], others have observed no effect of the incretin in rodent islets using biochemical detection methods [111]. Since ATP dynamics and/or cell heterogeneity may mask actions of incretin on metabolism, the effects of GLP-1 on intracellular free ATP:ADP were monitored with cellular resolution by expressing the recombinant probe Perceval throughout the first few layers of rodent and human islets [8, 55, 112]. Using these methods, we found that GLP-1 engages a metabolically coupled subnetwork of beta cells to amplify insulin secretion, an action that is dependent upon Ca^{2+} influx and elevations in cAMP [55]. Of note, in these studies, beta cells within mouse islets responded coordinately to GLP-1 with synchronous ATP:ADP oscillations, whereas human islets exhibited more random dynamics. Thus, the regulation of beta cell–beta cell metabolic connectivity may potentially contribute to the disparate actions of incretin in rodents and man, although confirmation of this will require simultaneous measures of Ca^{2+} and ATP:ADP in islets from both species.

Glucose- and GLP-1-regulated connectivity: Ca^{2+} signals

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

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

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

Genes and connectivity

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

TCF7L2

TCF7L2 is a member of the canonical Wnt-signaling pathway and a transcriptional partner for beta-catenin. Individuals who possess a single nucleotide polymorphism (SNP), rs7903146, in intron 3 of the *TCFL72* gene on chromosome 10, have an increased risk of developing T2DM, with an OR of 1.45 for the T allele [127–130]. This is believed largely to be due to defects in insulin secretion (insulin sensitivity is slightly impaired in T allele carriers), as well as a markedly (∼50 %) attenuated incretin effect [127, 131, 132] (though see [125] for a discussion of a role for hepatic glucose handling). Although the subject of debate, these results have subsequently been confirmed in conditional rodent models and human islets. Thus, TCF7L2 silencing leads to impaired insulin secretion from isolated mouse and human islets [133, 134], and deletion of *Tcf7l2* throughout the pancreas or selectively in the beta cell causes glucose intolerance [135, 136], particularly after oral glucose administration, with the observed effects increasing with age or exposure to an HFD. Of note, a further study failed to detect any effects on glycemia of deleting *Tcf7l2* in the adult beta cell, although this report was restricted to examination of intraperitoneal glucose tolerance in young (<12 weeks) animals [137]. GLP-1-stimulated insulin secretion is strongly inhibited by *Tcf7l2* elimination in vitro [134, 135], the latter due largely to reduced GLP-1R expression and defects in the exocytotic apparatus [133, 135, 138, 139]. Interestingly, when investigated in dissociated islets, TF7L2 knockdown leads to a slight potentiation of glucose-induced Ca^{2+} increases [133, 140], although only single (or clusters) of beta cells were studied, precluding analysis of synchrony or coordination. By contrast, ablation of the *Tcf7l2* gene selectively in the beta cell through Ins1*Cre*-directed recombination of *flox*'d alleles impairs these increases when assessed in the intact islet setting [136]. The reasons for these differences remain

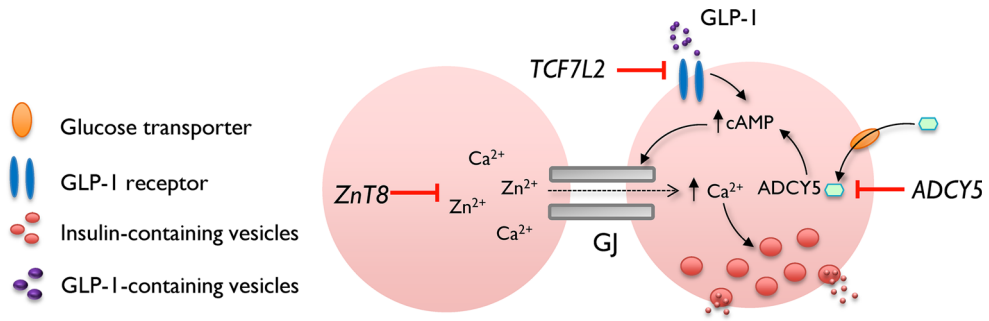

Fig. 3 Potential mechanisms by which T2D-associated genes may alter beta cell connectivity. *ZnT8* gene variants disrupt cytosolic Ca^{2+} and Zn^{2+} handling, and both of these ions are required for normal GJ activity. *ADCY5* gene variants decrease glucose-stimulated cAMP rises, a second messenger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

obscure but suggest that either silencing in nonbeta cells in the former case, or altered beta cell–beta cell interactions in the latter, are at play. Of note, *Tcf7l2* silencing in INS1 beta cells lowers the expression of Ca^{2+} channel subunits [141], suggesting that TCF7L2 may exert control, either directly or indirectly, over the Ca^{2+}-signaling machinery. Of relevance, when studied in islets from mice maintained on an HFD, glucose-stimulated beta cell connectivity in *Tcf7l2* null animals was significantly reduced versus that of control animals [136] (manuscript submitted). Of note, this alteration was not associated with any changes in GJ mRNA expression, though may conceivably involve changes in Cx36 protein abundance.

ADCY5

ADCY5 gene products encode isoform V of the adenylate cylase family, a type III Ca^{2+}-inhibited enzyme tasked with generation of cAMP [142, 143], a second messenger involved in glucoregulation as part of the "amplifying" pathway [144]. Whereas other isoforms predominate in the rodent islet, ADCY5 is among the most abundant members of this family in human beta cells [26, 145]. The T2DM-associated SNP rs11708067 on chromosome 3 lies within intron 3 of the *ADCY5* gene and is associated with increased fasting glucose and 2-h glucose, but not oral glucose responses [146], with an OR of 1.23 for the major A-allele [147]. Using lentiviral shRNA approaches to silence gene and protein expression in human islets, we have recently shown that ADCY5 is required for the coupling of glucose but not incretin to insulin secretion [148]. Although the former is partly due to impaired insulin processing (i.e., proinsulin → insulin conversion) [149], islets depleted for ADCY5 also displayed impaired glucose- but not GLP-1-induced increases in cAMP, and consequent impairments in glucose-induced metabolism (ATP:ADP ratios). Moreover, ADCY5-silenced islets showed more stochastic long-term evolutions in coordinated beta cell activity following glucose exposure [148]. By contrast, GLP-1-regulated connectivity was normal, suggesting that ADCY5 is unlikely to link incretin signaling to cAMP generation and beta cell communication. Thus, ADCY5 preferentially affects glucose-induced human islet dynamics, possibly through cAMP, which has been shown to increase GJ conductance and trafficking [22, 73, 150], although this has only been so far demonstrated in rodent tissues.

ZnT8

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

It should be noted that the studies concerning *ADCY5*, *TCF7L2* and beta cell connectivity were conducted on models in which expression has essentially been eliminated (through gene silencing or genomic deletion). It is likely that any phenotype observed in vivo in man is a consequence of more subtle cellular changes coupled with exposure to a permissive environment. It remains to be seen whether similar effects can be recapitulated in tissue obtained from normoglycemic donors harboring specific risk alleles. Lastly, even the strongest GWAS hits contribute only marginally (though in a statistically significant manner) to T2DM risk, and effects of gene variants on beta cell coordination are presently of uncertain importance in the absence of defined mechanisms/targets.

Rescuing beta cell connectivity during T2DM

Since the intraislet regulation of insulin release may be altered by both genes and the environment to reduce insulin secretion, beta cell connectivity may represent a novel target for the pharmaceutical restoration of functional beta cell mass. While upregulated GJ-signaling provides a logical starting point for the enhancement of beta cell connectivity, investigation of Cx36-modulating compounds has so far been complicated by their off-target effects. Notwithstanding, a recent study has described a panel of seventeen molecules that increase beta cell–beta cell communication, and further screening is warranted to validate their activity profiles and specificity [160]. In

addition, atlases of both GPCR and paracrine factor expression/secretion have been reported for human and rodent islets [94, 161], potentially accelerating the elucidation and development of putative candidates for manipulation of beta cell connectivity. Alternatively, personalized medicine/deep-phenotyping approaches [162] could be used to identify individuals where the beneficial effects of GLP-1 and GIP to enhance beta cell connectivity may be exploited [27, 28]. For example, carriers of ADCY5 risk alleles are predicted to respond well to the insulin-raising actions of the incretins, as this gene preferentially impacts glucose action [148]. By contrast, obese subjects would potentially benefit more from the pro-communicatory effects of the sulfonylureas due to altered GLP-1- and GIP-signaling inputs [27, 163, 164].

Future perspectives

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

Summary

The three-dimensional organization of beta cells into islets produces a gain of function in insulin release by fine-tuning beta cell intercommunication. Each islet operates as a self-supported signaling unit in which the spatiotemporally precise propagation of information between neighboring and distant cell ensembles is facilitated by GJ, neural, and paracrine communications. Using imaging approaches together with statistical methods borne from graph theory, the flow of information throughout the beta cell population can be monitored online and mapped. Pertinently, coordinated activity in rodent islets appears to be driven and orchestrated by a subpopulation of beta cells, and wiring density can be increased by both glucose and incretin to stimulate hormone release. We, therefore, propose that, alongside "cell autonomous" effects, environmental and genetic insults may target the intraislet regulation of insulin secretion to precipitate beta cell dysfunction and glucose intolerance, contributing to the risk of developing T2DM.

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Conflict of interest The authors have nothing to disclose.

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