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Pancreas islets in metabolic signaling - focus on the beta-cell

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1. ABSTRACT

The Islets of Langerhans form a nutrient sensing network spread throughout the pancreas. They are tightly connected to the source organ, the intestine, and the target organs - liver, muscle, and fat cells. The expression of a unique set of proteins enables beta cells, the most frequent islet cell type, to detect elevated blood glucose levels and secrete insulin accordingly. Clustered beta-cells achieve tighter regulation of glucose-induced insulin secretion by coordination through cell surface proteins. They also adjust their secretory capacity and flow to avoid being damaged. The immediate reaction of the beta cell to nutrients is regulated by translational mechanisms, while longer term adaptations involve changes in transcription. Glucose increases overall protein synthesis in beta-cells but selectively boosts translation of some secretory proteins including insulin. This may be mediated through recognition of RNA motifs in the untranslated regions of those messengers. If essential molecular components of this nutrient sensing system are broken or fail due to repeated stress, beta cells malfunction, which on a larger scale manifest as diseases like diabetes mellitus.

2. PANCREATIC ISLET MICROORGAN

2.1. The pancreas develops from endoderm

The pancreas develops from the innermost of the three germ layers called the endoderm. After the gut tube has formed in the embryo, several buds grow below the section that widens to become the stomach. These buds extend and branch to generate the liver, the gallbladder, and the pancreas rudiments (Figure 1).

A dorsal and a ventral bud make up the pancreas (1). They develop in an area of the gut tube that does not express the morphogen sonic hedgehog (2), otherwise present in all of the tube. The area is also characterised by the expression of pancreatic and duodenal homeobox 1 (Pdx1, also known as Ipf1) (3, 4). Many more signals act in concert to permit and instruct pancreas progenitors. The dorsal bud grows to make up the bulk of the mature pancreas. The ventral bud shares access to the gut with the liver and gallbladder and eventually rotates to fuse with the dorsal pancreas precursor. Dorsal and ventral ducts connect in 90% of humans and in 70% the dorsal connection to the intestine grows back, leaving only the joint duct with the

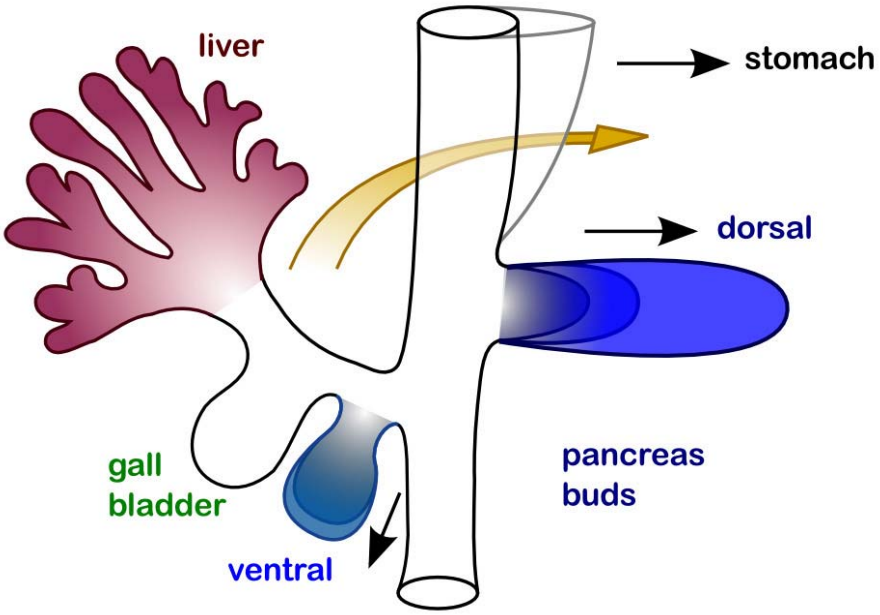

Figure 1. A ventral and a dorsal bud (both blue) fuse after rotation (yellow arrow) to form the pancreas. Black arrows indicate growth directions. The second ventral bud is omitted because it typically regresses. (CCby)

liver (5). However, there are species and individual variations.

The cells of the branching pancreatic buds differentiate according to their functions. The cells at the end of the branches form a cap of acinar (Latin: berry) cells, producing digestive juice. The cells lining the branches become ductal cell, transporting the products of the acinar cells to the intestine. During extension a special group of cells detaches from the branches and assembles into small colonies called pancreatic islets. These cells will eventually become the signalling centres of the pancreas. See (6, 7) for more detailed reviews of pancreas development.

2.2. The pancreas digests and signals the nutritional state

The pancreas has 2 functions. First, it secretes molecules into the intestine to facilitate digestion. Second, the peptides secreted by its islet cells signal to other organs involved in metabolism.

An adult pancreas weighs between 70-110 g (average 80 g) (8). 80% of the organ is dedicated to produce and deliver digestive enzymes and hydrogen carbonate (HCO_3^-). Hydrogen carbonate is a basic chemical which neutralises the hydrochloric acid (HCl) produced in the stomach. Digestive proteases, nucleases, and lipases are synthesised by the acinar cells of the pancreas to break down nutrients. Together with the neutralising agent the enzymes are collected in pancreatic ducts and finally secreted into the duodenum. The last millimetres of the pancreatic duct are typically shared with the gallbladder duct which transports the alkaline and fat-emulsifying bile from the liver. Bile facilitates the work of the pancreatic lipases.

A comparatively small but vital part of about 2% or 1-2 g of pancreatic mass (8) secretes instead into the blood stream to signal to receptive organs. It is a group of cells organised into tiny spheres or islets, which send peptides via adjacent blood vessels to the liver and from there to the heart and the rest of the body.

Islets are composed of 3 major, here defined as above 5% of all islet cells, and several minor cell types. Averaged over the total human pancreas, islet cells produce either insulin (67%, standard deviation (SD) = 9), glucagon (10%, SD=5), somatostatin (3%, SD=1), or pancreatic polypeptide (19%, SD=8) (9). A separate study estimates the frequency of ghrelin at 1% (10).

These figures, however, hide huge variations between distinct regions of the pancreas, between genders, and between species. The ventral bud develops into the posterior head of the pancreas which is about a tenth of the entire organ by mass and contains notably different islets (9). In rat, 1 in 5 islet cells in this region produces pancreatic polypeptide (PP) (11). In humans, 8 out of 10 cells synthesise PP and are therefore the dominant cell type (9, 8). The development of the pancreas from two buds results in two distinct regions with different islet types. This inhomogeneity of the pancreas, is the reason that two recent studies reported a different average islet composition. One study did not stain for PP (12), the other focused only on the dorsal, PP-poor lobe (13).

In addition, there are pronounced differences between species. Mice have more insulin and less glucagon cells compared to humans. Mouse islet cells also segregate to produce an insulin cell core and a mantle containing the

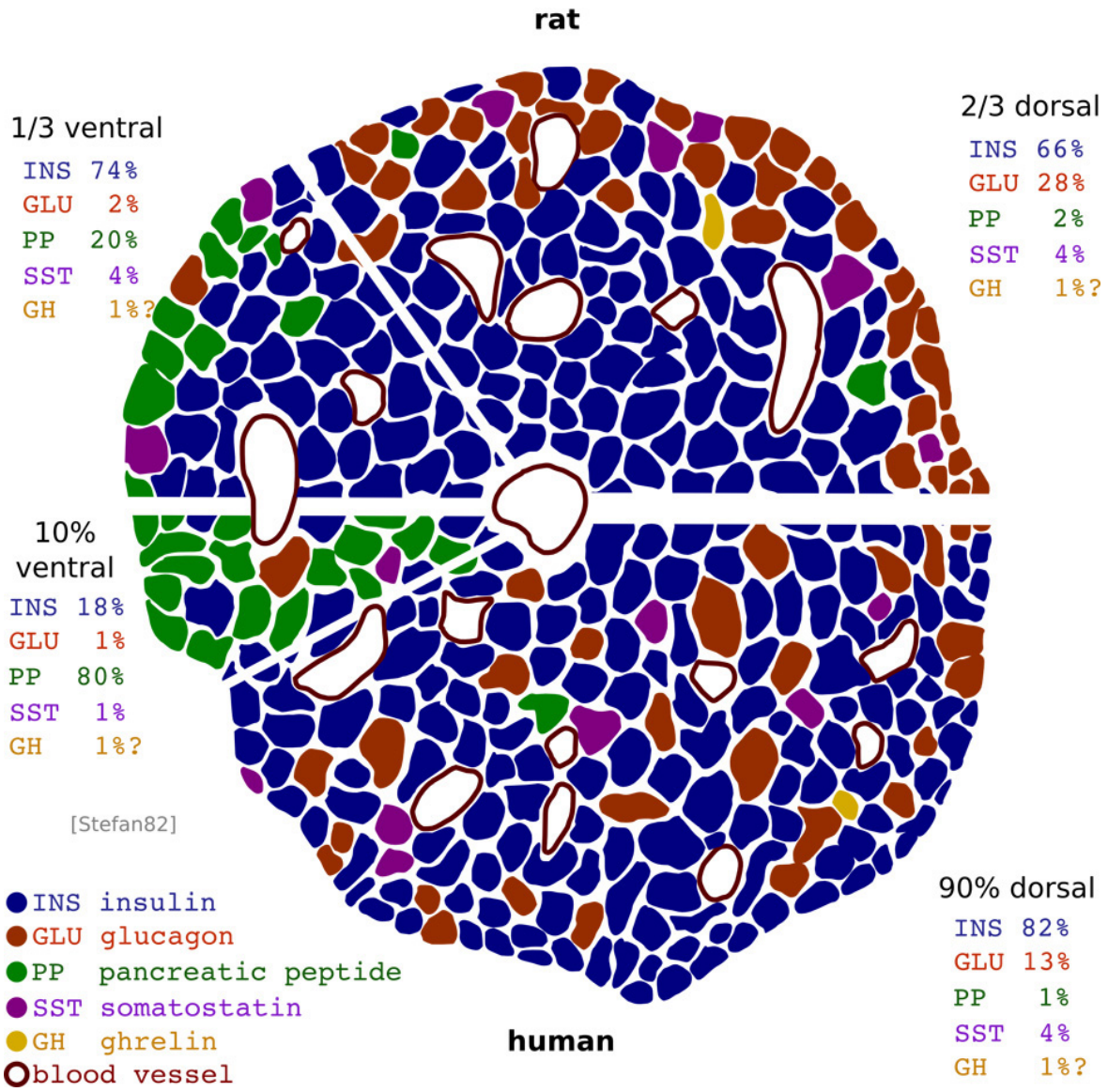

Figure 2. Human (9) and rat islets (11) are shown. For each organism the proportion of and difference between ventral and dorsal islets are highlighted. (CCby)

remaining cell types, while in humans the distribution appears random (12,13). A significant enrichment of PP cells has been observed in males compared to females (9) (Figure 2).

Thus, the majority of pancreatic cells works in meal digestion while a tiny but crucial minority of islet cells sends signals to other collaborating organs to regulate metabolism and specifically to prevent blood glucose excursions.

2.3. Islets are strategically connected for sensing and signaling

A control centre for the nutritional state of the body would have to be well connected to the major transport system, the blood circulation. In addition, it may be advantageous to be close to participating organs in terms of blood flow. Both is the case for the pancreatic islets.

Islets are very well irrigated by small blood vessels. Compared to the digestive pancreas, islet cells receive about 10x the amount of blood per mass (14). In addition, islet capillaries are larger (5 vs. 4 micrometres) and the vessel-lining endothelium shows a remarkable accumulation of small pores (14). These so called fenestrae (Latin windows) increase the permeability (15) and conceivably facilitate the access of insulin after expulsion from the producing cell to the blood stream (16).

This special capillary system in and around islets is probably set up by bi-directional signaling between growing vessel and maturing islet cells. The removal or

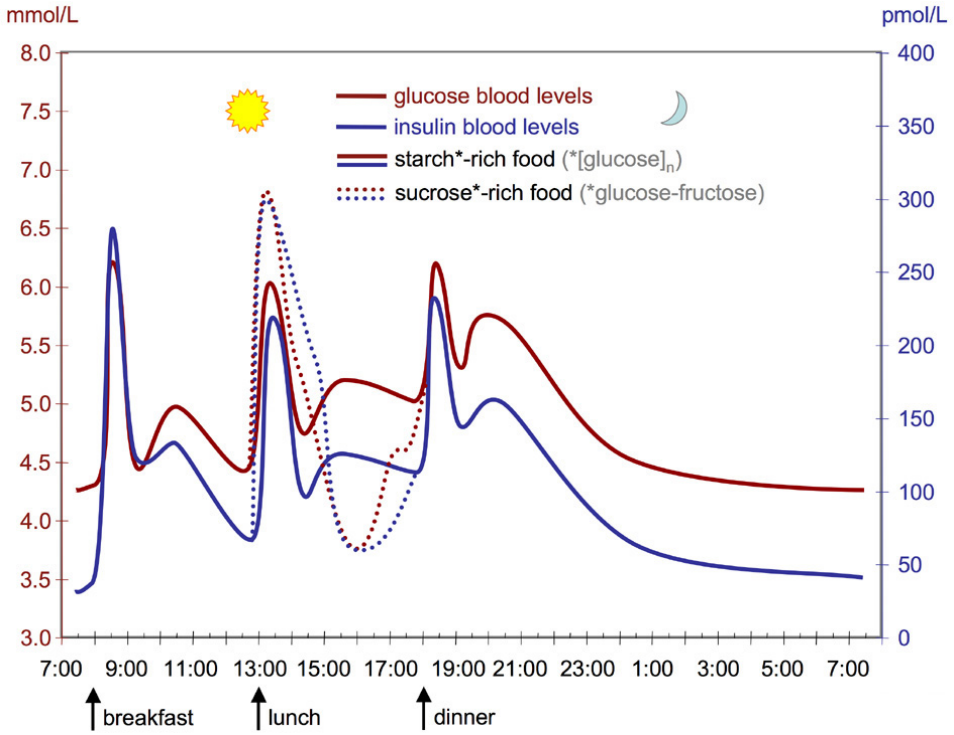

Figure 3. Blood insulin closely follows glucose. A meal rich in table sugar (dashed line) at lunch instead of a starch-rich diet (solid line) increases the amplitude. Based on (22). (CCby)

misplacement of blood vessels leads to abnormal insulin expression (17). One of the signals, if not the main one, seems to be the vascular endothelial growth factor A (VEGF-A) as seen by insulin cell-specific (18) or pancreas-specific knockout in mice (19), and reviewed in (20).

Besides the dense vascularisation of the islets the overall position in the circulatory system is notable. Partly deoxygenated blood from the gut, the spleen, and the pancreas makes up three quarters of the blood supply of the liver (hepatic portal vein) with only one quarter being fresh and coming directly from the heart. The liver, an important metabolic organ and glucose storage site, is thus the first stop for both nutrients absorbed through the gut and nutritional signals from the pancreatic islets.

3. NUTRIENT SENSING BY THE INSULIN CELL

3.1. beta-cells prevent excess blood glucose by secreting insulin

Shortly after a meal, the concentrations of food breakdown products like monosaccharides, amino acids, short peptides, and triacylglycerols start to increase in the circulation. beta-cells secrete insulin to induce other organs to take up these metabolites and reduce their liberation of similar molecules.

One of the metabolites that quickly accumulates in the blood after food ingestion is glucose. Its concentration in the blood is tightly regulated because excursions can quickly starve or damage cells and, in extreme cases, threaten the life of the affected organism. In humans, fasting blood glucose is maintained between 3.5 and 7 mmol/L (60-130 mg/dl, rounded). Diabetes mellitus, if unmedicated, causes glucose peaks beyond the 7 mmol/L limit, especially after food intake. Excessive blood glucose leads to increased urination due to overload of the kidney blood filtration system (the origin of the word diabetes) followed by thirst. In the long-term, elevated glucose also causes direct tissue damage by reacting non-enzymatically with biomolecules leading to their malfunction (21).

The insulin cell population of the pancreatic islets prevents high glucose concentration by sensing its levels and secreting the glucose-lowering hormone insulin accordingly. A diagram of how blood glucose and insulin levels change during the day is shown (Figure 3).

3.2. Insulin cells may have evolved to recognise glucose as major stimulus for insulin secretion

In humans, when comparing meals of various combinations of sugar, protein, and fat, only glucose (75 g) causes blood insulin levels to rise from a baseline of around 20-30 pmol/L to a 30 min-maximum of 250-300 pmol/L and back to basal in a few hours. Fat or fat+protein meals only cause a modest insulin increase to 50 and 60 pmol/L, respectively (23).

Glucose is the strongest natural stimulus of insulin secretion, because it was most likely a principal food component during evolution. Glucose is the monomer unit of the common storage polymer starch in vegetables

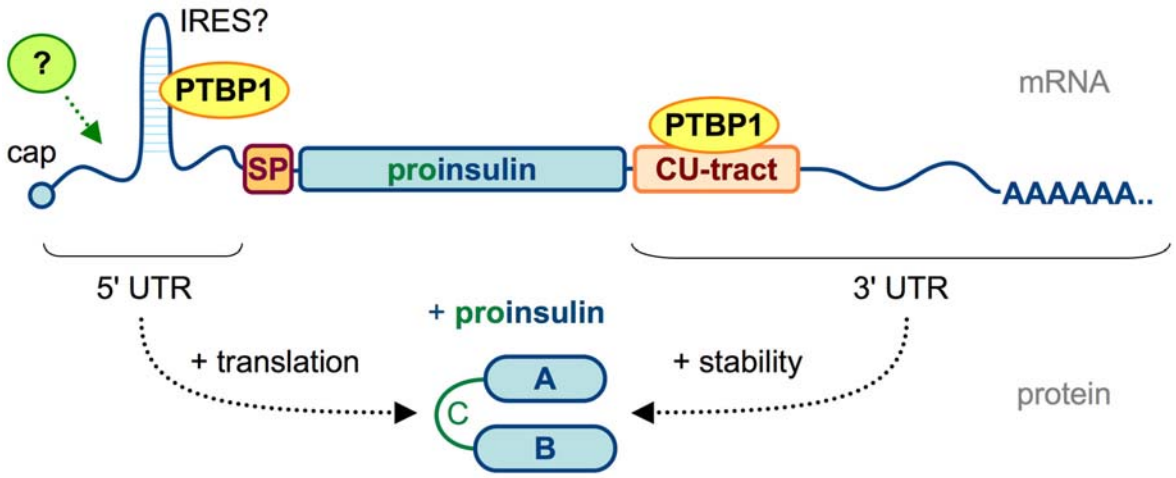

Figure 4. PTBP1 (97) and a yet unidentified protein (82) control insulin protein synthesis in response to glucose. (CCby)

and along with fructose the major ingredient of fruits. It may be impossible to ascertain the composition of the original pre-civilisation food our digestive systems adapted to. Nevertheless, some deductions can be made from the annual food intake of our closest relatives, the great apes, which is made up almost entirely of plants (24). At the same time, it has been suggested that meat due to its high nutrient concentration was important in human evolution (24).

In addition to glucose, the insulin cells of the pancreatic islets react to other breakdown products of food like other monosaccharides, amino acids, and fatty acids. For example, fructose alone does not stimulate insulin release but increases its synthesis (25) and can augment glucose-induced insulin secretion (26). Palmitate, leucine, and arginine but not alanine double the insulin secretion of isolated rat islets pre-incubated at 1.1 mmol/L glucose (27).

3.3. Specialisations in glucose and lactate metabolism

No glucose receptor has been found to date, despite it being a simple model to explain glucose sensing in the insulin cell. Instead, the metabolic products of glucose and other nutrients within the beta-cell trigger a sequence of molecular events that culminate in insulin secretion.

The first step is entry of glucose into the cytosol of the insulin cell. High glucose outside rapidly equilibrates with intracellular glucose via GLUT2-facilitated diffusion. The GLUT2 channel protein is only expressed in beta-cells (28, 29), the liver, to a lesser degree in the kidneys (30), in intestinal absorptive cells (31), and in some brain neurons (32). beta-cells specifically express this glucose transporter because it does not require mobilisation by insulin, unlike GLUT4, expressed in muscle and fat tissue. Also, its low substrate affnity with a Km of 17 mmol/L (33) ensures a high rate of glucose influx, proportional to blood glucose concentrations up to 10 mmol/L. Placement of GLUT2 in the outer membrane is a crucial step to a fully functional beta-cell. This is seen by the fact that in glucose-insensitive neonatal rat beta-cells GLUT2 is mostly found in intracellular vesicles (34). GLUT2 also transports glycerol-phospho-inositol (35) and glucosamine (36).

Immediately after entering the cytoplasm, glucose is phosphorylated and, as a consequence, cannot leave the cell through GLUT2 anymore. The responsible hexokinase subtype, commonly called glucokinase, is specific for insulin and liver cells (37). Its highest affinity substrates are glucose and mannose (38) and it displays an intriguing cooperative behaviour despite being a monomer and having only 1 reaction site (39).

Two important properties distinguish glucokinase from the other hexokinases, allowing it to function as glucose sensor. It has a lower affinity for glucose with a Km of 6 mmol/L (40) which is in the middle of the normal blood glucose range of 4-10 mmol/L (72-180 mg/dl). Other hexokinases are already at maximum speed at this concentration. Also, glucokinase is not inhibited by its product, glucose-6-phosphate (40). This allows continued activity despite high glycolysis load and links the flow through the pathway to extracellular levels of glucose.

The importance of GLUT2 and glucokinase for the proper function of insulin and other cells can also be seen by their involvement in disease. GLUT2 mutations, despite many failed attempts to link polymorphisms to diabetes, were eventually linked to a related disease called Fanconi-Bickel syndrome, which is characterised by glycogen accumulation and impairment of glucose utilisation (41). Glucokinase mutations can result in an increased threshold for glucose-stimulated insulin secretion (42) and have been observed in several families with inherited forms of diabetes (MODY) (43). The possibility of glucokinase-activating drugs to improve glucose balance is being investigated (44). Glucokinase and its activators are reviewed in more detail in (45).

GLUT2 and glucokinase expression sets apart the insulin cell from the majority of cell types in the body but does not explain the difference to liver cells. Lactate

metabolism, however, is distinctive. Liver cells take up lactate and use it to synthesise glucose (Cori cycle). Insulin cells, on the other hand, express almost no lactate transporter (46) or lactate dehydrogenase (47). Indeed, overexpression of lactate dehydrogenase A in insulinoma cells disturbs metabolism and insulin secretion (48).

With the lactate and gluconeogenesis routes closed, almost all glucose-derived carbon is channelled down into the citrate cycle. To feed all the pyruvate into the cycle, insulin cells express 7x more pyruvate carboxylase compared to glucagon cells (49). Most of the glucose carbons are terminally catabolised to generate ATP and CO2 but about a quarter is taken out of the citrate cycle to synthesise new protein (49).

See also the review (50) for a more detailed discussion.

3.4. Ion flux triggers insulin granule release

The first stage of signal transduction in the beta-cell is a cascade of increasing metabolite concentrations starting with glucose, through pyruvate, and finally ATP and other nucleotides. The second stage of signal transduction is electric. The bridge between the metabolic stage and the electrical one is a potassium channel, which closes in response to increased ATP.

The so called KAT P channel is made up of 4 central subunits of Kir6.2 (gene name KCNJ11) and 4 subunits of sulphonylurea receptor (SUR1). The Kir6.2 subunits form the potassium channel, while the SUR1 membrane proteins surround and influence the pore (51). Sulphonylureas are a group of oral anti-diabetes drugs which act by partially closing the channel thus stimulating insulin release (52, 53). The primary ATP recognition site is located on Kir6.2 while SUR1 is required for sensitivity to sulphonylureas and diazoxide and for activation by Mg-ADP (54). For a more detailed review of channel function see (55).

Mutations in both Kir6.2 and SUR1 can cause deregulation of insulin secretion and, consequently, glucose balance. For example, Kir6.2 alterations interfering with the ability of the channel to close can block glucose-induced insulin secretion and lead to neonatal diabetes (56). On the other side of the spectrum, Kir6.2 mutations can cause excessive insulin secretion and depressed blood glucose (57, 58). See (59) for in-depth review.

A recent study suggests that the link between metabolic and electric stage of signal transduction might be more immediate than previously assumed. It was shown that the glycolytic enzymes glyceraldehyde dehydrogenase (GAPDH) and pyruvate kinase are not only found in a complex with Kir6.2 but also directly block the channel (60).

When KAT P channels close due to increasing ATP concentrations, the basal -70 mV difference across the membrane partially collapses. This causes voltage-gated calcium channels to open which triggers transient fusion of granules with the outer beta-cell membrane and insulin release.

Insulin cells express several calcium channel subtypes which appear to be linked to the different phase of insulin secretion. If CaV1.2 is deleted in mouse islets, the whole-cell Ca^{2+} current is only halved but the first phase is almost completely abolished (61). On the other hand, deletion of the CaV2.3 gene selectively suppressed the late phase without influencing the early phase (62). So, while type 1 channels are involved in both phases, type 2 channels may selectively control the second phase of insulin secretion. For a very detailed review of voltage-gated calcium channels see (63).

3.5. AMPK activation in the liver reduces blood glucose

New control systems are being discovered and added to the existing picture. AMP-activated protein kinase (AMPK) has recently been implied in the regulation of glucose-induced insulin secretion. Overexpression of an artificially active form almost completely suppresses glucose to insulin secretion coupling (64). Three AMPK activating kinase have been discovered in yeast. If all three are knocked out (or if AMPK is deleted), mutant yeast can no longer grow on other sugars besides glucose (65). The finding that the widely used diabetes drug metformin appears to work by activating AMPK (66) supports the importance of AMPK in balancing glucose levels. In 2006, a more specific activator was found and may become available for treatment (67).

However, AMPK activators seem to work primarily via the liver and not via the beta-cell. This hypothesis is backed by a liver specific knockout of LKB1, an upstream activating kinase of AMPK. The resulting mice show elevated blood glucose, resistance to metformin treatment, and increased expression of genes involved in glucose and lipid synthesis (68). It also emphasises the point that glucose homeostasis is achieved through the cooperation of pancreatic islets and various cell types with the liver being the most important.

AMPK may still be important for beta-cell signaling, since AMPK is also activated by calcium-dependent kinase kinase (69). This could allow AMPK to activate downstream proteins in response to both elevated AMP and calcium.

4. FAST REACTIONS TO NUTRIENTS

Cells like the organism they form adjust to the environment on different time scales. There are immediate changes after external cues (insulin secretion) and gradual changes that only become obvious with time (changes in expression patterns, proliferation). No clear boundary can be drawn between the two but a rough categorisation helps to understand the insulin cell.

4.1. The short-term activation of the insulin cell by nutrients is mainly translational

When investigating the regulation of a gene like insulin, it is logical and common to start with the regulation of transcription because it is the first step in expression and

thus the most economical to regulate. However, the control of insulin levels in the short term appears to be a special case.

In a landmark paper, it was demonstrated that in isolated rat islets, incubated in 25 and 2.8 mmol/L glucose for 1 hour, proinsulin protein levels shoot up tenfold at high glucose while the amount of proinsulin mRNA remains the same (70). This is a clear demonstration that, in the time frame examined, insulin is under translational control: the insulin protein level is increased by stimulating translation of the pre-existing proinsulin messenger RNA pool of 0.3 ng per rat islet (70). An earlier study had shown that the glucose-induced boost of insulin protein synthesis does not require mRNA synthesis within the first 45 min. Blocking transcription with actinomycin D only slowed the accumulation of insulin protein after that time point (71).

Nevertheless, its is clear that insulin mRNA levels and stability are adjusted by the beta-cell proportionally to extracellular glucose levels in the longer term. Rats starved for 3 days show only 15-20% of insulin mRNA levels compared to fed controls (72). In isolated islets, insulin mRNA stability is decreased after several hours incubation in 3.3 mM versus 17 mM. Also, the insulin mRNA pool increases with glucose concentration after a day-long incubation (73). It has also been shown that the relative amount of insulin mRNA is increased 5-fold after 4 h stimulation with 28 mM compared to 2.8 mM (74).

Recent evidence from our lab (75) indicates that insulin mRNA half-life is 16 h in rodent islets and thus much shorter than previous estimates of 29 h (73). It was also found that insulin mRNA levels drop off sharply in INS1 cells after about 2 h without glucose, but that this decrease can be prevented by elevating cAMP levels (75).

It appears that the regulation of insulin in the beta-cell retraces the secretory pathway with increasing time frame. Immediately after glucose stimulation, the control resides mostly downstream in protein synthesis and RNA stabilisation. Later, control moves upstream from post-transcriptional to transcriptional mechanisms.

4.2. The beta-cell translates mostly insulin mRNA which is recruited to the ER by elevated glucose

The insulin cell is an astonishing specialist for the production of insulin. Within normal blood glucose range, the ratio of insulin to total synthesis is between 1/3 at 3 mmol/L and almost 1/2 at 7 mmol/L glucose in isolated insulin cells (76). In isolated rat islets the effect seems to be even more pronounced with over half of new protein synthesis by isotope integration being insulin at high glucose (70). Taking into account that insulin is a tiny protein of only 5.8 kg/mol (kDa) and that islets contain other cell types, almost all mRNAs translated in beta-cells encode insulin.

Insulin mRNA waits pre-loaded with ribosomes. Glucose induces its transport to the endoplasmic reticulum (ER) and the association of additional ribosomes for protein synthesis. A subcellular fractionation study of the MIN6 insulinoma cell line at low and high glucose showed that glucose induces the reduction of the fraction of cytosolic mRNA while increasing the amount in the membrane-bound fraction. mRNAs were shown to be ER-associated by removal of cytosolic mRNAs after digitonin treatment. mRNAs of other insulin granule components, like proprotein convertase 2 (PC2) and carboxypeptidase E (CPE), also relocate but less than insulin messenger since a larger fraction is already present on the ER. The migration starts within minutes after glucose stimulation and continued over the period of one hour. While no ribosome-free insulin mRNA was detected, more ribosomes were found on insulin, PC2, and CPE mRNAs at high glucose (77).

4.3. Translational activation is both overall and gene specific

In response to nutrients the insulin cells activates its translation. The overall speed of translation is increased but certain mRNAs are selectively boosted, among them insulin.

Overall activation of translation might be controlled by glucose-induced dephosphorylation of eukaryotic initiation factor 2a (eIF2a) via protein phosphatase 1 (78). Dephosphorylated eIF2a is released from sequestration by eIF2B and increases overall mRNA translation (79). The simple hypothesis that less overall translation via eIF2a phosphorylation protects the beta-cell was refuted by treatment of isolated rat beta-cells with salubrinal, a PP1 inhibitor, which led to decreased insulin secretion and apoptosis (80). Instead, the ratio of eIF2a-P to eIF2a is high (1.2) in islets after a 2 h incubation with 2 mmol/L glucose, low (<1) after 2 h in 5 mmol/L, and around 1 for culturing with 10 mmol/L glucose (81).

GLP1 signaling might also be involved in the overall induction of translation since the GLP1 receptor agonist exendin4 also increases eIF2a dephosphorylation/activation in isolated rat islets (82). In an ER stress mouse model overexpressing calmodulin, treatment with exendin4 protects and improves the action of insulin cells (83).

Another common mechanism for overall increase of protein synthesis is the release of eIF4e from sequestration by its binding protein. When the binding protein is phosphorylated, eIF4e is free to associate with the mRNA 5' cap and induce translation. However, it appears this mechanism is not important in the beta-cell, since glucose does not stimulate eIF4e or induces phosphorylation of its binding protein (84).

In addition to a broad acceleration of protein synthesis in glucose-stimulated beta-cells, insulin and several insulin granule mRNAs are translated even more actively. The overall boost in protein synthesis is about 3-fold, while the translation of proinsulin mRNA is amplified about 8x by glucose (85). The difference is due to the interplay of specific features of this mRNA and a suitable set of translation control proteins in the beta-cell.

Experiments with artificial mRNAs lacking either or both untranslated regions (UTRs) of preproinsulin demonstrated that glucose-induced translation increase is most dramatic with both UTRs present. It is notable with only the 5' UTR, but absent with only the 3' UTR. The study also showed that insulin UTRs cannot mediate the same translational effect in liver cells. There, no increase in protein synthesis by glucose was observed and the 3' UTR drastically reduced protein levels compared to the SV40 equivalent (86).

Together with insulin, glucose prompts the rapid translation of other insulin granule components, including the proinsulin cleaving enzymes PC1 and PC2 (87, 88, 89), but not the carboxypeptidase E (89). The granule transmembrane protein ICA512 is also increasingly synthesised (90, 91). Chromogranin A, a protein with affinity for the cholesterol-rich membrane of the secretory granule and a possible role in packaging (92), is also upregulated by glucose (93).

4.4. Gene-specific activation is achieved by PTBP1 and other proteins recognising UTR motifs

Polypyrimidine tract binding proteins (PTBPs) are a family of proteins involved in exon repression during splicing in the nucleus (94, 95, 96) and stabilisation and ribosome recruitment for selected mRNAs in the cytosol. Thus, PTBPs can increase protein levels indirectly, by prolonging mRNA life time, and directly, by stimulating translation.

A xenopus homologue of PTBP1, VgRBP60, controls the position of Vg1 RNA in the oocyte (97). PTBP1 may have a similar function in mammalian cells but direct evidence is lacking.

For example, in the neurosecretory cell line PC12, which bears many similarities to hormone-secreting beta-cells, PTBPs are required for efficient differentiation. The growth of nerve terminals is stunted, if PTBP1 and PTBP2 are reduced by RNAi and thereby prevented from stabilising beta-actin mRNAs in the growth zone (98).

The mRNA effects of PTBP1 are adjusted by moving out a larger fraction of the protein from the nucleus to the cytosol. The export signal is the phosphorylation of Ser16 by the cAMP-induced protein kinase A (PKA) (99, 98). The same mechanism has also been observed in the beta-cell model INS1 (75).

Once in the cytoplasm PTBP1 can bind to a CU-rich sequence in the 3' UTR of proinsulin mRNA, an effect augmented by glucose. If the PTBP1 binding site is mutated proinsulin mRNA is destabilised (101). Only inhibition of mTOR reduced PTBP1 binding to insulin mRNA (102). Another study adds that mTOR inhibition only partially blocks glucose-induced protein synthesis (84). It is surprising that PKA inhibition had no effect, despite its probable role in PTBP1 recruitment from the nucleus.

Our lab has shown that PTBP1 boosts several insulin granule proteins, including insulin itself. In the case of ICA512, PTBP1 binding decreases 3' UTR mRNA decay. For the insulin-processing prohormone convertase 2 (PC2), which is packaged into insulin granules, mRNA translation is increased strongly by its 5' UTR, somewhat by its 3' UTR, and significantly less by RNAs with mutated PTB binding sites. Overall, knockdown of PTBP1 via RNAi reduces the levels of several granule proteins and slashes cell insulin content to about one third (100).

Insulin and insulin granule mRNAs seem to share properties that allow for targeted stabilisation and translation by RNA-binding proteins like PTBP1 in response to glucose. These features appear to have evolved in the regulatory RNA sequences before and after the protein-coding region.

In 2007, an intriguing new regulatory element in the 5' UTR of proinsulin mRNA was published. A yet unknown factor binds to a conserved region around 40-48 nt from the 5' end. The association parallels glucose stimulation. Removal of the binding sequence in the RNA results in loss of glucose stimulation (85). An earlier study reported a rare insulin splice variant in humans which is more readily translated than the prevalent form (103). Interestingly, the alternative splicing breaks the binding sequence of the unknown factor. The factor bound to its proinsulin mRNA target might work as a ribosome block lifted by glucose, similar to the mechanism of iron response.

4.5. Does the insulin cell employ IRES, known mostly from viruses?

The most common and best studied mechanism of translation initiation in eukaryotes requires a 7-methyl-guanosine cap attached to the 5' nucleotide of an mRNA via a triphosphate bridge.

There is an ongoing debate about how important the alternative mode of translation start is for normal gene expression.

It is known from viruses that an alternative mechanism of starting protein synthesis exists (104, 105). Some viruses can recruit the 40S small ribosomal subunit to cap-less mRNA via an intricately folded internal ribosome entry site (IRES) in the 5' UTR. In a current model for dicistroviruses, the IRES RNA structure was proposed to mimic the Met initiator tRNA (105).

Internal translation start further requires the binding of auxiliary proteins to the IRES which together with the RNA help to attract the ribosomal halves. PTBP1 is one of those factors (106, 107, 108, 109, 110, 111, 112).

The involvement of PTBPs in IRES translation has been dissected using artificial IRES constructs in combination with PTBP1 (113, 114). These studies uncovered several very intriguing properties of the system. A simple short stem-loop is sufficient to initiate translation. CCU rather than CUU repeats were the most efficient PTBP1 binding sites. The pyrimidine repeats can be on either side of the hairpin. Unexpectedly, single mismatches

in the RNA stem increase translation initiation. A minimal distance of about 30 nt is required between the stem and the start codon AUG supposedly for the ribosome to land. Shorter splice variants of PTBP1 and PTBP2 are more efficient than full-length PTBP1. And lastly and most novel, PTBP1 was shown to bind the double-stranded stem RNA contrary to common belief which assumes PTBPs to be single-stranded RNA binding proteins.

The authors of the studies stress the importance of IRES-mediated translation not only in viral but also in normal cellular context by estimating that 1 out of 10 genes employ this mechanism of translation (114). The relevance for the beta-cells though, is still awaiting further investigation.

5. LONGER-TERM INSULIN CELL ADAPTATIONS

Beyond the translational mechanisms activated shortly after nutrient stimulation, the insulin cell displays many slower adaptations to its environment. These generally rely more on transcription than the fast reaction mentioned above. Examples are the control of ER load, granule counting, and cell cooperation.

5.1. Capacity and throughput of the secretory pathway need to be regulated

The beta-cell is a high-volume production unit for insulin. An average mouse beta-cell contains 13000 insulin granules which occupy more than 10% of the total cell volume (115). Each granule is estimated to contain 200'000 insulin molecules (116).

However, it is also a seasonal factory. If nutrient levels are high, the production is accelerated. If they are low, protein synthesis proceeds only at a basal level. The beta-cell therefore requires mechanisms to promptly increase the volume of protein synthesis as well as the capacity of the secretory organelles to properly fold and transport the wave of glucose-induced proteins.

An important ER regulatory system involves the ER transmembrane kinase PERK (117). PERK phosphorylates and thus changes the activity of eIF2a (79), which was already mentioned regarding broad control of translation above. Its importance in glucose balance is highlighted by the finding that mutations in the gene where associated with a recessively hereditary form of diabetes, the Wolcott-Rallison syndrome (118).

Deletion of PERK in mice resulted in swollen ER, activation of the stress transducer IRE1a/ERN1, increased apoptosis, and diabetes (119). Blocking eIF2a phosphorylation by mutating its target serine residue resulted in a similar yet more severe phenotype (120), which suggests that PERK phosphorylation of eIF2a can be partially compensated by other kinases. In 2006, a study of PERK knockouts at different stages of mouse development showed that only the early not the late mutants showed less insulin expression and malformed islets. PERK may thus be crucial for beta-cell precursor expansion and differentiation (121). This appears to refute the hypothesis that ER stress, unmitigated by PERK/eIF2a signalling, leads to insulin cell death in this form of diabetes. Instead it moves the time of PERK requirement forward to prenatal islet development.

It does not exclude that similar ER signals are indeed essential for pancreatic islets in adults. The WFS1 protein might be part of such a mechanism. It takes its name from the Wolfram syndrome, an autosomal recessive combination of diabetes, blindness, and deafness. The WFS1 gene was first correlated with the disease in two linkage studies (122, 123). The insulin cells of the knockout mouse were progressively lost (124), a finding confirmed in the insulin-cell specific deletion (125). Later it was discovered that WFS1 is upregulated by glucose and by ER stress via IRE1a and PERK (126). IRE1a activation by auto-phosphorylation also responds to glucose which leads to increased WFS1 transcription and increases insulin synthesis (127).

It may seem contradictory that IRE1a activation has also been reported in conjunction with decrease in insulin synthesis although through downstream XBP1 not WFS1 (128). But both observations may represent different signal cascades which share the component IRE1a. At first, glucose may be stimulating insulin synthesis via WFS1. After prolonged exposure, however, it may reduce insulin production via activated XBP1 (129).

5.2. Granules may be counted via ICA512 cleavage during fusion

It is logical that the beta-cells would have evolved a mechanism to detect how much insulin it has secreted and adjust new protein synthesis accordingly. This type of feedback may indeed exist and probably involves a granule transmembrane protein called ICA512 named after its property as an islet cell autoantigen (ICA).

Insulin granules are probably transported over long distance on tubulin tracks (130) before they arrive near their destination at the peripheral actin meshwork (131). When granules are not linked to the cytoskeleton by motors, they can be anchored via their membrane protein ICA512 and beta2-synthrophin to the actin cortex (132).

When intracellular Ca^{2+} increases during glucose activation, granules are mobilised and fuse transiently with the outer membrane to release their cargo. At the same time, the protease μ-calpain is activated by elevated calcium levels and cleaves away a cytosolic fragment from ICA512. This part is now no longer bound to the granule and was shown to move to the nucleus where it binds the sumoylating enzyme PIAS gamma. Transfection of the insulinoma cell line INS1 with the fragment strongly increases insulin mRNA levels (91). In a subsequent study, the cytosolic fragment was shown to bind the tyrosine phosphorylated transcription factor STAT5 and prevent its dephosphorylation. This enhances the transcription of insulin granule genes including ICA512 itself. Sumoylation of ICA512 by PIAS gamma, in turn, may reverse this process by decreasing the binding of ICA512 to STAT5 (133).

The importance of ICA512 (IA-2) has been confirmed by its knockout mouse, which shows abnormally

low insulin levels in a glucose tolerance test (134). Also, insulinoma cells overexpressing ICA512 contain 3x more insulin granules and consequently release more insulin upon glucose stimulation (135).

It may thus be that the amount of the ICA512 cytosolic tail is proportional to secreted insulin and increases insulin and insulin granule protein transcription.

6. CELL COOPERATION IN NUTRIENT SENSING

6.1. Appropriate insulin secretion requires cooperation with the digestive and nervous system

Increasing catabolite levels in the blood after meal ingestion are not the only stimuli for insulin release. This is clearly demonstrated by the fact that intravenously injected glucose causes only about a third of the blood insulin increase of a similar amount of glucose absorbed through the gut (136). The digesting gut signals to pancreatic islets via hormones and nervous signals. Glucose-dependent insulinotropic polypeptide (GIP) (137) and glucagon-like peptide 1 (GLP1) (138, 139) are examples of gut hormones that increase insulin secretion.

Insulin and glucagon secretion are also controlled by nerves and peptide release from neighbouring cells. Sympathetic and parasympathetic nerves innervate the islets. Sympathetic nerves stimulate insulin release via beta-adrenergic receptors and inhibit via alpha-adrenergic receptors, whilst parasympathetic vagal nerves stimulate both insulin and glucagon release. The neural input serves to stimulate insulin secretion in anticipation of food entering the gastrointestinal tract. For details see these reviews (140, 141).

beta-cells detect small nutrient biomolecules with glucose being the strongest stimulus. These pieces of information are integrated with nervous signals and levels of gut hormones like GIP and GLP1 to generate a suitable insulin output in the healthy state.

6.2. Groups of beta-cells show better insulin switching and action than separate cells

It appears that beta-cell coordination is required to achieve an OFF state with low insulin secretion at low glucose. Isolated insulin cells under the same conditions leak more insulin. A cell group also has a higher ON state and displays unique waves of insulin secretion.

An early study from 1989 (142) showed that cooperation between islet cells is important for proper function. Two recent papers have analysed this behaviour. Islets from connexin36 -/- mice lack gap junction, which usually connect neighbouring insulin cells. This leads to a lack of pulsed insulin secretion but does not lower the total amount released. It does, however, increase the basal leakage of insulin at low glucose (143). On the other end of the spectrum, insulinoma MIN6 cells show increased insulin secretion after glucose exposure when they are clustered as compared to separated (144).

Islet coordination has also been investigated by modelling. Glycolysis (145) and insulin secretion occur in pulses (42). Equal amounts of insulin affect target tissue more if their concentration is oscillating at the normal frequency of a few minutes, a pattern which is impaired in type 2 diabetes. See the review (146).

A simulation of electrical and metabolic oscillations shows coordination between islets cells and between islets through the blood and the liver (147). It has predictive power but still lacks experimental confirmation.

Another group investigated the changing size distribution of islets in mice 6 weeks and 5 months after birth and found a skewed distribution with a maximum around 100 cells per islet (148). They compare this to a previous model which combines thermal noise and channel opening probability to predict electrical behaviour of a group of insulin cells (149). Based on both they hypothesise that only islets beyond a minimal size can coordinate their electrical activity and that the most frequently observed size is in fact the point where addition of further cells does not improve cooperativity.

6.3. Insulin cells cooperate via surface protein signals

Adjacent beta-cells signal to each other via a pair of membrane proteins. The transmembrane receptor tyrosine kinase EphA recognises its ligand, the GPI-anchored ephrin-A, which leads to signalling to both cells involved. A mechanism which involves Rac and actin can downregulate insulin secretion in the cell carrying the receptor, while it has the opposite effect in the cell carrying the ligand (150). Ephrin-A EphA contact signalling thus provides insulin cells with a way to decrease their basal insulin secretion while augmenting their peak release. How the opposing effects of this mechanism are balanced remains to be elucidated.

6.4. beta-cells attract blood vessels which in turn improve insulin secretion

The interplay between endothelial cells, which form blood vessels, and islet precursor cells is known from pancreas development (see section 2.3). It was recently demonstrated that endothelial cells build the basement membrane between them and the adjacent insulin cells. They are attracted by VEGF-A secreted from insulin cells and reply with a signal mediated through the secreted protein laminin which induces insulin cell proliferation and insulin expression (151).

For an excellent graphic representation of the confusing multitude of beta-cell signaling pathways see (152).

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Abbreviations: AMPK -AMP-activated protein kinase, CCby - creative commons attribution license v3 attached to figure, CPE - carboxypeptidase E, eIF -eukaryotic initiation factor, GAPDH -glyceraldehyde dehydrogenase, GIP - glucose-dependent insulinotropic polypeptide, GLP1 - glucagonlike peptide 1, GLUT -glucose transporter, GPI - glyco-phosphatidyl-inositol, IA-2 -islet autoantigen 2 = ICA512 -islet cell autoantigen 512, IRE1 -inositol requiring 1 = ERN1 -endoplasmic reticulum to nucleus signaling 1, IRES -internal ribosome entry site, mTOR -mammalian target of rapamycin, PC -proprotein convertase, Pdx1 - pancreatic and duodenal homeobox 1, PERK -PKR-like endoplasmic reticulum kinase, PIAS -protein inhibitor of activated STAT, PKA -cAMP-induced protein kinase A, PP -pancreatic polypeptide, PTBP -polypyrimidine-tract binding protein, SD -standard deviation, SP -signal peptide, STAT signal transducers and activators of transcription, SUR1 -sulphonylurea receptor 1, UTR -untranslated region, VEGF -vascular endothelial growth factor, WFS1 - Wolfram syndrome (gene) 1, XBP1 -X-box binding protein 1.

Key Words: Pancreas, dorsal pancreas, ventral pancreas, Islets of Langerhans, pancreatic islets, beta cells, insulin, alpha cells, glucagon, delta cells, pancreatic polypeptide, somatostatin, ghrelin, blood glucose, glucose homeostasis, hypoglycemia, hyperglycemia, hypoglycaemia, hyperglycaemia, diabetes mellitus, MODY, Fanconi-Bickel syndrome, Wolcott-Rallison syndrome, Wolfram syndrome, metabolism, GLUT2, glucose transporter 2, glucokinase, Cori cycle, lactate, K_{ATP} channel, Kir6.2, KCNJ11, SUR1, GAPDH, glyceraldehyde dehydrogenase, AMP-activated protein kinase, AMPK, post-translational control, translational control, mRNA stability, untranslated region, UTR, internal ribosome entry site, IRES, endoplasmic reticulum stress response, ER stress response, unfolded protein response, UPR, eukaryotic initiation factor 2a, eIF2a, PERK, IRE1a, WFS1, XBP1, ICA512, calpain, SUMO, sumoylation, STAT5, glucose-dependent insulinotropic polypeptide, GIP, glucagon-like peptide 1, GLP1, adrenergic neurons, sympathetic, parasympathetic, cooperativity, islet size, contact signaling, ephrin A, EphA, VEGF-A, vascularisation, Review

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