Review

The insulin secretory granule as a signaling hub

Jakob Suckale[1] and Michele Solimena[1,2]

[1] Molecular Diabetology, Paul Langerhans Institute Dresden, School of Medicine and University Clinic 'Carl Gustav Carus', Dresden University of Technology, Dresden 01307, Germany
[2] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden 01307, Germany

The insulin granule was previously thought of as merely a container, but accumulating evidence suggests that it also acts as a signaling node. Regulatory pathways intersect at but also originate from the insulin granule membrane. Examples include the small G-proteins Rab3a and Rab27a, which influence granule movement, and the transmembrane proteins (tyrosine phosphatase receptors type N) PTPRN and PTPRN2, which upregulate β-cell transcription and proliferation. In addition, many cosecreted compounds possess regulatory functions, often related to energy metabolism. For instance, ATP and γ-amino butyric acid (GABA) modulate insulin and glucagon secretion, respectively; C-peptide protects β-cells and kidney cells; and amylin reduces gastric emptying and food intake via the brain. In this paper, we review the current knowledge of the insulin granule proteome and discuss its regulatory functions.

The insulin granule has traditionally been viewed as a mostly inert delivery container acted upon by regulatory proteins. For two other organelles involved in the transport of secretory proteins (the ER and endosomes), this view has been upturned in light of recent discoveries. The ER has been established as the origin of the unfolded protein response, which warns other cell compartments of impending cell damage due to overloading of the protein production machinery [1]. Endosomes have been shown to be the major site of action for some transmembrane receptor-ligand complexes, which reside only transiently at the plasma membrane, and then during most of their lifetime signal from the endosome [2]. Similar to the ER and the endosome, there is mounting evidence that the insulin granule can no longer be viewed as a passive container. Instead, it functions as a hub for regulatory pathways and is the origin of important signals itself.

The source of insulin, if not from the needle

Insulin is the major hormonal signal for reducing blood glucose and storing energy in metabolites after eating. Under normal conditions and in adults, insulin is produced in significant amounts only by the β-cell, located within 100–200 μm cell clusters (islets) throughout the pancreas [3]. A few exceptions to the exclusivity of insulin production in the body have recently been discovered; for example, specialized thymic cells synthesize insulin [4] to ensure self-tolerance [5]. Insulin might also be produced in the developing brain [6,7], in which its receptor has been linked to axon guidance [8,9], but the connection between ligand and receptor remains to be confirmed.

Extensive studies in toxin-, diet- and mutation-induced rodent models of diabetes have shown that insulin synthesis can be activated in non-β-cells. Under these stress conditions, insulin expression was found mostly in bone marrow-derived cells but was also detected in the liver, fat and spleen [10]. Despite these exceptions, it holds true that all circulating insulin in a healthy adult originates from β-cells only. This extremely specialized cell dedicates up to half of its protein synthesis capacity to insulin production [11], and the newly made insulin is then packaged into insulin granules (Figure 1).

Structure of the insulin granule: a bag of surprises

The β-cell is a high-volume production unit for insulin. The average rodent β-cell has been estimated to contain ~10,000 insulin granules, corresponding to ~10–20% of the total cell volume [12,13]. Because each granule can store ~200,000 insulin molecules [14], an insulin cell is brimming with ~2 billion insulin molecules. Aldehyde-fixed insulin granules appear under EM as spherical organelles with a diameter of 300–350 nm [12,13], containing an electron-dense core separated from the surrounding membrane by a characteristic halo. The dense core is composed of tightly packed crystals containing six molecules of insulin stabilized by the coordination of one calcium and two zinc ions [15] in addition to a variety of soluble proteins and ions (Figure 1).

Maturation

Proinsulin must be processed to insulin to be fully functional [16,17]. Maturation is catalyzed by three proteases: prohormone convertase (PCsk) 1 and 2 and carboxypeptidase E (CPE) ([18]. According to pulse-chase experiments in isolated rat islets, proinsulin synthesis in the ER and passage to the Golgi takes around 20 min, and processing to insulin in post-Golgi vesicles increases linearly during a period of 1–2 hours [19]. Unlike constitutive vesicles, which are released by default, granules can then be stored for several days before they release their insulin content extracellularly in response to metabolic stimuli. A similar isotope study in mouse islets has estimated the (pro)insulin half-life and by inference, that of the insulin granule, to be ~3 days [20].

Corresponding author: Solimena, M. (Michele.Solimena@tu-dresden.de).

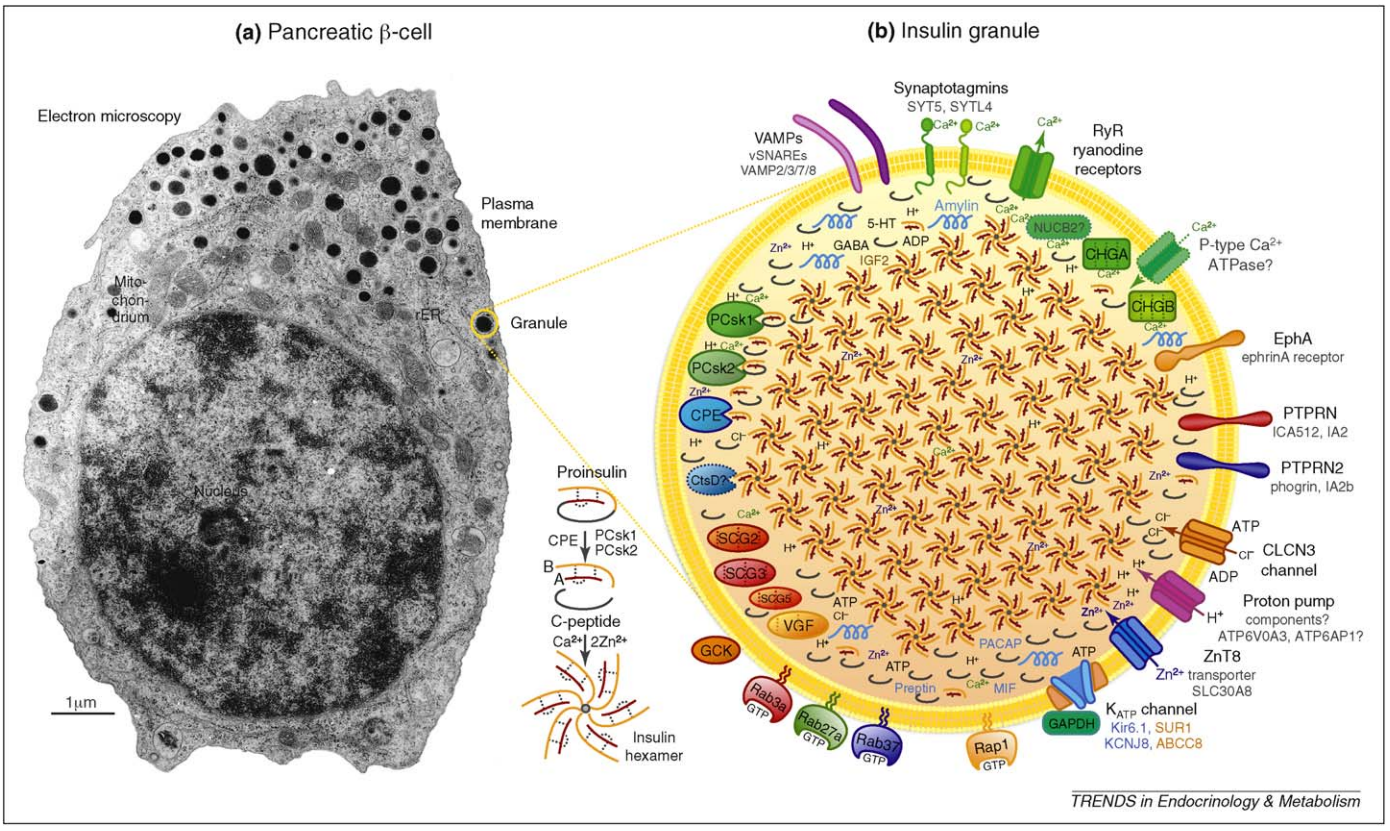

Figure 1. The insulin granule. **(a)** The pancreatic β-cell, as seen by EM, produces thousands of **(b)** small membrane-bound vesicles loaded with insulin. Apart from this principal component, these granules contain many proteins, small molecules and ions in the lumen, as well as several transmembrane proteins, channels and membrane-associated proteins. All proteins are labeled using standard names, except for insulin (INS), amylin (IAPP), ZnT8 (SLC30A8). Please refer to the HGNC or the NCBI for explanations. 5HT, 5-hydroxytryptamine (serotonin); A, insulin A peptide; B, insulin B peptide; GABA, γ–aminobutyric acid; IGF2, insulin-like growth factor 2; MIF, macrophage migration inhibitory factor; PACAP, pituitary adenylate cyclase-activating polypeptide;.

Heterogeneity

Crystallization of insulin increases with time, and two-thirds of granules younger than 2 days carry only the non-crystalline form of insulin, which is released more rapidly [21]. Interestingly, younger insulin granules preferentially undergo exocytosis, bypassing older granules [22,23]. The subcellular localization, the anchoring to the cytoskeleton and the modification/cleavage of granule membrane proteins further contributes to insulin vesicle heterogeneity.

The granule proteome

The total number of insulin vesicle proteins is often cited as ~150, a figure based on a spot count on a two-dimensional gel of gradient-purified insulin granules from a rat tumor [24]. In light of two recent proteomic studies using liquid chromatography tandem mass spectrometry (LC-MS/MS) on gradient purified granules from the rat insulinoma cell line INS1E, the total number of insulin granule proteins might have to be revised downward to about 50–100. The more stringent of the two studies employed affinity purification with VAMP2 antibodies in addition to selection by density, and identified ~50 proteins [25] (Table 1). Using only gradient centrifugation, the other study arrived at the much larger figure of ~130, inflated by numerous lysosomal and mitochondrial proteins [26] (Table 1). However, both studies only share 15 proteins, and major insulin granule components such as PCsk1 are missing. Nevertheless, several novel candidate granule proteins were discovered and await verification. The studies also represent a forward push against the technical challenges, which appear to lie mostly in the still insufficient purification of insulin granules. In the future, tandem affinity purification via tagged granule-restricted transmembrane proteins and fluorescent cargo-based sorting might improve purity. In addition, proteomic studies will have to be extended to primary insulin granules, for which the recently developed technique of quantitative MS analysis [27] should be employed.

Cargo functions

Insulin: the main passenger

The most important and abundant molecule of the β-cell granule is the hormone insulin, a 51 residue peptide that makes up 50–60% of total granule protein [24]. Although insulin is best known for its pivotal role in regulating glucose homeostasis and nutrient storage [28], signaling by insulin and the related insulin-like growth factor (IGF)1 can also contribute to the development of cancer, and thus are the target of novel anticancer drugs [29]. In addition to its direct metabolic role, the effect of insulin on the brain has been increasingly studied, and very interesting connections between insulin levels and appetite, synaptic plasticity, memory and mood have been uncovered [30] (Figure 2).

Table 1. Insulin granule proteomes

Proteomic study	Detected polypeptides as categorized in the paper with minor changes[a]	Comments
MS/MS of gradient-purified rat insulinoma cell line INS1E granules [26]	(57 luminal protein types)	
	32 hydrolases (e.g. **Cpe**, Cpn1, **Pcsk2**, *Ctsb*, Ctsl, ***Ctsd***, *Ctsf*, Scpep1, *chitobiase, hexosaminidases, sulfatases, glucosidase, mannosidase.*)	Many hydrolases are probably *lysosomal* contaminants (*italics*)
	19 secreted (e.g. **Ins1**, **Ins2**, **Chga**, **Chgb**, **Scg2**/3/5, Pcsk1n, Clu, Fn1, Nptx1, **Nucb2**, Prdx4, Stc1, Tcn2, Wif1, *Epdr1, Psap*)	Scg5, Pam, Pcsk1n re-categorized
	6 other (*Cst3*, **Pdia3**, Sod1, Gc, Hspe1, Mdh2)	Mitochondrial: Hspe1, Mdh2
	(60 membrane protein types)	
	14 Rabs (**1a**/b, **2a**, **3a**/c/d, 5/b/c, 7, 10, 14, 35, 37)	
	6 ATPases (*Atp6v0d1, Atp6v1a/b1/d/g1/h*)	ATPases lysosomal?; Atp6ap1 known from [91]
	1 ATPase associated protein (Atp6ap2)	
	4 Vamps (Vamp2/3/7/8)	
	35 other (e.g. Pam, Ptprn, Ptprn2, Dnajc5, Gnb2, Syt5, Sytl4, Vdac1)	
	(10 other incl. 14–3–3s: Ywhag, Ywhaq, **Ywhaz** & **Gapdh**; 4 novel proteins)	
MS/MS of gradient and synaptobrevin 2 (=Vamp2) antibody-purified INS1E granules [25]	(55 proteins in total; article states 51?)	Vamps might have been removed by immunopurification; **Sytl4** mentioned in text
	7 hormones/granins (**Ins1, Ins2, Scg2, Chga, Chgb**, Vgf)	
	3 proteases (**Cpe, Pcsk2, Ctsd**)	
	11 chaperones (Hspa5, **Pdia3**/6, Tra1, Hsc70-ps1, Hsp90ab1, P4hb, Hyou1)	Novel granule chaperones or ER contaminants?
	12 metabolic enzyme (cytosolic: Acly, Aldoa, Eno1, **Gapdh**, Pkm2; mitochondrial: Atp5a1/b, Aco2, Cox5b, Got2; lysosomal: *Gaa*; ER: Prkcsh)	Insulin granule candidates with previously known location
	4 vesicle transport (**Rab1a, Rab2a, Rab3a**, Tmed10)	New category, not in paper
	5 cytoskeletal proteins (Acta1, Actb, Tuba1a)	Cytoskeletal coprecipitation?
	4 signaling proteins (Gnao1, Gnal, Gnb4, **Ywhaz**)	Gnb2 found in above
	4 protein synthesis (Rpl18/23a, Eef1a1, Eef2)	Eef2 recategorized
	2 metal binding (Calr, **Nucb2**)	Calr also a chaperone
	2 nucleic acid binding (Hnrnpk, Npm1), 1 ion transport (Atp1a1)	Npm1 typically nucleolar

[a]Overlaps in bold. Acly, ATP citrate lyase: synthesis of cytosolic acetyl-CoA and lipid synthesis; Aco2, mitochondrial aconitase hydratase 2: citrate cycle; Acta1/b, actin alpha1/beta: cytoskeleton; Aldoa, aldolase A: glycolysis; Atp1a1, ATPase; Na+/K+ transporting; alpha 1 polypeptide: ATP-driven antiporter catalytic subunit; Atp5a1/b, ATP synthase H+ transporting mitochondrial F1 complex alpha1/beta polypeptide: part of mitochondrial F_1F_0 ATP synthase; Atp6v0d1, ATPase H+ transporting lysosomal 38kDa V0 subunit d1: part of proton pathway; Atp6v1x, ATPase H+ transporting lysosomal V1 subunit x: ATP hydrolysis; Atp6ap2, ATPase lysosomal accessory protein 2 = renin receptor: interacts w renin and vacuolar ATPase; Calr, calreticulin: ER calcium-binding chaperone; Chga, chromogranin A: precursor to bioactive peptides; Chgb, chromogranin B = Scg1: precursor?; chromogranin C = Scg2; Clu, clusterin: unknown; Cox5b, cytochrome c oxidase subunit Vb: terminal oxidase in mitochondrial electron transport; Cpe, carboxypeptidase E: proinsulin processing; Cpn1, carboxypeptidase N polypeptide 1: C-terminal basic protease; Cst3, cystatin 3: Cys protease inhibitor incl. Ctsb/h/l, Ctsb/f and cathepsin B/F: prob. lysosomal protease; Ctsl/d: prob. lysosomal and granule proteases; Dnajc5, DnaJ homolog subfamily C member 5: neurotransmitter release; Eef1a1, eukaryotic translation elongation factor 1 alpha 1; Eef2, eukaryotic translation elongation factor 2; Eno1, enolase 1 alpha: glycolysis and regulator; Epdr1, ependymin-related protein 2; Fn1, fibronectin 1: extracellular matrix; Gaa lysosomal alpha-glucosidase: glycogen breakdown (pred.); Gapdh, glyceraldehyde-3-phosphate dehydrogenase = G3pdh: cytosolic glycolysis and nuclear transcription; Gc, vitamin D binding protein; Gnal, guanine nucleotide binding protein alpha stimulating: G protein known from olfaction; Gnao1, guanine nucleotide binding protein (G protein) alpha activating activity polypeptide O: G protein subunit; Gnb2, Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2: G protein subunit; Gnb4, guanine nucleotide binding protein beta polypeptide 4: G protein subunit; Got2, glutamate oxaloacetate transaminase 2: mitochondrial Asp aminotransferase; Hnrnpk, heterogeneous nuclear ribonucleoprotein K: mRNA/DNA binding protein and regulator; Hsc70-ps1, heat shock cognate protein 70 pseudogene = Hsc72-ps1; Hsp90ab1, heat shock protein 90kDa alpha (cytosolic); class B member 1: cytosolic chaperone; Hspa5, heat shock 70 kDa protein 5 = Grp78 78 kDa glucose-regulated protein: ER chaperone and assembly of secreted proteins; Hspe1, heat shock 10kDa protein 1: mitochondrial matrix chaperone; Hyou1, hypoxia up-regulated 1: ER chaperone protecting from oxygen deprivation; Ins1/2, insulin 1/2: glucose-lowering hormone; Mdh2 mitochondrial malate dehydrogenase; Npm1, nucleophosmin (nucleolar phosphoprotein B23): nucleolar ribosome assembly and histone chaperone; Nptx1, neuronal pentraxin 1: uptake of synaptic material; Nucb2, nucleobindin 2: Golgi Ca^{2+} binding and homeostasis; P4hb, prolyl 4-hydroxylase beta polypeptide = Pdi(a1) protein disulfide isomerase: intra- and extracellular disulfide rearrangement/chaperone and aggregation; Pam,peptidyl-glycine alpha-amidating monooxygenase: activates peptides by amidation; Pcsk1 (PC1), proprotein convertase subtilisin/kexin type 1 = neuroendocrine convertase NEC1: prohormone esp. insulin processing; Pcsk1n, ProSAAS: PCsk1 inhibitor; Pcsk2, (PC2) = Nec2: proinsulin processing; Pdia3/6, protein disulfide-isomerase A3 (=Grp58)/A6: glycoprotein disulfide remodelling in ER; Pkm2, pyruvate kinase isozymes M1/M2: glycolysis (pred.); Prdx4, peroxiredoxin 4: redox/NFκB regulation; Prkcsh, protein kinase C substrate 80K-H: subunit of ER glucosidase 2; Psap prosapin, Sgp1 sulfated glycoprotein 1: lipid catabolism in lysosome and regulator; Rab, Ras-related GTP-binding protein: control of vesicle traffic; Rpl18, ribosomal protein L18; Rpl23a, ribosomal protein L23a; Ptprn, receptor-type tyrosine-protein phosphatase N (IA2)/N2 (IA2β): granule regulator; Scg2, secretogranin 2 = chromogranin C: cargo sorting and precursor to bioactive peptides; Scg3, unknown; Scg5 = Scg1 = neuroendocrine protein 7B2: Pcsk2 chaperon and peptide precursor; Sod1, superoxide dismutase; Stc1, stanniocalcin 1: calcium/phosphate-regulating protein; Syt5, synaptotagmin 5: calcium-dep. exocytosis; Sytl4, synaptotagmin-like 4 = granuphilin: calcium-dep. exocytosis; Tcn2, transcobalamin 2: cobalamin transport; Tmed10, transmembrane emp24-like trafficking protein 10: vesicle transport possibly ER to Golgi; Tra1, tumour rejection antigen gp96: ER chaperone; Tuba1a, tubulin alpha 1A: cytoskeleton; Vamp, vesicle-associated membrane protein = synaptobrevin: vesicle docking and fusion; Vdac1, voltage-dependent anion-selective channel protein 1: V-gated anion channel; Vgf, neurosecretory protein VGF: cell-cell interactions and synaptogenesis; Wif1, Wnt inhibitory factor 1; Ywhag/q/z, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma/theta/zeta polypeptide: versatile adapter proteins in signaling.

In addition to the distant effects of insulin, there is an intense debate over insulin autocrine signaling [31]. The majority of publications on the effect of insulin on its own transcription indicate a positive role, but a sizable minority of studies point instead to no measurable or even negative effects. The effect of insulin on its own secretion is even more contentious, with a slight majority of the reports indicating either no or negative effects. By contrast, the consensus regarding the positive role of insulin on β-cell proliferation and survival is almost unanimous, and β-cells express insulin receptors on their surface. However, studies observing an autocrine effect fall short of explaining how the β-cell can sense concentration differences and prevent the desensitization of its insulin receptors despite the very high local levels of insulin. Waves of insulin release have been proposed as a mechanism, but the effect is not yet sufficiently explored.

The C-peptide: countering damage

One 31 residue C-peptide is produced for every proinsulin cleaved, and thus constitutes the other most abundant cargo of the insulin granule. In mature granules with a crystalline core, most C-peptide is found in the soluble phase at the periphery of the lumen. The C-peptide was

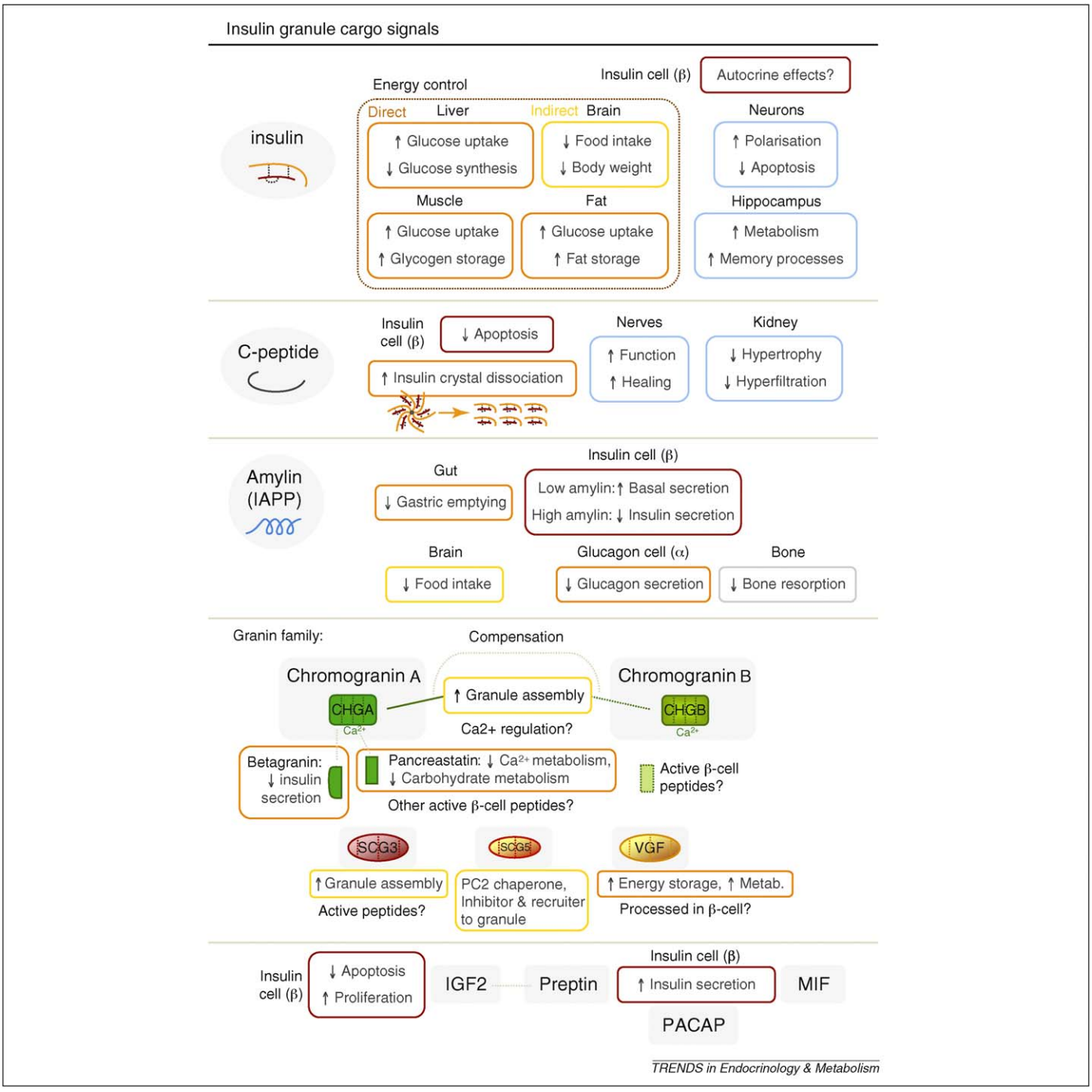

Figure 2. Insulin granule cargo signals. The cargo proteins of the insulin granule have a wide variety of effects on the β-cell itself, on neighboring cells in the islet and on distant cells. Signals regulating energy control are color-coded: orange for direct effects, yellow for indirect effects. Seemingly unrelated effects are in blue, and autocrine effects are in purple. Note that insulin signaling to the β-cell is controversial (see main text). The granins are cleaved (broken lines) to give rise to several peptides (shown below the precursor, e.g. betagranin is derived from CHGA). Peptides have separate functions to their precursors, but many effects relate to energy metabolism. It is still a matter of debate which peptides are produced from the granins of the insulin granule.

initially investigated for insulin-like effects but no such activity was discovered. However, C-peptide was subsequently shown to enhance insulin action by speeding up the dissociation of its hexameric form [32], and to reduce β-cell apoptosis [33]. It has also been proposed that C-peptide improves kidney and nerve function, particularly by reducing glomerular hyperfiltration [34] and reversing nociceptive nerve damage [35]. These findings would therefore suggest that the β-cell packages a molecule alleviating complications of elevated glucose with a molecule reducing blood sugar.

Modulating peptides

In addition to its main cargo of insulin and C-peptide, insulin granules contain small amounts of other peptide hormones. Islet amyloid polypeptide (IAPP; also known as amylin) is a 37 residue peptide, cosecreted with insulin at a variable ratio of ~1:3 [36], and known to inhibit glucagon

release and influence insulin secretion depending on concentration: low amylin increases basal insulin release, whereas higher concentrations inhibit glucose-induced insulin secretion [37]. In addition, amylin influences distant body function related to food; specifically, it inhibits gastric emptying similar to glucagon-like peptide (GLP)1 [38] and decreases food intake [39]. It also inhibits bone resorption [40], an interesting finding considering that osteoblasts stimulate β-cell proliferation [41]. In addition, similar to the amyloid precursor protein in Alzheimer's disease, amylin can misfold and cause β-cell death [42].

IGF2 is another peptide hormone that is probably contained in the insulin granule, based on data from cell lines and isolated islets [43]. IGF2 binding to the IGF1 receptor on the β-cell forms an autocrine feedback loop enhanced by GLP1 [43] that increases β-cell proliferation [44]. Similar to the granins, proIGF2 is processed into several peptides. In addition to IGF2, the proprotein gives rise to preptin, which was purified from the granules of an insulinoma cell line and shown to increase insulin secretion in this model [45], and also to stimulate primary osteoblast proliferation [46]. Another direct potentiator of β-cells is macrophage migration inhibitory factor (MIF), which colocalizes with insulin and increases insulin secretion in an islet perifusion model [47]. Likewise, pituitary adenylate cyclase-activating polypeptide (PACAP) was localized to insulin granules by EM and potentiates glucose-induced insulin secretion [48]. In summary, the insulin granule packs several peptide hormones that modulate the activity of the β-cell itself, and in many cases, distant cells also. It will be important to quantify their effects and to test whether these findings hold across species boundaries.

Granins

The chromogranin (CHG) secretogranin (SCG) protein family currently contains two evolutionarily related chromogranins and five heterogeneous secretogranins. All are acidic, heat-stable proteins found in the secretory granules of neuroendocrine cells. Insulin granules contain CHGA, CHGB (=SCG1), SCG2 (=CHGC), SCG3, SCG5 and VGF (=SCG7) [26,25] (Figures 1b, 2). Based on cell line experiments, it had been proposed that full-length CHGA is essential for granule biogenesis in the β-cell [49], but mouse null mutants demonstrated that other granins can compensate for its loss, resulting in only minor β-cell defects and normal blood glucose levels [50]. A separate study confirmed that CHGA knockout mice have normal blood glucose, despite having fewer islets with a decreased β-cell ratio and reduced plasma insulin levels [51]. Regarding the paralog CHGB, null mice have shown that the molecule is not required for granule formation but plays an important role in the release not only of insulin but also other islet hormones such as glucagon and somatostatin [52].

Granin-derived peptides

In addition to the function of the full-length protein, granins are cleaved by the same convertases that cleave proinsulin, namely PCsk1 (PC1) and PCsk2 (PC2) [53,54], giving rise to active peptides. Potentially, chromogranin A can give rise to about 13 peptides, of which only a few (e.g. betagranin [55] and pancreastatin [56]) have been confirmed to be made in the insulin granule. Many of the CHGA-derived peptides have functions related to energy metabolism. Betagranin [55] and pancreastatin inhibit insulin secretion [57]; the latter also reduces liver glycogen synthesis [58] and shifts insulin sensitivity from liver to muscle tissue [59]. CHGA 4–16 affect nociceptive neurons and modulate gut mobility [60]. Other CHGA-derived peptides seem to have unrelated functions and are probably not produced by the β-cell. Vasostatin 1, for example, is secreted by immune cells and has antimicrobial properties [61]. In summary, it has become clear that granins, despite their redundancy, are important for granule assembly and hormone secretion, although further studies are needed to separate clearly the effects of full-length protein versus derived peptides. We also anticipate the analysis of CHGA/CHGB double knockout mice and other granin null mutants.

GABA and ATP: stop and go

In addition to secreting peptide hormones, the β-cell produces and secretes small amounts of neurotransmitters that can affect the β-cell itself and/or other nearby cells. For example, GABA, a classic inhibitory neurotransmitter of the nervous system, is also found in about 15% of insulin granules [62]. Upon co-release with insulin, it reduces glucagon secretion from adjacent α-cells as shown in isolated rodent islets [63]. Because glucagon opposes many actions of insulin, its inhibition by GABA amounts to an increase in the effects of insulin.

Other small molecules cosecreted with insulin are ATP and ADP. Once in the extracellular space, they bind to several surface receptors, which send feedback signals back to the β-cell [64]. Extracellular ATP, for example, increases insulin secretion via binding to the Ca^{2+}-transporting P2X purinergic receptors, a process positively modulated by zinc [65]. Contrary to the stimulatory effect of ATP via P2X, a knockout mouse model for the G protein-coupled $P2Y_1$ receptor indicates an inhibitory function of ATP on insulin secretion [66]. Whether ATP is stimulatory or inhibitory might be determined by its concentration, with small amounts increasing insulin secretion and large amounts having the opposite effect [67].

After release from the granule into the intracellular space, ATP is rapidly degraded by ectonucleoside hydrolases to adenosine, which is presumably detected by the adenosine receptor A1. In isolated islets, adenosine has been shown to reduce insulin secretion [68]. Taken together, ATP exerts both positive and negative effects on insulin secretion, depending on its concentration and degradation dynamics.

Zinc: zooming to the forefront

After the discovery of the zinc transporter SLC30A8 (ZnT8) as a risk factor for type 2 diabetes and as an autoantigen in type 1 diabetes, much attention has focused on this small ion. ZnT8 KO mice have insulin crystallization defects, but synthesize and release insulin normally and achieve glucose homeostasis [69,70]. The hypothesis that Zn^{2+} released from insulin granules inhibits glucagon release from neighboring α-cells remains controversial, with the

majority of studies in favor [71,72] but others against [73]. It is possible that the role of the zinc transporter in diabetes risk lies at least partially in zinc supporting insulin signal transduction [74].

Membrane control

The insulin granule membrane is the control point for its biogenesis, transport, storage and exocytosis. To accomplish these crucial functions, numerous peripherally associated and integral membrane proteins, including channels, transporters, anchors and fusion factors, are targeted to its membrane (Figure 3). Most of these components have now been identified and characterized, but a few remain elusive. More recently, it has also become clear that many protein functions are influenced by lipid components in the membrane bilayer.

Assembly via transmembrane anchors, tethers and aggregation

The insulin granule is formed in the trans-Golgi network (TGN) by the concerted effect of several processes (Figure 3a): the presumptive interaction of cargo with intrinsic and peripheral membrane proteins such as granins; the interaction of proteins with the lipid environment; the progressive aggregation and precipitation of cargo peptides as a result of acidification and increase in Ca^{2+}; and the concomitant removal of non-granule components from the TGN.

Insulin granules contain several transmembrane proteins such as PTPRN, PTPRN2 and possibly peptidyl-α-amidating monooxygenase (PAM) [26]. Several regions of PTPRN2 have been shown to be important for its localization to the granule and possibly for membrane budding processes through its binding to the clathrin-associated protein AP2 [75,76].

Other proteins are peripherally associated with the luminal leaflet of the granule membrane and could facilitate the sorting of cargo proteins and their assembly. SCG3, for example, binds to the cholesterol-rich granule membrane [77]. The N-terminal loops of CHGA [78] and CHGB [79] have affinity for membranes, and have been implicated in the anchoring of these proteins, and thereby the granule cargo, to the membrane. Evidence that CPE [80] and PCsk1 [81] have a single transmembrane helix at their C-terminus has not been corroborated [82].

Several granule content proteins bind each other or aggregate, which further aids assembly. CHGA and CHGB spontaneously aggregates in the presence of Ca^{2+} and Zn^{2+} [83], SCG3 can bind to CHGA [84], whereas endoproteolytic conversion of proinsulin into insulin by PCsk1 facilitates the sorting and retention of this hormone into the granules [85]. Both the luminal [86] and cytosolic domains of PTPRNs can form homodimers [87,88], although the significance of these dimers for granule biogenesis has not been shown. Thus, membrane association and aggregation work together in the formation of insulin granules.

Ion channels of the insulin granule

A pH of 5.5 is required for optimal function of the proteases acting on proinsulin [19,89]. Therefore, granule maturation occurs with the acidification of its lumen. The complete subunit composition of the insulin granule proton pump has not yet been unequivocally identified, although the a3 isoform of V-ATPase, Atp6v0a3 [90], and the accessory subunit Atp6ap1 (Ac45) [91] have been shown to be important for H^+ import into the granule lumen.

The transport of only a few protons across the granule membrane would rapidly create a charge difference that halts further acidification. CLCN3 chloride channels were shown to dissipate this difference and play a role in priming granules for exocytosis in wild-type mouse β-cells [92] and subsequently in CLCN3 knockout mice [93,94]. As Cl^- transport increases with the ATP:ADP ratio, it could directly link the energy status of the cell with granule priming.

The granule lumen contains several other ions, among which calcium is prominent, because of its high intragranular concentration and importance in triggering exocytosis. Several studies have addressed the question of whether granular Ca^{2+} plays a signalling role, but convincing evidence has yet to emerge. For example, inositol 1,4,5-trisphosphate (IP_3) transmembrane receptors (ITPR) might be involved in the IP_3-gated release of Ca^{2+} from granular stores. In the related neuroendocrine chromaffin cell granule, ITPR interacts with CHGA and CHGB [95], both of which bind Ca^{2+} at low granular pH. In addition, CHGA has been shown to activate the ITPR in reconstituted liposomes and thereby increase its Ca^{2+} release activity [96]. However, the insulin granule contains no ITPRs, according to immuno-EM [97] and IP_3 activation experiments [98]. By contrast, the Ca^{2+} release from β-cell vesicles, possibly granules, via ryanodine receptors (RyR) is supported by a vesicle-associated membrane protein (VAMP)2–aequorin reporter in mouse insulinoma MIN6 cells [99]. Apart from the possibility of Ca^{2+} release from the granule, it has been proposed that granules associate with L-type voltage-gated Ca^{2+} channels in the outer membrane of the β-cell to form a microenvironment conducive to exocytosis [100] (Figure 3c). This enables the cell to achieve high local Ca^{2+} concentrations with only a few channels in the vicinity of the signal target (the insulin granule). Interestingly, prolonged treatment of β-cells with fatty acids disperses these high Ca^{2+} zones and reduces insulin secretion [101,102]. How insulin granules are tethered to Ca^{2+} channels remains to be determined.

K_{ATP} channels composed of channel forming Kir6.2 subunits (*KCNJ11*) and regulatory SUR1 subunits (*ABCC8*) are best known for their role in converting the glucose-induced increase of the ATP:ADP ratio into plasma membrane depolarization. A study observed that most K_{ATP} channels are not actually located at the cell surface, as in the canonical model of glucose-induced insulin secretion, but instead are in the insulin granule membrane [103]. However, further experiments argue against a signaling role for K_{ATP} channels in the granule and instead suggest that insulin granules might merely deliver K_{ATP} channels to the plasma membrane [104], where they are involved in the transduction of a cytosolic ATP/ADP to an electrical signal.

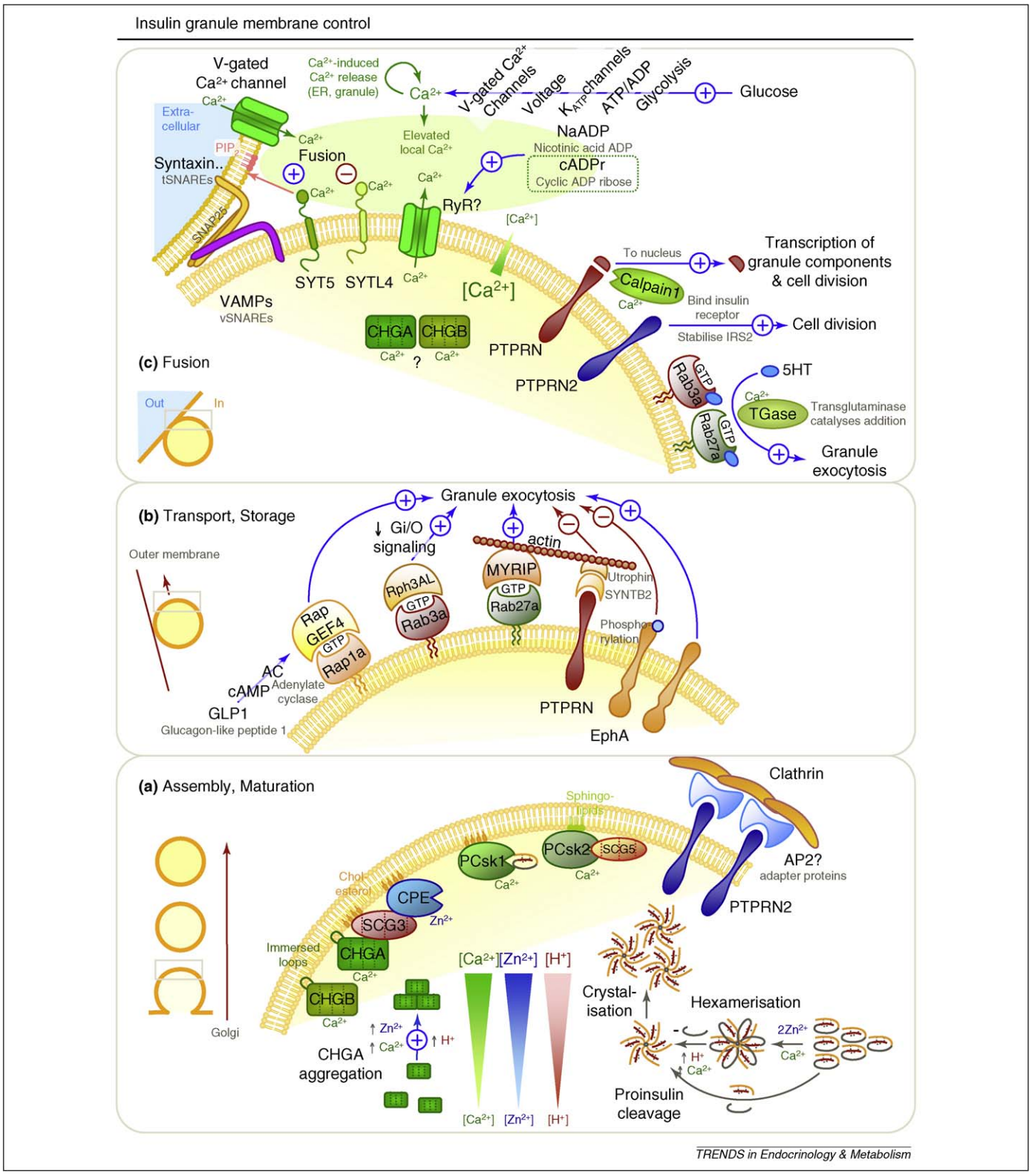

Figure 3. Insulin granule membrane control. From the bottom up, this figure shows **(a)** the assembly and maturation of the insulin granule, **(b)** its transport and storage and, finally, **(c)** its fusion with the plasma membrane. Membrane-associated proteins of the insulin granule fulfill important regulatory functions at all stages. The same proteins can have different roles at different stages of the life cycle of the granule. Standard protein names are used throughout. cAMP, cyclic adenosine monophosphate; $G_{i/o}$, cAMP-inhibiting G-protein coupled receptor (GPCR); IRS2, insulin receptor substrate 2. PIP_2, phosphatidylinositol 4,5-bisphosphate.

Granule traffic from Golgi to the surface

After assembly at the Golgi, insulin vesicles need to be transported to the surface for release. During the journey, the lumen is acidified and cargo molecules mature. In response to metabolic signals, particularly a rise in cytosolic Ca^{2+}, granules fuse with the outer membrane and release their content.

The best known vesicle traffic wardens are the numerous, small timer proteins called Rabs (Figure 3b/c), which switch from an inactive GDP state to an active GTP state.

Many so-called effector proteins work in concert with Rabs. Rabs are delivered and retrieved from membranes, to which they associate via prenyl groups that are otherwise sequestered by binding proteins in the cytosol. Proteomic studies in INS1 cells [26,25] have consistently identified Rab1a, Rab2a and Rab3a to be associated with vesicles. However, Rab1 and 2 are known transport regulators between the ER and the Golgi [105], so their detection is probably a result of ER vesicles not removed by the purification procedures employed. By contrast, Rab3a is known as a secretory vesicle regulator, and the interaction of Rab3 and its effector rabphilin 3A-like (Rph3al or Noc2) stimulates insulin secretion [106]. Although Rab27a was not identified in the proteomic studies described in Table 1, this protein is important for exocytosis and binds to cortical actin through its effector Myrip (Slac2c) [107]. Interestingly, intracellular 5-hydroxytryptamine (serotonin) increases insulin secretion when covalently bound to Rab3a and Rab27a during exocytosis, which renders them constitutively active [108]. A similar small GTPase called Rap1 potentiates insulin secretion in response to elevated cAMP levels sensed by its binding partner Rapgef4 (Epac2) [109]. In addition to the Rabs, transmembrane proteins anchor insulin granules to the cytoskeleton or its motor proteins. For example, PTPRN binds to the actin network via syntrophin [110], whereas the cytosolic tail of PAM affects the cytoskeleton via kalirin [111].

The ultimate step of vesicle fusion with the outer membrane is mediated by pairs of rod-like membrane proteins called SNAREs (soluble N-ethylmaleimide-sensitive factor attachment proteins): one in the vesicle (v) membrane and one in the target membrane. The insulin granule carries several vSNAREs and related regulatory synaptotagmins (Table 1), which react to increased calcium levels and bind to several other proteins of the fusion complex.

Feedback mechanisms from membrane proteins

In addition to the self-regulatory effects of β-cell granule cargo such as insulin and ATP, several granule transmembrane proteins influence functions beyond the immediate mechanisms required to assemble, transport, store and exocytose insulin granules.

The levels of the catalytically inactive protein tyrosine phosphatase PTPRN (IA2, ICA512) in the β-cells are increased by glucose, cAMP and insulin [112]. When PTPRN reaches the plasma membrane during insulin exocytosis, it is cleaved by the Ca^{2+}-activated protease calpain 1 [113]. The resulting cytoplasmic fragment moves to the nucleus, where it binds to the tyrosine phosphorylated signal transducer and activator of transcription (STAT)5, increases transcription of secretory granule genes [114] and increases β-cell proliferation [115]. In the cytosol, the fragments serve the additional function of displacing the actin linker β2-syntrophin from its full-length counterpart on the granule, thereby increasing granule mobility and exocytosis [88]. The homologous PTPRN2 has recently also been shown to regulate β-cell growth by binding to the insulin receptor at the plasma membrane and thereby increasing the stability and presumably the signaling through insulin receptor substrate (IRS)2 [116].

The transmembrane receptor tyrosine kinase Ephrin (Eph)A is also found in the insulin granule membrane. Once the granule reaches the cell surface, EphA on one cell engages its ligand ephrin-A on the surface of a juxtaposed β-cell, sending opposing insulin secretory signals into both. Under low glucose conditions, inhibitory EphA signals dominate and reduce basal exocytosis. Under high glucose, in turn, the balance is skewed towards stimulatory ephrin-A signals by dephosphorylating EphA, thereby increasing exocytosis. Taken together, this mechanism allows a cooperative of β-cells to both reduce basal and increase stimulated insulin secretion compared with that of a single cell [117].

All the above pathways involve integral membrane proteins of the insulin granule that recognize tyrosine phosphorylation motifs. They move and are stored with the granule, but their signaling function is only triggered upon exocytosis and is dependent on the local environment, thus enabling the β-cell to detect and react to insulin granule exocytosis.

The granule as a regulatory node

In the previous sections, we have described several molecules and pathways that demonstrate how the insulin granule of the β-cell acts as a signaling hub. The insulin container itself, the granule membrane, is studded with proteins such as Rap1, Rab3/27, PTPRN and EphA, which regulate exocytosis by various, partially unknown mechanisms (Figure 3). In the case of EphA, a large fraction of molecules typically resides in the granule membrane, where its unphosphorylated form increases granule exocytosis, whereas its phosphorylated form has the inverse effect [117].

It is important to note that the location of several insulin granule membrane proteins, such as the PTPRNs and the Rabs, crucially changes their signaling function. For example, PTPRN in the cytosol inhibits exocytosis by tethering granules to the cytoskeleton, whereas its cleavage at the plasma membrane gives rise to a cytosolic fragment that increases granule mobility [88], transcription [113] and β-cell proliferation [115].

Similar to the granule membrane, the inside is also loaded with signaling compounds, many of them generated by proteolytic processing, like insulin itself (Figure 2). When the granule content is poured out into the intracellular space between islet cells, many components, such as amylin, IGF2, zinc and ATP, affect adjacent cells [37] and the β-cell itself [43,65]. In addition, many of the cargo elements might have distal effects, such as preptin on bone [46] and amylin on the brain [65], which are often also related to energy metabolism (e.g. the appetite-lowering effect of amylin [39]).

We conclude that the insulin granule is an organelle in which many regulatory pathways cross and converge, and furthermore is the origin of several signals that regulate both the β-cell activity and affect neighboring and distant cell types. The concert of this multitude of signals ensures the coordination of several cellular processes and tissues to achieve nutrient homeostasis.

Acknowledgments
We thank Yanmei Liu, Silvia Brambillasca, Hassan Mziaut and Eckhard Lammert for improving the review with their suggestions. This work was in part supported by a grant from the German Federal Ministry of Education and Research (BMBF) to MS within the German Diabetes Competence Network (KKNDm). JS is the recipient of a MedDrive grant from the Medical Faculty at Dresden Univ. of Technology.

References

1 Fonseca, S.G. *et al.* (2009) Endoplasmic reticulum stress in beta-cells and development of diabetes. *Curr. Opin. Pharmacol.* 9, 763–770
2 Miaczynska, M. *et al.* (2004) Not just a sink: endosomes in control of signal transduction. *Curr. Opin. Cell Biol.* 16, 400–406
3 Suckale, J. and Solimena, M. (2008) Pancreas islets in metabolic signaling–focus on the beta-cell. *Front. Biosci.* 13, 7156–7171
4 Derbinski, J. *et al.* (2001) Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol.* 2, 1032–1039
5 Fan, Y. *et al.* (2009) Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J.* 28, 2812–2824
6 Deltour, L. *et al.* (1993) Differential expression of the two nonallelic proinsulin genes in the developing mouse embryo. *Proc. Natl. Acad. Sci. U. S. A.* 90, 527–531
7 Devaskar, S.U. *et al.* (1994) Insulin gene expression and insulin synthesis in mammalian neuronal cells. *J. Biol. Chem.* 269, 8445–8454
8 Song, J. *et al.* (2003) Axons guided by insulin receptor in Drosophila visual system. *Science* 300, 502–505
9 Rajala, A. *et al.* (2008) Loss of neuroprotective survival signal in mice lacking insulin receptor gene in rod photoreceptor cells. *J. Biol. Chem.* 283, 19781–19792
10 Kojima, H. *et al.* (2004) Extrapancreatic insulin-producing cells in multiple organs in diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2458–2463
11 Schuit, F.C. *et al.* (1988) Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells. *Proc. Natl. Acad. Sci. U. S. A.* 85, 3865–3869
12 Dean, P.M. (1973) Ultrastructural morphometry of the pancreatic beta-cell. *Diabetologia* 9, 115–119
13 Straub, S.G. *et al.* (2004) Stimulation of insulin release by glucose is associated with an increase in the number of docked granules in the beta-cells of rat pancreatic islets. *Diabetes* 53, 3179–3183
14 Howell, S.L. (1984) The mechanism of insulin secretion. *Diabetologia* 26, 319–327
15 Dunn, M.F. (2005) Zinc-ligand interactions modulate assembly and stability of the insulin hexamer – a review. *Biometals* 18, 295–303
16 Yu, S.S. and Kitbachi, A.E. (1973) Biological activity of proinsulin and related polypeptides in the fat tissue. *J. Biol. Chem.* 248, 3753–3761
17 Galloway, J.A. *et al.* (1992) Biosynthetic human proinsulin. Review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. *Diabetes Care* 15, 666–692
18 Steiner, D.F. *et al.* (2009) A brief perspective on insulin production. *Diabetes. Obes. Metab.* 11 (Suppl 4), 189–196
19 Davidson, H.W. *et al.* (1988) Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic beta cell via two distinct site-specific endopeptidases. *Nature* 333, 93–96
20 Marsh, B.J. *et al.* (2007) Regulated autophagy controls hormone content in secretory-deficient pancreatic endocrine beta-cells. *Mol. Endocrinol.* 21, 2255–2269
21 Michael, D.J. *et al.* (2006) Pancreatic beta-cells secrete insulin in fast- and slow-release forms. *Diabetes* 55, 600–607
22 Halban, P.A. (1982) Differential rates of release of newly synthesized and of stored insulin from pancreatic islets. *Endocrinology* 110, 1183–1188
23 Gold, G. *et al.* (1982) Evidence that glucose "marks" beta cells resulting in preferential release of newly synthesized insulin. *Science* 218, 56–58
24 Hutton, J.C. *et al.* (1982) Isolation and characterisation of insulin secretory granules from a rat islet cell tumour. *Diabetologia* 23, 365–373
25 Hickey, A.J.R. *et al.* (2009) Proteins associated with immunopurified granules from a model pancreatic islet beta-cell system: proteomic snapshot of an endocrine secretory granule. *J. Proteome Res.* 8, 178–186
26 Brunner, Y. *et al.* (2007) Proteomics analysis of insulin secretory granules. *Mol. Cell Proteomics* 6, 1007–1017
27 Cox, J. and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* 26, 1367–1372
28 Saltiel, A.R. and Kahn, C.R. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414, 799–806
29 Pollak, M. (2008) Insulin and insulin-like growth factor signalling in neoplasia. *Nat. Rev. Cancer* 8, 915–928
30 McNay, E.C. (2007) Insulin and ghrelin: peripheral hormones modulating memory and hippocampal function. *Curr. Opin. Pharmacol.* 7, 628–632
31 Leibiger, I.B. *et al.* (2008) Insulin signaling in the pancreatic beta-cell. *Annu. Rev. Nutr.* 28, 233–251
32 Shafqat, J. *et al.* (2006) Proinsulin C-peptide elicits disaggregation of insulin resulting in enhanced physiological insulin effects. *Cell Mol. Life Sci.* 63, 1805–1811
33 Bugliani, M. *et al.* (2007) Effects of C-peptide on isolated human pancreatic islet cells. *Diabetes Metab. Res. Rev.* 23, 215–219
34 Samnegård, B. *et al.* (2005) C-peptide prevents glomerular hypertrophy and mesangial matrix expansion in diabetic rats. *Nephrol. Dial. Transplant* 20, 532–538
35 Kamiya, H. *et al.* (2006) C-Peptide reverses nociceptive neuropathy in type 1 diabetes. *Diabetes* 55, 3581–3587
36 Ogawa, A. *et al.* (1990) Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. *J. Clin. Invest.* 85, 973–976
37 Akesson, B. *et al.* (2003) Islet amyloid polypeptide inhibits glucagon release and exerts a dual action on insulin release from isolated islets. *Regul. Pept.* 111, 55–60
38 Young, A.A. *et al.* (1996) Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7-36) NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. *Metabolism* 45, 1–3
39 Rushing, P.A. *et al.* (2000) Amylin: a novel action in the brain to reduce body weight. *Endocrinology* 141, 850–853
40 Dacquin, R. *et al.* (2004) Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo. *J. Cell Biol.* 164, 509–514
41 Lee, N.K. *et al.* (2007) Endocrine regulation of energy metabolism by the skeleton. *Cell* 130, 456–469
42 Khemtémourian, L. *et al.* (2008) Recent insights in islet amyloid polypeptide-induced membrane disruption and its role in beta-cell death in type 2 diabetes mellitus. Exp. Diabetes Res. 2008, DOI: doi:10.1155/2008/421287
43 Cornu, M. *et al.* (2009) Glucagon-like peptide-1 protects beta-cells against apoptosis by increasing the activity of an IGF-2/IGF-1 receptor autocrine loop. *Diabetes* 58, 1816–1825
44 Cornu, M. *et al.* (2010) Glucagon-like peptide-1 increases beta-cell glucose competence and proliferation by translational induction of insulin-like growth factor-1 receptor expression. *J. Biol. Chem.* 285, 10538–10545
45 Buchanan, C.M. *et al.* (2001) Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet beta-cells and enhances insulin secretion. *Biochem. J.* 360, 431–439
46 Cornish, J. *et al.* (2007) Preptin, another peptide product of the pancreatic beta-cell, is osteogenic in vitro and in vivo. *Am. J. Physiol. Endocrinol. Metab.* 292, E117–122
47 Waeber, G. *et al.* (1997) Insulin secretion is regulated by the glucose-dependent production of islet beta cell macrophage migration inhibitory factor. *Proc. Natl. Acad. Sci. U. S. A.* 94, 4782–4787
48 Portela-Gomes, G.M. *et al.* (2003) PACAP is expressed in secretory granules of insulin and glucagon cells in human and rodent pancreas. Evidence for generation of cAMP compartments uncoupled from hormone release in diabetic islets. *Regul. Pept.* 113, 31–39
49 Kim, T. *et al.* (2001) Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis. *Cell* 106, 499–509
50 Hendy, G.N. *et al.* (2006) Targeted ablation of the chromogranin a (Chga) gene: normal neuroendocrine dense-core secretory granules

and increased expression of other granins. *Mol. Endocrinol.* 20, 1935–1947
51 Portela-Gomes, G.M. *et al.* (2008) The importance of chromogranin A in the development and function of endocrine pancreas. *Regul. Pept.* 151, 19–25
52 Obermüller, S. *et al.* (2010) Defective secretion of islet hormones in chromogranin-B deficient mice. *PLoS ONE* 5, e8936
53 Udupi, V. *et al.* (1999) Prohormone convertase-1 is essential for conversion of chromogranin A to pancreastatin. *Regul. Pept.* 83, 123–127
54 Hoflehner, J. *et al.* (1995) Processing of secretogranin II by prohormone convertases: importance of PC1 in generation of secretoneurin. *FEBS Lett.* 360, 294–298
55 Schmid, G.M. *et al.* (2007) Inhibition of insulin secretion by betagranin, an N-terminal chromogranin A fragment. *J. Biol. Chem.* 282, 12717–12724
56 Arden, S.D. *et al.* (1994) The post-translational processing of chromogranin A in the pancreatic islet: involvement of the eukaryote subtilisin PC2. *Biochem. J.* 298, 521–528
57 Tatemoto, K. *et al.* (1986) Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 324, 476–478
58 Sánchez-Margalet, V. and Goberna, R. (1994) Pancreastatin inhibits insulin-stimulated glycogen synthesis but not glycolysis in rat hepatocytes. *Regul. Pept.* 51, 215–220
59 Gayen, J.R. *et al.* (2009) A novel pathway of insulin sensitivity in chromogranin A null mice: a crucial role for pancreastatin in glucose homeostasis. *J. Biol. Chem.* 284, 28498–28509
60 Ghia, J. *et al.* (2004) The effect of a chromogranin A-derived peptide (CgA4-16) in the writhing nociceptive response induced by acetic acid in rats. *Life Sci.* 75, 1787–1799
61 Lugardon, K. *et al.* (2000) Antibacterial and antifungal activities of vasostatin-1, the N-terminal fragment of chromogranin A. *J. Biol. Chem.* 275, 10745–10753
62 Braun, M. *et al.* (2007) Corelease and differential exit via the fusion pore of GABA, serotonin, and ATP from LDCV in rat pancreatic beta cells. *J. Gen. Physiol.* 129, 221–231
63 Bailey, S.J. *et al.* (2007) Glucose-dependent regulation of gamma-aminobutyric acid (GABA A) receptor expression in mouse pancreatic isl*et al*pha-cells. *Diabetes* 56, 320–327
64 Petit, P. *et al.* (2009) P2 purinergic signalling in the pancreatic beta-cell: control of insulin secretion and pharmacology. *Eur. J. Pharm. Sci.* 37, 67–75
65 Richards-Williams, C. *et al.* (2008) Extracellular ATP and zinc are co-secreted with insulin and activate multiple P2X purinergic receptor channels expressed by islet beta-cells to potentiate insulin secretion. *Purinergic Signal.* 4, 393–405
66 Léon, C. *et al.* (2005) The P2Y(1) receptor is involved in the maintenance of glucose homeostasis and in insulin secretion in mice. *Purinergic Signal.* 1, 145–151
67 Verspohl, E.J. *et al.* (2002) Effect of purinergic agonists and antagonists on insulin secretion from INS-1 cells (insulinoma cell line) and rat pancreatic islets. *Can. J. Physiol. Pharmacol.* 80, 562–568
68 Bertrand, G. *et al.* (1989) Membrane and intracellular effects of adenosine in mouse pancreatic beta-cells. *Am. J. Physiol.* 257, E473–478
69 Lemaire, K. *et al.* (2009) Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. *Proc. Natl. Acad. Sci. U. S. A.* 106, 14872–14877
70 Nicolson, T.J. *et al.* (2009) Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. *Diabetes* 58, 2070–2083
71 Ishihara, H. *et al.* (2003) Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. *Nat. Cell Biol.* 5, 330–335
72 Slucca, M. *et al.* (2010) ATP-sensitive K+ channel mediates the zinc switch-off signal for glucagon response during glucose deprivation. *Diabetes* 59, 128–134
73 Ravier, M.A. and Rutter, G.A. (2005) Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells. *Diabetes* 54, 1789–1797
74 Jansen, J. *et al.* (2009) Zinc and diabetes–clinical links and molecular mechanisms. *J. Nutr. Biochem.* 20, 399–417
75 Torii, S. *et al.* (2005) Cytoplasmic transport signal is involved in phogrin targeting and localization to secretory granules. *Traffic* 6, 1213–1224
76 Wasmeier, C. *et al.* (2002) The lumenal domain of the integral membrane protein phogrin mediates targeting to secretory granules. *Traffic* 3, 654–665
77 Hosaka, M. *et al.* (2005) Interaction between secretogranin III and carboxypeptidase E facilitates prohormone sorting within secretory granules. *J. Cell Sci.* 118, 4785–4795
78 Taupenot, L. *et al.* (2002) Identification of a novel sorting determinant for the regulated pathway in the secretory protein chromogranin A. *J. Cell Sci.* 115, 4827–4841
79 Glombik, M.M. *et al.* (1999) The disulfide-bonded loop of chromogranin B mediates membrane binding and directs sorting from the trans-Golgi network to secretory granules. *EMBO J.* 18, 1059–1070
80 Dhanvantari, S. *et al.* (2002) Carboxypeptidase E, a prohormone sorting receptor, is anchored to secretory granules via a C-terminal transmembrane insertion. *Biochemistry* 41, 52–60
81 Arnaoutova, I. *et al.* (2003) The prohormone processing enzyme PC3 is a lipid raft-associated transmembrane protein. *Biochemistry* 42, 10445–10455
82 Stettler, H. *et al.* (2005) Proprotein convertase PC3 is not a transmembrane protein. *Biochemistry* 44, 5339–5345
83 Jain, R.K. *et al.* (2002) In vitro aggregation of the regulated secretory protein chromogranin A. *Biochem. J.* 368, 605–610
84 Hosaka, M. *et al.* (2004) Secretogranin III binds to cholesterol in the secretory granule membrane as an adapter for chromogranin A. *J. Biol. Chem.* 279, 3627–3634
85 Kuliawat, R. *et al.* (2000) Proinsulin endoproteolysis confers enhanced targeting of processed insulin to the regulated secretory pathway. *Mol. Biol. Cell* 11, 1959–1972
86 Primo, M.E. *et al.* (2008) Structure of the mature ectodomain of the human receptor-type protein-tyrosine phosphatase IA-2. *J. Biol. Chem.* 283, 4674–4681
87 Gross, S. *et al.* (2002) Multimerization of the protein-tyrosine phosphatase (PTP)-like insulin-dependent diabetes mellitus autoantigens IA-2 and IA-2beta with receptor PTPs (RPTPs). Inhibition of RPTPalpha enzymatic activity. *J. Biol. Chem.* 277, 48139–48145
88 Trajkovski, M. *et al.* (2008) Regulation of insulin granule turnover in pancreatic beta-cells by cleaved ICA512. *J. Biol. Chem.* 283, 33719–33729
89 Orci, L. *et al.* (1986) Conversion of proinsulin to insulin occurs coordinately with acidification of maturing secretory vesicles. *J. Cell Bio.* 103, 2273–2281
90 Sun-Wada, G. *et al.* (2006) The a3 isoform of V-ATPase regulates insulin secretion from pancreatic beta-cells. *J. Cell Sci.* 119, 4531–4540
91 Louagie, E. *et al.* (2008) Role of furin in granular acidification in the endocrine pancreas: identification of the V-ATPase subunit Ac45 as a candidate substrate. *Proc. Natl. Acad. Sci. U. S. A.* 105, 12319–12324
92 Barg, S. *et al.* (2001) Priming of insulin granules for exocytosis by granular Cl(-) uptake and acidification. *J. Cell Sci.* 114, 2145–2154
93 Deriy, L.V. *et al.* (2009) The granular chloride channel ClC-3 is permissive for insulin secretion. *Cell Metab.* 10, 316–323
94 Li, D. *et al.* (2009) Suppression of sulfonylurea- and glucose-induced insulin secretion in vitro and in vivo in mice lacking the chloride transport protein ClC-3. *Cell Metab.* 10, 309–315
95 Yoo, S.H. *et al.* (2001) Localization of three types of the inositol 1,4,5-trisphosphate receptor/Ca(2+) channel in the secretory granules and coupling with the Ca(2+) storage proteins chromogranins A and B. *J. Biol. Chem.* 276, 45806–45812
96 Thrower, E.C. *et al.* (2002) Activation of the inositol 1,4,5-trisphosphate receptor by the calcium storage protein chromogranin A. *J. Biol. Chem.* 277, 15801–15806
97 Ravazzola, M. *et al.* (1996) Inositol 1,4,5-trisphosphate receptor subtype 3 in pancreatic islet cell secretory granules revisited. *Proc. Natl. Acad. Sci. U. S. A.* 93, 2745–2748
98 Mitchell, K.J. *et al.* (2001) Dense core secretory vesicles revealed as a dynamic Ca(2+) store in neuroendocrine cells with a vesicle-associated membrane protein aequorin chimaera. *J. Cell Biol.* 155, 41–51

99 Mitchell, K.J. *et al.* (2003) Ryanodine receptor type I and nicotinic acid adenine dinucleotide phosphate receptors mediate Ca2+ release from insulin-containing vesicles in living pancreatic beta-cells (MIN6). *J. Biol. Chem.* 278, 11057–11064
100 Barg, S. *et al.* (2001) Fast exocytosis with few Ca(2+) channels in insulin-secreting mouse pancreatic B cells. *Biophys. J.* 81, 3308–3323
101 Hoppa, M.B. *et al.* (2009) Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca(2+) channels from secretory granules. *Cell Metab.* 10, 455–465
102 Collins, S.C. *et al.* (2010) Progression of diet-induced diabetes in C57BL6J mice involves functional dissociation of Ca2(+) channels from secretory vesicles. *Diabetes* 59, 1192–1201
103 Geng, X. *et al.* (2003) The insulin secretory granule is the major site of K(ATP) channels of the endocrine pancreas. *Diabetes* 52, 767–776
104 Varadi, A. *et al.* (2006) Intracellular ATP-sensitive K+ channels in mouse pancreatic beta cells: against a role in organelle cation homeostasis. *Diabetologia* 49, 1567–1577
105 Zerial, M. and McBride, H. (2001) Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2, 107–117
106 Matsumoto, M. *et al.* (2004) Noc2 is essential in normal regulation of exocytosis in endocrine and exocrine cells. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8313–8318
107 Waselle, L. *et al.* (2003) Involvement of the Rab27 binding protein Slac2c/MyRIP in insulin exocytosis. *Mol. Biol. Cell* 14, 4103–4113
108 Paulmann, N. *et al.* (2009) Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol.* 7, e1000229
109 Shibasaki, T. *et al.* (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19333–19338
110 Ort, T. *et al.* (2000) The receptor tyrosine phosphatase-like protein ICA512 binds the PDZ domains of beta2-syntrophin and nNOS in pancreatic beta-cells. *Eur. J. Cell Biol.* 79, 621–630
111 Mains, R.E. *et al.* (1999) Kalirin, a multifunctional PAM COOH-terminal domain interactor protein, affects cytoskeletal organization and ACTH secretion from AtT-20 cells. *J. Biol. Chem.* 274, 2929–2937
112 Löbner, K. *et al.* (2002) Different regulated expression of the tyrosine phosphatase-like proteins IA-2 and phogrin by glucose and insulin in pancreatic islets: relationship to development of insulin secretory responses in early life. *Diabetes* 51, 2982–2988
113 Trajkovski, M. *et al.* (2004) Nuclear translocation of an ICA512 cytosolic fragment couples granule exocytosis and insulin expression in {beta}-cells. *J. Cell Biol.* 167, 1063–1074
114 Mziaut, H. *et al.* (2006) Synergy of glucose and growth hormone signalling in islet cells through ICA512 and STAT5. *Nat. Cell Biol.* 8, 435–445
115 Mziaut, H. *et al.* (2008) ICA512 signaling enhances pancreatic beta-cell proliferation by regulating cyclins D through STATs. *Proc. Natl. Acad. Sci. U. S. A.* 105, 674–679
116 Torii, S. *et al.* (2009) Gene silencing of phogrin unveils its essential role in glucose-responsive pancreatic beta-cell growth. *Diabetes* 58, 682–692
117 Konstantinova, I. *et al.* (2007) EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. *Cell* 129, 359–370