



Insulin granule biogenesis and exocytosis

Muhmmad Omar-Hmeadi¹ · Olof Idevall-Hagren¹

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Abstract

Insulin is produced by pancreatic β -cells, and once released to the blood, the hormone stimulates glucose uptake and suppresses glucose production. Defects in both the availability and action of insulin lead to elevated plasma glucose levels and are major hallmarks of type-2 diabetes. Insulin is stored in secretory granules that form at the trans-Golgi network. The granules undergo extensive modifications en route to their release sites at the plasma membrane, including changes in both protein and lipid composition of the granule membrane and lumen. In parallel, the insulin molecules also undergo extensive modifications that render the hormone biologically active. In this review, we summarize current understanding of insulin secretory granule biogenesis, maturation, transport, docking, priming and eventual fusion with the plasma membrane. We discuss how different pools of granules form and how these pools contribute to insulin secretion under different conditions. We also highlight the role of the β -cell in the development of type-2 diabetes and discuss how dysregulation of one or several steps in the insulin granule life cycle may contribute to disease development or progression.

Keywords Diabetes · Lipids · β -Cell · Insulin

Introduction

Insulin is the major blood-glucose lowering hormone, and it acts by promoting glucose uptake and storage and by suppressing glucose production. A single cell-type, the β -cell, is solely responsible for all insulin production and secretion. These cells are located within the islets of Langerhans, micro-organs scattered throughout the pancreas, where it is mixed with other endocrine cells involved in blood glucose regulation. Insulin is stored in large, dense-core granules and is released to the circulation in response to elevated plasma glucose concentrations. Insulin secretion is largely controlled at the level of the β -cell, and a single β -cell, taken out of the pancreas, retains the ability to release insulin in response to glucose. This process begins with glucose uptake and metabolism to ATP that subsequently closes ATP-sensitive K^+ -channels, resulting in membrane depolarization, opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx and insulin granule exocytosis. Insulin granules can be divided into two functionally distinct pools; one containing granules that

are release competent and one containing granules that are not. The release competent granules are found immediately adjacent to the plasma membrane and their release gives rise to a sharp increase in plasma insulin known as the first phase of secretion. This granule pool is gradually depleted during prolonged stimulation, and new granules need to be recruited to sustain insulin secretion and maintain blood glucose homeostasis. This process involves both mobilization of granules from the larger reserve pool and de novo generation of insulin granules. Failure to appropriately secrete insulin results in impaired blood glucose control and is a hallmark of type-2 diabetes (T2D). In this review, we describe the molecular steps controlling granule formation at the Golgi and the subsequent granule maturation, transport, docking to and fusion with the plasma membrane. We highlight the role of lipids in multiple regulatory steps in insulin granule biogenesis and release and also discuss how defects in granule biogenesis and release contribute to diabetes development.

Preproinsulin synthesis

The mammalian insulin gene encodes a single chain precursor protein, preproinsulin, which matures into active insulin through a series of proteolytic reactions. The β -cell-specific

✉ Olof Idevall-Hagren
olof.idevall@mcb.uu.se

¹ Department of Medical Cell Biology, Uppsala University, Biomedical Centre, Husargatan 3, 75123 Uppsala, Sweden

expression of insulin is achieved by a glucose-dependent transcriptional program [1]. Post-transcriptional mechanisms, such as stabilization of insulin mRNA by mRNA-binding proteins, also contribute to glucose-effects on insulin production [2], as does translational mechanisms, including stimulation of translation initiation and elongation [3]. As proinsulin mRNA is translated, the N-terminal signal peptide is recognized by signal recognition particles that direct the ribosome to the ER and facilitate proinsulin translocation across the ER membrane [3, 4], where the signal peptide is removed [5, 6]. The resulting proinsulin molecules fold with the help of chaperones, including Glucose-regulated protein 94 (GRP94) [7], and form stable hexamers through interactions with zinc ions [8]. After passing the quality control checkpoint, proinsulin is transported to the cis-face of the Golgi apparatus via the ER-Golgi interface compartment. Following modifications by Golgi-resident enzymes, the proinsulin molecules reach the trans-Golgi network (TGN).

trans-Golgi network sorting of proinsulin and formation of immature secretory granules

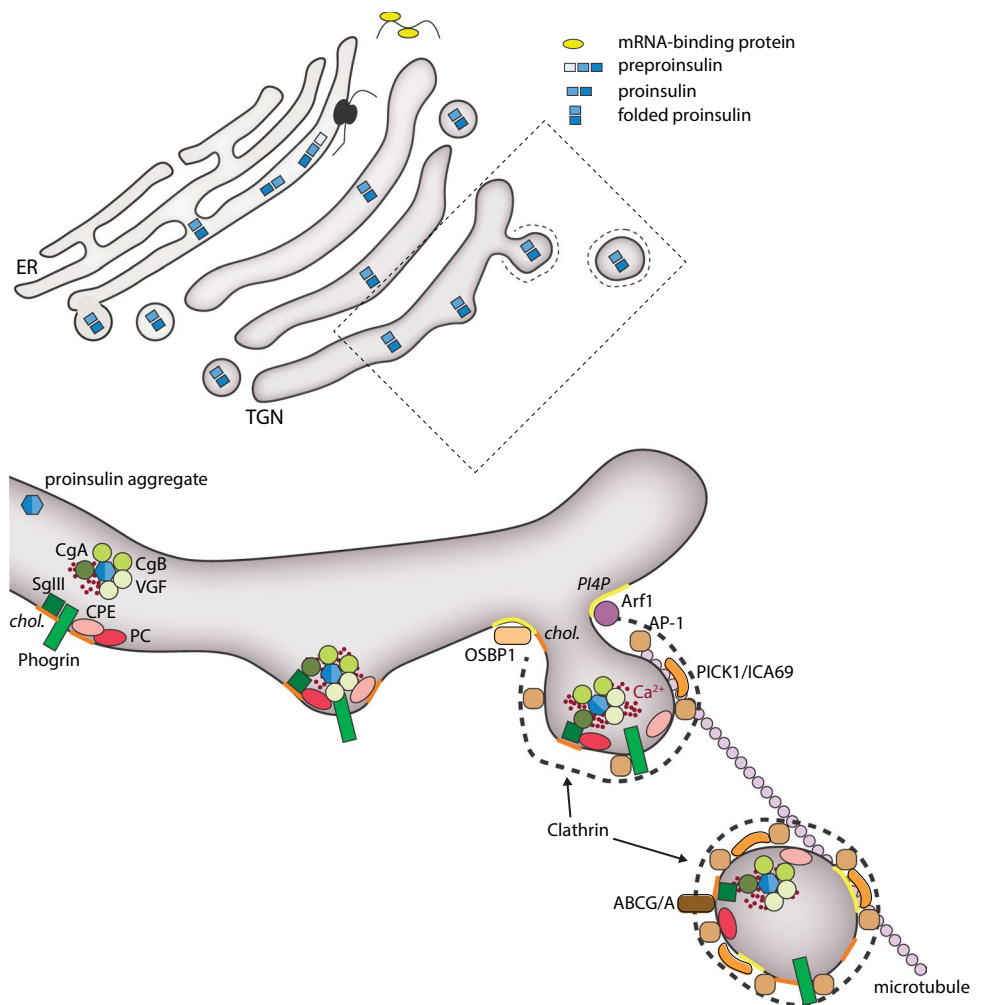
Insulin secretory granule (ISG) cargo, including proinsulin, is packaged into nascent granules that bud off from the TGN. Consensus regarding how sorting of ISG cargo is achieved is lacking, and the topic has been extensively debated over the last 30 years. The disagreement concerns whether sorting of proinsulin and other granule components occurs by sequestration in the TGN or by retention in the ISG. There is experimental evidence to support both modes of sorting, and perhaps they are not mutually exclusive but operate in parallel. Irrespective of model, the outcome is the formation of ISG with a composition that differs from that of the TGN.

Soluble proteins destined for ISG can form large aggregates in the presence of millimolar Ca^{2+} and weakly acidic pH [9, 10]. Such conditions exist both in the TGN and ISG, and these aggregates facilitate the condensation of bioactive peptides, such as proinsulin [9]. There is general consensus that aggregation of proinsulin occurs and that this aggregation is important for hormone maturation. However, it is not clear if this condensation occurs in the TGN or in the immature ISG [11]. Numerous proteins of importance for proinsulin condensation have been identified, including chromogranin A (CHGA), chromogranin B (CHGB) and VGF. Mice lacking CHGB exhibit reduced glucose-stimulated insulin secretion, and similar defects are seen in islets and clonal β -cells following transient knockdown of CHGB [12, 13]. Mechanistically, loss of CHGB does not affect ISG biogenesis but it impairs

proinsulin processing, leading to reduced insulin content [12]. In contrast, transient knockdown of CHGB impairs ISG biogenesis in clonal β -cells [13]. This difference is likely due to compensatory upregulation of other members of the granin family [14]. β -Cells lacking VGF also exhibit defect proinsulin processing and ISG biogenesis [15]. Noteworthy, the overexpression of a single aggregate-inducing protein is sufficient to induce formation of aggregate-containing vesicles in cells lacking a regulated secretory pathway [16], indicating that at least the aggregating capacity of these proteins is functionally redundant. Such redundancy may also help to explain variable outcomes in loss of function studies. Sorting of ISG cargo also occurs independent of aggregation through interactions with the TGN membrane. This involves both interactions with trans-membrane sorting receptors, such as phogrin (IA-2/2 β) [17–21], and with lipids in the TGN or ISG membranes. Whereas sorting of proinsulin to the ISG largely depends on aggregation, sorting of the enzymes involved in its processing to biologically active insulin, including carboxypeptidase E (CPE) and the prohormone convertases PC1/3 and PC2, depends on membrane interactions [22–24]. How sorting through membrane interaction occurs is not fully understood, but it seems to depend on the high cholesterol content (50–70 mol%) of the TGN and ISG membrane [25, 26]. Most ISG cholesterol likely originates from the TGN membrane and depend on directed cholesterol delivery via oxysterol-binding protein 1 (OSBP-1) and members of the ATP-binding cassette (ABC) cholesterol transporters. Indeed, loss of function experiments has confirmed roles of these proteins in ISG biogenesis [25–28]. The TGN membrane is also rich in phosphatidylinositol 4-phosphate (PI4P), a lipid that is required for the formation of ISGs [29–31]. PI4P, together with active, GTP-bound Arf1, recruits AP-1 and other coat proteins [32], resulting in the assembly of a clathrin coat around the budding ISG [33]. Diacylglycerol (DAG) is another lipid that accumulates in the TGN, where it generates membrane curvature that facilitates ISG budding [34, 35] and promotes fission through activation of PKD [36]. The fission process also depends on Golgi-derived microtubules which provide a scaffold for the budding ISG [37] (Fig. 1).

In summary, the formation of ISG at the TGN depends on the coordinated activity of membrane-localized and soluble proteins and their interactions with specific membrane lipids. Surprisingly little research has focused on elucidating the early steps of ISG biogenesis, and most of it has focused on how insulin and its processing enzymes are delivered to the granules. Information regarding to what extent other proteins key to ISG maturation and release are enriched on the granules already at the site of formation is largely missing.

Fig. 1 Insulin granule formation at the *trans*-Golgi network. Proinsulin is sequestered to the secretory granule budding site through interactions with proteins like Chromogranin A/B (CgA/B) and VGF, and together these form large aggregates. Additional sorting is provided by membrane-bound proteins like Phogrin and Secretogranin 3 (SgIII), and this occurs in parallel with sorting of proinsulin-processing proteins (PC1/3, PC2 and CPE). Insulin granule budding depends on the action of Arf1 and the membrane bud is stabilized by clathrin, the clathrin-adaptor AP-1, and BAR-domain proteins (PICK1 and ICA69) and is facilitated by high concentrations of the phospholipid PI4P



Insulin secretory granule maturation

The TGN plays an important role in defining the content of ISG, but the sorting process also continues through clathrin-mediated membrane retrieval after granule formation [38–40]. The clathrin-mediated sorting at the ISG is thought to be important for removal of proteolytic enzymes and proteins destined for other organelles, and results in a reduction in ISG size [41, 42]. The ISG additionally undergo a series of maturation steps, where acidification and removal of coat proteins coincide with the conversion of proinsulin to insulin [39, 40, 43]. The entire maturation process, estimated from pulse-chase experiments of radio-labelled proinsulin, takes around 3 h [44]. This is much shorter than the half-life of insulin, which is around 3 days [45], consistent with most insulin being stored in a reserve pool of granules.

The ISG lumen is acidified in a glucose-dependent manner [46] through the action of the vesicular ATP-dependent proton pump (V-ATPase), which in turn increases the activity of the proinsulin converting enzymes PC1/3 and PC2 [39, 44, 47]. These enzymes cleave off a fragment (C-peptide)

from proinsulin, converting it into insulin. Both C-peptide and insulin are subsequently used as substrates by CPE, which removes basic residues at the respective cleavage site [48]. The acidification starts shortly after budding from the TGN and occurs over a time-period of around 30 min, during which proinsulin is converted to insulin and the clathrin coat is lost [43, 49]. The mechanism that controls uncoating of ISG is relatively poorly characterized, at least in part because this pool of clathrin-coated vesicles is difficult to separate from clathrin-coated endocytic vesicles of plasma membrane origin. One elegant study followed the lifetime of TGN-derived clathrin-coated vesicles in kidney epithelial cells by rapid 3D imaging and found that these vesicles had a lifetime of around 40 s [50]. This is in sharp contrast to the estimated lifetime of the clathrin-coated ISG, but similar to the lifetime of an endocytic clathrin-coated vesicle [51]. It is possible that the lifetime of clathrin-coated ISG in β -cells is longer, but it still puts into question the importance of clathrin uncoating for the correct maturation of insulin [41, 52]. Mechanistically, the uncoating of ISG likely involves the adaptor protein Auxilin and the cytosolic heat shock cognate

protein complex 70 (Hsc70) [53]. Auxilin is recruited to the clathrin-coated granules, likely via binding to phosphoinositides, and subsequently recruits Hsc70, which initiates ATP-dependent removal of clathrin and other coat proteins [53–55]. The clathrin coat stabilizes the ISG, but this function is also shared with other proteins, including the BAR domain proteins PICK1 and ICA69 [56]. Loss of either of these proteins results in impaired proinsulin processing and in reduced synthesis of mature insulin, possibly through impaired sorting of PC1/3 to the ISG [32, 57].

Insulin is stored as a hexamer inside the uncoated ISG through the interaction with calcium and zinc ions. Part of the Zn^{2+} comes from the Golgi and ER [58], but ISG are also equipped with Zn^{2+} transporters, where ZnT8 is particularly well-studied, since mutations in its gene, SLC30A8, are associated with increased susceptibility to T2D [59]. Loss of function studies have revealed a role of ZnT8 in the regulation of proinsulin processing and ISG biogenesis, but the phenotypes are typically mild and its importance for glucose homeostasis is not firmly established [60]. In fact, recent work suggests that loss of function mutations in ZnT8 might even protect against T2D [61]. Interestingly, the high-risk mutation in ZnT8 is a gain-of-function mutation that increases Zn^{2+} transport [62]. These observations suggest that reducing the activity of ZnT8 might be considered a future therapeutic approach to the treatment of T2D, although care must be taken, since massive impairment of Zn^{2+} uptake strongly inhibits insulin production and secretion [63].

Lipids play important roles in ISG maturation. The cholesterol content of insulin granules is high, at least in part through the action of granule-localized ABC cholesterol transporters [64, 65]. β -Cells lacking the ABCG1/ABCA12 cholesterol transporters or treated with cholesterol synthesis inhibitors present with enlarged ISG and exhibit reduced glucose-stimulated insulin secretion [64–66]. The mechanism of cholesterol action is not clear, but it has been shown that cholesterol transport is needed to protect newly formed ISG against lysosomal degradation [28]. However, excess cholesterol also impairs β -cell function, causing ISG enlargement and impaired functionality by interfering with the localization of exocytic proteins to the ISG [67]. It seems that maintaining cholesterol levels within a narrow range is required for normal ISG biogenesis in β -cells. Indeed, alterations in cholesterol homeostasis have been described in T2D models [64]. In addition to cholesterol and its transporting proteins, steroidogenic acute regulatory protein-related lipid transfer protein 10 (STARD10), which transports phospholipids, has also been implicated in ISG biogenesis [68]. High risk alleles for T2D have been mapped to the *stard10* genomic locus, and risk allele carriers present with reduced STARD10 mRNA levels. Moreover, β -cell-specific loss of STARD10 results in impaired glucose-stimulated insulin

secretion [68]. It appears that STARD10 affects insulin secretion both at the level of ISG biogenesis and at more distal steps, but without knowledge of its cellular localization and lipid preference it is difficult to understand its role in β -cells. Phospholipids have also been more directly implicated in ISG maturation. The levels of PI4P are high on the ISG surface, at least in part via the presence of ISG-localized PI4-kinases [69], but the lipid somehow also needs to be removed by the action of the 4'-phosphatase Sac2 prior to ISG docking at the plasma membrane [70]. Such dynamic changes in the concentration of a phosphoinositide are reminiscent of the well-characterized phosphoinositide cascades that drive membrane and cargo trafficking in the endolysosomal compartment [71], and may indicate that there are similarities between the mechanisms that control cargo uptake and release in secretory cells [72]. Mass spectrometric analysis has revealed dramatic glucose-induced changes in the phosphoinositide composition of ISG; however, it is not known which lipid species that are affected [73] (Fig. 2).

Insulin secretory granule transport

Acute stimulation of insulin secretion primarily involves the release of granules already present at the plasma membrane. Prolonged stimulation requires mobilization of granules from the reserve pool through a mechanism that depends on glucose and a granule transport machinery [74]. There are two main transport routes in cells; actin filaments and microtubules. Filamentous actin (F-actin) is dynamically regulated by glucose and influence insulin secretion at numerous stages, including granule transport to the plasma membrane [75, 76]. Myosin Va is an F-actin-dependent transport protein that binds ISG through interactions with granophilin, Rab27a and Rabphilin [76–78]. Rab27a also binds to Exophilin-8/MyRIP, which in turn anchors ISG to the cortical F-actin network via interactions with Myosin VII, thereby stabilizing granules at the plasma membrane [79]. F-actin filaments are important for short-range movement of ISG close to the plasma membrane, whereas long-range movements instead involve kinesin-dependent microtubule transport [80]. ATP derived from glucose metabolism promotes microtubule-dependent movement of ISG, and interference with kinesin-1 function results in the selective suppression of sustained insulin secretion [80–82]. Interestingly, microtubule-dependent ISG transport primarily involves newly synthesized ISG, indicating that the granule membrane composition is altered with ageing to prevent binding to microtubules [83]. Kinesin-1 attaches to ISG via an adaptor protein, and it is, therefore, easy to envisage how changes in granule membrane composition can affect granule mobility. The identity of the adaptors and cargo proteins involved in microtubule-dependent transport are not known,

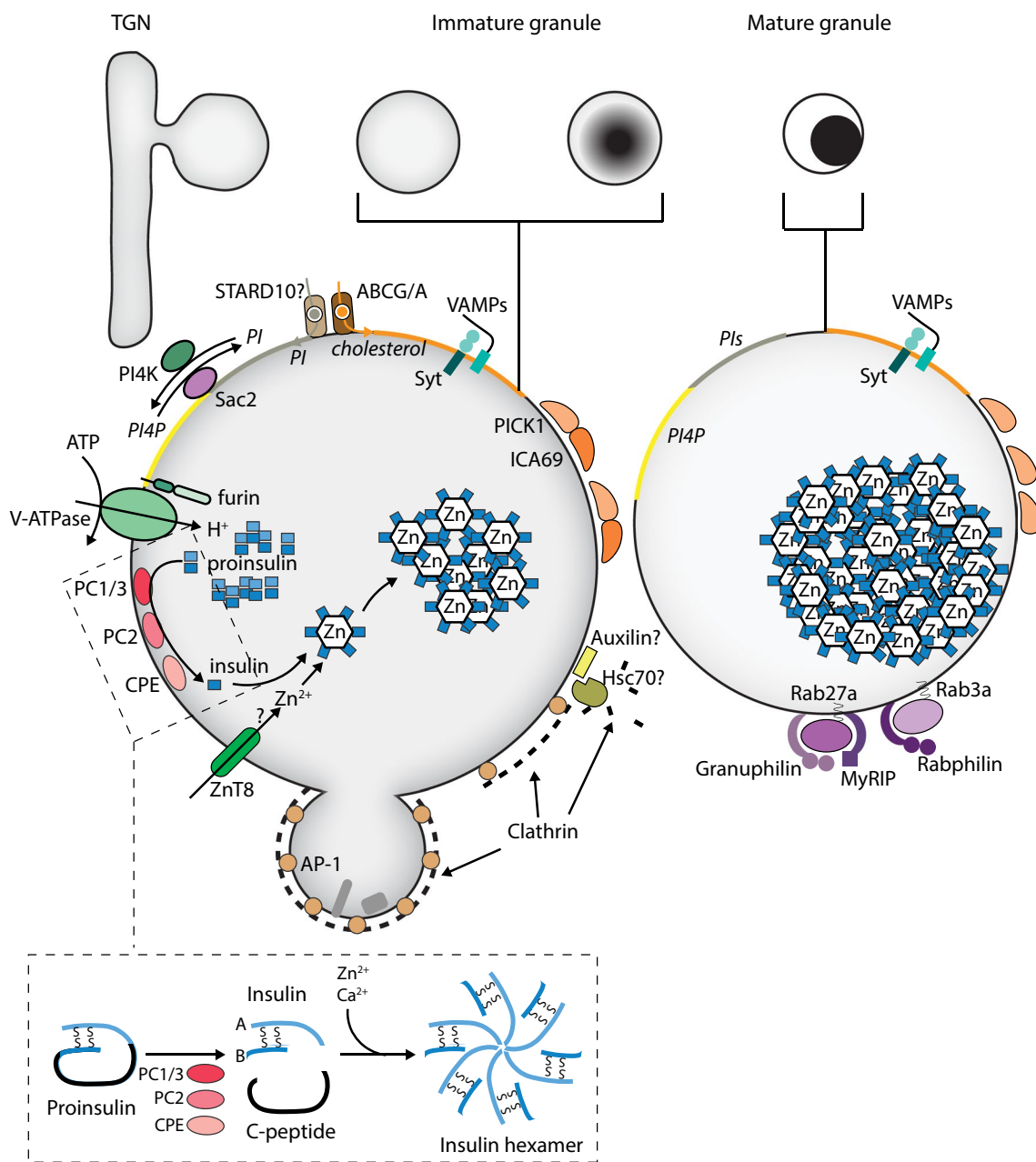


Fig. 2 Insulin granule maturation. Proinsulin processing to insulin occurs inside the granule. The proteolytic conversion of insulin requires granule acidification by the H⁺-transporting V-ATPase, which in turn activates the prohormone-processing enzymes (PC1/3, PC2 and CPE). Insulin is stored as a hexamer in complex with Zn²⁺, and at least some of the Zn²⁺ accumulation occurs via ZnT8-mediated transport. Granule-localized cholesterol transporters (ABCG/A) and phosphoinositide-transporting (STARD10) and modulating (PI4K,

Sac2) proteins are responsible for changes in the lipid composition of the granule. During maturation, the granule shrinks due to clathrin-dependent retrieval of membrane and cargo for sorting or degradation. Maturation also involves removal of the clathrin coat, which likely depends on the action of Auxilin and Hsc70, and acquisition of factors required for granule transport and docking, such as Rab27a and Rab3 and their corresponding effector proteins (Granuphilin and Rabphilin)

although both Rab3a and Rab27a have been shown to be involved in axonal transport of synaptic vesicle precursors in neurons [84]. Recently, it has been proposed that microtubules may also play a negative role in insulin secretion regulation by retrograde transport of ISG from the plasma

membrane [85]. The regulation of ISG transport is clearly complicated, and the relative importance of F-actin, microtubules and random diffusion for maintaining appropriate rates of insulin secretion is still not understood. Determining the mechanisms that control insulin granule attachment to motor

proteins and investigating the possibility that the mobility of different granules pools is governed by different transport mechanisms could help to answer these questions (Fig. 3).

Dynamics of insulin secretion

Numerous dietary components, such as glucose, proteins and fatty acids, can stimulate insulin secretion. Glucose is nevertheless considered the major physiological stimulus for insulin secretion, and the other components mostly act through amplification of the glucose-triggered secretory response [86]. Insulin secretion in response to a step increase in glucose concentration occurs in a biphasic manner composed of a transient first phase, lasting only a few minutes, followed by a prolonged, sustained second phase built up of regular pulses. This release kinetics has been observed at different organizational levels, from the portal vein of the perfused rat pancreas to individual β -cells [87–90], indicating that β -cell-intrinsic mechanisms are responsible for the biphasic secretory response. A common explanation is that exocytosis of different functional granule pools gives rise to the two phases. Morphologically, electron microscopy has shown that initial stimulation of insulin secretion selectively depletes ISG that are in the vicinity of the plasma membrane [91]. The granules undergoing fusion are classified into the readily releasable pool (RRP), which constitute a subset of plasma membrane-docked granules that are primed with a fully assembled exocytosis machinery. The vast majority (98%) of ISG belongs to a reserve pool located deeper within the β -cell, and release of these granules requires trafficking and recruitment to the same release sites. Consequently,

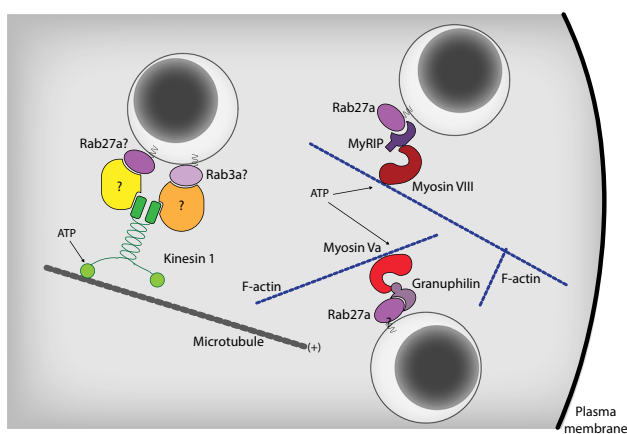


Fig. 3 Insulin granule transportation. Long-range transport of insulin granules occurs along microtubules and depend on the anterograde motor protein Kinesin-1. Short-range transport close to the plasma membrane instead occurs along F-actin tracks and depends on interactions between mature insulin granule proteins and the motor proteins Myosin Va and Myosin VIII

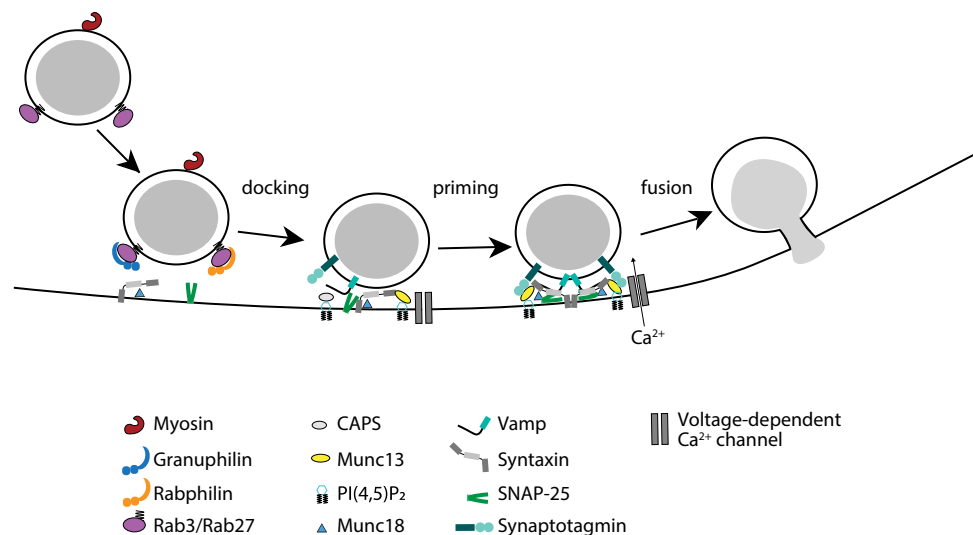
the second phase of insulin secretion is much slower and long-lasting [91–95]. Electrophysiological characterization has confirmed the presence of two populations of granules with different release kinetics; a small population of around 100 granules that are rapidly released (RRP), and a larger population with a slower release rate [95]. Similar conclusions were also drawn from modelling studies [96]. In light of the above-mentioned findings, the prevailing hypothesis is that the RRP is largely responsible for the first phase of secretion, while sustained secretion relies on recruitment of granules from the reserve pool [93, 97]. Studies addressing the molecular mechanisms of biphasic insulin secretion have relied heavily on the use of glucose or non-physiological secretagogues. Importantly, biphasic insulin secretion is also observed *in vivo* in humans after oral intake of a mixed meal (glucose, protein and fat), demonstrating that the biphasic release kinetics is physiological [98]. The first phase of insulin secretion plays an important role in postprandial glucose homeostasis, and it is often lost or reduced in early stages of type-2 diabetes (T2D; see later sections) [99] (Fig. 4).

Release probability is determined at the level of the insulin secretory granule

The morphologically visible separation of ISG into a docked and undocked pool suggests that β -cells have mechanisms for targeting granules to the plasma membrane. The challenge is to understand the molecular basis for these functionally defined granule pools. Live-cell imaging during phasic insulin secretion has resulted in a model, where the ISG are not distinguished based on their physical location *per se* but by their modes of exocytosis [96, 97, 99, 100]. Primary exocytosis occurs through a process involving ISG docking to the plasma membrane, followed by priming and Ca^{2+} -dependent release. This is generally believed to be the most important mode of ISG release. In newcomer granule exocytosis, ISG instead immediately fuse with the plasma membrane with either minimal or no residence time at the plasma membrane [99]. Moreover, kiss-and-run exocytosis, where ISG briefly contact the plasma membrane through a transient fusion pore, has also been described in β -cells [101]. ISG can also undergo compound exocytosis, involving the simultaneous release of several granules that had fused with each other inside the cytoplasm [102].

The SNARE model of exocytosis dictates that the t-SNARE proteins SNAP-25 and Syntaxin in the plasma membrane interact with the v-SNARE protein VAMP on the ISG membrane to form a SNARE complex. Exocytosis also depends on accessory proteins, including Munc18, Munc13, Rabs and active zone proteins, such as RIMs, Piccolo and Bassoon (reviewed in [74]). Differences in SNARE complex composition determines the mode of exocytosis. Primary

Fig. 4 Insulin granule exocytosis. The three main stages of insulin granule exocytosis at the plasma membrane, docking, priming, and fusion are regulated by interactions between specific sets of proteins and lipids (see text for details)



exocytosis is mediated by VAMP2, syntaxin1A, and SNAP-25, and depends on Munc18-1, while newcomer granule exocytosis involves Munc18b/c activation of syntaxin3/4, SNAP-25 and VAMP8 [103–107]. The importance of the different modes of exocytosis for normal β -cell function is not clear, but they may contribute to the strength and plasticity of insulin secretion.

Insulin secretory granule docking

Docked granules are physically immobilized at specialized docking sites at the plasma membrane [95, 108–110]. Several molecular interactions have been shown to play essential roles in docking-site formation. Small GTPases of the Rab family allow ISG to tether at the correct target membrane. The granules that successfully tether to their release sites are equipped with Rab3, which interacts with Rab3-interacting molecule RIM2 α at the plasma membrane [111]. This is followed by the clustering of the SNARE protein syntaxin1 and its binding partner munc18-1, which together initiate and orchestrate the building of the docking site at the plasma membrane [112]. Munc18-1 binds syntaxin1 in a dormant “closed” conformation, where the SNARE domain is not accessible, thus preventing early recruitment of other SNARE proteins [113–116]. Both syntaxin1 and munc18-1 are required for stable ISG docking at the plasma membrane [117, 118]. Syntaxin clustering also depends on cholesterol [119] and other membrane lipids [120]. In particular, PI(4,5)P₂ has been proposed to have a role in organizing the plasma membrane by acting, together with SNARE proteins, as beacons for incoming granules [121, 122]. Indeed, both syntaxin-1 and PI(4,5)P₂ have been shown to form microdomains in cell-free membranes and fixed cells that at least partially colocalize with docked granules

[123–125]. However, their presence in the plasma membrane of living cells is controversial and may depend on cell type and experimental methods [126–128]. However, selective removal of PI(4,5)P₂ at docking sites affects tethering and docking [129].

Insulin secretory granule priming and release

Docking is required, but not sufficient, for exocytosis. Morphological analyses indicate that a few hundred granules are docked at the plasma membrane. However, most of these granules are not released upon stimulation but must undergo a maturation process known as priming. The pool of docked granules is likely the main depot for granule priming and replenishment of the RRP [130]. Priming transforms the granules to a state of readiness for fusion, and involves all the molecular rearrangements and ATP-dependent protein and lipid modifications that take place after docking but before fusion. However, the key reactions defining priming are not fully elucidated. Priming is thought to involve conformational changes and partial formation of the core SNARE complex [131, 132]. Munc13 and Ca²⁺-dependent activator protein in secretion (CAPS) are thought to ensure proper assembly of the SNARE complex [133], and to attract Ca²⁺ channels to the release site [134]. They catalyse the transition of closed syntaxin-1 into its open conformation, leading to partially zippered SNARE complexes involving SNAP-25 and VAMP2 [135]. The docking factor Munc18-1 is also required for priming by facilitating the Munc13-dependent SNARE assembly and serving as a template that enables syntaxin-1 and VAMP2 interactions [135, 136]. Munc13, together with Munc18, also prevent NSF-dependent disassembly of the docking complex [133]. CAPS-1/2

are also essential components of the priming machinery, and they contain a sequence stretch with homology to the priming domain of Munc13 [137]. The partially formed SNARE complex is stabilized by complexin [138] and tomosyns [139] that prevent spontaneous exocytosis. Upon Ca^{2+} influx, ISG undergo exocytosis in a manner dependent on Synaptotagmin and Doc2B. Both proteins are equipped with Ca^{2+} -binding C2 domains which also exhibit distinct binding properties for SNARE proteins, Munc18 and phospholipids in the plasma membrane [140, 141]. Activation of these Ca^{2+} sensors releases the exocytic clamp and enable full SNARE zippering and granule fusion with the plasma membrane.

Lipids, in particular $\text{PI}(4,5)\text{P}_2$, plays important roles during granule priming and release. For example, the ATP requirement for priming involves $\text{PI}(4,5)\text{P}_2$ synthesis [142–144]. It has also been shown that the number of primed granules and the rate of sustained secretion relates to $\text{PI}(4,5)\text{P}_2$ levels [69, 127, 145]. This is likely due to $\text{PI}(4,5)\text{P}_2$ -dependent recruitment and activation of cytosolic proteins, e.g., CAPS and Munc13, at specific locations on the plasma membrane [137, 146–148]. It is worth to mention that β -cells are polarized within islets, and ISG exocytosis is directed towards the vasculature. These cellular domains are enriched in proteins involved in cell adhesion (F-actin, E-cadherin and integrins) [149] and associated with neuronal presynaptic proteins (RIM2 α , ELKS, Liprin, and Piccolo) [150]. In isolated cells, polarized secretion is maintained to some extent. Spatial patterning of ISG recruitment and fusion occurs at polarized sites especially when the SNARE clusters also include Ca^{2+} channels [134, 151]. Local activation of integrin signalling mimics the vascular face of the cell and contribute to polarized secretion [152, 153]. Further work is required to understand the role of this active zone-like organization in regulating physiological insulin secretion.

Do abnormalities in the β -cell secretory machinery contribute to type-2 diabetes?

The functional impairment of β -cells in T2D has been the topic of intense investigation for decades. Many factors involved in T2D pathogenesis have been identified, including inflammatory stress, ER stress, metabolic and oxidative stress, amyloid stress, changes in the structural integrity of the islet and defects in the insulin secretory machinery [154]. The relative importance of these different factors is not known and, due to the strong genetic component of T2D, may even vary from one individual to another. In this review we have chosen to focus on the link between T2D and defects in the insulin secretory machinery. There is evidence that defects in the ISG release machinery, together with

changes in metabolism and electrical activity, contribute to the functional β -cell decline in T2D. This disease is characterized by the loss of biphasic glucose-stimulated insulin secretion, both in vivo and in isolated islets. Loss of the first phase is one of the earliest manifestations of T2D [155], and might be explained by inadequate β -cell function [156, 157]. β -Cells from T2D organ donors exhibit decreased numbers of docked granules, at least in part due to downregulation of docking factors, such as and Syntaxin-1, Munc18, Munc13, Rim2, Rab3a, and Rabphilin3a [158], and recent advances in single-cell sequencing are likely to expand this list [159]. Moreover, ISG docking in T2D β -cells also loose spatial control and occurs at random locations at the plasma membrane, indicating the absence of a specific docking signal [151]. The reduced docking results in impaired ISG exocytosis in these cells [151, 158]. The ability of antidiabetic drugs, such as sulfonylureas, to restore biphasic insulin secretion indicates that β -cell defects also result from impaired electrical activity [160, 161]. It also highlights the role of the metabolic changes that accompany T2D, since the inability of glucose to fully depolarize the β -cell is the principal reason for the loss of biphasic insulin secretion. T2D also disrupts the architectural organization of individual ISG release sites. For example, the coupling of L-type Ca^{2+} -channels to the release site at the plasma membrane is lost in diabetic β -cells, leading to slowed exocytosis that is de-synchronized with Ca^{2+} -influx [134]. Altered clustering of Ca^{2+} channels and Syntaxin-1 has also been observed after long-term culture in elevated free fatty acids or glucose [162]. Together with previously discussed alterations ISG biogenesis and maturation in T2D, these observations highlight the central role of the β -cell secretory machinery in T2D development and progression.

Concluding remarks

Extensive work over the past century has brought about remarkable advance in our understanding of the nature of ISG biogenesis and secretion. It has also been firmly established that defects in ISG biogenesis and release are hallmarks of type-2 diabetes. However, many challenges still remain to fully understand insulin physiology. A major open question is what drives changes in β -cell function and insulin secretion in type-2 diabetes. It is currently not clear to what extent loss of insulin secretion is a primary defect or a consequence of, e.g., metabolic changes. Understanding this relationship will be crucial when devising new strategies to improve or restore β -cell function. Other important questions worth further exploring are the importance of the active zone-like organization for insulin secretion and to what extent, if any, different modes of exocytosis contribute to the pathology of T2D. Further studies are also needed to

shed light on insulin granule heterogeneity and turnover to fully understand the dynamics of insulin secretion. Another aspect that deserves further investigation is the role of the local microenvironment in regulating insulin secretion from β -cells, including auto- para- and juxtacrine interactions within the islets and cell polarization and the generation of cellular domains. Such experiments will require studies of β -cells within intact islets, ideally in vivo, something that has only recently been made possible thanks to the development of sophisticated imaging techniques and model systems. These techniques, together with single-cell approaches to identify diabetes genes and powerful genetic techniques for target validation may finally enable complete mapping of the insulin secretory granule pathway, a route where both detours and shortcuts may result in diabetes development or progression.

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