Chapter 3

ER Stress, Secretory Granule Biogenesis, and Insulin

Michiko Saito and Yoko Shiba

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76131

Abstract

Insulin is secreted from pancreatic β-cells, and the high demand of insulin biosynthesis is known to cause β-cell dysfunction in patients with type 2 diabetes mellitus. The insulin biosynthetic pathway has been extensively studied and is still an exciting area for future studies. In this chapter, first, we focus on proinsulin biosynthetic pathway in the endoplasmic reticulum (ER) and recent progress of our knowledge about ER stress. We discuss about how ER stress is involved in the development of diabetes. Second, we focus on the formation of insulin secretory granules. The biogenesis of secretory granules has been explored for several decades; however, it still has been debated and has yet to be understood. We review the current knowledge about the secretory granules and discuss about the problems for future studies.

Keywords: insulin, islets of Langerhans, ER stress, Golgi, secretory granules, biosynthetic pathway

1. Introduction

Pancreatic β-cells synthesize insulin and secrete it in response to the increase of blood glucose levels. Insulin is synthesized as proinsulin in the endoplasmic reticulum (ER) and transported to the Golgi apparatus. In the trans-Golgi network (TGN), insulin becomes hexamer, and then packaged into secretory granules (**Figure 1**). In secretory granules, proinsulin is processed to form mature insulin and C-peptide. By the stimulation of high glucose concentration in blood, insulin granule is exocytosed and insulin and C-peptide are secreted into blood.

Pancreatic β-cells are located in islets of Langerhans. In pancreas, there are islet-like cell clusters that are stained differently from other parts of pancreatic tissues (**Figure 2**). It was named as islets of Langerhans, from the name of the person who found this structure. Paul Langerhans found this structure in his doctoral thesis. The cell clusters appeared to be different

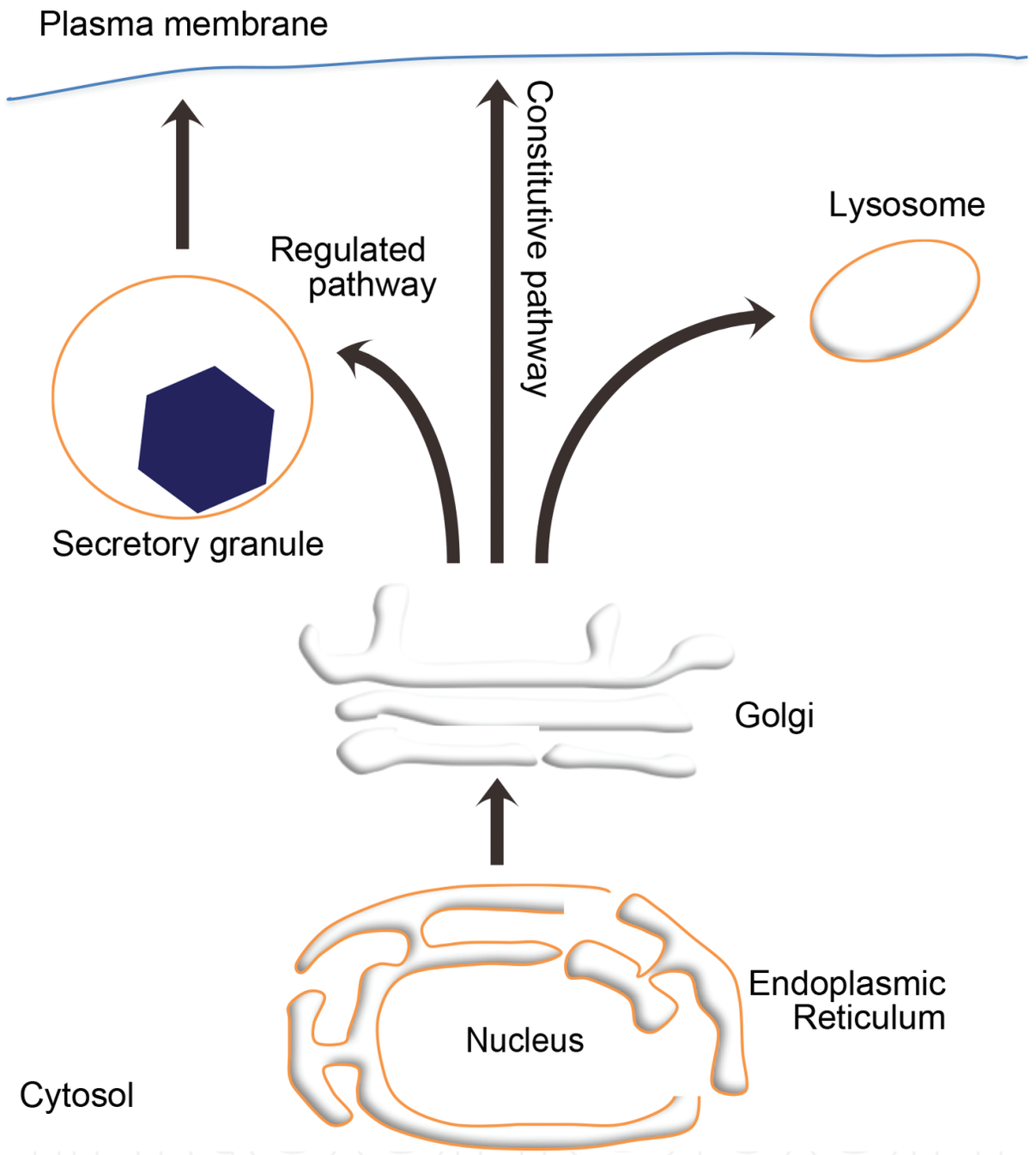

Figure 1. Biosynthetic pathway of secretory proteins. Intracellular transport in mammalian cells. Insulin is synthesized in the endoplasmic reticulum (ER), transported to the Golgi apparatus, and then packaged into secretory granules (SGs). Upon stimulation, SGs fuse with the plasma membrane (PM) and insulin is secreted. Insulin follows regulated secretory pathway in β-cells.

from the cells that secrete pancreatic enzymes, but he did not know what the function of this structure was. As he also found the cells that have dendrites in skin, his name is used for these cells as Langerhans cells, the dendritic cells [1].

Pancreas contains exocrine cells that secrete digestive enzymes including amylase and trypsin, and endocrine cells that secrete hormones including insulin and glucagon. The ratio of exocrine

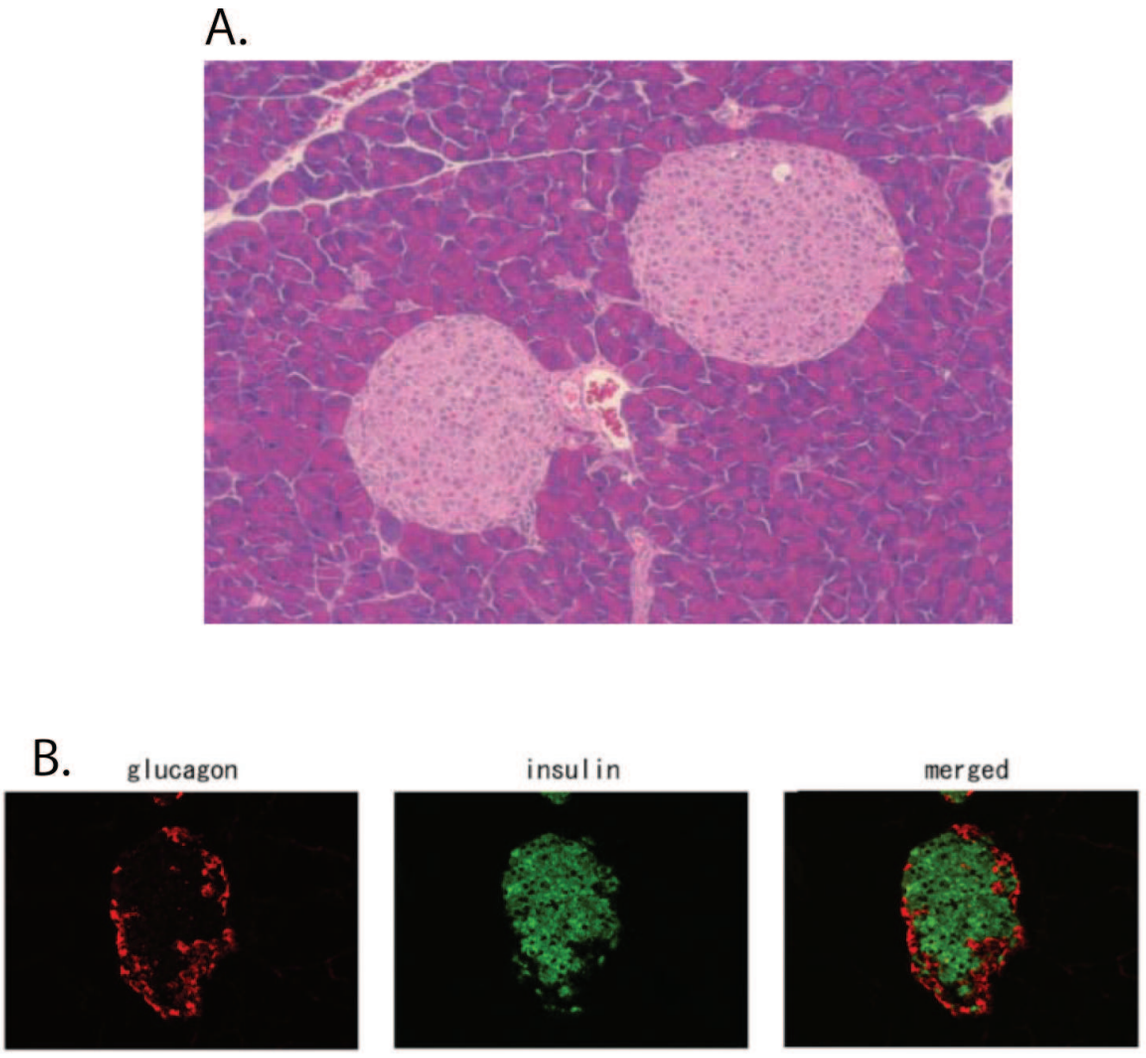

Figure 2. Islets of Langerhans in mouse. (A) Pancreatic segments from adult male mouse were stained by Hematoxylin-Eosin (HE). The lightly stained regions are islets of Langerhans. The densely stained regions that surround islets contain exocrine cells. (B) Islet of Langerhans captured by Immunofluorescence Pancreatic segments from adult male mouse were fixed and stained by anti-insulin antibody (green) and anti-glucagon antibody (red). Pancreatic β-cells that secrete insulin accumulate in the center, whereas α-cells that secrete glucagon are located in periphery.

and endocrine cells is approximately 9:1, meaning that less than 10% of cells are endocrine cells located in islets of Langerhans scattered in pancreas. Islets of Langerhans contain α, β, δ, ε, and PP cells. There are a million of islets of Langerhans in pancreas in human and 200–300 in adult mouse. In islets of Langerhans, 15–20% of cells are α-cells, 75–80% are β-cells, 5% are δ-cells, 1% are ε-cells, and 4% are PP cells. α-cells secrete glucagon that raises the blood glucose levels, β-cells secrete insulin that is the only hormone to decrease the blood glucose levels, δ-cells secrete somatostatin that inhibits the secretion of insulin and glucagon, ε-cells promote appetite and secrete ghrelin that inhibits insulin secretion, and PP cells secrete pancreatic peptide whose function is yet to be understood. In rodents, islets of Langerhans have a clear mantle core structure in which β-cells are located in the center of islets of Langerhans and surrounded by α, δ, ε, and PP cells (**Figure 1**). When rat islets are trypsinized and maintained in culture medium, the cells reassembled into aggregates that have a similar organization of intact islets; β-cells are

located in the center and the other cells are in the periphery [2]. Human islets of Langerhans do not have such clear structures. β-cells are mixed with the other cells. In avian pancreas, α-cells are in the center of islets. In zebrafish, their pancreas shares the basic structure with mammalian pancreas [3]. Recent studies show that zebrafish is a good model to study pancreatic development and diabetes mellitus [4–7].

2. ER stress and insulin

2.1. Proinsulin translation and folding in the ER

Human has INS gene as only insulin gene, whereas rodents have INS1 and INS2 genes for insulin. Human insulin gene encodes preproinsulin that has 110 amino acids containing N-terminal signal peptide following B chain, C-peptide, and A chain. Preproinsulin mRNA translation begins in the cytosol in pancreatic β-cells, and the signal peptide is recognized by signal recognition particle (SRP) to translate proinsulin across the membrane of the ER. In the ER, signal peptide is cleaved by signal peptidase to produce proinsulin that has 86 amino acids consisting of B chain, C-peptide, and A chain (**Figure 3**). Proinsulin is folded in the ER by chaperones including protein disulfide isomerase (PDI) family and BiP. Molecular chaperons and PDIs bind to the hydrophobic regions of proteins to promote folding and inhibit the aggregation of proteins [8]. Proinsulin has three disulfide bonds in A6–A11, A7–B7, and A20–B19 (**Figure 3**) [9]. *N*-glycosylation is often used as a marker for proper folding of newly synthesized proteins in the ER; however, proinsulin does not have *N*-glycosylation site.

2.2. ER stress

The high demand of insulin synthesis under a high plasma glucose condition causes ER stress that could cause β-cell dysfunction. Generally, the secretory proteins and transmembrane proteins are folded and acquire a variety of modification in the ER. Environmental and genetic factors affect protein folding in the ER. If protein folding is inhibited, unfolded proteins accumulate in the ER, leading to ER stress. Cells that sense ER stress cause unfolded protein response (UPR) that includes the inhibition of general protein translation, the induction of expression of ER chaperons, and ER-associated degradation (ERAD). UPR is a cellular response to recover ER homeostasis. In mammalian cells, there are three ER stress sensors, PERK, IRE1α, and ATF6 (**Figure 4**).

Protein kinase RNA-like ER kinase (PERK) is type-I transmembrane protein that localizes in the ER. Under ER stress, PERK undergoes autophosphorylation to be activated and oligomerized. Oligomerized PERK phosphorylates translation initiation factor, eIF2 α-subunit to inhibit protein translation. The inhibition of protein translation attenuates the accumulation of unfolded ER proteins as well as ER stress [10]. On the other hand, this inhibition of protein translation promotes the translation of ATF4, a transcription factor that induces genes related to apoptosis, amino acid metabolism, and antioxidants. Still, when cells cannot deal with ER stress even by these measures and ER stress is continued, ATF4 induces the transcription of C/EBP homologous

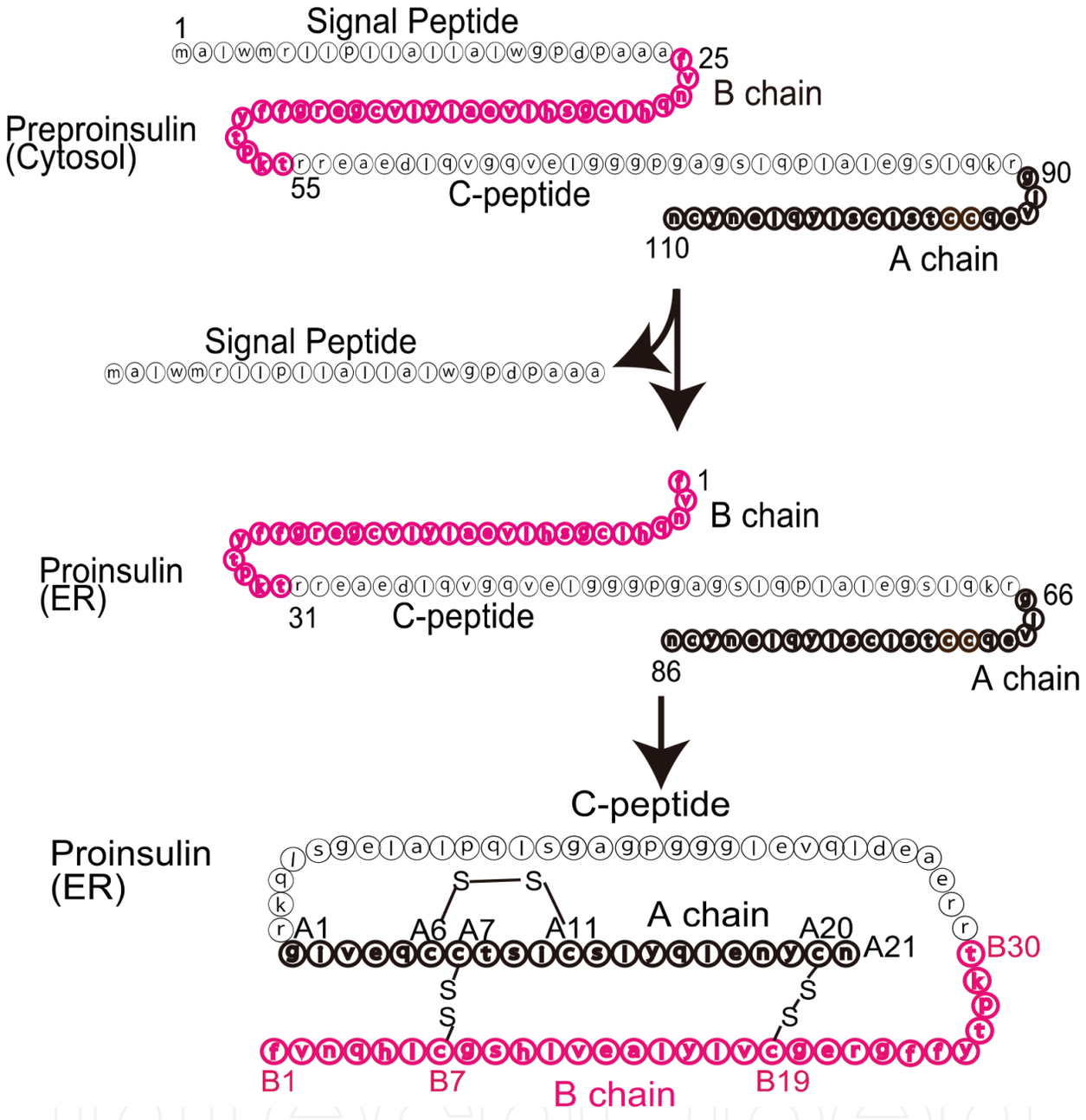

Figure 3. Insulin biosynthesis in the ER. The schematic structure of human insulin is shown. Insulin has signal peptide in its N-terminus followed by B chain, C-peptide, and A chain. Insulin mRNA translation is initiated in the cytosol as preproinsulin and cotranslationally inserted to the ER. Signal peptide is cleaved by endopeptidase during insertion into the ER and proinsulin is generated. In the ER, proinsulin is folded by three disulfide bonds of A and B chain.

proteins (CHOP/GADD153). CHOP and ATF4 form a heterodimer that induces the transcription of each downstream genes and promotes apoptosis [11, 12].

Inositol-requiring enzyme 1 (IRE1) has two isoforms: IRE1α that is expressed ubiquitously [13] and IRE1β that is expressed in goblet cells that secrete mucin in the digestive tract and lung [14, 15]. IRE1α is the type-I transmembrane protein localized in the ER. IRE1α has kinase and ribonuclease domains in its cytoplasmic region, and its lumenal domain has the binding site

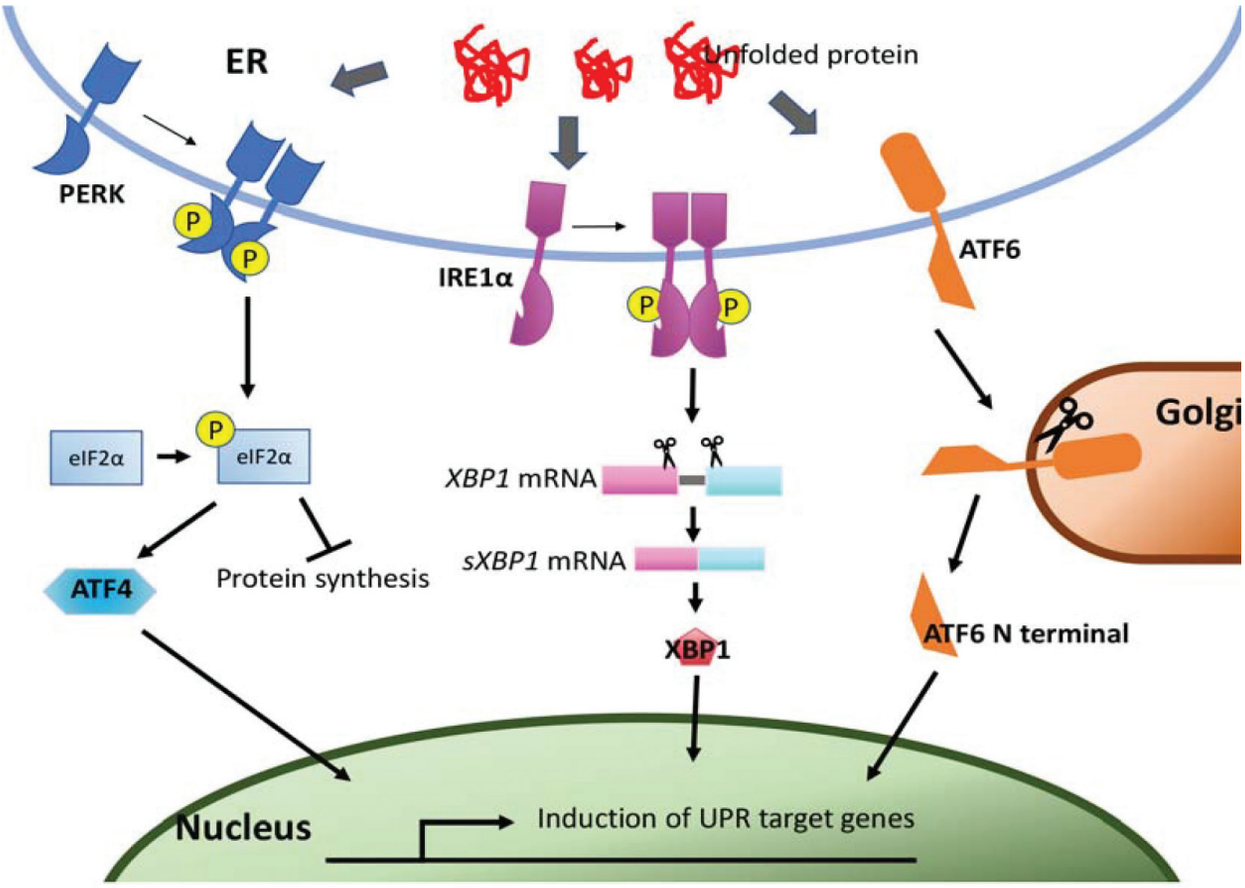

Figure 4. ER stress and activation of the unfolded protein response (UPR) pathways in mammalian cells. Accumulation of unfolded protein in the ER can be recognized by ER stress sensors, IRE1α, PERK and ATF6. These proteins are activated and mediate ER stress response, including inhibition of translation and induction of transcription of ER chaperones.

for BiP. In normal condition, IRE1α is in inactive form and binds to BiP, as well as other ER-stress sensors PERK and ATF6. In ER stress condition, BiP is released from IRE1α and IRE1α forms oligomer to be in an active form [16–21]. Autophosphorylation of the kinase domain in cytoplasmic region of IRE1α activates the ribonuclease (RNase) domain in the C-terminal region of cytoplasmic domain of IRE1α [22–25]. The activated RNase domain cleaves the precursor form of XBP1 (XBP1 unspliced; XBP1u mRNA) in specific two sites to produce mature XBP1 mRNA (XBP1 spliced; XBP1s mRNA) that produces functional transcription factor to induce the transcription of genes related to ER chaperones, ERAD, and lipid metabolism to recover ER homeostasis [26, 27].

Activating transcription factor 6 (ATF6) is a type-II transmembrane protein functioning as a transcription factor. ATF6 localizes in the ER but has the Golgi-localizing signal in its lumenal region that is inhibited in normal condition by binding to BiP. Under ER-stress condition, BiP is released from the lumenal region of ATF6, and its Golgi-localizing signal is exposed to transport ATF6 to the Golgi apparatus [28, 29]. In the Golgi, site 1 protease (S1P) and site 2 protease (S2P) cleave the transmembrane region of ATF6 and produce ATF6 that has only cytoplasmic region containing DNA-binding site [30, 31]. The cleaved ATF6 is translocated

into the nucleus to function as a transcription factor that induces the transcription of genes related to ER chaperones [10, 32]. Prolonged ER stress promotes ATF6 to bind to ATF4 to induce the transcription of CHOP, resulting in apoptosis [33–35].

2.3. ER stress and insulin

Pancreatic β-cells are specialized cells to synthesize and secrete a large amount of insulin. Insulin biosynthesis in pancreatic β-cells accounts for 10–50% of total protein synthesis [36, 37]. Therefore, the burden to the ER (ER stress) in pancreatic β-cells is constitutively high even in physiological condition. It is also known that pancreatic β-cells are sensitive against oxidative stress and hypoxia [38] as well as ER stress. The expression level of glutathione peroxidase, an antioxidant, is very low in pancreatic β-cells; therefore, pancreatic β-cells are sensitive to oxidative stress [39]. The islets of Langerhans are surrounded by blood vessels and supplied with nutrients and oxygen. Hypoxia affects insulin secretion of pancreatic islets and the survival rate of grafted islets [40, 41].

In type-II diabetes, it was reported that pancreatic β-cell mass is decreased [42, 43]. Huang et al. reported that the rat model of type-II diabetes expressing human islet amyloid polypeptide (hIAPP) showed the decrease of β-cell mass due to β-cell apoptosis, and the proteins related to ER stress including CHOP is highly expressed in β-cells [44]. The relationship between ER stress and diabetes has been studied by a variety of animal models and human genetic diseases. Akita mouse, another mouse model of diabetes named by Akio Koizumi in Akita University, has a single mutation in insulin 2 gene. Although there are no gross defects in the transcription of the wild-type insulin 2 allele and the two alleles of insulin 1, the phenotype of a single mutation of insulin 2 is dominant. Insulin 2 gene in Akita mouse has tyrosine instead of cysteine 96 (C96Y), and the mutated proinsulin does not form the disulfide bond between A chain and B chain (A7–B7). The mutated proinsulin cannot be transported to the Golgi apparatus and its secretion is inhibited [45]. The mutated proinsulin is accumulated in the ER that causes UPR to result in the induction of the expression of GRP78, XBP1, and CHOP. Eventually, pancreatic β-cells die by apoptosis. The necessity of ER stress for β-cell death was demonstrated by the delay of the development of diabetes in mouse produced by crossing Akita mouse with CHOP–knock-out mouse [46]. It was reported that human also has the same mutation [47].

Wolcott-Rallison syndrome (WRS) is caused by the malfunction of Eif2ak3 gene that encodes PERK [48]. WRS is an autosomal-recessive disorder that has neonatal diabetes, epiphyseal dysplasia, osteoporosis, and growth retardation. Patients with WRS have the point mutation in the kinase domain of PERK or the mutation that causes the deletion mutant of PERK. The mutation causing kinase dead of PERK develops diabetes after several months of birth, whereas the mutation that still maintains kinase activity of PERK delays the development of diabetes after 30 months. As well as WRS, PERK knock-out mice showed the secretory defects in many tissues causing diabetes and growth defects [49–51]. Furthermore, the knock-in mice having the mutation of phosphorylation site of eIF2α, the downstream molecule of PERK signaling, are unable to inhibit translation leading to over-synthesis of insulin and resulting in the dysfunction of pancreatic β-cells and β-cell death [52].

ATF6 knock-out mice do not show gross defects in normal diet, whereas high-fat diet causes the dysfunction of pancreatic β-cells [53, 54]. Furthermore, strong ER stress promotes the death of pancreatic β-cells [55, 56].

The knock-out mice of IRE1α specifically deleted in pancreatic β-cells cause diabetic phenotype [57, 58]. The mRNA levels of preproinsulin are not impaired; however, the protein level of proinsulin and mature insulin decreases, and protein and mRNA levels of five PDI protein families, PDI, PDIR, P5, ERp44, and ERp46, also decrease. These results indicate that these five PDI families are involved in proinsulin folding downstream of IRE1α, and upregulation of these PDI families could be the next approach for the treatment of diabetes.

3. Biogenesis of insulin secretory granules

After reaching the Golgi from the ER, secretory proteins are sorted in the *trans*-Golgi network (TGN) (**Figure 1**). One of the secretory pathways is the constitutive pathway in which proteins are constitutively secreted. When there is no sorting signal, proteins are thought to follow this pathway in mammalian cells. By contrast, another secretory pathway is the regulated pathway in which secretory proteins are packaged into the immature secretory granules (ISGs) (**Figure 5**). ISGs mature into mature secretory granules (MSGs), then MSGs are fused with the plasma membrane (PM) upon the stimulation of secretagogues to secrete the contents of MSGs. Proinsulin follows the regulated secretory pathway after the Golgi apparatus.

Secretory proteins destined for regulated pathway are segregated from other proteins and packaged into ISGs. This is termed as sorting by entry. On the other hand, in the process of formation and maturation of ISGs, other proteins are eliminated from ISGs. It is termed as sorting by exit, or sorting by retention [59–61].

3.1. Proinsulin transport to immature secretory granules

The molecular mechanisms of proinsulin sorting in the TGN are yet to be understood. It is thought that the selective aggregation of proinsulin occurs in the TGN [62, 63]. Insulin secretory granules contain a clear electron-dense core structure suggesting that insulin is crystallized in the granules. In pituitary AtT-20 cells, insulin granules can be formed by transfecting insulin gene, and hemagglutinin that flows in a constitutive pathway is segregated from the dense-core structure. Hemagglutinin is distributed evenly through the Golgi stacks as well as proinsulin; however, they are segregated after the TGN [62]. Therefore, the proinsulin sorting from constitutive pathway could occur in the TGN.

The sorting receptor that recognizes proinsulin and transport proinsulin into ISGs remains unidentified [59]. Carboxypeptidase E (CPE), an enzyme involved in insulin processing, was proposed to play a role as the sorting receptor [64]; however, the islets from mice that lost CPE by its mutation showed that insulin is efficiently secreted by secretagogues as well as in control islets, whereas the constitutive secretion of insulin remains as low as 1% similar to that in control islets [65]. Therefore, the possibility that CPE plays a role as a sorting receptor in

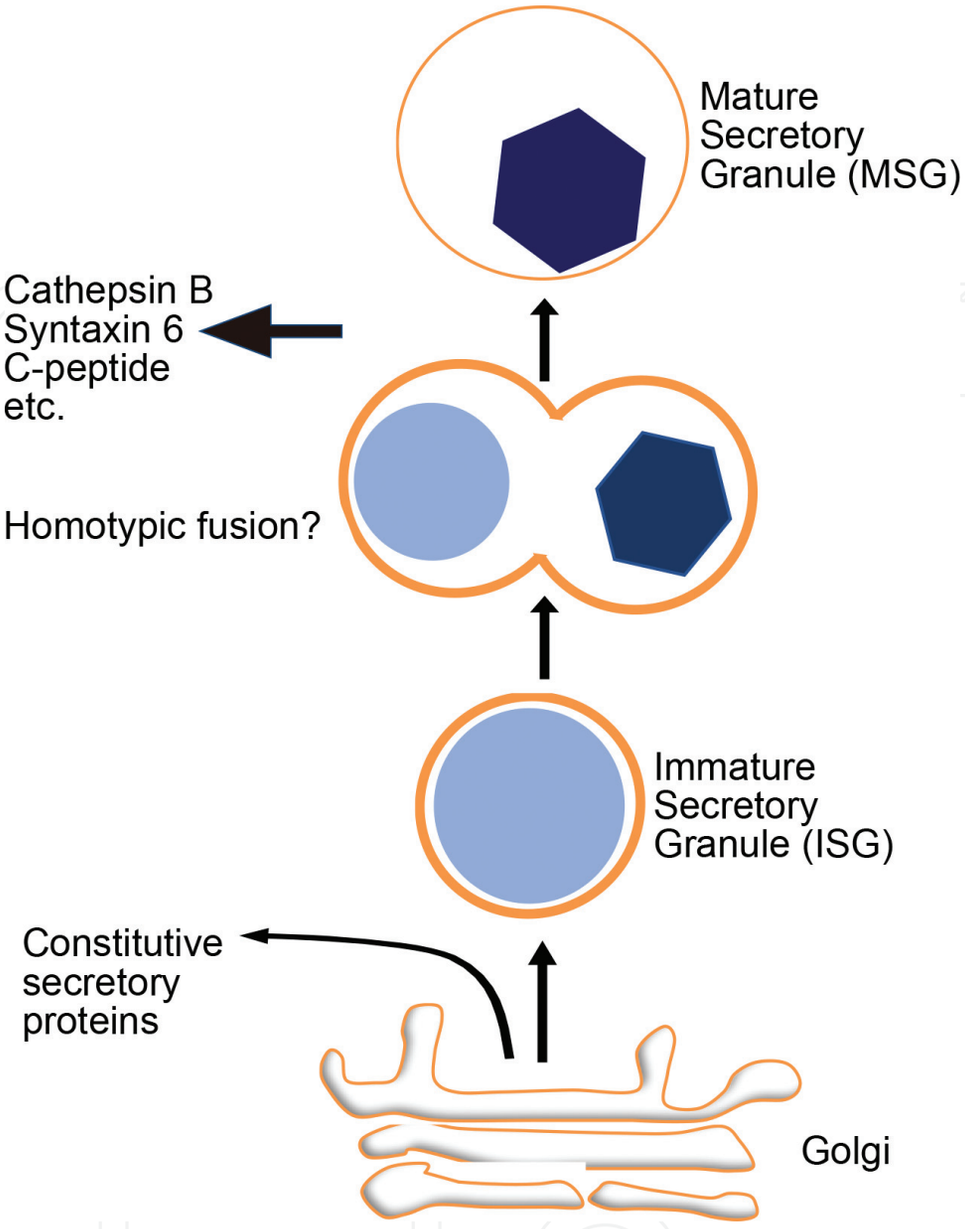

Figure 5. Insulin Secretory Granule (SG) formation. After folded in the ER, proinsulin is transported to the Golgi apparatus, then packaged into immature secretory granules (ISG) from the *trans*-Golgi network (TGN). ISGs mature into mature secretory granules (MSGs). There are several steps to make SGs; segregation from constitutive secretory proteins, possibly homotypic fusion and removal of proteins not required for MSGs.

pancreatic β-cells is questionable [59]. While research to find out the sorting receptor has been going on, another possibility was proposed; cargo aggregation/oligomerization is the sorting signal for ISGs [60, 61, 66].

It is proposed that aggregated proteins directly bind to lipid micro-domain in the TGN membranes and these micro-domains could be recognized by cytosolic machineries [59, 67]. Secretory granules (SGs) contain a high amount of cholesterol, and the depletion or addition of cholesterol affects glucose-stimulated insulin secretion (GSIS) [68]. Secretogranin III is one of

the components of SGs and known to bind to cholesterol-rich membranes [69]. Although the role of secretogranin III in SG biogenesis in mice is not clear [70], it could be important to investigate the role of cholesterol.

There is an interesting phenomenon using SEGFP that is the green fluorescent protein (GFP) having a signal peptide in its N-terminus. In cultured insulin-secreting cells (INS-1 cells), SEGFP is sorted to secretory granules and secreted by secretagogues similar to insulin, whereas secreted alkaline phosphatase (SEAP), a model protein of constitutive pathway, is constitutively secreted [71]. SEGFP forms oligomer by a disulfide bond, and its oligomerization could unexpectedly function as a sorting signal to ISGs. The results may support the idea that cargo oligomerization itself, rather than specific sequences on cargo, is required for sorting. The involvement of lipids or other sorting proteins in this case is unclear.

Zinc and calcium ions play important roles in insulin oligomerization. Structural studies showed that insulin forms a dimer, and in the presence of zinc and calcium ions, it forms a hexamer [72, 73]. It is thought that the concentration of zinc and calcium ions rises in the TGN [9], and these ions are enriched in SGs [74, 75]. The oligomerization regions of insulin and proinsulin are essentially the same with or without C-peptide [72]. The cleavage of C-peptide from proinsulin hexamer decreases the solubility of insulin hexamer leading to crystallization of insulin in mature secretory granules (MSGs). Insulin crystals are thought to be stable and can be stored in MSGs for a long time without being degraded [73]. ZnT8 zinc transporter, the product of *SLC30A8* gene, is highly expressed in pancreatic β-cells, and the combined deletion of ZnT8 and ZnT7 inhibits GSIS [76]. However, ZnT8 mutation is protective against type 2 diabetes [77]. The precise function of ZnT8 in insulin biosynthetic pathway and its relationship with the development of diabetes remains unclarified.

Although the molecular mechanisms of sorting are yet to be understood, recent studies revealed the molecules to be involved in the fission process of SGs from the TGN. Arfaptin 1 has a lipid-binding domain termed Bin/Amphiphysin/Rvs (BAR) domain that binds to a curved membrane structure [78, 79] and implicated in a regulating membrane fission [80]. Arfaptin-1 binds to small GTP-binding proteins, Arf1- and Arf-like protein 1 (Arl1), and recruited to the Golgi membrane by a GTP-bound form of Arf1 and Arl1 [81, 82]. Arfaptin 1 is phosphorylated by Protein Kinase D (PKD) that is activated by diacylglycerol (DAG) enriched in the neck of budding vesicles [83]. Non-phosphorylated mutant of Arfaptin 1 (S132A) or PKD inhibitor blocks insulin SG fission from the TGN [84]. The expression of Arfaptin 1 (S132A) or Arfaptin 1 depletion inhibits GSIS. As Arfaptin-1 was reported to be involved in other transport pathways [82, 85, 86], the specificity of Arfaptin 1 in SG biogenesis needs to be carefully addressed. Although Arfaptin 1 is proposed to play a role in membrane fission [84, 87], it could be interesting to investigate the upstream molecules of Arfaptin 1 to look for the sorting machinery for SG biogenesis.

3.2. Maturation of secretory granules

3.2.1. Insulin processing

The excursion of C-peptide decreases the solubility of insulin hexamer and causes insulin crystallization within SGs [73]. Proinsulin is processed into mature insulin by prohormone

convertases (PC1/3 and PC2) and carboxypeptidase E (CPE) [88] (**Figure 6**). PC1 (also known as PC3) cleaves 32–33 junction between B chain and C-peptide, and then CPE removes 31, 32 arginine residues. The intermediate form of proinsulin that is cleaved in B–C junction but is yet to be cleaved in A–C junction is termed as des-31, 32 split proinsulin. PC2 cleaves 65–66 junction between A chain and C peptide, and CPE removes 64, 65 lysine and arginine residues to form another intermediate termed des-64, 65 split proinsulin [9]. The cleavage of B–C junction tends to occur first before the cleavage of A–C junction [89]. PC1/3 and PC2 are Ca^{2+}- and pH-dependent endopeptidases. The optimal pH of both enzymes is pH 5.5 [90, 91]. The pH at the TGN is reported to be ~6.0 [92]. The pH of ISGs varies from 5.5 to 7.0 and the pH of

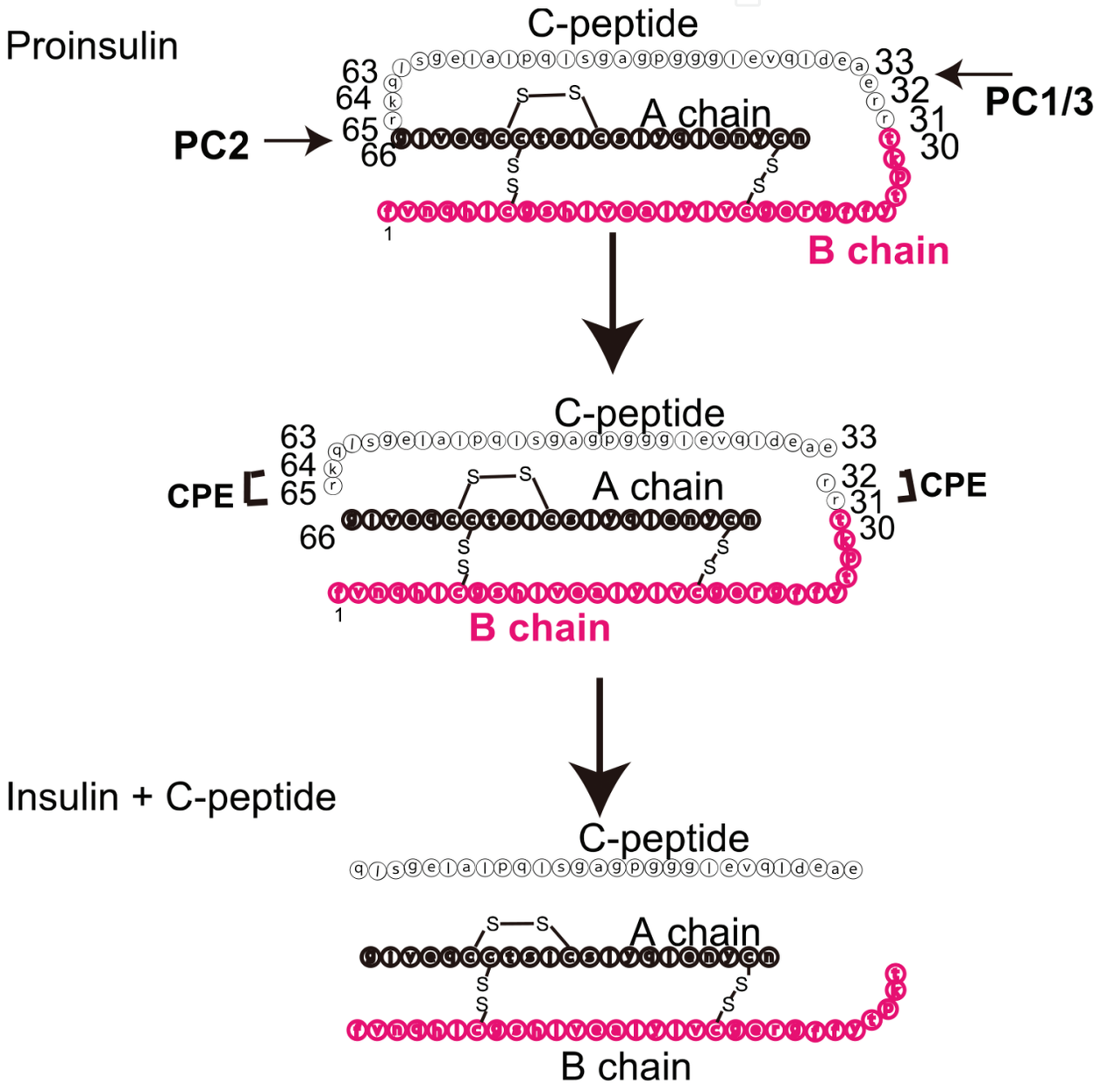

Figure 6. Proinsulin processing. Proinsulin processing is thought to be initiated in the TGN and continue to undergo in ISGs. Prohormone convertases PC1/3 and PC2 cleave C-peptide from proinsulin then Carboxypeptidase E (CPE) removes di-basic residues (Arg-Arg or Arg-Lys) to produce mature insulin and C-peptide.

MSGs is ~5.0 [89]. PC1/3 could be active in the TGN as well as in ISGs [93], whereas PC2 is thought to be active in ISGs and MSGs [91, 94].

3.2.2. Sorting by retention

As ISGs mature, ISGs produce vesicles to remove proteins that are not required for MSGs, whereas insulin is retained in MSGs. For example, ISGs are known to produce constitutive-like vesicles that contain excess C-peptide than insulin to be secreted [95, 96]. Also, in contrast to constitutive secretory proteins, lysosomal enzymes are thought to be segregated from ISGs in pancreatic β-cells [97, 98]. Generally, lysosomal enzymes are synthesized in the ER as well as secretory proteins and transported to the Golgi apparatus (**Figure 1**). In the TGN and endosomes, lysosomal enzymes are recognized by mannose 6-phosphate receptors (MPRs) and then packaged into clathrin-coated vesicle (CCVs). Clathrin is a coat protein that forms a cage-like structure to produce CCVs in the post-Golgi compartment [99]. AP-1 is a clathrin adaptor that binds to MPRs and clathrin and mediates to form clathrin/AP-1-coated vesicles [100, 101]. Proinsulin ISGs have clathrin and AP-1 on their surfaces as well as MPRs [98]. Also, MSGs lose the signal of cathepsin B, a lysosomal protease, whereas ISGs still have a strong cathepsin B signal. These results suggest that lysosomal enzymes recognized by MPRs are removed from ISGs [98].

3.2.3. Homotypic fusion

Syntaxin 6, a SNARE protein that is reported to be important for the homotypic fusion of ISGs in neuroendocrine cells [102], is also removed from insulin ISGs [98]. In neuroendocrine cells, it is thought that homotypic fusion plays an important role in SG maturation, and the fusion machineries required for homotypic fusion are different than that required for MSGs fusion to the PM [67, 102]. It is proposed that membrane fusion machinery is remodeled in the end of ISG maturation. The role of Syntaxin 6 and homotypic fusion in insulin granule maturation is not clear [103]. However, recent study showed that homotypic fusion could also be important in insulin granule maturation [104]. In islets from HID-1 KO mice, Vamp-4, another SNARE protein that is proposed in ISG-derived vesicle fusion to the PM in neuroendocrine cells [67], is mislocalized. Proinsulin processing and acidification are delayed, and by 3D electron microscopy, there are less homotypic fusion events [104]. Although the role of HID-1 and Vamp-4 in homotypic fusion in pancreatic β-cells should be addressed in the future, it is possible that homotypic fusion might also be important for insulin granule formation.

The MSGs are fused with the PM, and insulin and C-peptide are secreted upon stimulation. For details about the exocytosis of insulin granules, see the review articles [59, 105, 106].

4. Conclusion

Because of the importance of insulin in diabetes mellitus, insulin secretory pathway has been extensively studied. Recent advance in the understanding of biosynthetic pathway reveals the importance of ER stress in β-cell dysfunction and novel machineries of secretory granule biogenesis. However, still many questions remain. What are the mechanisms by which ER-stress

sensors regulate proinsulin translation and folding? Is it relevant to prevent β-cell death by preventing UPR? The inhibition of CHOP has been studied to prevent β-cell death for the treatment of diabetes [46, 107–109]; however, it should be addressed carefully that even if β-cells survive by preventing CHOP, and too much accumulation of unfolded proteins in the ER may prevent normal proinsulin folding and would not support the function of islets of Langerhans. Decreasing the continuous high demand of insulin synthesis is anyway the primary importance for diabetes; then thinking about how to support proinsulin folding, packaging proinsulin into secretory granules, and elimination of unfolded proteins from β-cells would help for developing new treatments of diabetes.

Author details

Michiko Saito[1] and Yoko Shiba[2]*

*Address all correspondence to: shibay@iwate-u.ac.jp

1 Bio-Science Research Center, Kyoto Pharmaceutical University, Kyoto, Japan

2 Faculty of Science and Engineering, Iwate University, Morioka, Japan

References

[1] Ronald Kahn C, Weir G, King G, Jacobson A, Smith R, Moses A. Joslin's Diabetes Mellitus. 14th ed. Lippincott Williams & Wilkins; 2004

[2] Halban PA et al. Spontaneous reassociation of dispersed adult rat pancreatic islet cells into aggregates with three-dimensional architecture typical of native islets. Diabetes. 1987;**36**(7):783-790

[3] Kinkel MD, Prince VE. On the diabetic menu: Zebrafish as a model for pancreas development and function. BioEssays. 2009;**31**(2):139-152

[4] Zang L, Shimada Y, Nishimura N. Development of a novel zebrafish model for type 2 diabetes mellitus. Scientific Reports. 2017;**7**(1):1461

[5] Tehrani Z, Lin S. Antagonistic interactions of hedgehog, Bmp and retinoic acid signals control zebrafish endocrine pancreas development. Development. 2011;**138**(4):631-640

[6] Kimmel RA, Meyer D. Zebrafish pancreas as a model for development and disease. Methods in Cell Biology. 2016;**134**:431-461

[7] Prince VE, Anderson RM, Dalgin G. Zebrafish pancreas development and regeneration: Fishing for diabetes therapies. Current Topics in Developmental Biology. 2017;**124**:235-276

[8] Otero JH, Lizak B, Hendershot LM. Life and death of a BiP substrate. Seminars in Cell & Developmental Biology. 2010;**21**(5):472-478

[9] Liu M et al. Proinsulin entry and transit through the endoplasmic reticulum in pancreatic beta cells. Vitamins and Hormones. 2014;**95**:35-62

[10] Yoshida H et al. A time-dependent phase shift in the mammalian unfolded protein response. Developmental Cell. 2003;**4**(2):265-271

[11] Han J et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nature Cell Biology. 2013;**15**(5):481-490

[12] Harding HP et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Molecular Cell. 2000;**6**(5):1099-1108

[13] Tirasophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes & Development. 1998;**12**(12):1812-1824

[14] Martino MB et al. The ER stress transducer IRE1beta is required for airway epithelial mucin production. Mucosal Immunology. 2013;**6**(3):639-654

[15] Tsuru A et al. Negative feedback by IRE1beta optimizes mucin production in goblet cells. Proceedings of the National Academy of Sciences of the United States of America. 2013; **110**(8):2864-2869

[16] Bertolotti A et al. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nature Cell Biology. 2000;**2**(6):326-332

[17] Kimata Y et al. Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins. Molecular Biology of the Cell. 2003;**14**(6):2559-2569

[18] Kimata Y et al. A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. The Journal of Cell Biology. 2004;**167**(3):445-456

[19] Kimata Y et al. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. The Journal of Cell Biology. 2007;**179**(1):75-86

[20] Korennykh AV et al. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009;**457**(7230):687-693

[21] Li H et al. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(37):16113-16118

[22] Welihinda AA et al. Protein serine/threonine phosphatase Ptc2p negatively regulates the unfolded-protein response by dephosphorylating Ire1p kinase. Molecular and Cellular Biology. 1998;**18**(4):1967-1977

[23] Papa FR et al. Bypassing a kinase activity with an ATP-competitive drug. Science. 2003; **302**(5650):1533-1537

[24] Han D et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell. 2009;**138**(3):562-575

[25] Ali MM et al. Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response. The EMBO Journal. 2011;**30**(5):894-905

[26] Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell. 1997;**90**(6):1031-1039

[27] Yoshida H et al. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;**107**(7):881-891

[28] Shen J et al. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Developmental Cell. 2002;**3**(1):99-111

[29] Shen J et al. Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response. Molecular and Cellular Biology. 2005;**25**(3):921-932

[30] Wang Y et al. Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. The Journal of Biological Chemistry. 2000;**275**(35):27013-27020

[31] Chen X, Shen J, Prywes R. The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. The Journal of Biological Chemistry. 2002;**277**(15):13045-13052

[32] Ye J et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Molecular Cell. 2000;**6**(6):1355-1364

[33] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nature Reviews. Molecular Cell Biology. 2007;**8**(7):519-529

[34] Mori K. Signalling pathways in the unfolded protein response: Development from yeast to mammals. Journal of Biochemistry. 2009;**146**(6):743-750

[35] Kimata Y, Kohno K. Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells. Current Opinion in Cell Biology. 2011;**23**(2):135-142

[36] Schuit FC, In't Veld PA, Pipeleers DG. Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells. Proceedings of the National Academy of Sciences of the United States of America. 1988;**85**(11):3865-3869

[37] Van Lommel L et al. Probe-independent and direct quantification of insulin mRNA and growth hormone mRNA in enriched cell preparations. Diabetes. 2006;**55**(12):3214-3220

[38] Eizirik DL et al. Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(20):9253-9256

[39] Robertson RP, Harmon JS. Pancreatic islet beta-cell and oxidative stress: The importance of glutathione peroxidase. FEBS Letters. 2007;**581**(19):3743-3748

[40] Dionne KE, Colton CK, Yarmush ML. Effect of oxygen on isolated pancreatic tissue. ASAIO Transactions. 1989;**35**(3):739-741

[41] Carlsson PO et al. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes. 2001;**50**(3):489-495

[42] Butler AE et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;**52**(1):102-110

[43] Rahier J et al. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, Obesity & Metabolism. 2008;**10**(Suppl 4):32-42

[44] Huang CJ et al. High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. Diabetes. 2007;**56**(8):2016-2027

[45] Wang J et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. The Journal of Clinical Investigation. 1999; **103**(1):27-37

[46] Oyadomari S et al. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. The Journal of Clinical Investigation. 2002;**109**(4):525-532

[47] Stoy J et al. Insulin gene mutations as a cause of permanent neonatal diabetes. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(38): 15040-15044

[48] Delepine M et al. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nature Genetics. 2000;**25**(4):406-409

[49] Harding HP et al. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/-mice reveals a role for translational control in secretory cell survival. Molecular Cell. 2001;**7**(6): 1153-1163

[50] Zhang W et al. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. Cell Metabolism. 2006;**4**(6):491-497

[51] Gao Y et al. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Molecular and Cellular Biology. 2012;**32**(24):5129-5139

[52] Scheuner D et al. Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. Nature Medicine. 2005;**11**(7):757-764

[53] Yamamoto K et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Developmental Cell. 2007;**13**(3):365-376

[54] Usui M et al. Atf6alpha-null mice are glucose intolerant due to pancreatic beta-cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. Metabolism. 2012;**61**(8):1118-1128

[55] Song B et al. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. The Journal of Clinical Investigation. 2008;**118**(10):3378-3389

[56] Fonseca SG et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. The Journal of Clinical Investigation. 2010;**120**(3):744-755

[57] Hassler JR et al. The IRE1alpha/XBP1s pathway is essential for the glucose response and protection of beta cells. PLoS Biology. 2015;**13**(10):e1002277

[58] Tsuchiya Y et al. IRE1-XBP1 pathway regulates oxidative proinsulin folding in pancreatic β cells. The Journal of Cell Bioligy. Published Online: 5 March; 2018

[59] Hou JC, Min L, Pessin JE. Insulin granule biogenesis, trafficking and exocytosis. Vitamins and Hormones. 2009;**80**:473-506

[60] Molinete M et al. Trafficking/sorting and granule biogenesis in the beta-cell. Seminars in Cell & Developmental Biology. 2000;**11**(4):243-251

[61] Tooze SA. Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. Biochimica et Biophysica Acta. 1998;**1404**(1–2):231-244

[62] Orci L et al. The trans-most cisternae of the Golgi complex: A compartment for sorting of secretory and plasma membrane proteins. Cell. 1987;**51**(6):1039-1051

[63] Chanat E, Huttner WB. Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. The Journal of Cell Biology. 1991;**115**(6):1505-1519

[64] Cool DR et al. Carboxypeptidase E is a regulated secretory pathway sorting receptor: Genetic obliteration leads to endocrine disorders in Cpe(fat) mice. Cell. 1997;**88**(1):73-83

[65] Irminger JC et al. Proinsulin targeting to the regulated pathway is not impaired in carboxypeptidase E-deficient Cpefat/Cpefat mice. The Journal of Biological Chemistry. 1997;**272**(44):27532-27534

[66] Tooze SA, Martens GJ, Huttner WB. Secretory granule biogenesis: Rafting to the SNARE. Trends in Cell Biology. 2001;**11**(3):116-122

[67] Kogel T, Gerdes HH. Maturation of secretory granules. Results and Problems in Cell Differentiation. 2010;**50**:1-20

[68] Tsuchiya M et al. Cholesterol biosynthesis pathway intermediates and inhibitors regulate glucose-stimulated insulin secretion and secretory granule formation in pancreatic beta-cells. Endocrinology. 2010;**151**(10):4705-1476

[69] Hosaka M, Watanabe T. Secretogranin III: A bridge between core hormone aggregates and the secretory granule membrane. Endocrine Journal. 2010;**57**(4):275-286

[70] Maeda Y et al. Impaired processing of prohormones in Secretogranin III-null mice causes maladaptation to an inadequate diet and stress. Endocrinology. 2018;**159**(2):1213-1227

[71] Molinete M et al. Trafficking of non-regulated secretory proteins in insulin secreting (INS-1) cells. Diabetologia. 2000;**43**(9):1157-1164

[72] Dodson G, Steiner D. The role of assembly in insulin's biosynthesis. Current Opinion in Structural Biology. 1998;**8**(2):189-194

[73] Dunn MF. Zinc-ligand interactions modulate assembly and stability of the insulin hexamer – A review. Biometals. 2005;**18**(4):295-303

[74] Howell SL, Montague W, Tyhurst M. Calcium distribution in islets of Langerhans: A study of calcium concentrations and of calcium accumulation in B cell organelles. Journal of Cell Science. 1975;**19**(2):395-409

[75] Zalewski PD et al. Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. The Journal of Histochemistry and Cytochemistry. 1994;**42**(7):877-884

[76] Syring KE et al. Combined deletion of Slc30a7 and Slc30a8 unmasks a critical role for ZnT8 in glucose-stimulated insulin secretion. Endocrinology. 2016;**157**(12):4534-4541

[77] Flannick J et al. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nature Genetics. 2014;**46**(4):357-363

[78] Peter BJ et al. BAR domains as sensors of membrane curvature: The amphiphysin BAR structure. Science. 2004;**303**(5657):495-499

[79] Gehart H, Ricci R. Saving the neck from scission. Communicative & Integrative Biology. 2013;**6**(2):e23098

[80] Boucrot E et al. Membrane fission is promoted by insertion of amphipathic helices and is restricted by crescent BAR domains. Cell. 2012;**149**(1):124-136

[81] Kanoh H, Williger BT, Exton JH. Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. The Journal of Biological Chemistry. 1997;**272**(9):5421-5429

[82] Man Z et al. Arfaptins are localized to the trans-Golgi by interaction with Arl1, but not Arfs. The Journal of Biological Chemistry. 2011;**286**(13):11569-11578

[83] Szule JA, Fuller NL, Rand RP. The effects of acyl chain length and saturation of diacylglycerols and phosphatidylcholines on membrane monolayer curvature. Biophysical Journal. 2002;**83**(2):977-984

[84] Gehart H et al. The BAR domain protein Arfaptin-1 controls secretory granule biogenesis at the trans-Golgi network. Developmental Cell. 2012;**23**(4):756-768

[85] Williger BT, Ostermann J, Exton JH. Arfaptin 1, an ARF-binding protein, inhibits phospholipase D and endoplasmic reticulum/Golgi protein transport. FEBS Letters. 1999; **443**(2):197-200

[86] Huang LH et al. Arfaptin-1 negatively regulates Arl1-mediated retrograde transport. PLoS One. 2015;**10**(3):e0118743

[87] Anitei M et al. Spatiotemporal control of lipid conversion, actin-based mechanical forces, and curvature sensors during clathrin/AP-1-coated vesicle biogenesis. Cell Reports. 2017; **20**(9):2087-2099

[88] Steiner DF. The proprotein convertases. Current Opinion in Chemical Biology. 1998;**1**:31-39

[89] Orci L et al. pH-independent and -dependent cleavage of proinsulin in the same secretory vesicle. The Journal of Cell Biology. 1994;**126**(5):1149-1156

[90] Hutton JC. Insulin secretory granule biogenesis and the proinsulin-processing endopeptidases. Diabetologia. 1994;**37**(Suppl 2):S48-S56

[91] Bailyes EM et al. Differences between the catalytic properties of recombinant human PC2 and endogenous rat PC2. The Biochemical Journal. 1995;**309**(Pt 2):587-594

[92] Demaurex N et al. Mechanism of acidification of the trans-Golgi network (TGN). In situ measurements of pH using retrieval of TGN38 and furin from the cell surface. The Journal of Biological Chemistry. 1998;**273**(4):2044-2051

[93] Bailyes EM et al. A member of the eukaryotic subtilisin family (PC3) has the enzymic properties of the type 1 proinsulin-converting endopeptidase. The Biochemical Journal. 1992;**285**(Pt 2):391-394

[94] Lamango NS et al. The proteolytic maturation of prohormone convertase 2 (PC2) is a pH-driven process. Archives of Biochemistry and Biophysics. 1999;**362**(2):275-282

[95] Arvan PKR, Prabakaran D, Zavacki AM, Elahi D, Wang S, Pilkey D. Protein discharge from immature secretory granules displays both regulated and constitutive characteristics. The Journal of Biological Chemistry. 1991:14171-14174

[96] Kuliawat RAP. Protein targeting via the "constitutive-like" secretory pathway in isolated pancreatic islets: Passive sorting in the immature granule compartment. The Journal of Cell Biology. 1992;**3**:521-529

[97] Kuliawat RAP. Distinct molecular mechanisms for protein sorting within immature secretory granules of pancreatic beta-cells. The Journal of Cell Biology. 1994;**126**(1):77-86

[98] Klumperman J et al. Mannose 6-phosphate receptors are sorted from immature secretory granules via adaptor protein AP-1, clathrin, and syntaxin 6-positive vesicles. The Journal of Cell Biology. 1998;**141**(2):359-371

[99] Robinson MS. Forty years of Clathrin-coated vesicles. Traffic. 2015;**16**(12):1210-1238

[100] Le Borgne R, Hoflack B. Protein transport from the secretory to the endocytic pathway in mammalian cells. Biochimica et Biophysica Acta. 1998;**1404**(1–2):195-209

[101] Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: New twists in the tale. Nature Reviews. Molecular Cell Biology. 2003;**4**(3):202-212

[102] Wendler FPL, Urbé S, Tooze SA. Homotypic fusion of immature secretory granules during maturation requires syntaxin 6. Molecular Biology of the Cell. 2001;**12**(6):1699-1709

[103] Kuliawat R et al. Syntaxin-6 SNARE involvement in secretory and endocytic pathways of cultured pancreatic beta-cells. Molecular Biology of the Cell. 2004;**15**(4):1690-1701

[104] Du W et al. HID-1 is required for homotypic fusion of immature secretory granules during maturation. eLife. 2016:**5**

[105] Rorsman P, Braun M. Regulation of insulin secretion in human pancreatic islets. Annual Review of Physiology. 2013;**75**:155-179

[106] Izumi T, Kasai K, Gomi H. Secretory vesicle docking to the plasma membrane: Molecular mechanism and functional significance. Diabetes, Obesity & Metabolism. 2007;**9**(Suppl 2): 109-117

[107] Gurlo T et al. CHOP contributes to, but is not the only mediator of, IAPP induced beta-cell apoptosis. Molecular Endocrinology. 2016;**30**(4):446-454

[108] Yang Y et al. Transcription factor C/EBP homologous protein in health and diseases. Frontiers in Immunology. 2017;**8**:1612

[109] Nam DH et al. CHOP deficiency ameliorates ERK5 inhibition-mediated exacerbation of Streptozotocin-induced hyperglycemia and pancreatic beta-cell apoptosis. Molecules and Cells. 2017;**40**(7):457-465