Zinc and Diabetes

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Abstract

Zn^{2+} ions are essential for the normal processing and storage of insulin and altered pancreatic insulin content is associated with all forms of diabetes mellitus. Work of the past decade has identified variants in the human *SLC30A8* gene, encoding the zinc transporter ZnT8 which is expressed highly selectively on the secretory granule of pancreatic islet β and α cells, as affecting the risk of Type 2 Diabetes. Here, we review the regulation and roles of Zn^{2+} ions in islet cells, the mechanisms through which *SLC30A8* variants might affect glucose homeostasis and diabetes risk, and the novel technologies including recombinant targeted zinc probes and knockout mice which have been developed to explore these questions.

Abbreviation

ISG: Insulin Secreting Granules; T2D: Type 2 Diabetes; T1D: Type 1 Diabetes;

Diabetes mellitus is a common metabolic disease, affecting approximately 9% of the adult population worldwide. It is characterized by high circulating glucose levels over a prolonged period of time which leads to long-term health complications [1]. Type 1 Diabetes (T1D) involves the autoimmune destruction of insulin-secreting β cells while Type 2 Diabetes (T2D) is linked to both a decrease of insulin release and deficient hormone action on targeted organs [2].

The links among Zn^{2+}, diabetes and insulin were established in the 1930s, a decade after the discovery of the hormone, when a study showed crystallized insulin contains Zn^{2+} and that Zn^{2+}, along with other metal ions, could reversibly trigger insulin crystallization [3]. Zinc was later demonstrated to prolong insulin action when co-injected with the hormone [4]. These early studies, as well as much more recent findings showing association between T2D risk and the inheritance of gene variants encoding a critical β cell Zn^{2+} transporter, ZnT8 [5] have generated considerable interest in the role of Zn^{2+} in diabetes aetiology and as a possible therapeutic target [6].

1. Zinc and the diabetic patient

Scott and Fisher were the first to report a direct link between zinc and diabetes in patients. While assessing the insulin content in the pancreas of diabetic patients compared to non-diabetic cadavers, they showed that in the former group zinc content was reduced by 75 % [7].
Epidemiological studies also demonstrated that diabetes and whole body zinc status are associated [8-11]. In T1D and T2D patients, serum zinc levels are significantly decreased [9-11], this being associated with an increased zinc urinary loss [8].

 Zinc may have important anti-oxidant properties, as it acts as a cofactor of the superoxide dismutase enzyme which regulates the detoxification of reactive oxygen species, regulating the expression and protecting against the oxidative stress induced by chronic hyperglycaemia (for review, [12]). Furthermore, zinc inhibits alpha-ketoglutarate-dependent mitochondrial respiration suggesting that Zn^{2+} can interfere with mitochondrial antioxidant production and may also stimulate production of reactive oxygen [13].

Zinc supplementation has been shown to improve T2D symptoms, both in mice and diabetic patients. Studies on obese *ob/ob* mice showed a positive effect as attenuating the hyperinsulinemia and fasting hyperglycaemia of the animals [14]. Accordingly, dietary zinc supplementation attenuated hyperglycemia and hyperinsulinemia in *db/db* mice [15].

Correspondingly, several studies have assessed the effect of a dietary zinc supplementation in T1D and T2D patients (for review, [16]). The effects on the diabetic phenotype vary but globally, scientists observed beneficial effects especially in decreasing HbA1c levels and cholesterol. However, it is still unclear if dietary zinc supplementation would have an effect in preventing the disease in adults with insulino-resistance [17], and it has been shown recently that supplementation may affect the insulin response differentially, depending on the patient genotype for the *SLC30A8* gene encoding the zinc transporter ZnT8 [18, 19] (see below).

2. Zn^{2+} in the pancreatic β cell

The healthy pancreas has a particularly high zinc content and this is greatly decreased in diabetic patients [7]. In fact, most of the zinc within the pancreas is contained in the β cells, and concentrated within the dense core insulin secreting granules (ISG) where the total concentration is between 10 and 20 microM [20, 21]. Importantly, Zn^{2+} ions are an essential factor for both insulin processing and storage.

2.1 Zinc in insulin processing and storage

The mature insulin molecule is a 6 kDa protein composed of two polypeptide chains designated as A and B that are joined by two pairs of disulphide bonds with an additional intramolecular disulphide bond in the A chain. Insulin mRNA is translated inititally to produce an inactive preproinsulin molecule consisting of the two chains connected by the C-peptide and a signal peptide attached to its N-terminus. Preproinsulin is first synthesized within the lumen of the rough endoplasmic reticulum (RER), where the signal peptide is cleaved, forming the proinsulin. Proinsulin is then transported to the trans-Golgi network and packaged into secretory granules where maturation takes place. Proinsulin is cleaved by the prohormone convertases (PC1/3 and PC2, encoded by *PCSK1* and *PCSK2*) to produce C-peptide and a native insulin molecule (for review see [22]). Insulin is stored initially as a monomer and then forms dimer as it accumulates in the maturating granules. In the presence of zinc, concentrated in the maturating vesicles *via* its transport by the zinc transporter ZnT8 [23] (see below), the dimers aggregate to form a hexamer around two Zn^{2+} ions. The hexamerization process reduces insulin solubility and triggers its crystallisation, increasing the storage capacity of the insulin secreting vesicles [24, 25]. Concomitantly, pH is decreased in the maturating granules via a proton pump (<pH 6) as insulin hexamers are unstable at neutral pH. When insulin is secreted into the extracellular medium during exocytosis, hexamers are rapidly converted into active monomers as the pH increases. This process thus also liberates significant concentrations of Zn^{2+} into the circulation, potentially with actions independent to those of insulin [25, 26].

2.2 Zinc homeostasis in the β cell

High free Zn^{2+} concentrations are toxic for mammalian cells but Zn^{2+} also needs to be readily available to perform numerous functions, notably as an enzyme cofactor or structural component of proteins (approximately 3,000 genes within the human genome present a Zn^{2+} binding domain) [27], particularly in the context of the β cell. Cellular Zn^{2+} homeostasis must therefore be tightly regulated and three protein families are involved: the metallothioneins (MTs), the zinc importers (ZIP, *SLC39A*) and the zinc transporters (ZnT, *SLC30A*).

While MTs bind Zn^{2+} ions directly, transporters regulate intracellular free Zn^{2+} levels by sequestration or recruitment from organelles or the extracellular media. Ten members of the ZnT family have been identified (ZnT1 to 10 – encoded by SL30A1 – 10) and 14 members of the ZiP family (ZiP1-14, encoded by SLC39A1 – 14). The expression of specific ZiPs and ZnTs is tissue-dependent and related to specific cellular functions [28]. For the purposes of this review we focus on transporters expressed in the pancreatic β cell.

2.2.1 ZnT family

ZnT family members transport Zn^{2+} from the cytosol into organelles or towards the extracellular space. ZnT transporters are members of the cation diffusion facilitators (CDFs) super family, having 6 transmembrane domains and with both the amino and carboxi termini located in the cytosolic side [29].

Several ZnT are expressed in the β cell (Figure 1, for review [30]). ZnT1, ubiquitously present in tissues, is the only member of the family localised at the plasma membrane, exporting zinc to the extracellular media [31]. ZnT5 and ZnT7 are also expressed [32, 33]: ZnT5 localizes with the Golgi apparatus and secretory vesicles, whereas ZnT7 resides mostly in the perinuclear region, consistent with its Golgi localization (see Figure 1). ZnT6 mRNA was detected in the islets of Langerhans [34], its functional heterodimerization with ZnT5 has been described [35], and its presence in purified β cells from both mouse [36] and human [37, 38] confirmed by massive parallel sequencing (RNASeq).

ZnT3 is responsible in neurons for the uptake of Zn^{2+} into synaptic vesicles [39]. ZnT3 (SLC30A3) protein has been reported in the islets, and in the pancreatic β cell line INS-1E but not in a corresponding pancreatic α-cell line, α-TC6 [40-42]. While ZnT3 mRNA was reported in purified human β cells in one study [38], levels were zero, or at the limit of detection, in samples from several subjects in another [37]. ZnT3 mRNA was similarly barely

detected by RNASeq in purified mouse β cells [36], in line with our findings in mouse islets [34]. The role of this particular transporter in controlling β cell Zn^{2+} thus remains unclear.

Finally, ZnT8 expression is largely restricted to the pancreas, where it is expressed exclusively, and at similar levels, in β and α cells in both mouse and man [43, 44]. In β cells, ZnT8 transports Zn^{2+} from the cytosol to the ISG and thus plays a critical a role in hormone storage as described above. It is the only Zn^{2+} transporter showing such dramatic tissue-specific expression and its expression level is the highest of all isoforms in both β and α cells, making it the most strongly expressed Zn^{2+} transporter in the islet [30]. The relationship between ZnT8/*SLC30A8* variants and T2D risk will be described in more detail later.

2.2.2 ZIP family

ZIP transporters are responsible for zinc transport into the cytoplasm, with either influx from the extracellular space or from intracellular organelles. ZIPs are thus localised to the plasma membrane (for most family members) and/or on the membranes of the nucleus, ER and Golgi apparatus (ZIP7, ZIP13, ZIP9), and lysosomes and vesicles (ZIP13, ZIP3, ZIP8) [45]. In the mouse pancreatic islet mRNA expression, albeit quite low in some cases, has been detected for most of the ZIP family members, ZIP6 ZIP7 and ZIP9 being the most expressed [30]. Concordantly, ZIP6 and ZIP7 are prominent zinc influx transporters in murine and human primary β cells [34, 46]. ZIP6 and ZIP7 localise in these cells predominantly at the ER but also at the plasma membrane (Figure 1). Interestingly, in response to glucose, ZIP6 colocalisation coefficient with a membrane marker increases, suggesting the transporter translocates from the ER to the plasma membrane [46]. ZIP6 and ZIP7 also show, alongside with ZIP8, an increase in mRNA levels when murine β cells are treated with 16.7 mM glucose for 24 hours [34]. Thus, β cells may adapt Zn^{2+} transporter expression and localisation to modulate Zn^{2+} content in response to glucose stimulation. In support of this view, modulation of ZIP6 and ZIP7 expression in the pancreatic β cell line MIN6 significantly alters cytosolic Zn^{2+} influx. Functionally, ZIP6 and ZIP7 knockdown leads to impaired insulin secretion [46].

2.2.3 Metallothioneins

Members of the metallothionein (MT) family are intracellular, low molecular weight, cysteine-rich proteins. Ubiquitous in eukaryotes, MTs have unique structural characteristics and possess potent metal-binding and redox capabilities. Four isoforms have been described, the most widely expressed isoforms in mammals being MT1 and MT2. Among the metals they associate with, MTs bind Zn^{2+} with high affinity but can also function as Zn^{2+} donors to other Zn^{2+} binding proteins [25, 47, 48].

MT1 and MT2 are the major isoforms found in the pancreas and in the β cell specifically [30, 34]. In response to high glucose (16.7 mM for 24 h), the cytosolic Zn^{2+} concentration is increased and is associated with an increase in MT1 and MT2 expression. This demonstrates that, as for ZIP6 and ZIP7, glucose stimulation of β cells regulates the expression of genes involved in Zn^{2+} homeostasis [34].

In addition to the members of the three protein families described above, other proteins have been described as having a role in controlling zinc homeostasis and transport. Notably in the β cell, the voltage gated calcium channel (VGCC), transporting Ca^{2+} in the intracellular space in response to glucose which triggers insulin secretion [49], have been reported to transport Zn^{2+} concomitantly [50]. Indeed, inhibited Zn^{2+} uptake in β cells under stimulatory conditions were reported with little or no change in Zn^{2+} accumulation under low glucose conditions. However, this result was not reproduced by a later study using a different method of free Zn^{2+} concentration measurement [34] (and see below for further details).

2.3 Free Zn^{2+} concentrations in the β cell

We know that β cells are especially rich in Zn^{2+} ions and that 70% of total zinc is contained in insulin-secreting vesicles [20]. But until quite recently, the data available on free Zn^{2+} concentrations within these cells and in their intracellular organelles were sparse, due to the lack of accurate tools with which to perform such measurements. The development of genetically encoded Förster resonance energy transfer (FRET) sensors with appropriate affinities (ie. in the low nanomolar range) has recently allowed the measurement of free Zn^{2+} concentration in living cells with high precision and spatial accuracy. Two main families of FRET-based Zn^{2+} sensors have been developed, namely the Zap-sensors by the Palmer group [51], and the eCALWYsensors by the Merkx group and ourselves [52]. Free cytosolic zinc concentrations measured using these FRET-sensors were approximately in the same range as those measured with chemical probes or hybrid sensors, and it is generally agreed now that free zinc is around a few hundreds of picomolar [53-55]. Both FRET sensor families have been targeted to intracellular organelles such as the ER, the nucleus, the mitochondria or the Golgi apparatus by in-frame fusion with suitable targeting motifs [51, 56-58]. Of note, concentrations measured in the ER and in the mitochondria varied greatly depending on the FRET or hybrid sensors used, discrepancies that still have to be explained [51, 55-57].

Using the eCALWY-4 probe to measure cytosolic and organellar free zinc concentrations in different cell types, we observed that the cytosolic Zn^{2+} concentration of primary β cells or

different β cell lines was between 0.4 and 1.5 nM, similar to other cell types tested (Figure 1) [34, 56]. However, in response to 24 h treatment with high glucose (16.7 *vs* 3 mM), the cytosolic free Zn^{2+} concentration was doubled, this being associated with the up-regulation of Zn^{2+} homeostasis genes as described above [34]. Conversely, deprivation of O_2 for 24 h lowered free cytosolic Zn^{2+} concentrations in mouse and human islet cells. Hypoxia similarly decreased ZnT8 mRNA expression in islets, and immunoreactivity in β cells [59]. The link between the lack of ZnT8 and lower free Zn^{2+} cytosolic concentration has clearly been established as Zn^{2+} concentrations are lower in β cells from β cell specific ZnT8 knock-out mice [60]. The basis of this paradoxical finding is, however, not fully understood.

Recently, a new generation of Zn^{2+} sensors has been developed by Merkx and colleagues, in collaboration with this laboratory, called eZinCh2. Vamp2-eZinCh2 is the first genetically-encoded sensor targeted to ISGs which shows a response to calibration conditions in these organelles and has allowed us to measure a free Zn^{2+} concentration in this compartment of ~ 120 nM in non-stimulated β cells (Figure 1) [61].

2.4 Zn^{2+} imaging to assess β cell mass in vivo?

The healthy pancreas has a very high zinc content because of its high concentration present in β cells. In comparison, zinc ions are less abundant in the exocrine tissue [62]. In the T2D, the decline in β cell function is associated with an overall decrease in overall β cell mass [63]. But measuring β cell mass *in vivo* by classical methods (such as MRI or PET) is challenging given (1) islets represent only ~1% of the pancreas volume and (2) the absence of a suitable contrast reagent. Thus, dual-modal zinc probes, based on transition metal chelates capable of binding zinc ions could be of clinical value as imaging tools [64].

3. ZnT8 and diabetes risk

3.1 ZnT8 and T2D: GWAS studies

Recent genome-wide association studies (GWAS) have provided a powerful new means of identifying genetic factors associated with (and presumably driving) diabetes risk. The very first comprehensive GWAS for T2D thus demonstrated a link between T2D development and ZnT8 activity, since as a non-synonymous variant (rs13266634) in the *SLC30A8* gene is enriched in diabetic patients [5]. This single nucleotide polymorphism (SNP) rs1326663 causes the replacement of an arginine residue with a tryptophan (R325W) in the cytosolic C-terminus of the transporter. Possession of the risk variant of rs13266634 is associated with a

17% increase in disease risk per allele [65]. R325 allele carriers also present an increased proinsulin:insulin ratio during an oral glucose tolerance test [66], an impaired insulin secretion during an intravenous glucose tolerance test [67] and lower β cell function (HOMA-B assessment) [68].

Subsequent studies identified five other SNPs, all located in exon13 and with one also causing an amino-acid change at position 325 [69, 70]. The other four SNPs are all located in the 3' translated region two of which (rs3802177 and rs11558471) are in strong linkage disequilibrium with rs13266634 [69]. However, it is not clear if possession or not of these SNPs has an impact on glucose homeostasis parameters (e.g. fasting blood glucose, glucose tolerance etc.) [66-68, 71, 72].

Unexpectedly, a recent study by Flannick et al. identified 12 rare protein-truncating variants statistically associated with a 65% *decrease* in T2D risk. Among the variants, the two most common sequences failed to express a stable protein. Thus, *SLC30A8* haploinsufficiency leading to a lower expression of the transporter appears from these studies to protect against T2D [73].

3.2 ZnT8 structure and variants activity

ZnT8 is located at the membrane of ISG [44] and possesses, in common with other members of the ZnT family, six transmembrane-spanning domains with both the amino and carboxy-termini located on the cytosolic side of the granule (Figure 2). A crystal structure of mammalian ZnT8 is not yet available; consequently, the data on protein structure-function relationships and effect of the SNPs on them are based on the bacterial homolog YiiP, which has 51.8% sequence homology with the mammalian protein [74, 75]. Arginine/Tryptophan-325 is located in an area involved in transporter homodimerization but as the side chains point away from the dimerization interface, it is unlikely that the R325W substitution prevents dimerization or Zn^{2+} binding (Figure 2) [76, 77]. However, it is conceivable that the positive charge of the R-side chain may hamper molecular interactions, for instance with unidentified Zn^{2+}-binding proteins which might interact with ZnT8 to facilitate zinc transport. Supporting this view, we found by overexpressing either isoform in a β cell line and measuring zinc in vesicles with Zinquin that in presence of supraphysiological zinc concentration, zinc accumulation into granules was significantly higher for W- versus R-form. Thus, the risk variant R325 is less active in transporting zinc in β cells than the protective variant W325 [77]. Similar data were also obtained by Kim et al. using isolated secretory granules and radiotracers (^{65}Zn) [78].

These findings are, nonetheless, difficult to reconcile with those of Flannick and colleagues which implied that *SLC30A8* haploinsufficiency is protective against T2D. The basis of this apparent contradiction is not yet understood, though various possible explanations are explained in detail elsewhere [6] and below.

3.3 ZnT8 studies in vivo

To further investigate the role ZnT8 *in vivo* in the maintenance of glucose homeostasis, several groups (including ours) have developed ZnT8 null mouse models, either with global deletion or deletion restricted largely or exclusively to the β cell depending on the Cre delete strain deployed (for review see [27]). Although variations between the models are observed, and may be attributed to differences in genetic background, housing conditions, alterations in the microbiome etc [79], in the majority of cases null mice display impaired or unaltered glucose tolerance: no study reported an improvement in this metabolic parameter.

Striking granule abnormalities were observed by electron microscopy in ZnT8-depleted β cells, notably that granules lacked the characteristic insulin dense cores, whilst many ISGs contained a "rod-like" structure [60, 77, 80]. Moreover, islet and β cell free Zn^{2+} was decreased in both global and β cell specific ZnT8 null animals [60, 77, 81].

A recent study by Tamaki et al. [82] showed that mice with β cell ZnT8 specific deletion had lower peripheral insulin concentrations whereas insulin secretion from the isolated perfused pancreas was increased. In an elegant set of experiments this group also showed that the apparently contradictory results could be explained by a role for secreted zinc in inhibiting insulin internalization and degradation by the liver. These findings thus uncovered a more complex role for ZnT8 in glucose homeostasis, and in particular in tissue-tissue interactions, than previously thought. Importantly, these complex interactions might contribute to the differences in the impact of genetic deletion of ZnT8 in mice versus reduction in levels (by 50 %) in rare allele carriers [73] or inheritance of risk variants (R325W) [77].

The effects of exposure to a high fat diet as a means of promoting the development of T2D in mice have been studied in global and β cell-specific ZnT8 KO animals [83]. β cell specific null mice displayed similar bodyweights to controls but insulin secretion was impaired and these animals and were glucose-intolerant compared to control littermates. Global KO animals fed a high fat diet showed increased weight gain and became both glucose-intolerant and insulin-resistant. Thus, expression of ZnT8 in extra-pancreatic tissue seems to be crucial for regulating bodyweight. In this laboratory, we deleted ZnT8 using a highly

specific β cell Ins1Cre driver line, which produces no detectable recombination in the hypothalamus [84], thus avoiding potential complications resulting from deletion in appetite centres which occurs after the use of the rat insulin promoter (RIP2Cre) driver line [85]. Confirming previous observations, these animals, depleted for ZnT8 highly specifically in the β cell, presented with impaired glucose tolerance, abnormal granule morphology, reduced cytosolic zinc concentration in the β cells and lower zinc secretion [60]. These findings thus confirm a β cell autonomous action of ZnT8 manipulation.

Finally, we have also generated a transgenic mouse cell line overexpressing the human W325 variant of ZnT8 specifically in the β cells. Increased ZnT8 expression was associated with improved glucose tolerance. Interestingly, we were able to ascribe the improvement not to increased insulin secretion but rather to enhanced Zn^{2+} secretion, possibly via an autocrine/paracrine on neighbouring cells (see below) and via an inhibition of insulin clearance as described by Tamaki and colleagues [82].

4. ZnT8 in the alpha cell

As mentioned above, ZnT8 mRNA is present in pancreatic α cells and ZnT8 immunoreactivity was detected in glucagon-positive cells by immunocytochemical analysis of human and mouse islets. Analysis of gene expression by microarray and qPCR revealed that, in α cells, ZnT8 is also the most expressed ZnT family member [77]. However, ZnT8 is not expressed in α cells from porcine pancreas, demonstrating clear differences between species. In the pig islet, α cell zinc homeostasis is thus regulated differently from humans and mice [86]. Knockdown of ZnT8 in the glucagonoma-derived α cell line αTC1.9 induced an increase in glucagon mRNA expression and a decrease by 70% in stimulated glucagon secretion. Reversibly, when overexpressing the ZnT8 R325 or W325 variants, the cells displayed reduced glucagon content associated with a 50% lower glucagon secretion [87]. However, up to now, few studies have investigated the role of ZnT8 in glucagon secretion *in vitro* and *in vivo*. Global ZnT8 null mice had normal fasting glucagon levels and stimulated glucagon secretion from isolated islets was comparable to control mice [88]. A further study found similarly normal fasting levels and glucose homeostasis in alpha-cell-specific ZnT8 KO mice [81].

In line with those results, we observed in a later study no effect of ZnT8 deletion selectively in α cells on glucose homeostasis or fasting glucagon. However, we observed in these more recent studies that KO mice displayed enhanced responses to hypoglycaemia *in vivo* and

increased glucagon secretion from isolated islets [89]. Conversely, transgenic mice expressing ZnT8 selectively in the adult mouse α cell display impaired glucagon secretion in response to lowered glucose levels (Solomou, A *et al*, manuscript under revision).

5. Zn^{2+} secretion and action on insulin target tissues

5.1 Effects of secreted Zn^{2+}

During insulin secretion, free zinc is released in the extracellular media and has been shown to have an autocrine effect on β cells. Indeed, secreted zinc feedback negatively at the level of the ATP-sensitive K$^+$ channels (K$_{ATP}$) and Ca^{2+} channels to reduce insulin secretion [90, 91]. Zinc also has a paracrine effect on neighbouring cells, notably on α cells where it can inhibit glucagon secretion [88]. Thus, the cessation of zinc secretion under hypoglycaemic conditions could act as a trigger for glucagon secretion from α cells at proximity [92, 93].

As demonstrated in β cell-specific ZnT8 KO mice, zinc secreted from islets also regulates hepatic insulin clearance mediated by clathrin-dependent internalisation of the insulin receptor [82].

5.2 Tools for imaging Zn^{2+} secretion during insulin release

Exploiting the fact that Zn^{2+} is co-released with insulin during secretion, we have developed fluorescent, small molecule cell surface-attached Zn^{2+} indicators for monitoring induced exocytotic release, including ZIMIR ("Zinc indicator for imaging insulin release"). These probes display a robust fluorescence enhancement on Zn^{2+} chelation and have been successfully used to detect insulin secretion in real time with subcellular resolution [94-96].

5.3 Zn^{2+} action on insulin target tissues

The phosphorylation of insulin receptor (IR) following its binding to insulin leads to the triggering of two main pathways, and stimulate then glucose transport, glycogen synthesis, lipogenesis, etc [27].

Action of zinc on insulin-targeted tissue was first reported with a study describing an increased rate of lipogenesis in rat adipocytes treated with high zinc concentrations [97]. In skeletal muscle cells, zinc increased the phosphorylation of the insulin receptor substrate-1 (IRS1) phosphorylation [98]. Additionally, zinc treatment of 3T3-L1 cells and rat adipocytes induced the phosphorylation of IR but also the protein kinase B, key enzyme of the insulin

response pathway [99]. The former phosphorylation depends on inhibition by zinc of protein tyrosine phosphatase 1B (PTP1B), and the enzyme is one of the most studied targets of the insulin-mimetic effects of zinc [100].

Conclusions

From its role in the processing and storage of insulin to its insulin-mimetic effects, Zn^{2+} is implicated at different levels in the regulation of glucose homeostasis in mammals. This was recently emphasized by the discovery that variants of the ISG zinc transporter ZnT8 are associated with T2D risk/protection. Although raising controversies that remain to be resolved, this new information suggests that modulation of ZnT8 activity may provide an interesting new target for diabetes therapies which can be used in a personalized manner to treat the aetiological deficiency in carriers of risk alleles.

Acknowledgements

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Figure legends

Figure 1: Zn^{2+} homeostasis in the β cell.

β cells express several ZnT and ZiP zinc transporters that localise either at the plasma or organelles membranes. ZnT8 is specific to pancreatic cells, highly express in β cells and is located at the ISG membrane. Cytosolic and organellar free zinc concentrations were determined using genetically-encoded FRET sensors. Values indicated were measured with eCALWY-4 sensors (white, [56]) and Vamp2-eZinCh2 (yellow, [61]). Modified from [27].

Figure 2: ZnT8 structure and function.

The transporter encoded by *SLC30A8* possesses 6 transmembrane domains. ZnT8 is located within the membrane of ISG and it concentrates zinc ions inside the vesicle. GWAS studies identified a non-synonymous SNP causing the replacement of an arginine residue with a tryptophan at position 325 (R325W). R325 carriers have an increased risk of developing T2D. Modified from [27].
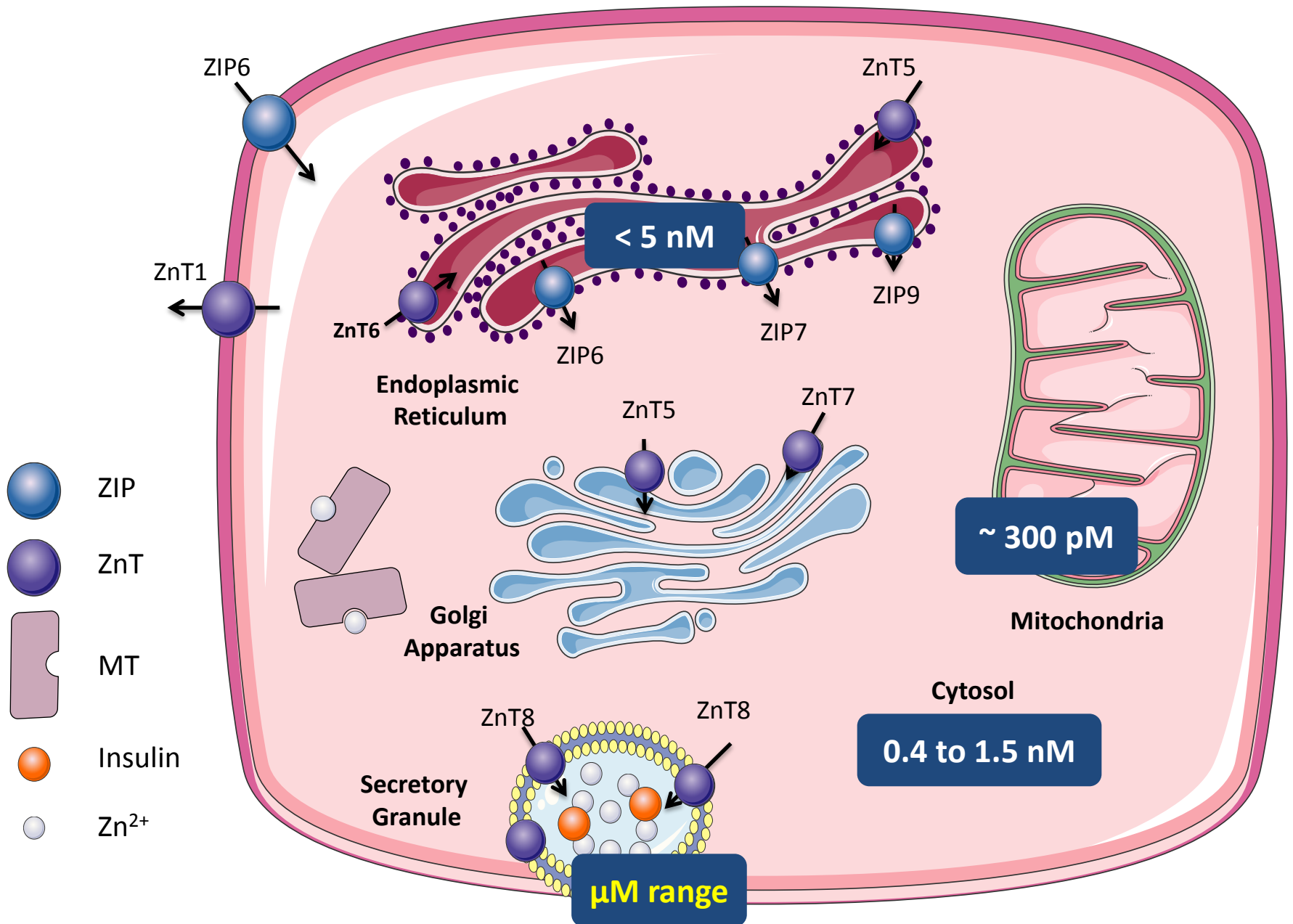
Figure 1

Figure 2