BioMetals (2005) 18:313–317
DOI 10.1007/s10534-005-3687-9

ZnT-8, a pancreatic beta-cell-specific zinc transporter

Fabrice Chimienti, Alain Favier & Michel Seve*
*Laboratoire des lésions des acides nucléiques, DRFMC/SCIB/LAN, CEA/Grenoble, 17, Rue des martyrs, 38054, GRENOBLE CEDEX 9, Grenoble, France; *Author for correspondence (Tel: +33-4-38789060; Fax: +33-4-38785090; E-mail: seve@drfmc.ceng.cea.fr)*

Key words: beta cell, insulin, Langerhans islets, pancreas, zinc, zinc transport, zinc transporter

Abstract

The zinc content in the pancreatic beta cell is among the highest of the body. Zinc appears to be an important metal for insulin-secreting cells as insulin is stored inside secretory vesicles as a solid hexamer bound with two Zn^{2+} ions per hexamer. Zinc is also an important component of insulin secretion mechanisms and is likely to modulate the function of neighbouring cells via paracrine/autocrine interactions. Therefore beta cells undoubtedly need very efficient and specialized transporters to accumulate sufficient amounts of zinc in secretion vesicles. We report here the discovery and the characteristics of a new zinc transporter, ZnT-8, belonging to the CDF (Cation Diffusion Facilitator) family and expressed only in pancreatic beta cells. This transporter, localized in secretion vesicles membrane, facilitates the accumulation of zinc from the cytoplasm into intracellular insulin-containing vesicles and is a major component for providing zinc to insulin maturation and/or storage processes in insulin-secreting pancreatic beta cells. We discovered mammalian orthologs (rat, mouse, chimpanzee, and dog) and found these ZnT-8 proteins very similar (98% conserved amino acids) to human ZnT-8, indicating a high conservation during evolution.

Introduction

Zinc is an essential component of many proteins and is necessary to almost if not all cell functions (Vallee & Falchuk 1993). The zinc content in the pancreatic beta cell is among the highest of the body (Zalewski *et al*. 1994). Therefore zinc appears to be an important metal for insulin-secreting cells, and it may serve as a mediator of insulin storage and secretion (Chausmer 1998). Here, we review the principal aspects of the knowledge regarding zinc homeostasis in insulin-secreting cells and present a new beta cell-specific zinc transporter.

SLC30 family zinc transporters

Zinc homeostasis depends on the uptake and export of zinc by specialized proteins (for review see (Chimienti *et al*. 2003, Eide 2004)). The two main components involved in zinc homeostasis are metallothioneins and zinc transporters (Liuzzi & Cousins 2004). Metallothioneins are implied in intracellular zinc storage and trafficking (Maret 2003). Zinc transporters ensure zinc carriage across biological membranes. The SLC39 family of proteins (Zips proteins) allows intracellular uptake of zinc (Eide 2004), while the SLC30 family (ZnTs proteins) permits cellular efflux of zinc into extracellular matrix, or into intracellular vesicles. In mammalian cells, 10 homologous SLC30 proteins, named ZnT-1 to -10 have been described (Seve *et al*. 2004). These proteins are members of the CDF (Cation Diffusion Facilitator) family, and share the same predicted structure, with six membrane-spanning domains and a histidine-rich intracellular loop between helices IV and V, except for ZnT-6 which retains a serine-rich loop from prokaryotic transporter CZCD (Huang *et al*. 2002) and ZnT-10 which bears a basic amino acid-rich loop. ZnT-1 and -2, located either in the plasma

membrane or in acidic endosomal/lysosomal vesicles, respectively, confer zinc resistance by ensuring zinc efflux from the cytoplasm (Palmiter & Findley 1995, Palmiter *et al*. 1996a). ZnT-3 and ZnT-4, cloned from human, also localized in intracellular vesicles, but are more dedicated to secretion pathways (Palmiter *et al*. 1996b, Murgia *et al*. 1999). ZnT-3 is tissue specific and mainly located in brain, in the membranes of zinc-rich synaptic vesicles within mossy fiber boutons of hippocampus (Wenzel *et al*. 1997) and in testis (Palmiter *et al*. 1996b). Conversely, ZnT-4 is largely expressed (Liuzzi *et al*. 2001). However, higher levels of ZnT-4 were found in brain and epithelial cells, and this transporter has been shown to be essential in mammary epithelia for regulating milk zinc content (Huang & Gitschier 1997). More recently ZnT-4 has been shown to have a role in mast cell zinc homeostasis (Ho *et al*. 2004). ZnT-5 is a zinc transporter mainly localized in the Golgi apparatus and ubiquitously expressed at higher levels in pancreas, liver, kidney, muscle and heart (Kambe *et al*. 2002). ZnT-5 null mice have been shown to suffer from osteopenia and male-specific sudden cardiac death, suggesting that ZnT-5 protein plays an important role in maintenance of the cells involved in the cardiac conduction system (Inoue *et al*. 2002). Two other transporters, ZnT-6 and -7, described very recently, are expressed in various tissues and both localized to the Golgi apparatus (Huang *et al*. 2002, Kirschke & Huang 2003). The intracellular distribution of ZnT-6 and ZnT-4 has been shown to be regulated by zinc, while exposure of ZnT-7-expressing cells to zinc causes an accumulation of zinc in the Golgi apparatus. More recently, ZnT-5 and -7 have been shown to be needed for the activation of Glycosylphosphatidylinositol (GPI)-anchored zinc-requiring enzymes (Suzuki *et al*. 2005). Few information is available for ZnT-9, also described as HUEL (Human Embryonic Lung cDNA, or *C4orf1*), which displays nuclear translocation in a cell cycle-dependent manner (Sim del *et al*. 2002) and ZnT-10 (Seve *et al*. 2004). The role of ZnT-8 will be described below.

Zinc and insulin secretion

Insulin is generally believed to be stored inside secretory vesicles as a solid hexamer bound with two Zn^{2+} ions per hexamer (Emdin *et al*. 1980, Gold & Grodsky 1984, Dodson & Steiner 1998). Insulin, produced and stored in pancreatic beta cells, is released by exocytosis in response to external stimuli, such as elevated glucose concentration. When exocytosis of insulin occurs, insulin granules fuse with the beta cell plasma membrane and release their content, insulin as well as zinc into the circulation (Qian *et al*. 2000). Moreover, there appears to be a complex relationship between zinc and both type 1 and type 2 diabetes, since several complications of diabetes may be mediated through oxidative stress, amplified in part by zinc deficiency (Chausmer 1998). Hence, zinc is an important component of insulin storage and secretion mechanisms. Free zinc will also be produced in the islet when the insulin crystals are dissolved on exocytosis of β-cells. Therefore zinc is also likely to modulate the function of neighbouring cells via paracrine/autocrine interactions. A recent report indicates that zinc released from activated insulin-secreting beta cells is implied in the suppression of glucagon-secreting activity of neighbouring alpha cells (Ishihara *et al*. 2003). Hence beta cells undoubtedly need very efficient and specialized transporters to accumulate sufficient amounts of zinc in secretion vesicles.

A new zinc transporter bringing zinc to insulin vesicles

We recently identified and cloned a pancreatic specific zinc transporter belonging to the SLC30 protein family, named ZnT-8, and localized in insulin secretory granules (Chimienti *et al*. 2004). This new zinc transporter (Genbank accession number AY117411) may be a major component for providing zinc to insulin maturation and/or storage processes in insulin-secreting pancreatic beta cells. ZnT-8, coded by the gene *SLC30A8* (chromosome 8q24.11), is a 369 amino acids protein. ZnT-8 was predicted to adopt the same topology as the other ZnT proteins with 6 transmembrane helixes. In addition, the predicted sequence contains a histidine-rich domain between the fourth and the fifth helix. ZnT-8 sequence is closely related to ZnT-2, ZnT-3, and ZnT-4 (Seve *et al*. 2004), all of which have already been involved in secretory/synaptic vesicles transport and lysosomal/endosomal zinc storage in different cells. We demonstrated that *SLC30A8*

expression could be detected only from pancreas cDNA sample. We also found an expression in the insulin-secreting INS-1 cell lines, human fetal pancreas and human Langerhans islets. As demonstrated by confocal immunofluorescence microscopy assay, ZnT-8 was co-localized with the insulin granules secretory pathway Altogether these data suggest an important role for ZnT-8 in insulin secretion/synthesis (Chimienti *et al*. 2004).

Mammals' orthologs

After identification and cloning of the human ZnT-8 sequence (NP_776250), we searched for orthologs in other mammal species in the available databases. We found proteins similar to human ZnT-8 in rat, mouse (NP_766404), chimpanzee (XP_519918), and dog. All proteins have the same genomic organisation, with 8 exons and 7 introns. These mammalian ZnT-8 protein sequences were aligned using the ClustalW program (Thompson *et al*. 1994). All proteins share 98% conserved amino acids and 70% identical residues (Figure 1a), reflecting a high degree of sequence homology. Therefore ZnT-8 is highly conserved during evolution, suggesting a central role for zinc transport within pancreatic beta cells. Using the amino acids alignment, a phylogenetic tree for the mammalian ZnT-8 sequences was calculated by the neighbour-joining method (Figure 1b). Bootstrap analysis is based on multiple re-sampling of the original data and is the most common method of estimating the degree of confidence in the topology of phylogenetic trees (Xia & Xie 2001). From this analysis, as expected, we can delineate two subgroups: human, chimp and dog ZnT-8 are closely related, specially for the human and chimpanzee proteins, whereas rodents ZnT-8 (mouse and rat) are much more related to each other than to other species.

Role of ZnT-8 in beta cell physiology

Zinc is known to play physiologically a key role in the synthesis and action of insulin, and its homeostasis is modified in the pathogenic state of diabetes (for review see (Chausmer 1998)). Zinc is required for insulin synthesis and storage (Emdin *et al*.

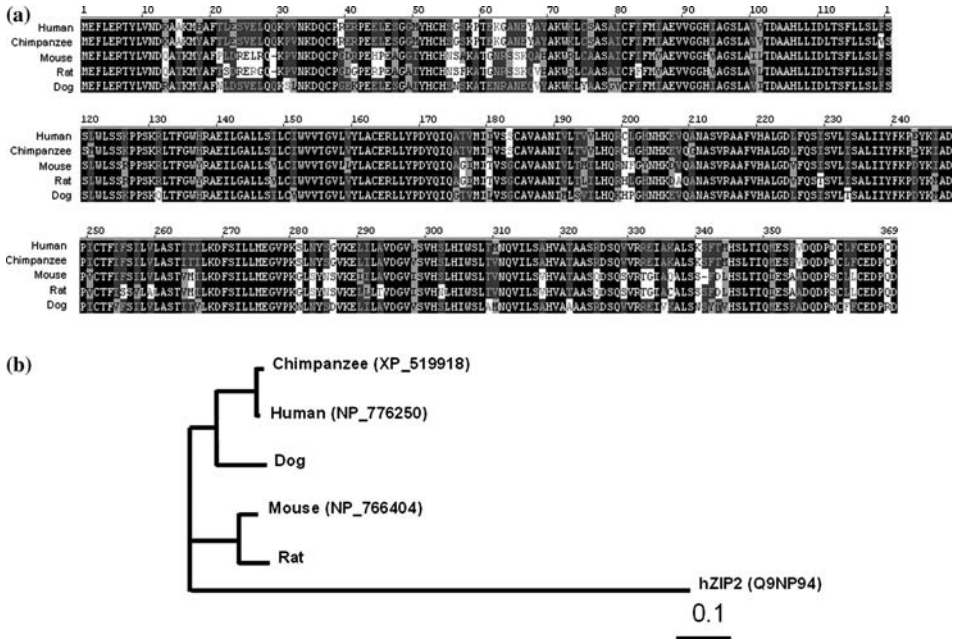

Figure 1. Alignment and phylogeny of ZnT-8 ortholog proteins. (a) Amino acid alignment of ZnT-8 protein sequences identified in *Homo sapiens* (human, NP_776250), *Pan troglotydes* (chimpanzee, XP_519918), *Mus musculus* (mouse, NP_766404), *Rattus norvegi*cus (rat) and *Canis familiaris* (dog) were performed with ClustalW program. Black boxes correspond to conserved residues in all the sequences while grey and white boxes correspond to partially conserved residues. (b) For phylogenetic analysis ZnT-8 protein sequences were aligned using the clustalW program. Bootstrapping (2000 replicate sets) and calculation of the consensus tree were done with the DAMBE program. The scale of the branch length is given in amino acid substitutions per site.

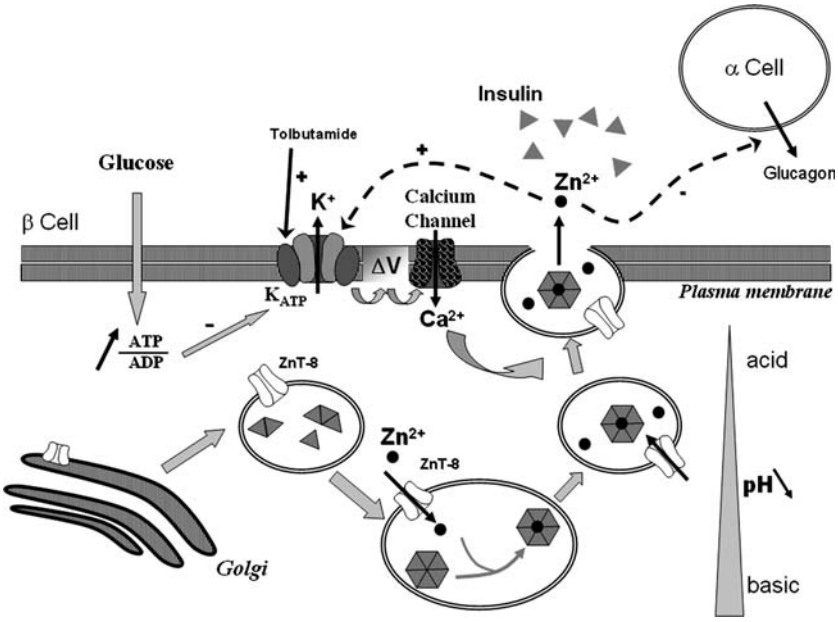

Figure 2. Mechanism of insulin secretion and hypothetic role of ZnT-8 and zinc ions in the pancreatic beta cell. Accumulation of zinc in vesicles occurs through the whole process of insulin maturation, storage and secretion. Zinc insulin is stored inside secretory vesicles as a solid hexamer bound with two Zn^{2+} ions per hexamer. Zinc is also an important component of insulin secretion mechanisms through an autocrine action and modulates the function of neighbouring cells via paracrine interactions.

1980), and thus large amounts of zinc are released with insulin after glucose stimulation (Gold & Grodsky 1984, Qian *et al*. 2000). During insulin synthesis, we propose that the presence of ZnT-8 in insulin vesicles is necessary to allow zinc to be incorporated within these vesicles and to facilitate the formation of zinc-insulin solid hexamers. Additionally, considering the anatomical structure of the Langerhans islets, it is likely that the beta cells also modulate the function of neighbouring cells via paracrine/autocrine interactions (Ishihara *et al*. 2003). There is precedence for such a paracrine role of zinc from work in the central nervous system, where it has been shown that synaptically released zinc functions as a conventional synaptic neurotransmitter or neuromodulator (Frederickson & Bush 2001). Moreover, a specific zinc transporter, ZnT-3, has been described in these secretory neurons (Palmiter *et al*. 1996b). Thus, zinc is of prime importance for both insulin synthesis/secretion by beta cells and islet cells paracrine/autocrine communication.

Typically, insulin is secreted from beta cells after glucose stimulation (Figure 2). Glucose-induced increase of ATP/ADP ratio will then block ATP-dependent potassium (KATP) channels, thereby provoking plasma membrane depolarization (Tarasov *et al*. 2004). Therefore, voltage-sensitive calcium channels open. Influx of $Ca^{(2+)}$, and thus increase in the $[Ca^{(2+)}]_i$ is the main trigger for fusion of insulin vesicles to the plasma membrane and thus insulin secretion. Recent works demonstrate that zinc regulates this pathway at many levels, i.e. on KATP channels (Bancila *et al*. 2004), in insulin synthesis and storage (Dodson & Steiner 1998) and at the alpha cell level (Ishihara *et al*. 2003).

Conclusion

In conclusion, zinc has a significant role in all processes of insulin trafficking, i.e. synthesis, storage and secretion, and is also important in Langerhans islet cells communication. Additionally, the hypozincemia frequently associated with diabetes can have deleterious effects for pancreatic beta cells (Chausmer 1998). We describe here a Langerhans islets-specific zinc transporter expressed in beta cells, which facilitates accumulation of zinc from the cytoplasm into intracellular vesicles. Thus, ZnT-8 may be a major component for providing zinc to insulin maturation and/or storage processes in insulin-secreting pancreatic

beta cells. However further work is needed to clarify the exact molecular mechanism by which ZnT-8 acts in beta cells.

Acknowledgements

This work has been supported by a grant from the Programme de Toxicologie Nucléaire Environnementale (http://www.toxnuc-e.org).

References

Bancila V, Cens T, Monnier D, *et al*. 2004 Two SUR1-specific histidine residues mandatory for zinc-induced activation of the rat KATP channel. *J Biol Chem*, published online ahead of print December 21, 2004.

Chausmer AB. 1998 Zinc, insulin and diabetes. *J Am Coll Nutr* **17**, 109–115.

Chimienti F, Aouffen M, Favier A, Seve M. 2003 Zinc homeostasis-regulating proteins: new drug targets for triggering cell fate. *Curr Drug Targets* **4**, 323–338.

Chimienti F, Devergnas S, Favier A, Seve M. 2004 Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. *Diabetes* **53**, 2330–2337.

Dodson G, Steiner D. 1998 The role of assembly in insulin's biosynthesis. *Curr Opin Struct Biol* **8**, 189–194.

Eide DJ. 2004 The SLC39 family of metal ion transporters. *Pflugers Arch* **447**, 796–800.

Emdin SO, Dodson GG, Cutfield JM, Cutfield SM. 1980 Role of zinc in insulin biosynthesis. Some possible zinc-insulin interactions in the pancreatic B-cell. *Diabetologia* **19**, 174–182.

Frederickson CJ, Bush AI. 2001 Synaptically released zinc: physiological functions and pathological effects. *Biometals* **14**, 353–366.

Gold G, Grodsky GM. 1984 Kinetic aspects of compartmental storage and secretion of insulin and zinc. *Experientia* **40**, 1105–1114.

Ho LH, Ruffin RE, Murgia C, *et al*. 2004 Labile zinc and zinc transporter ZnT4 in mast cell granules: role in regulation of caspase activation and NF-kappaB translocation. *J Immunol* **172**, 7750–7760.

Huang L, Gitschier J. 1997 A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nat Genet* **17**, 292–297.

Huang L, Kirschke CP, Gitschier J. 2002 Functional characterization of a novel mammalian zinc transporter, ZnT6. *J Biol Chem* **277**, 26389–26395.

Inoue K, Matsuda K, Itoh M, *et al*. 2002 Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene, Znt5. *Hum Mol Genet* **11**, 1775–1784.

Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB. 2003 Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. *Nat Cell Biol* **5**, 330–335.

Kambe T, Narita H, Yamaguchi-Iwai Y, *et al*. 2002 Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic beta cells. *J Biol Chem* **277**, 19049–19055.

Kirschke CP, Huang L. 2003 ZnT7, a novel mammalian zinc transporter, accumulates zinc in the Golgi apparatus. *J Biol Chem* **278**, 4096–4102.

Liuzzi JP, Blanchard RK, Cousins RJ. 2001 Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. *J Nutr* **131**, 46–52.

Liuzzi JP, Cousins RJ. 2004 Mammalian zinc transporters. *Annu Rev Nutr* **24**, 151–172.

Maret W. 2003 Cellular zinc and redox states converge in the metallothionein/thionein pair. *J Nutr* **133**, 1460S–1462S.

Murgia C, Vespignani I, Cerase J, Nobili F, Perozzi G. 1999 Cloning, expression, and vesicular localization of zinc transporter Dri 27/ZnT4 in intestinal tissue and cells. *Am J Physiol* **277**, G1231–G1239.

Palmiter RD, Cole TB, Findley SD. 1996 ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *Embo J* **15**, 1784–1791.

Palmiter RD, Cole TB, Quaife CJ, Findley SD. 1996 ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc Natl Acad Sci USA* **93**, 14934–14939.

Palmiter RD, Findley SD. 1995 Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *Embo J* **14**, 639–649.

Qian WJ, Aspinwall CA, Battiste MA, Kennedy RT. 2000 Detection of secretion from single pancreatic beta-cells using extracellular fluorogenic reactions and confocal fluorescence microscopy. *Anal Chem* **72**, 711–717.

Seve M, Chimienti F, Devergnas S, Favier A. 2004 In silico identification and expression of SLC30 family genes: an expressed sequence tag data mining strategy for the characterization of zinc transporters' tissue expression. *BMC Genomics* **5**, 32.

Sim del LC, Yeo WM, Chow VT. 2002 The novel human HUEL (C4orf1) protein shares homology with the DNA-binding domain of the XPA DNA repair protein and displays nuclear translocation in a cell cycle-dependent manner. *Int J Biochem Cell Biol* **34**, 487–504.

Suzuki T, Ishihara K, Migaki H *et al*. 2005 Zinc transporters, ZnT5 and ZnT7, are required for the activation of alkaline phosphatases, zinc-requiring enzymes that are GPI-anchored to cytoplasmic membrane. *J Biol Chem* **280**, 637–643.

Tarasov A, Dusonchet J, Ashcroft F. 2004 Metabolic regulation of the pancreatic beta-cell ATP-sensitive K + channel: a pas de deux. *Diabetes* **53**(Suppl 3), S113–S122.

Thompson JD, Higgins DG, Gibson TJ. 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Vallee BL, Falchuk KH. 1993 The biochemical basis of zinc physiology. *Physiol Rev* **73**, 79–118.

Wenzel HJ, Cole TB, Born DE, Schwartzkroin PA, Palmiter RD. 1997 Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. *Proc Natl Acad Sci USA* **94**, 12676–12681.

Xia X, Xie Z. 2001 DAMBE: software package for data analysis in molecular biology and evolution. *J Hered* **92**, 371–373.

Zalewski PD, Millard SH, Forbes IJ, *et al*. 1994 Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. *J Histochem Cytochem* **42**, 877–884.