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ZnT3: a zinc transporter active in several organs

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Abstract The review collects the emerging information about zinc transporter 3 (ZnT3). ZnT3 has been associated with Alzheimer's disease, airway diseases and diabetes. ZnT3 was discovered and cloned in 1996. Since then, the major interest in the protein has been in its ability to transport zinc into pre-synaptic vesicles of glutamatergic neurones and its role during the development of amyloid β plaques in Alzheimer's disease. Increasing evidence suggests that ZnT3 is present in various cell types like different cell types in the brain, cells from adipose tissue, beta-cells from pancreatic islets, epithelial cells, cells from testis, prostate cancer cells and cells from retina. The expression of ZnT3 is regulated by age, hormones, fatty acids, zinc chelation, and glucose.

Keywords Slc30A3 · ZnT3 · Zinc transport · Apoptosis · Alzheimer's disease · Diabetes

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Introduction

Zinc is an essential metal present in all biological tissue. It plays a fundamental role in physiology, both for cellular metabolism and for the regulation of the expression of numerous genes. Zinc is a co-factor or component of more than 300 metalloenzymes and is utilized for catalysing reactions or stabilising protein structures. Zinc is redox-stable due to a filled d-orbital hence the zinc ion is a perfect metal cofactor for reactions that require a redox-stable ion (Butler 1998; McCall et al. 2000). The human body contains 2–3 g zinc, and nearly 90% is found in muscle and bone (Wastney et al. 1986). Besides that, brain, testis and pancreatic islets are rich in zinc. In these organs, zinc appears to be critical for cognitive functions, germ cell maturation, and insulin processing.

Disturbances in zinc metabolism is related to the development of a number of diseases such as asthma, diabetes and Alzheimer's disease causing increased focus on the transport mechanisms for zinc (Devirgiliis et al. 2007). Zinc homeostasis is maintained by two different solute-linked carrier families, Zinc Transporters (ZnT or SLC30A) and ZRT/IRT-related Proteins (ZIP or SLC39A). ZnTs are responsible for the efflux of zinc from the cytoplasm into cellular compartments or to the extracellular matrix. ZIPs transport zinc in the opposite direction. Ten ZnT genes and 14 ZIP genes have been identified in the human genome and zinc transport activity has been demonstrated for 7 ZnTs and 9 ZIPs (Cousins et al. 2006).

ZnT3 characteristics

Znt3 was cloned in 1996 based on a screening of a mouse genomic λ library using rat Znt2 cDNA as a probe. The rat-Znt3 amino acid sequence shares a 52% homology with the mouse-Znt2 (Palmiter et al. 1996). The human-ZnT3 protein has 388 amino acids and shares 86 and 87% homologies with rat- and mouse-Znt3, respectively. The gene for human ZnT3 is localized on chromosome 2 p23.3. The protein is predicted to have six transmembrane domains enclosing a pore lined with a histidine-rich loop (Gaither and Eide 2001) (Fig. 1). Based on studies of the zinc transporter in yeast the transport mechanism of ZnT3 is suggested to function via an electrogenic antiport, exchanging one hydrogen ion for one zinc ion (MacDiarmid et al. 2002; Kambe et al. 2004). According to the SignalP software program, the ZnT3 peptide does not contain any localization signal peptides (Bendtsen et al. 2004). It was recently found that the intracellular localization of ZnT3 depends of the redox status of the cell. Oxidative stress induces dimerization of Znt3 and increases the subcellular targeting of Znt3 to the synaptic-like microvesicles (SLMV) in the endocrine tumour cell line originating from rat adrenal medulla, PC12 cells. This homo-oligomerization mechanism depends on a redox-regulated covalent tyrosine dimerization of Y357 and Y372 (Salazar et al. 2009). Dityrosine bond formation increases with aging, cellular stress, UV-irradiation and diseases e.q. atherosclerosis (Garcia-Castineiras et al. 1978; Giulivi and Davies 1994; Heinecke 2002). In addition, dityrosine bonds have been associated with the oligomerization of amyloid β in Alzheimer's disease (Atwood et al. 2004). Interestingly, according to National Center for Biotechnology Information, NCBI, (www.NCBI.nlm.nih.gov) ZnT3 have three SNPs. One of these SNPs is the negatively charged Y372 which has the ambiguity code R372. This SNP could hypothetically reduce the dimerization of Znt3 and thereby obstruct the SLMV localization and zinc transporting function of Znt3 during oxidative stress conditions. A minor change, Y372F, has proven to impair dimerization, targeting to SLMV, zinc transport and cell viability (Salazar et al. 2009).

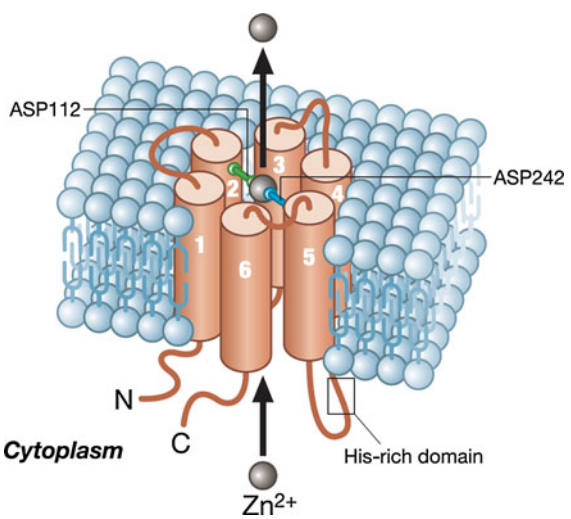

Fig. 1 The topology of ZnT3. Modified from (Eide 2006; Kambe et al. 2004). ZnT3 has six transmembrane domains (TMs) with a cytoplasmic histidine-rich loop between TM4 and TM5. The highly conserved TM2 and TM5 are the binding sites for zinc in the bacterial form of ZnTs (YiiP) (Wei and Fu 2006)

ZnT3 in the brain

The brain has been considered the primary localization for ZnT3. Several tissues from different organs from mice (intestine, kidney, liver, spleen, seminal vesicles, testis, submaxillary glands, tongue and brain) have been investigated for the presence of Znt3 RNA. The presence of Znt3 RNA investigated by Palmiter et al. was restricted to tissues from brain and testis (Palmiter et al. 1996), and by electron microscopy Znt3 was found at the membranes of glutamatergic and tyrosin-hydroxilase positive synaptic vesicles in the zinc-enriched terminals in neural tissue (Wenzel et al. 1997; Wang et al. 2003). Znt3 expression has also been detected in the epithelial cells of the choroid plexus (Wang et al. 2005), the Bergman glial cells of mouse cerebellar cortex (Wang et al. 2004) and the postganglionic neurones of mouse superior cervical ganglia (Wang et al. 2003). The zinc transporting capacity of Znt3 was investigated in Znt3 knock-out mice which turned out to have diminished zinc content in the hippocampus and cortex when compared to wild type mice. ZnT3s zinc transporting mechanism in vesicles was thought to be active only in neurones since other cell types like pancreatic beta-cells and seminiferous tubule cells from the testis maintained their reactive zinc content in Znt3 knock-out mice (Cole et al. 1999).

ZnT3 in different cell types

Recent studies have challenged the hypothesis that ZnT3 is exclusively located in brain and testes (Table 1). In female rats, the *Znt3* transcript is down-regulated in duodenal epithelial cells as a response to chronic metabolic acidosis (Wongdee et al. 2009). In addition, human airway epithelial cells express Znt3 (Ackland et al. 2007), and Znt3 is present in pancreatic islets from mice. Despite a modest gene expression, Znt3 protein is detectable in the islets, most likely in the beta-cells since Znt3 mRNA and protein has been detected in the pancreatic beta-cell line INS-1E but not in a corresponding pancreatic alpha-cell line, α-TC6 (Gyulkhandanyan et al. 2008; Smidt et al. 2009). Znt3 is present in adipose tissue where the expression of Znt3 is down-regulated in visceral fat from lean persons compared to subcutaneous fat, and the transcription of Znt3 is diminished in subcutaneous fat from obese persons compared to subcutaneous fat from lean persons (Smidt et al. 2007). Finally, Znt3 protein has been detected in the light-adapting part of the mouse retina (Redenti and Chappell 2004).

Interaction with other proteins

The interaction between ZnT3 and other proteins is a new important field in the characterization of ZnT3s function. In PC12 neuroendocrine tumour cells from the rat adrenal medulla, Znt3 is targeted to distinct synaptic vesicles by Adaptor Protein-3 (AP-3) (Salazar et al. 2004) in a complex including other proteins e.g. phosphatidylinolistol-4-Kinase Type II α (Salazar et al. 2005). Phosphatidylinolistol-4-Kinase Type II α regulates AP-3 since knock-down of phosphatidylinolistol-4-Kinase Type II α reduces the presence of AP-3 in PC12 cells (Salazar et al. 2005). In pancreatic beta-cells phosphatidylinositol 4-kinases serve as metabolic sensors and regulate priming of secretory granules (Olsen et al. 2003) but interactions with Znt3 or AP-3 have yet not been established. Another connection seems to exist between *Znt3* and *Znt8* gene expression in INS-1E cells since conditions leading to an up-regulation of *Znt3* expression (high glucose concentration or DEDTC treatment) cause a down-regulation of *Znt8* expression (Smidt et al. 2009). Specifically, knock-down of ZnT3 leads to an up-regulation of ZnT8 expression and vice versa (Petersen et al. 2011).

ZnT3 and age

Notably, the age of the animal is an important factor when scanning tissue for *Znt3* gene expression. In isolated pancreatic islet from Sprangue–Dawley and Wistar BBDR rats the gene expression of *Znt3* varies

Table 1 The presence of ZnT3 mRNA or protein in various cell types

Cell type		Reference
Brain: Glutamatergic synaptic vesicles	RNA and protein	(Palmiter et al. 1996; Wenzel et al. 1997; Wang et al. 2003)
Brain: Chorid plexus; epithelial cells	Protein	(Wang et al. 2004)
Brain: Cerebellar cortex; Bergman glial cells	Protein	(Wang et al. 2005)
Brain: Cervical ganglia; postganglionic neurons	Protein	(Wang et al. 2003)
Brain: Neuroblastoma	RNA and protein	(Suphioglu et al. 2010)
Retina	Protein	(Redenti and Chappell 2004)
Pancreatic islet	RNA and protein	(Smidt et al. 2009)
Pancreatic beta cell	RNA and protein	(Smidt et al. 2009)
Testis	RNA	(Palmiter et al. 1996)
Duodenal epithelial	RNA	(Wongdee et al. 2009)
Airway epithelial	RNA	(Ackland et al. 2007)
Adipose tissue	RNA	(Smidt et al. 2007)
Prostate cancer cell	RNA	(Iguchi et al. 2004)

with age revealing a moderate gene expression at 10 days of age but a low gene expression at 5 weeks of age. In puppies at day 3 and in adult rats *Znt3* gene expression was not detectable in the islets (Clifford and MacDonald 2000). Likewise, *Znt3* is not expressed in isolated pancreatic islets from CD1 female mice at 10–12 week of age (Bellomo et al. 2011). Other studies have investigated the expression of Znt3 in isolated pancreatic islets. Wijesekara et al. found no Znt3 gene expression whereas Gyulkandanyan et al. detected a minor *Znt3* gene expression in islets isolated from mice. The ages of the mice tested by Wijesekara et al. and Gyulkandanyan et al. were not outlined in the text but due to the findings by Clifford and MacDonald differences in age could be a reasonable explanation to the inconsistency of the *Znt3* gene expression in these two studies. (Wijesekara et al. 2009; Gyulkhandanyan et al. 2008; MacDonald et al. 2007). In addition, an age-dependant down-regulation of ZnT3 protein levels is found in the cerebral cortex from mice and humans and with a further decline in ZnT3 protein level in people suffering from Alzheimer's disease (Adlard et al. 2010).

Gonadal steroids

A number of studies have focused on the regulatory effect of gonadal steroids on ZnT3 expression. Androgens negatively regulate Znt3 expression as illustrated by comparing the gene expression of Znt3 from the human androgen-responsive LNCaP cells, a prostate cancer cell line, with the Znt3 gene expression from the LNCaP-derived androgen-independent AIDL cells. The androgen-mediated down-regulation of Znt3 expression was further found by stimulating LNCaP with a synthetic androgen (R1881/metyltrienolone) (Iguchi et al. 2004). In female mice undergoing ovariectomy the Znt3 protein level increased in the brain whereas estrogen supplementation reduced the Znt3 protein level. These changes correlate with transcriptional changes of the δ subunit of AP-3 which are proposed to modulate the levels of *Znt3* (Lee et al. 2004). Sex differences in the development of Alzheimer's disease have been described in transgenic mice expressing human amyloid protein precursor (hAPP). These mice were crossed with mice lacking or harbouring Znt3 (hAPP+ :Znt3−/−, hAPP+ :Znt3+/+, respectively). The studies show, that female hAPP+ :Znt3+/+ mice have higher levels of synaptic zinc, insoluble amyloid β and plaques than their male counterparts; this sex difference is not seen in hAPP+ :Znt3−/− mice (Lee et al. 2002).

Regulation of ZnT3 by nutrients and ambient zinc level

Nutrients and ambient zinc levels may influence the expression of *Znt3*. The omega-3 fatty acid, alpha-linolenic acid (ALA) an essential nutrient that is not synthesized by mammals, down-regulates the gene expression of Znt3 and the synthesis of ZnT3 protein in the human neuroblastoma cell line M17 (Suphioglu et al. 2010). Chelating intracellular zinc with TPEN for 7 days has no effect on the protein level of Znt3 in mouse brain but reduces the level of labile zinc ions with up to 35% (Cho et al. 2010). In the pancreatic beta-cell line, INS-1E, intracellular zinc chelation leads to an up-regulation of *Znt3* transcription as do high glucose concentrations (16 mM) compared to low glucose concentration (5 mM) (Smidt et al. 2009). Due to the different experimental conditions in these investigations it is difficult compare these studies and they do not clearly elucidate to what extent zinc chelation affects the expression of *Znt3*.

ZnT3 and cell death

Zinc has an ambiguous role in the regulation of apoptosis since zinc can be either pro- or anti-apoptotic depending on its cellular concentration. Both zinc deprivation and excess can induce apoptosis in the same cell line (Haase et al. 2001; Watjen et al. 2002; Formigari et al. 2007; Cummings and Kovacic 2009; Plum et al. 2010). The anti-apoptotic effect of zinc may be conducted by a decrease in the Bax/Bcl-2 ratio and an inhibition of caspase-3, 6, 7, and 8 by zinc. On the contrary, the pro-apoptotic effect of zinc includes the enhancement of the cellular level of Bax and an increase in the translocation of Bax to mitochondria with an elevated release of cytochrome-c from mitochondria as a consequence (Plum et al. 2010; Feng et al. 2008). The expression of zinc transporters are influenced by the

zinc concentration (Devergnas et al. 2004) and this influence can either reinforce the deleterious effect or counteract the harmful effect caused by changes in the zinc concentration.

Down-regulation of ZnT3 by the omega-3 fatty acid ALA leads to a decrease of zinc in synaptic vesicles and a decreased apoptosis as measured by a decrease in the Caspase-3 protein level in a human neuroblastoma cell line, M17 (Suphioglu et al. 2010). Furthermore, ZnT3 is suggested to play a role in the regulation of apoptosis in the human airway epithelial cell line, A549, possibly by controlling zinc fluxes. Znt3 gene expression is seven fold up-regulated by petroleum diesel emissions particulate matter (PBEP), a substance causing activation of Caspase-3 and thereby apoptosis (Ackland et al. 2007). In INS-1E cells, *Znt3* gene expression is up-regulated by hyperglycemia or zinc chelation induced by DEDTC which in turn leads to an increased cell death as measured by DNA fragmentation and an increase in the gene expression ratio of Bax/Bcl-2 (Smidt et al. 2009). By contrast, specific knock-down of *Znt3* in INS-1E by siRNA transfection leads to an increase in apoptosis as measured by DNA fragmentation (Petersen et al. 2011). A possible protecting role of ZnT3 is demonstrated in mice exposed to a traumatic brain injury where Znt3 knock-out mice appear more vulnerable and reveal a significant higher number of apoptotic neurones compared to control mice (Doering et al. 2010).

ZnT3 and Alzheimer's disease

The pathology of Alzheimer's disease is characterised by the accumulation of amyloid fibrils known as amyloid β in the neuronal cortex and hippocampus and by tau hyperphosphorylation leading to neurofibrillary tangles. Presynaptic glutamatergic vesicles contain high levels of zinc which is imported by ZnT3 localized in the vesicles. Under normal conditions, zinc acts as a co-transmitter on postsynaptic receptors such as the glutamate receptor, NMDAR, the neurotrophic tyrosine kinase receptor type 2, TrkB and the zinc-sensing receptor ZnR (Fig. 2) (Sensi et al. 2009). In Alzheimer's disease, it has been suggested that an abnormal regulation of free zinc levels is involved in the aggregation of amyloid β leading to clusters of amyloid plaques (Selkoe

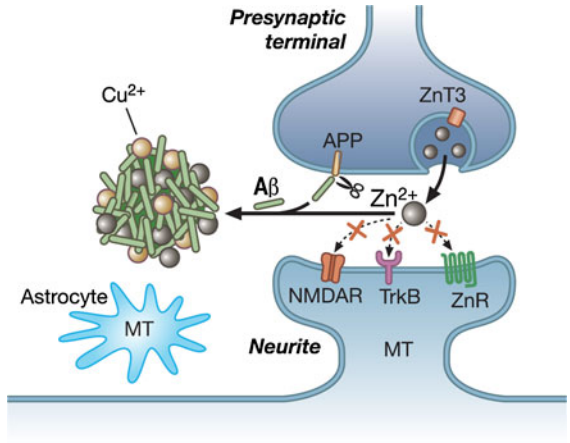

Fig. 2 Proposed role for zinc in amyloid plaque formation. Presynaptic glutamatergic vesicles contain ZnT3 which imports zinc into the synaptic vesicles. Along with glutamate zinc ions are released. Zinc ions react with the glutamate receptor, NMDAR, the neurotrophic tyrosine kinase receptor type 2, TrkB, and the zinc-sensing receptor ZnR. Metallothionein, MT, present in astrocytes and in postsynaptic neurones, binds zinc in a reversible manner. Amyloid precursor protein, APP, is synthesised and processed upon normal neuronal activity leading to soluble amyloid β. Post-synaptically released zinc and copper react with amyloid β which leads to the formation of insoluble amyloid plaques. Adapted from (Sensi et al. 2009)

1998; Walsh et al. 2000; Askanas and Engel 2001; Cherny et al. 2001). This abnormal regulation of zinc is suggested to occur in two phases. First, an inhibition of zinc uptake postsynaptically leads to alterations in zinc homeostasis. The related increase in presynaptic zinc leads to enhanced oligomerization and a concomitant insolubility of amyloid β (Sensi et al. 2009). Second, the dysregulation of zinc homeostasis is caused by an inhibition of the zinc export leading to elevated levels of intracellular zinc. For a full review see (Sensi et al. 2009). Recently, Deshpande et al. demonstrated that vesicular released zinc is critical for a synaptic localization of amyloid β since Znt3 knock-out mice had reduced synaptic localization of amyloid β oligomerization compared to wild-type mice (Deshpande et al. 2009). These finding correlates with a study performed by Lee et al. in 2002 in which a brain-protecting mechanism by Znt3 knock-out was demonstrated in Znt3 knock-out mice. These mice had significantly fewer and smaller plaques and lower zinc concentrations in the hippocampus compared to control mice (Lee et al. 2002). Zhang et al. showed that post-mortem tissues

of cerebral cortex from patients suffering from Alzheimer's disease had abundant expression of ZnT3 in the zinc containing plaques and amyloid-angiopathy diseased vessels (Zhang et al. 2008). In addition, Znt3 protein is predominantly located and widely distributed in the plaques in the cerebellum of the amyloid β protein precursor (APP)/presenilin 1 (PS1) transgenic mouse (Zheng et al. 2010). These findings may support the theory that ZnT3 is involved in the formation of senile plaques and cerebral amyloid angiopathy. On the contrary, another study demonstrated that in human post mortem brain tissue Znt3 transcription is 45–60% down-regulated in cortical regions of people with diagnosed Alzheimer's disease (Beyer et al. 2009). This result is supported by the findings from Adlard et al. who found a decline in the ZnT3 level of people suffering from Alzheimer's disease compared to controls (Adlard et al. 2010). In addition, Znt3 knock-out mice had significantly impaired cognitive functions at the age of 6 months but not at 3 months leading to the final conclusion that the phenotype of Znt3 knock-out mice emerges with aging (Adlard et al. 2010).

ZnT3 and diabetes

Human ZnT3 protein shares a 44% homology with ZnT8, a zinc transporter which is essential for insulin crystallization in pancreatic beta-cells (Kirchhoff et al. 2008; Lemaire et al. 2009; Nicolson et al. 2009). The role of ZnT3 in the pathophysiology of diabetes was investigated recently in a study in which Znt3 knock-out mice and control mice were treated with streptozotocin. The results of this study revealed that Znt3 knock-out mice had impaired glucose metabolism compared to control mice during conditions of severe beta-cell stress (Smidt et al. 2009). Furthermore, siRNA knock-down of *Znt3* lead to a decrease in insulin secretion from INS-1E cells (Smidt et al. 2009; Petersen et al. 2011). It has been hypothesised that zinc may, as in the CNS, catalyse the aggregation of amyloid-like fibrils produced from islet amyloid polypeptide (IAPP) and that insulin may counteract this by inhibiting the formation of amyloid-like fibrils (Westermark et al. 1996). Consequently, zinc homeostasis in beta-cells may have consequences for the development of amyloidosis in beta-cells. The effect of ZnT3 on the formation of amyloid β in beta-cells needs to be clarified, but it may be possible that the link between ZnT3 and amyloid-related pathologies such as type 2 diabetes is present in beta-cells as well as in the hippocampus, cerebellum and other regions of the brain (Zheng et al. 2010; Stoltenberg et al. 2007).

Conclusion

It seems clear that ZnT3 is expressed in various cell types including cells outside the brain. The expression of ZnT3 is age-dependant at least in the cerebral cortex and in pancreatic islets. Furthermore, ZnT3 expression is regulated in various cell types by different stimuli. Znt3 expression in adipose tissue is down-regulated by unhealthy conditions such as obesity, whereas apoptosis-mediating zinc chelation, PBEP treatment or hyperglycemia increase the Znt3 transcription level. ZnT3 most likely influences glucose metabolism since knock-out and knock-down of Znt3 deteriorates glucose metabolism in vivo and decrease insulin secretion in vitro. In addition, androgens and estrogens down-regulate *Znt3* expression in prostate and in cerebrum. The effect of estrogen possibly contributes to a higher incidence of women suffering from Alzheimer's disease. In conclusion, the role of ZnT3 in the regulation of zinc homeostasis in various organs and diseases is currently being described and while the link between ZnT3 and development of Alzheimer's disease is suggested by several studies the involvement of ZnT3 in other diseases needs further investigation.

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