



## Expression profiles of zinc transporters in rodent placental models

Nagayoshi Asano<sup>a</sup>, Masuo Kondoh<sup>a,\*</sup>, Chiaki Ebihara<sup>a</sup>, Makiko Fujii<sup>a</sup>,  
Tsuyoshi Nakanishi<sup>b</sup>, Michael J. Soares<sup>c</sup>, Emi Nakashima<sup>d</sup>,  
Keiichi Tanaka<sup>b</sup>, Masao Sato<sup>e</sup>, Yoshiteru Watanabe<sup>a</sup>

<sup>a</sup> Department of Biopharmaceutics and Pharmaceutics, Showa Pharmaceutical University, Tokyo 194-8543, Japan

<sup>b</sup> Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka 565-0871, Japan

<sup>c</sup> Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66103, USA

<sup>d</sup> Department of Pharmaceutics, Kyoritsu College of Pharmacy, Tokyo 105-8512, Japan

<sup>e</sup> Department of Public Health, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

Received 23 April 2004; received in revised form 2 July 2004; accepted 2 July 2004

Available online 21 August 2004

### Abstract

Zinc is a vital metal that is a structural and functional component of many proteins. The precise mechanism of zinc transport in the placenta remains unclear. In this study, we investigated the expression of zinc transporters (ZnTs) in the mouse placenta and in two rat trophoblast cell lines, TR-TBT cells, which are syncytiotrophoblast cells of the labyrinth zone, and Rcho-1 cells, which retain trophoblast cell features and differentiate into trophoblast giant cells of the junctional zone. All of the ZnTs that have been identified in mice (ZnT1–7) were detected in the mouse placenta by RT-PCR. The expression profiles of ZnTs in the placenta during pregnancy were different. The mRNA levels of ZnTs, with the exception of ZnT7, did not change during pregnancy. The ZnT7 mRNA level in placenta was elevated during pregnancy. In TR-TBT cells, ZnT1, ZnT3 and ZnT4 were detected by RT-PCR analysis. In Rcho-1 cells, all of the ZnTs that have been identified in rats (ZnT1–4) were detected by RT-PCR analysis. There were no differences between the mRNA expression levels of ZnT family members in undifferentiated Rcho-1 cells and differentiated Rcho-1 cells. This is the first report of expression profiles of ZnTs during differentiation of the placenta in the mouse placenta and rat placental cell models.

© 2004 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** TR-TBT; Rcho-1; Zinc transporter; Mouse placenta

**Abbreviations:** Zn, Zinc; ZnT, Zn transporter; RT-PCR, Reverse transcription-polymerase chain reaction; PLP-A, Prolactin-like protein A; FBS, Fetal bovine serum; ICP-MS, Inductively coupled plasma-mass spectrometer

\* Corresponding author. Tel.: +81 42 721 1556; fax: +81 42 723 3585.

E-mail address: [masuo@ac.shoyaku.ac.jp](mailto:masuo@ac.shoyaku.ac.jp) (M. Kondoh).

## 1. Introduction

Zinc (Zn) is an essential trace element needed for the normal growth and development of the fetus. Zn is required for the functions of Zn-containing proteins in the regulation of gene expression and protein synthesis, intracellular protein trafficking and immune responses (Wildman and Medeiros, 2000). A deficiency in maternal Zn can cause biochemical and functional abnormalities in the fetus (Beach et al., 1983). Essential nutrients are transported from the maternal body to the fetus across the placenta (Knipp et al., 1999). While transplacental transporters of amino acids, fatty acids and peptides have been characterized (Knipp et al., 1999; Takahashi et al., 2001), Zn transport in the placenta is not fully understood.

The intracellular Zn level is strictly regulated by several mechanisms, including Zn transporters and Zn-binding proteins (Liuzzi et al., 2001). Recent studies have indicated that intracellular Zn homeostasis is regulated by the Zn transporter (ZnT) family proteins (McMahon and Cousins, 1998; Kambe et al., 2002; Huang et al., 2002; Kirschke and Huang, 2003). All ZnT family proteins have six transmembrane domains and a histidine-rich loop between transmembrane domains IV and V, where Zn may be bound by histidines and subsequently transported across the membrane. Seven ZnT family members have been identified. Their expression profiles and functions are different (McMahon and Cousins, 1998; Kambe et al., 2002; Huang et al., 2002; Kirschke and Huang, 2003). ZnT1 is involved in zinc efflux across the plasma membrane and is expressed in most tissues of mice (Liuzzi et al., 2001; Palmiter and Findley, 1995). ZnT2–7 are located on intracellular vesicular membranes and facilitate Zn accumulation in vesicles (Kambe et al., 2002; Huang et al., 2002; Kirschke and Huang, 2003; Cousins and McMahon, 2001). The expression profiles of ZnT2–7 are tissue-specific. ZnT2 is expressed in the small intestine, kidney, placenta and liver (Liuzzi et al., 2001), while ZnT3 is expressed in the brain and testes (Palmiter et al., 1996a,b). ZnT4 is abundantly expressed in the brain and mammary glands (Huang and Gitschier, 1997), and ZnT5 is abundantly expressed in the pancreas (Kambe et al., 2002). ZnT6 is expressed in the liver, kidney, brain and small intestine, and ZnT7 is expressed in the lung and small intestine (Huang et al., 2002; Kirschke and Huang, 2003). A genetic ap-

proach has been used to analyze the functions of some ZnT family members. ZnT3-deficient mice lack Zn within synaptic vesicles of neurons (Cole et al., 1999), and ZnT4 is deficient in the lethal milk mouse (Huang and Gitschier, 1997). Moreover, osteopenia and male-specific sudden cardiac death in mice lacking ZnT5 have been reported (Inoue et al., 2002). Thus, the functions of ZnT family members vary according to tissue type.

The placenta is essential for the maintenance of pregnancy in mammals. Trophoblast cell lineages are principal sources of placental functions, including the secretion of steroid and peptide hormones (Shiota et al., 1991; Soares et al., 1991). Zn might play a pivotal role in the placenta and trophoblasts. Indeed, Zn uptake has been observed in human trophoblast cells and human placental microvillous membrane vesicles (Vargas Zapata et al., 2000; Aslam and McArdle, 1992; Mas and Sarkar, 1991). Recent studies have found that ZnT1, ZnT2 and ZnT4 are expressed in rat placenta (Liuzzi et al., 2001, 2003). However, there have been no previous reports of ZnT family member expression profiles in experimental models of the mouse placenta and trophoblast cell lines. In the rat placenta, there are four types of differentiated trophoblast cells: syncytiotrophoblast cells, glycogen cells, spongiotrophoblast cells and giant cells. Trophoblast giant cells are thought to play a principal role in the endocrinology of the rat placenta (Yamamoto et al., 1994). These different lineages originate from stem cells of trophodermal origin (Gardner and Beddington, 1988). Rat Rcho-1 cells, which were derived from a spontaneous choriocarcinoma, can be induced to differentiate into trophoblast giant cells *in vitro* (Faria and Soares, 1991). Thus, these cells have been widely used for biochemical and genetic analyses of trophoblast cell differentiation (Yamamoto et al., 1994; Hamlin and Soares, 1995; Oda et al., 2001; Ohgane et al., 2002). On the contrary, syncytiotrophoblast cells are generally accepted to be the main type of trophoblast cells regulating the transport of nutrients and waste in the placenta (Knipp et al., 1999). Kitano et al. (2002) established the rat syncytiotrophoblast cell line TR-TBT and showed that TR-TBT is a good model for analysis of the placental transport of nutrients and chemicals.

In the present study, we investigated the expression profiles of ZnT family members in experimental

models of the placenta, including the murine placenta, Rcho-1 cells and TR-TBT cells.

## 2. Materials and methods

### 2.1. Materials

C57BL/6J mice (weighing 30 g) were obtained from Japan Laboratory Animals Inc. (Tokyo, Japan). NCTC-135 medium and TRIzol reagent were purchased from Sigma-Aldrich (St Louis, MO) and Invitrogen (Gaithersburg, MD), respectively. Primers for PCR were obtained from TaKaRa Bio (Kyoto, Japan). All of the reagents used in this study were of research grade.

### 2.2. Animals and experimental protocol

The mice received humane care throughout the experiments according to the guidelines of Showa Pharmaceutical University. Mice were housed in stainless steel cages with a 12 h light/12 h dark cycle at 25 °C and were given ad libitum access to commercial mouse chow (CE-2, Clea Japan, Inc., Japan) and water. Female mice were housed individually with a male mouse, and mating was confirmed by the presence of a copulatory plug. Day 0 of gestation was determined as the day on which the presence of a copulatory plug was observed. On days 10, 14 and 18 of gestation, mice were anesthetized with ether. Then the abdominal cavity was opened, and the fetus was removed from the uterus. Whole blood was obtained through the abdominal aorta, and maternal serum was separated by centrifugation. The placenta was also collected. The fetuses and the placenta were frozen immediately in liquid N<sub>2</sub>, and stored at –80 °C until use. The serum was stored at –20 °C until used for determination of Zn levels.

### 2.3. Cell cultures

Rcho-1 cells were maintained in growth medium (NCTC-135 supplemented with 20% fetal bovine serum (FBS), 26 mM sodium bicarbonate, 1 mM sodium pyruvate, 50 mM 2-mercaptoethanol, 10 mM Hepes) in a 5% CO<sub>2</sub> atmosphere at 37 °C. The cells were routinely maintained in sub-confluent conditions.

In the experiments, the cells were seeded into a culture dish in a sub-confluent condition, and differentiation of Rcho-1 cells was induced by continuous culture with a change of medium every 2 days. Morphology of Rcho-1 cells gradually changed to differentiated giant cells, and prolactin-like protein A (PLP-A), a sign of trophoblast giant cells, was expressed in the cells after 12 days of culture (Faria and Soares, 1991). Thus, cells on days 2 and 12 of culture were used as undifferentiated and differentiated trophoblast cells, respectively. TR-TBT (clone 18d-1) cells were established as described previously (Kitano et al., 2002). TR-TBT cells were cultured in DMEM supplemented with 10% FBS and 15 µg/ml endothelial cell growth factor from bovine brain (bECGF; Boehringer Mannheim, Mannheim, Germany) at 33 °C in a humidified atmosphere of 5% CO<sub>2</sub>.

### 2.4. Measurement of Zn levels

The fetus was homogenized in 50 mM Tris-HCl (pH 8.0), and proteins of the resultant homogenates were digested by treatment with a mixture of acids (HNO<sub>3</sub>:HClO<sub>4</sub>:H<sub>2</sub>SO<sub>4</sub> = 8:2:1 in volume) at 130 °C for 3 h and then by additional incubation in a mixture of acids (HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub> = 5:1 in volume) at 170 °C for 12–18 h. Zn levels in the digested samples and serum were measured using an inductively coupled plasma-mass spectrometer (ICP-MS) (Hewlett Packard 4500). The amounts of Zn were represented as the concentration of Zn per mg of protein. Protein assays were performed using a Bio-Rad protein assay kit.

### 2.5. RT-PCR analysis

Expression of ZnT family was determined by reverse transcription-polymerase chain reaction (RT-PCR). The primers used in this study for mouse placenta are listed in Table 1, and those used for rat trophoblasts are listed in Table 2. Total RNAs were extracted from mouse placenta, Rcho-1 cells and TR-TBT cells with TRIzol reagent, and RT-PCR reactions were performed using a commercially available kit (TaKaRa RNA PCR kit (AMV) according to the manufacturer's protocols (TaKaRa Bio, Kyoto Japan). The PCR products were electrophoresed in a 3% agarose gel and then stained with ethidium bromide. PCR conditions were

Table 1  
Primers for RT-PCR analysis of ZnT expression in placentas of mice

Gene		Sequence (5' to 3')	PCR product length (bp)
ZnT1	Sense primer	CCTGGGCTTCrTCTCTAGATTG	634
	Antisense primer	TGTCTTGAAA GGTTGTTCTG	
ZnT2	Sense primer	TGAGGTGCCTACTTGCTCCT	352
	Antisense primer	TTCCAGCTGCTCCCTGTA	
ZnT3	Sense primer	CCTGGTCTCTGTACTGTCCATCT	300
	Antisense primer	GATCACGAA CAGCTGTGAAGTC	
ZnT4	Sense primer	CCATCCTCATCTACTTCAAGCC	494
	Antisense primer	CAGAGGCAA CATGGT AAGTGAG	
ZnT5	Sense primer	GGAAGCGCCTCAAATCTATGCT	256
	Antisense primer	TACATTCAAAAATGGCTTGGCACA	
ZnT6	Sense primer	GATAGCCTGGCAGTGAGAGCTG	282
	Antisense primer	TACATTCAAAAATGGCTTGGCACA	
ZnT7	Sense primer	CACACCAGGAGAGCACTGAA	365
	Antisense primer	TCAGCCCTCCAACCTGAGACT	
GAPDH <sup>a</sup>	Sense primer	ACCACAGTCCATGCCATCAC	452
	Antisense primer	TCCAC CACCCTGTTGCTGTA	

<sup>a</sup> Used as an internal RNA control.

Table 2  
Primers for RT-PCR analysis of ZnT expression in rat trophoblast cells

Gene		Sequence (5' to 3')	PCR product length (bp)
ZnT1	Sense primer	CCCAGCTTCATACATGCAGGTG	997
	Antisense primer	CCTTGCTCTTCTCCCTATATGCTC	
ZnT2	Sense primer	GAACCTGCCCTCTGTTGAACTG	423
	Antisense primer	AACATGGTGTCCCCTTTGATCTC	
ZnT3	Sense primer	TGACTATCACATTGAGCGGGT	479
	Antisense primer	TGTGCAGAGGCAACGTGGTAA	
ZnT4	Sense primer	GATCGGAGAGCTGTAGGTGGATAC	398
	Antisense primer	TCGGAGAATTGAAGGTA GGGAG	
PLP-A	Sense primer	TCCrGGACAGTCCTCTTGC	410
	Antisense primer	TCTTTATGGCATCCAGGAGC	
GAPDH <sup>a</sup>	Sense primer	TGAAGGTCGGTGTCAACGGATTGGC	983
	Antisense primer	CATGTAGGCCATGAGGTCCACCAC	

<sup>a</sup> Used as an internal RNA control.

as follows: 60 s at 95 °C, 45 s at 56 °C, 45 s at 72 °C for indicated cycles in placenta from mice; rat ZnT1–4, 20 s at 95 °C, 30 s at 59 °C, 40 s at 72 °C for indicated cycles; rat GAPDH 10 min at 95 °C, 1 min at 60 °C, 2 min at 72 °C for indicated cycles; rat PLP-A, 1 min at 94 °C, 1 min at 57 °C, 1 min at 72 °C for indicated cycles.

## 2.6. Statistical analysis

The significant difference was calculated using one-way ANOVA followed by Dunnett's test. The level of significance was set at  $P < 0.05$ .

## 3. Results

### 3.1. Expression of ZnT family members in the mouse placenta

Mice are widely used as a model to analyze Zn metabolism in organisms, and seven members of the ZnT family have been identified in mice (Kirschke and Huang, 2003). Therefore, we used RT-PCR to investigate the expression of ZnT1–7 in the mouse placenta. Representative results of the ZnT mRNA expression pattern in mouse placenta are shown in Fig. 1. All members of the ZnT family were detected in the placenta.

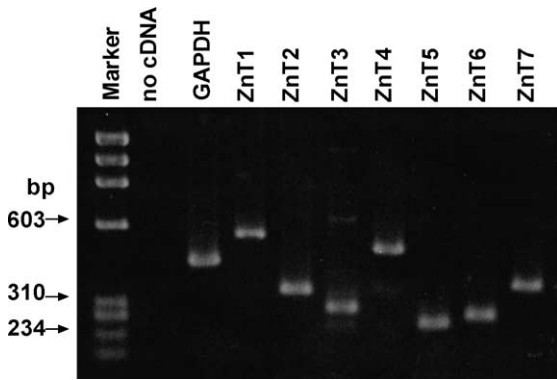


Fig. 1. Expression profiles of Zn transporters in the mouse placenta. Total RNA extracted from the mouse placenta (day 14 of gestation) was reverse-transcribed. The resultant cDNA was amplified by PCR as described in Section 2. There were 20 PCR cycles for GAPDH, 24 cycles for ZnT1, 40 cycles for ZnT2 and ZnT3, 26 cycles for ZnT4–6 and 28 cycles for ZnT7. The PCR products were electrophoresed on 3% agarose gels and visualized by ethidium bromide staining.

However, the detection of ZnT3 required 40 cycles of PCR, while all other ZnT family members could be detected after 30 or less cycles of PCR.

Zn plays an important role in growth, development and reproduction. Zn deficiency during pregnancy and lactation has adverse effects in laboratory animals, causing congenital malformations, embryonic death and fetal death (Hurley, 1980). So, we examined the expression profiles of ZnT mRNA in the placenta during pregnancy (Fig. 2). The expression of all ZnTs, with the exception of ZnT3, was detected. There were no changes in the expression levels of ZnT1, ZnT2, ZnT4, ZnT5 and ZnT6 during pregnancy, but the expression level of ZnT7 was increased during pregnancy. ZnT7 was first detectable on day 12 of gestation (data not shown). Changes in Zn levels in the maternal serum and embryo during pregnancy are shown in Fig. 3A and B. Embryonic Zn concentrations were monitored from day 12 of gestation until the end of pregnancy. Zn levels in maternal serum decreased in the early period of pregnancy (up to day 14 of gestation) and then increased to the level at day 0 of gestation. Changes in embryonic Zn concentration were opposite to those in maternal serum. Embryonic Zn concentrations on days 16 and 18 of gestation were decreased compared to the concentrations on days 12 and 14 of gestation. These results suggest that ZnT7 plays a role in the placenta in the late period of pregnancy.

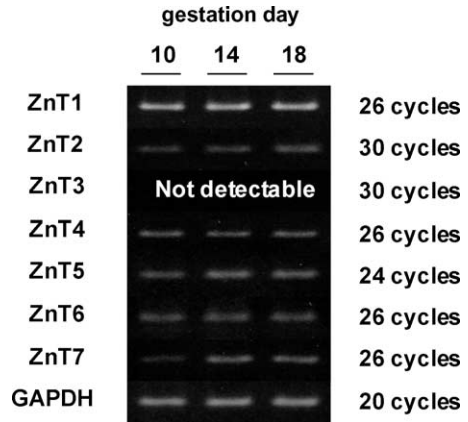


Fig. 2. Changes in the expression profiles of Zn transporters during pregnancy in mice. Total RNA was extracted from the mouse placenta on the indicated day of gestation. Then, the expression of ZnT family members was detected by RT-PCR as described in Section 2. The number of PCR cycles is indicated in the right panel. PCR products were electrophoresed on 3% agarose gels and visualized by ethidium bromide staining.

### 3.2. Expression of ZnT family members in rodent trophoblast cell lines

The placenta consists of various types of cells, including fetal endothelial cells, pericytes, villous macrophages and trophoblasts. Trophoblasts play a pivotal role in the transport of substances between the maternal body and the fetus (Stulc, 1989). Rodent trophoblast cell lines are readily available and therefore convenient for studying Zn transport. To the best of our knowledge, there are only two rat trophoblast cell lines, Rcho-1 cells and TR-TBT cells. Trophoblast cells are classified as trophoblast giant cells, spongiotrophoblasts, glycogen cells or syncytiotrophoblast cells (Knipp et al., 1999). Rcho-1 cells and TR-TBT cells are trophoblast giant cells and syncytiotrophoblast cells, respectively (Faria and Soares, 1991; Kitano et al., 2002). We investigated the expression of ZnT family members in Rcho-1 cells and TR-TBT cells. Since Rcho-1 cells can spontaneously differentiate into trophoblast giant cells by continuous culture, these cells are often used as a model of placental differentiation (Faria and Soares, 1991). ZnT1–4 have previously been identified in the rat (Liuzzi et al., 2003). We detected the expression of ZnT1–4 in Rcho-1 cells and TR-TBT cells. As shown in Fig. 4A, some ZnT family members were detected in Rcho-1 cells by RT-PCR analy-

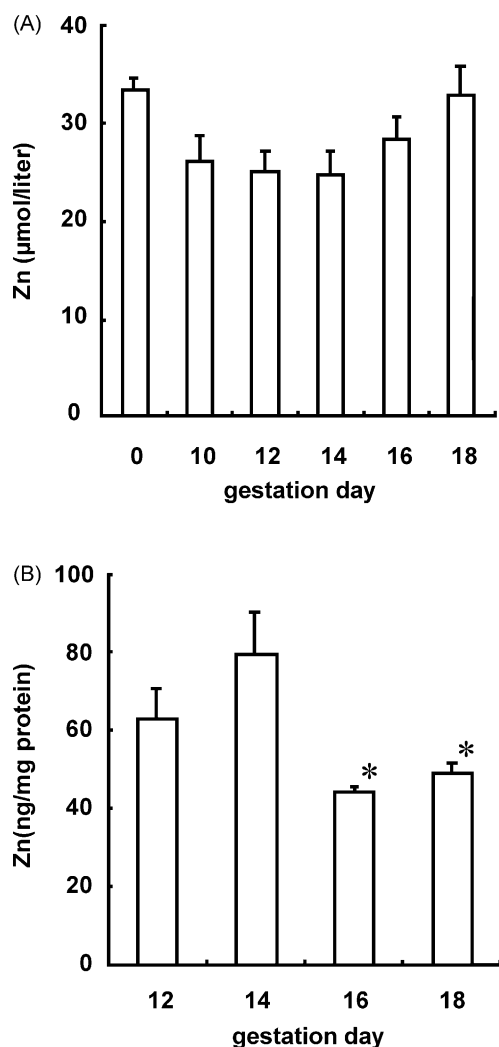


Fig. 3. Changes in Zn levels in maternal serum and the embryonic/fetal tissues during pregnancy. Maternal serum and embryos/fetuses were isolated on the indicated day of gestation, and the Zn levels of maternal serum (A) and embryos/fetuses (B) were determined by ICP-MS as described in Section 2. Data are means  $\pm$  SE ( $n = 3-6$ ). \* Significant difference from Zn levels at day 12 of gestation ( $P < 0.05$ ).

sis. However, the expression of ZnT2 mRNA was not detected in TR-TBT cells (Fig. 4B). Rcho-1 cells were seeded in culture dishes in a sub-confluent condition. Continuous culture for 12 days caused an elevation in the expression level of PLP-A, a sign of differentiation of Rcho-1 cells into trophoblast giant cells (Fig. 4C) (Faria and Soares, 1991). We used Rcho-1 cells that were seeded in a sub-confluent culture for 2 days and

that did not express PLP-A mRNA as undifferentiated cells. ZnT1–4 mRNA levels and cellular Zn levels did not change between undifferentiated Rcho-1 cells and differentiated Rcho-1 cells (Fig. 4C).

#### 4. Discussion

More than a dozen molecules involved in Zn transport have been identified by genetic and genomic studies in mammalian cells (Palmiter and Huang, 2004). In the present study, we investigated the expression profiles of ZnT family members during pregnancy in rat trophoblast cell lines and placentas of mice. Members of the ZnT family are able to decrease the cytoplasmic Zn concentration by transporting Zn out of cells or into intracellular compartments. Zn family proteins have similar membrane topologies consisting of six transmembrane domains and a histidine-rich loop between transmembrane domains IV and V, where Zn may be bound by histidines and subsequently transported across the membrane. Seven members of the ZnT family have been identified and characterized in mice (McMahon and Cousins, 1998; Kambe et al., 2002; Huang et al., 2002; Kirschke and Huang, 2003). The ZnT family members are expressed in a tissue-specific manner. For example, ZnT3 is only expressed in the brain and testes, and ZnT2 is widely expressed but not detectable in the liver, mammary gland, muscle, adipose, thymus and spleen (Palmiter et al., 1996a,b). In this study, we detected the expression of ZnT1–7 in the placentas of mice. The brain is the only other tissue in which all ZnT family members (ZnT1–7) have been detected (Palmiter and Huang, 2004). Thus, the placenta is the second tissue to be identified in which all ZnT family members have been detected. Expression profiles of ZnT family members are different during pregnancy. Since there is no biological or functional proof that the identified ZnT family proteins actually play roles in the placenta during pregnancy and in the differentiation of trophoblasts, our results cannot directly address the roles of the ZnT family in the placenta. However, the finding that the expression level of ZnT7 mRNA was elevated from day 14 of gestation in the mouse placenta suggests an association of ZnT7 with placental development. ZnT7 was determined to be a transporter of zinc from the cytoplasm into the Golgi apparatus

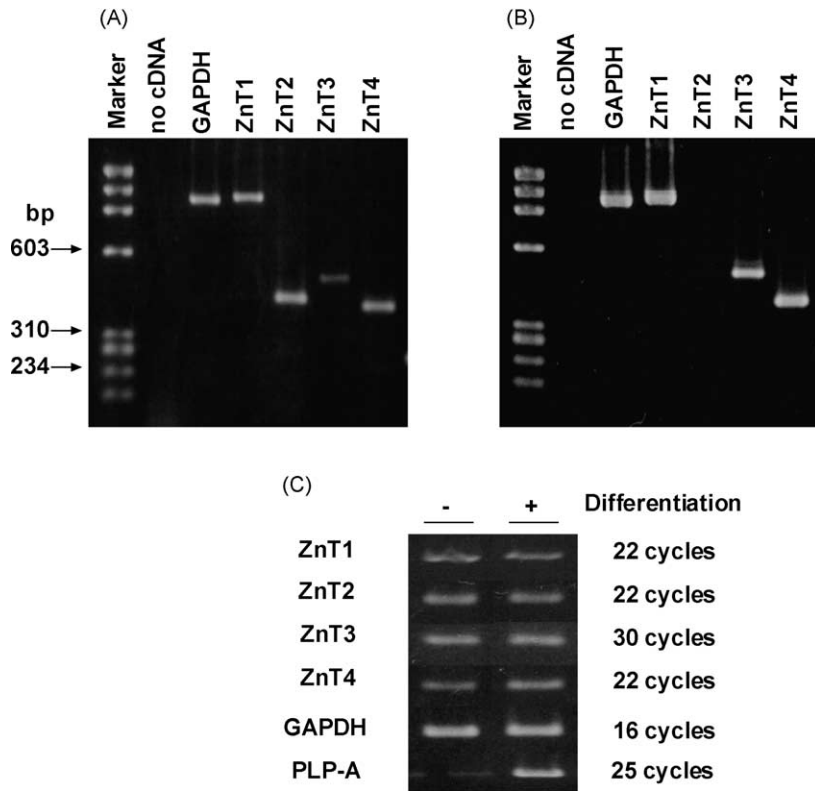


Fig. 4. Expression profiles of ZnT family members in Rcho-1 cells and TR-TBT cells. Total RNA was extracted from Rcho-1 cells that were cultured in a sub-confluent condition (A) and TR-TBT cells (B). The RNA was subjected to RT-PCR as described in Section 2. There were 23 PCR cycles for GAPDH, 33 cycles for ZnT1 and ZnT3 and 30 cycles for ZnT2 and ZnT4 in Rcho-1 cells. There were 25 PCR cycles for GAPDH and 33 cycles for ZnT1–4 in TR-TBT cells. Expression of ZnT family members in Rcho-1 cells that differentiated into trophoblast giant cells (C). Rcho-1 cells were seeded in 60 mm<sup>2</sup> plates in a sub-confluent condition. Differentiation of Rcho-1 cells was confirmed by giant cell morphology and by expression of PLP-A, a marker of trophoblast giant cells. Rcho-1 cells that were cultured for 2 days were used as undifferentiated cells, and Rcho-1 cells that were cultured for 12 days were used as differentiated cells. Total RNA extracted from undifferentiated and differentiated Rcho-1 cells was reverse-transcribed, and the resultant cDNA was amplified by PCR with the indicated number of cycles. The PCR products were electrophoresed on a 3% agarose gel and visualized by ethidium bromide staining.

(Kirschke and Huang, 2003). Considering the ability of the placenta to produce a wide variety of proteins, peptides and steroid hormones (Rama and Rao, 2003), the expression of ZnT7 in the late period of pregnancy may be involved in endocrine function of the placenta. A core promoter-binding protein (CPBP) is a zinc finger protein involved in the regulation of TATA box-less genes, such as those encoding pregnancy-specific glycoproteins (Slavin et al., 1999). Expression profiles of CPBP differed during pregnancy, and CPBP mRNA was strongly expressed from day 12.5 to 18.5 of gestation in the placentas of mice (Slavin et al., 1999). Thus, it may be interesting to investigate

whether ZnT7 regulates the activity of CPBP during pregnancy.

The placenta consists of various types of cells, including fetal endothelial cells, pericytes, villous macrophages and trophoblasts. Rodent trophoblasts are classified into trophoblast giant cells, spongiotrophoblasts, glycogen cells and syncytiotrophoblast cells (Knipp et al., 1999). In the present study, we characterized the expression profiles of the ZnT family members in a rat trophoblast giant cell model, Rcho-1 cells, and in a rat syncytiotrophoblast cell model, TR-TBT cells (Faria and Soares, 1991; Kitano et al., 2002). Although ZnT2 mRNA was detected in Rcho-1

cells, the expression of ZnT2 was deficient in TR-TBT cells. Syncytiotrophoblast cells are chiefly involved in regulating the transport of various substances between maternal and fetal compartments (Knipp et al., 1999). Trophoblast giant cells play an endocrine role in the placenta (Yamamoto et al., 1994). Expression of ZnT2 results in Zn accumulation in the intracellular acidic endosomes and the attenuation of cytotoxicity induced by transient rises in intracellular Zn levels (Palmiter et al., 1996a,b). Therefore, one possible explanation for different expression levels of ZnT2 in Rcho-1 cells and TR-TBT cells is that ZnT2 is not needed in syncytiotrophoblast TR-TBT cells for transport between maternal and fetal compartments. Synthesis of proteins, including hormones and proteins, in trophoblast giant cells requires intracellular uptake of excess Zn, and ZnT2 may therefore be expressed in order to attenuate cytotoxicity of elevation of intracellular Zn levels.

In summary, this is the first report to investigate the expression profiles of ZnT family members in rodent placental models. The results provide insights into the biology of the ZnT family in the placenta.

## Acknowledgements

The authors thank Dr. H. Mizuguchi (National Institute of Health Sciences, Japan) for his encouragement. This study was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

## References

- Aslam, N., McArdle, H.J., 1992. Mechanism of zinc uptake by microvilli isolated from human term placenta. *J. Cell Physiol.* 151, 533–538.
- Beach, R.S., Gershwin, M.E., Hurley, L.S., 1983. Persistent immunological consequences of gestation zinc deprivation. *Am. J. Clin. Nutr.* 38, 579–590.
- Cole, T.B., Wenzel, H.J., Kafer, K.E., Schwartzkroin, P.A., Palmiter, R.D., 1999. Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1716–1721.
- Cousins, R.J., McMahon, R.J., 2001. Integrative aspects of zinc transporters. *J. Nutr.* 130 (5S Suppl.), 1384S–1387S.
- Faria, T.N., Soares, M.J., 1991. Trophoblast cell differentiation: establishment, characterization, and modulation of a rat trophoblast cell line expressing members of the placental prolactin family. *Endocrinology* 129, 2895–2906.
- Gardner, R.L., Beddington, R.S., 1988. Multi-lineage ‘stem’ cells in the mammalian embryo. *J. Cell Sci. (Suppl.)* 10, 11–27.
- Hamlin, G.P., Soares, M.J., 1995. Regulation of deoxyribonucleic acid synthesis in proliferating and differentiating trophoblast cells: involvement of transferrin, transforming growth factor- $\beta$ , and tyrosine kinases. *Endocrinology* 136, 322–331.
- Huang, L., Gitschier, J., 1997. A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nat. Genetics* 17, 292–297.
- Huang, L., Kirschke, C.P., Gitschier, J., 2002. Functional characterization of a novel mammalian zinc transporter ZnT6. *J. Biol. Chem.* 277, 26389–26395.
- Hurley, L.S., 1980. *Developmental Nutrition*. Prentice-Hall, Englewood Cliffs, NJ.
- Inoue, K., Matsuda, K., Itoh, M., Kawaguchi, H., Tomoike, H., Aoyagi, T., Nagai, R., Hori, M., Nakamura, Y., Tanaka, T., 2002. Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene ZnT5. *Hum. Mol. Genet.* 11, 1775–1784.
- Kambe, T., Narita, H., Yamaguchi, Y., Hirose, J., Amano, T., Sugiura, N., Sasaki, R., Mori, K., Iwanaga, T., Nagao, M., 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, C.P., Huang, L., 2003. ZnT7, a novel mammalian zinc transporters, accumulates zinc in the Golgi apparatus. *J. Biol. Chem.* 278, 4096–4102.
- Kitano, T., Iizasa, H., Terasaki, T., Asashima, T., Matsunaga, N., Utoguchi, N., Watanabe, Y., Obinata, M., Ueda, M., Nakashima, E., 2002. Polarized glucose transports and mRNA expression properties in newly developed rat syncytiotrophoblast cell lines TR-TBTs. *J. Cell Physiol.* 193, 208–218.
- Knipp, G.T., Audus, K.L., Soares, M.J., 1999. Nutrient transport across the placenta. *Adv. Drug Deliver. Rev.* 38, 41–58.
- Liuzzi, J.P., Blanchard, R.K., Cousins, R.J., 2001. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. *J. Nutr.* 131, 46–52.
- Liuzzi, J.P., Bobo, J.A., Cui, L., McMahon, R.J., Cousins, R.J., 2003. Zinc transporters 1, 2 and 4 are differentially expressed and localized in rats during pregnancy and lactation. *J. Nutr.* 133, 342–351.
- Mas, A., Sarkar, B., 1992. Binding, uptake and efflux of  $^{65}\text{Zn}$  by isolated human trophoblast cells. *Biochim. Biophys. Acta.* 35–38.
- McMahon, R.J., Cousins, R.J., 1998. Mammalian zinc transporters. *J. Nutr.* 128, 667–670.
- Oda, M., Sun, W., Hattori, N., Tanaka, S., Shiota, K., 2001. PAL31 expression in rat trophoblast giant cells. *Biochem. Biophys. Res. Commun.* 287, 721–726.
- Ohgane, J., Hattori, N., Oda, M., Tanaka, S., Shiota, K., 2002. Differentiation of trophoblast lineage is associated with DNA methylation and demethylation. *Biochem. Biophys. Res. Commun.* 290, 701–706.
- Palmiter, R.D., Findley, S.D., 1995. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J.* 14, 639–649.
- Palmiter, R.D., Huang, L., 2004. Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. *Pflugers Arch.* 447, 744–751.

- Palmiter, R.D., Cole, T.B., Findley, S.D., 1996a. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *EMBO J.* 15, 1784–1791.
- Palmiter, R.D., Cole, T.B., Quaife, C.J., Findley, S.D., 1996b. ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14934–14939.
- Rama, S., Rao, A.J., 2003. Regulation of growth and function of the human placenta. *Mol. Cell Biochem.* 253, 262–268.
- Shiota, K., Sasaki, N., Hattori, N., Miura, R., Hasegawa, T., Guang, J.X., Noda, K., Takahashi, M., 1991. Cytosolic 20  $\alpha$ -hydroxysteroid dehydrogenase activity in spontaneous neoplasms in the dog and cat. *J. Vet. Med. Sci.* 53, 549–552.
- Slavin, D., Sapin, V., Lopez-Diaz, F., Jacquemin, P., Koritschner, N., Dastugue, B., Davidson, I., Chatton, B., Bocco, J.L., 1999. The Kruppel-like core promoter binding protein gene is primarily expressed in placenta during mouse development. *Biol. Rep.* 61, 1586–1591.
- Soares, M.J., Faria, T.N., Roby, K.F., Deb, S., 1991. Pregnancy and the prolactin family of hormones: coordination of anterior pituitary, uterine, and placental expression. *Endocr. Rev.* 12, 402–423.
- Stulc, J., 1989. Extracellular transport pathways in the haemochorial placenta. *Placenta* 10, 113–119.
- Takahashi, T., Utoguchi, N., Takara, A., Yamamoto, N., Nakanishi, T., Tanaka, K., Audus, K.L., Watanabe, Y., 2001. Carrier-mediated transport of folic acid in BeWo cell monolayers as a model of the human trophoblast. *Placenta* 22, 863–869.
- Vargas Zapata, C.L., Trugo, N.M., Donangelo, C.M., 2000. Zinc uptake by human placental microvillous membrane vesicles. *Biol. Trace Elem. Res.* 73, 127–137.
- Wildman, R.E.C., Medeiros, D.M., 2000. *Advanced Human Nutrition*. CRS Press LLC.
- Yamamoto, T., Roby, K.F., Kwok, S.C., Soares, M.J., 1994. Transcriptional activation of cytochrome P450 side chain cleavage enzyme expression during trophoblast cell differentiation. *J. Biol. Chem.* 269, 6517–6523.