Cloning and Characterization of a Novel Mammalian Zinc Transporter, Zinc Transporter 5, Abundantly Expressed in Pancreatic β Cells*

Taiho Kambe‡§, Hiroshi Narita†, Yuko Yamaguchi-Iwai‡, Junko Hirose, Tatsuaki Amano‡, Naomi Sugiyura, Ryozi Sasaki‡, Koshi Mori‡‡, Toshihiko Iwanaga‡‡, and Masaya Nagao‡

From the †Division of Integrated Life Science, Graduate School of Biosciences, Kyoto University, Kyoto 606-8502, Japan, the ‡Department of Food Science, Kyoto Women’s University, Kyoto, 605-8501, Japan, the §Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan, the ¶Department of Life Style Studies, School of Human Cultures, University of Shiga Prefecture, Shiga 522-8533, Japan, and the ‡‡Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido 060-0818, Japan

Intracellular homeostasis for zinc is achieved through the coordinate regulation of specific transporters engaged in zinc influx, efflux, and intracellular compartmentalization. We have identified a novel mammalian zinc transporter, zinc transporter 5 (ZnT-5), by virtue of its similarity to ZRC1, a zinc transporter of Saccharomyces cerevisiae, a member of the cation diffusion facilitator family. Human ZnT-5 (hZnT-5) cDNA encodes a 765-amino acid protein with 15 predicted membrane-spanning domains. hZnT-5 was ubiquitously expressed in all tested human tissues and abundantly expressed in the pancreas. In the human pancreas, hZnT-5 was expressed abundantly in insulin-containing β cells that contain zinc at the highest level in the body. The hZnT-5 immunoreactivity was found to be associated with secretory granules by electron microscopy. The hZnT-5-derived zinc transport activity was detected using the Golgi-enriched vesicles prepared from hZnT-5-induced HeLa/hZnT-5 cells in which exogenous hZnT-5 expression is inducible by the Tet-on gene regulation system. This activity was dependent on time, temperature, and concentration and was saturable. Moreover, zinc at a high concentration (10 mM) inhibited the growth of yeast expressing hZnT-5. These results suggest that ZnT-5 plays an important role for transporting zinc into secretory granules in pancreatic β cells.

Zinc is a trace element that is indispensable for life, because it is a key structural component of a large number of proteins such as metalloenzymes and zinc-dependent transcriptional factors (1, 2). In mammals, zinc is absorbed from the diet through intestinal epithelial cells and is transported into the blood, where most of it is bound by albumin and α2-macroglobulin (3). The circulating zinc is incorporated into all types of cells, and the cytosolic zinc is taken up by intracellular organelles.

The kinetic studies and characterization of zinc transport using a variety of both animal cell systems and membrane vesicles have suggested the transporter-mediated movement of zinc (3). Recently, cDNAs of several zinc transporters have been molecularly cloned from mammals, and most of them have been assigned to two metal transporter families, the cation diffusion facilitator (CDF) family that functions in zinc efflux from cytoplasm and the ZRT/IRT-related protein (ZIP) family that functions in zinc influx into cytoplasm (4–7). CDF family members, ZnT-1 to -4, have characteristics of membrane topol-ogy such as six predicted membrane-spanning domains, a cytoplasmic histidine-rich loop between membrane-spanning domains IV and V, and both amino and carboxyl termini thought to reside intracellularly (5). The histidine-rich loop may construct a metal-binding site. In mammals, ZnT-1 is a probable zinc transporter responsible for zinc efflux across the plasma membranes (8). ZnT-2 is found on late endosomes, where it appears to facilitate zinc transport into its vesicle compartment (9, 10). ZnT-3 is expressed in the brain (11) and is responsible for the accumulation of zinc in synaptic vesicles (12). ZnT-4 is expressed in intestinal epithelial cells (13) and mammary gland epithelia, where it is required for zinc transport into milk (14). Thus, ZnT-1 to -4 appear to be engaged in zinc efflux or organelle compartmentalization from cytoplasm.

The members of the ZIP family are predicted to have eight potential membrane-spanning domains and an intracellular loop with a histidine-rich region between membrane-spanning domains III and IV that may function as a metal-binding site as does the similar loop in CDF family members. Thus, three transporters (human Zip1 to -3) were identified in mammals (15–17). hZip1 and hZip2 are thought to be involved in zinc influx across the plasma membranes, because forced expression of these transporters in mammalian cells increased their zinc uptake (16, 17).

In addition to the biological functions in metalloenzymes and transcription factors, zinc plays unique roles in some specialized cells (18–20). In hippocampal neurons and pancreatic β cells, a large amount of zinc is accumulated in cytoplasmic vesicles/granules (11, 21). Zinc in synaptic vesicles is co-

Received for publication, January 28, 2002, and in revised form, March 19, 2002
Published, JBC Papers in Press, March 19, 2002, DOI 10.1074/jbc.M200910200

Printed in U.S.A.

This paper is available on line at http://www.jbc.org
Zinc Transporter Abundantly Expressed in Pancreatic β Cells

A

B

C

Homologous region to CDF family
secreted with neurotransmitters upon excitation; zinc is thought to modulate synaptic transmission (18). In pancreatic β cells, zinc binds with proinsulin in the secretory pathway to form zinc proinsulin hexamers, which are subsequently converted into zinc insulin microcrystals for storage in secretory granules (19, 22). It is believed that formation of zinc proinsulin hexamers facilitates intracellular transport and that formation of microcrystals causes accumulation of insulin at a supersaturating concentration, thereby providing a mechanism for an acute secretion of insulin in response to glucose. ZnT-3 has been shown to be responsible for zinc accumulation in synaptic vesicles (12), but nothing is known about the mechanism by which zinc enters secretory granules in β cells.

We previously showed transepithelial transport of several metal ions including zinc in polarized Madin-Darby canine kidney cells (23). This finding prompted us to search the mouse expressed sequence tag (EST) sequences homologous to ZRC1 (24), an S. cerevisiae zinc transporter that belongs to the CDF family. Further data base analysis and subsequent experiments resulted in identifying a novel zinc transporter, ZnT-5, that has a very long unique amino-terminal sequence, followed by carboxyl-terminal sequence significantly homologous to CDF family members. ZnT-5 was expressed abundantly in the human pancreas in association with secretory granules. Here we report the structural characteristics and localization of ZnT-5. We propose that ZnT-5 may be a transporter of zinc into secretory granules in β cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—HeLa Tet-on cells (gift from Dr. Kazuhiro Iwai) were maintained in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% Tet System Approved Serum (CLONTECH) and 200 μg/ml G418 (Nacalai, Kyoto, Japan). JAR, HeLa, HEp-2 (gift from Dr. Mitsuaki Tabuchi), and Hep3B cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Trace Scientific Ltd.). To establish stable transfectants, we co-transfected HeLa Tet-on cells with pTR-hygroycin-c and pTRE-CMVmin-hZnT-5 or pTRE-CMVmin-FLAG-hZnT-5 plasmids. Drug-resistant clones were screened in the presence of 150 μg/ml Hygromycin B (Nacalai). From the resistant clones, a HeLa/hZnT-5 cell line harboring the pTRE-CMVmin-hZnT-5 and a HeLa/FLAG-hZnT-5 cell line harboring the pTRE-CMVmin-FLAG-hZnT-5 were selected on the basis of the highest inducibility of expression of hZnT-5 in the presence of 2.5 μg/ml doxycycline (Dox). Immunohistochemical Studies—Zinc Transporter Abundantly Expressed in Pancreatic β Cells

Fig. 1. Sequence of hZnT-5 cDNA and protein. A, sequence of hZnT-5 cDNA. The putative start ATG codon and termination TAG codon are underlined. Small ORFs (5 and 13 amino acids long) upstream of start ATG are indicated by boxes. hZnT-5 is composed of 16 exons, and exon/intron gaps are indicated by vertical lines. These structures are completely conserved in mZnT-5. B, comparison of the hZnT-5 and mZnT-5 amino acid sequences. Identical residues are indicated by vertical lines. C, a hydropathy profile of hZnT-5 protein generated by the use of the algorithm and hydrophapy values of Kyte and Doolittle (37). The membrane-spanning domains predicted by the use of the PSORT II program (available on the World Wide Web at psort.ibms.u-tokyo.ac.jp) are designated with roman numerals. A histidine-rich region constituting potential metal-binding sites is indicated by a horizontal line. The region homologous to the members of CDF family in the carboxyl-terminal portion (six membrane-spanning domains) is separated by vertical broken lines.

Downloaded from http://www.jbc.org/ on July 24, 2018 by guest
Zinc Transporter Abundantly Expressed in Pancreatic β Cells

RESULTS

Identification of Human and Mouse cDNA Encoding ZnT-5—Data base comparisons of the mouse EST sequences with ZRC1 (24), a zinc transporter that belongs to CDF family, revealed several nucleotide fragments homologous to this transporter. Three of them were not found in known genes and partially overlapped. A further database search of contiguous sequences identified a 2.3-kb fragment that contained a putative open reading frame (ORF), an in-frame termination codon, and a canonical polyadenylation signal. The 2.3-kb fragment did not contain a translation initiation codon, suggesting that this fragment is the 3′ portion of a longer cDNA. We obtained human and mouse full-length cDNAs by the combination of rapid cDNA PCR screening and primer extension technique. Human cDNA is shown in Fig. 1A. Because this cDNA encodes a zinc transporter with homology to CDF family as described below, we hereafter refer to this protein as ZnT-5.

The ATG at position +202 was assumed to initiate an ORF encoding hZnT-5 of 765 amino acids (Fig. 1A). This initiation codon was preceded by a 200-bp untranslated region that contains two small ORFs that can encode two small peptides (3 and 13 amino acids long) by initiating at positions +103 and +152, respectively. Interestingly, this structure in the untranslated region is completely conserved in mZnT-5. mZnT-5 had 761 amino acids and showed high homology to hZnT-5 (94%; Fig. 1B). Hydropathy plot analysis of hZnT-5 showed the potential 15 membrane-spanning domains (Fig. 1C). The amino-terminal portion (~400 amino acid residues) containing nine membrane-spanning domains showed little homology to known proteins.

Northern blot analysis indicated that the hZnT-5 gene was ubiquitously expressed as 3- and 4-kb transcripts in human tissues (Fig. 2). High expression was seen in the pancreas, followed by the kidney and liver. hZnT-5 cDNA that we identified in this study corresponds to the 3-kb isoform. Analysis of the 4-kb transcript is under way in our laboratory.

Detection of hZnT-5 Protein by Anti-hZnT5 Monoclonal Antibody, 1-7B—A region of the carboxyl-terminal end of hZnT-5 was used as an antigen for antibody production. This carboxy-terminal region was fused with maltose-binding protein, and the fused protein was expressed in E. coli. Purified fusion protein was used as an antigen, and a hybridoma producing anti-hZnT-5 monoclonal antibody (1-7B mAb) was established. To demonstrate that hZnT-5 is a membrane protein, we carried out immunoblot analysis using the membrane and cytosolic fractions derived from some human cell lines (HEp-2, Hep3B, JAR, and HeLa cells) and HeLa/hZnT-5 cells in which exogenous hZnT-5 is inducible when cultured in the presence of Dox (Fig. 3A). The 1-7B mAb recognized a 55-kDa protein in membrane fractions, and no positive bands were found in cytosolic fractions. The molecular size of hZnT-5 calculated from cDNA is 84 kDa. A significant difference in the calculated molecular size and that found by immunoblot analysis raised the possibility that hZnT-5 may be cleaved to yield the carboxy-terminal protein detected by 1-7B mAb. To examine whether this is the case, we expressed the amino-terminal FLAG-tagged hZnT-5 protein in HeLa cells (HeLa/FLAG-hZnT-5) and detected the expressed hZnT-5 by an anti-FLAG antibody, M5. As Fig. 3B shows, FLAG-hZnT-5 migrated with a size of 55–60 kDa, which is similar to the size detected by the 1-7B mAb. Thus, the 55-kDa protein that reacts with 1-7B mAb is the intact hZnT-5.

Preferential Expression of hZnT-5 in Human Pancreatic β Cells—Since hZnT-5 mRNA was abundantly expressed in human pancreas (see Fig. 2B), we immunohistochemically examined the localization of hZnT-5. As shown in Fig. 4A, expression of hZnT-5 in the human pancreas can be almost completely superimposed on that of insulin. β cells expressed ZnT-5 much more abundantly than the other types of pancreatic cells; hZnT-5 was undetectable in other endocrine cell types includ-
Fig. 3. Immunoblot analysis with anti-hZnT-5 monoclonal antibody, 1-7B. A, hZnT-5 was present in membrane proteins. Immunoblot analysis was performed using the membrane proteins (mb) and cytosolic proteins (Cy) derived from some human cell lines (larynx carcinoma (HEp-2), hepatoma (Hep3B), placental trophoblast-derived choriocarcinoma (JAR), and cervix carcinoma (HeLa)) and HeLa/hZnT-5 cells cultured in either induced or uninduced conditions. hZnT-5 was detected only in the lanes of membrane proteins. B, the 55-kDa protein was intact hZnT-5 protein. Immunoblot analysis was performed with 1-7B mAb (lanes 1 and 2) or anti-FLAG antibody, M5 (lanes 3 and 4), using membrane proteins prepared from HeLa/hZnT-5 cultured in the induced condition (lanes 1 and 4) and HeLa/FLAG-hZnT-5 cells cultured in the induced condition (lanes 2 and 3). 20 μg of protein was loaded in each lane. The position of a protein size marker is indicated on the left.

Fig. 4. Preferential expression of hZnT-5 in human pancreatic β cells. A, double immunofluorescence staining of human pancreas was performed using 1-7B mAb and anti-insulin antibody. Note that hZnT-5 protein is specifically detected in insulin-containing β cells but not in other endocrine cell types or acinar cells. Scale bar, 50 μm. B, electron microscopic immunolabeling of β cells shows that hZnT-5 was localized in association with secretory granules. Immunogold particles for hZnT-5 are present on the perimeter of secretory granules (arrows). Scale bar, 1 μm.

ZnT-5 Acts as a Zinc Transport Protein—Since the carboxyl-terminal portion of hZnT-5 has a strong homology to zinc transporters belonging to the CDF family and hZnT-5 was abundantly expressed in pancreatic β cells in association with secretory granules, we tested whether hZnT-5 functions as a zinc transporter. We chose HeLa cells to find zinc transport activity of hZnT-5 because of their lesser expression of endogenous hZnT-5 among all tested human cell lines (data not shown). First, the subcellular localization of hZnT-5 was determined using HeLa/hZnT-5 cells cultured in the presence of Dox (hZnT-5-induced condition). The localization of hZnT-5 was indistinguishable from that of a marker protein of Golgi apparatus, GM130, by indirect immunoreactive staining (Fig. 5A) and immunoblot analysis (Fig. 5B), indicating that most of hZnT-5 is present in Golgi apparatus.

Next, because expression of hZnT-5 was increased 9-fold when HeLa/hZnT-5 cells were cultured in the induced condition (see Fig. 3A), we examined whether the induced HeLa/hZnT-5 cells accumulate zinc in Golgi apparatus more than did the uninduced cells using Zinquin, a zinc-specific fluorescence probe. No significant difference was found between the induced and uninduced cells (data not shown). Then we tried to detect zinc transport activity of hZnT-5 using Golgi-enriched vesicles prepared from total membranes of HeLa/hZnT-5 cells cultured in either hZnT-5-induced or uninduced condition. The membranes were fractionated by sucrose gradient centrifugation, and 5-fold enrichment of Golgi membranes was confirmed by immunoblot analysis (data not shown). hZnT-5-induced or uninduced vesicles were prepared from the enriched Golgi membranes (see “Experimental Procedures”) and used for 65Zn transport assays. Time course of 65Zn uptake into vesicles was investigated using the hZnT-5-induced vesicles or uninduced vesicles (Fig. 6A). The 65Zn uptake into the hZnT-5-induced vesicles was higher than that of uninduced vesicles at 37 °C, although no difference in the uptake was seen at 4 °C. The increased uptake observed in hZnT-5-induced vesicles at 37 °C is probably caused by the induced hZnT-5 protein. The uptake at 37 °C was much larger than that at 4 °C, indicating that 65Zn uptake is likely transporter-mediated accumulation into vesicles rather than cell surface binding. Fig. 6B shows that the rate of 65Zn uptake at 37 °C is concentration-dependent and saturable in both hZnT-5-induced vesicles and uninduced vesicles. The uptake rate in hZnT-5-induced vesicles at 37 °C was higher than that in uninduced vesicles. The exogenous hZnT-5-derived activity calculated by subtracting uptake rates of uninduced vesicles from those of hZnT-5-induced vesicles was also concentration-dependent and saturable. The saturation curve fitted Michaelis-Menten kinetics with an apparent Vmax of 15 nmol/mg protein/min.

In another study, we examined the growth dependence of hZnT-5-induced and uninduced HeLa/hZnT-5 cells on zinc in the medium but found no differences with the concentration of zinc (data not shown). Then, we examined the effects of hZnT-5 expression on yeast. The yeast expressing hZnT-5 grew normally in the normal medium (Fig. 6C, left) but showed poor growth in medium containing 10 mM zinc, which is the upper limit for the control yeast to grow (Fig. 6C, middle). A high iron concentration had no effect (Fig. 6C, right). It is noted that the growth of the yeast expressing the 5′-deleted hZnT-5 cDNA (ΔhZnT-5) in which the amino-terminal nine membrane-spanning domains have been deleted is unaffected by zinc at a high concentration (Fig. 6C, middle). This result indicates that hZnT-5 acts as a zinc transporter, although the mechanism responsible for the zinc-sensitive phenotype remains to be identified. A similar sensitive phenotype was seen in the presence of a high cobalt concentration (0.5 mM), suggesting that cobalt is a potential substrate of hZnT-5 (data not shown).
**DISCUSSION**

A data base search of DNA sequences homologous to a yeast zinc transporter and cloning of full-length cDNA revealed a novel protein, which appeared to be a zinc transporter consisting of 765 amino acid residues. The primary sequence was well conserved in human and mouse. Since its carboxyl-terminal portion (365 amino acid residues) contains six membrane-spanning domains, a structural characteristic of metal transporters in the CDF family, and is highly homologous to mammalian zinc transporters (ZnT-1 to -4) that belong to the CDF family, we refer to this transporter as ZnT-5. Functional assays of this protein using Golgi-enriched vesicles and yeast cells supported this classification. ZnT-5, however, has a very long amino-terminal portion (400 amino acid residues) with nine membrane-spanning domains. MSC2 (YDR205W) protein (a CDF family member) with zinc homeostasis function in yeast has been shown to contain 12 membrane-spanning domains (31). MSC2 and ZnT-5 are homologous in the carboxyl-terminal portions with six membrane-spanning domains but not in the amino-terminal portions. To our knowledge, there is only one protein homologous to the amino-terminal portion of ZnT-5 in *Caenorhabditis elegans* (CAB81912). The function of this *C. elegans* protein is unknown.

The functions of most members of the CDF family have been examined by overexpression experiments; i.e. the cells gain resistance to metal toxicity, indicating that the six membrane-spanning domain is sufficient to exhibit transporter activity. These facts raised the possibility that ZnT-5 may possess translocation activity and the six membrane-spanning domains are sufficient to exhibit transporter activity. This is unlikely because immuno blot analysis of the use of 1-7B mAb against the carboxyl-terminal fragment of ZnT-5 and the antibody against FLAG tagged at the amino terminus detected HZnT-5 with a similar molecular size. Furthermore, zinc at a high concentration inhibited the growth of yeast expressing the full-length HZnT-5 cDNA but not that of yeast expressing the 5'-deletion form of HZnT-5 cDNA (ΔHZnT-5).

CDF family members are found in both eukaryotes and prokaryotes (5, 32), and complementation assays between different species have been successful (14, 33). For example, expression of mouse ZnT-4 in Δzrc1 yeast that cannot grow in medium containing zinc at a high concentration due to insufficient efflux of zinc allows the yeast to grow. Expression of HZnT-5 in Δzrc1 yeast, however, could not reduce zinc toxicity (data not shown). Instead, wild-type yeast expressing HZnT-5 showed limited growth at a high zinc concentration. We attempted to determine the localization of HZnT-5 expressed in yeast, but unfortunately yeast contained protein(s) that cross-reacts with 1-7B mAb; the high background made it difficult to localize HZnT-5 in yeast. The mechanism making the yeast hypersensitive to zinc remains unknown. Nevertheless, a zinc-dependent phenotype of yeast expressing HZnT-5 provides additional support that ZnT-5 transports zinc. Gaither and Eide (17) have reported a similar finding that yeast expressing hZIP1, a mammalian zinc transporter belonging to the ZIP family, shows limited growth at a high zinc concentration. hZIP1 does not complement the Δzrt1/Δzrt2 double mutant yeast strain, which cannot grow in a zinc-limiting condition (17).

The intracellular concentration of free zinc is estimated to be at a nanomolar level (34), because the major part of zinc in cytoplasm is bound to proteins such as metallothionein. A *K* in value of ~0.25 μM that we calculated from the in vitro zinc transporter activity of HZnT-5 is relatively high, suggesting that free zinc may not be a real substrate of ZnT-5. A transport assay under conditions that mimic intracellular environments more faithfully is necessary. The addition of H* +* and ATP showed no effects in zinc uptake by HZnT-5-induced vesicles (data not shown).

![Fig. 5. hZnT-5 was localized in Golgi apparatus in HeLa/hZnT-5 cells cultured in ZnT-5-induced condition.](image-url)

A, hZnT-5 was co-localized with a marker protein of Golgi apparatus, GM130. HeLa/hZnT-5 cells cultured in ZnT-5-induced condition were fixed and double-immunostained with 1-7B mAb or GM130 polyclonal antibody, followed by secondary antibodies conjugated with Alexa 488 or Alexa 594, respectively. B, hZnT-5 protein prepared from HeLa/hZnT-5 cells cultured in ZnT-5-induced condition were fixed and double-immunostained with 1-7B mAb or GM130. HeLa/hZnT-5 cells cultured in ZnT-5-induced condition were fixed and double-immunostained with 1-7B mAb; the high background made it difficult to localize HZnT-5 in yeast. The mechanism making the yeast hypersensitive to zinc remains unknown. Nevertheless, a zinc-dependent phenotype of yeast expressing HZnT-5 provides additional support that ZnT-5 transports zinc. Gaither and Eide (17) have reported a similar finding that yeast expressing hZIP1, a mammalian zinc transporter belonging to the ZIP family, shows limited growth at a high zinc concentration. hZIP1 does not complement the Δzrt1/Δzrt2 double mutant yeast strain, which cannot grow in a zinc-limiting condition (17).

The intracellular concentration of free zinc is estimated to be at a nanomolar level (34), because the major part of zinc in cytoplasm is bound to proteins such as metallothionein. A *K* in value of ~0.25 μM that we calculated from the in vitro zinc transporter activity of HZnT-5 is relatively high, suggesting that free zinc may not be a real substrate of ZnT-5. A transport assay under conditions that mimic intracellular environments more faithfully is necessary. The addition of H* +* and ATP showed no effects in zinc uptake by HZnT-5-induced vesicles (data not shown).
Zinc Transporter Abundantly Expressed in Pancreatic β Cells

Zinc is not only a key structural component of a large number of zinc-containing proteins but also possesses important modulator functions in some specialized cells (1, 2, 18–20). One of these is the formation of zinc insulin microcrystals in secretory granules in pancreatic β cells. Zinc in these granules is believed to play two indispensable roles to store insulin in crystal forms (19, 22, 35); first, two zinc ions are bound with six proinsulin molecules to form a hexamer, which occurs just after endoplasmic reticulum export of proinsulin. Second, zinc promotes the granule core (crystal) formation, which occurs after proteolytic conversion from proinsulin to insulin in secretory granules. The extensive accumulation of zinc in pancreatic β cells suggests that these cells are equipped with the powerful machinery that drives zinc influx into secretory granules. However, which transporter handles zinc into endoplasmic reticulum and secretory granules is unknown. Recently, mRNAs of ZnT-1 to 4 in pancreatic islets were detected by RT-PCR techniques (36), but neither the cellular nor subcellular distribution of these transporter proteins in the pancreas is known. We found that hZnT-5 protein is abundantly expressed in human pancreatic β cells but not in glucagon-secreting α cells and most acinar cells. It is noteworthy that hZnT-5 is associated with secretory granules. From these results, we propose that ZnT-5 is responsible for transporting zinc into secretory granules to form insulin crystals. Since ZnT-5 is ubiquitously expressed, however, ZnT-5 probably plays a role in the movement of zinc in a variety of tissues.

Acknowledgments—We thank Drs. Kazuhiro Iwai and Mitsuaki Tabuchi for providing the cell lines used in this work and Dr. Nobuaki Nakamura for providing the GM130 polyclonal antibody. We are grateful to Mayuko Nanbu for technical assistance.

REFERENCES

1. Valle, B. L., and Falchuk, K. H. (1993) Physiol. Rev. 73, 79–118
2. Berg, J. M., and Shi, Y. (1996) Science 271, 1081–1085
3. Reyes, J. G. (1996) Am. J. Physiol. 270, C401–C410
4. Cousins, R. J., and McMahon, R. J. (2000) J. Nutr. 130, 13848–13878
5. Paulsen, I. T., and Saier, M. H., Jr. (1997) J. Membr. Biol. 156, 99–103
6. Eide, D. (1997) Curr. Opin. Cell Biol. 9, 573–577
7. Gueriniot, M. L. (2000) Biochim. Biophys. Acta 1465, 190–198
8. Palmiter, R. D., and Findley, S. D. (1995) EMBO J. 14, 639–649
9. Palmiter, R. D., Cole, T. B., and Findley, S. D. (1996) EMBO J. 15, 1784–1791
10. Kobayashi, T., Beuchat, M. H., Lindsay, M., Frias, S., Palmiter, R. D., Sakuraba, H., Parton, R. G., and Gruenberg, J. (1999) Nat. Cell Biol. 1, 113–118
11. Palmiter, R. D., Cole, T. B., Quazie, C. J., and Findley, S. D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14934–14939
12. Cole, T. B., Wenzel, H. J., Kafer, K. E., Schwartztkroin, P. A., and Palmiter, R. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1716–1721
13. Murgia, C., Vespignani, I., Cerese, J., Nobili, F., and Peruzzi, G. (1999) Am. J. Physiol. 277, G1231–G1239
14. Huang, L., and Gitschier, J. (1997) Nat. Genet. 17, 292–297
15. Costello, L. C., Liu, Y., Zou, J., and Franklin, R. B. (1999) J. Biol. Chem. 274, 17499–17504
16. Gaither, L. A., and Eide, D. J. (2000) J. Biol. Chem. 275, 5550–5564
17. Gaither, L. A., and Eide, D. J. (2001) J. Biol. Chem. 276, 22258–22264
18. Huang, E. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13386–13387
19. Dodson, G., and Steiner, D. (1998) Curr. Opin. Struct. Biol. 8, 189–194
20. Hershfinkel, M., Moran, A., Grossman, N., and Sekler, I. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 11749–11754
21. Zalewski, P. D., Millard, S. H., Forbes, I. J., Kapaniris, O., Slavotinek, A., Betts, W. H., Ward, A. D., Lincoln, S. F., and Mahadevan, I. (1994) J. Histochem. Cytochem. 42, 877–885
22. Huang, X. F., and Arvan, P. (1995) J. Biol. Chem. 270, 20417–20423
23. Nagao, M., Sugura, E., Kambe, T., and Sasaki, R. (1999) Biochem. Biophys. Res. Commun. 267, 289–294
24. Kamozono, A., Nishihara, M., Tanenishi, Y., Murata, K., and Kimura, A. (1989) Mol. Gen. Genet. 219, 161–167
25. Ikura, K., Nasu, T., Yokota, H., Tsuchiya, Y., Sasaki, R., and Chiba, H. (1988) Biochemistry 27, 2988–2993
26. Gossen, M., Freundlieb, S., Berger, G., Muller, G., Hilten, W., and Bujard, H. (1995) Science 268, 1766–1769
27. Morishita, E., Narita, H., Nishida, M., Kawashima, N., Yamagishi, K., Masuda, S., Nagao, M., Hatta, H., and Sasaki, R. (1996) Blood 88, 465–471
28. McMahon, R. J., and Cousins, R. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4841–4846
29. Balch, W. E., Dunphy, W. G., Braeol, W. A., and Rothman, J. E. (1984) Cell 39, 465–476
30. Asham, N., and McArthie, H. J. (1992) J. Cell. Physiol. 151, 533–538
31. Li, L., and Kaplan, J. (2004) J. Biol. Chem. 279, 5036–5043
32. van der Zaal, B. J., Neutelhuis, I. M., Wino, J. E., Chardonnens, A. N., Schat, H., Verkleij, J. A., and Hooykaas, P. J. (1997) Plant Physiol. 119, 1047–1055
33. Persans, M. W., Nienan, K., and Salt, D. E. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9995–10000
34. Williams, R. J. P. (1989) Zinc in Human Biology (Mills, C. F., ed) Springer-Verlag, New York
35. Arvan, P., and Castle, D. (1998) Biochem. J. 332, 583–610
36. Clifford, K. S., and MacDonald, M. J. (2000) Diabetes Res. Clin. Pract. 49, 77–85
37. Ryte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
Cloning and Characterization of a Novel Mammalian Zinc Transporter, Zinc Transporter 5, Abundantly Expressed in Pancreatic β Cells
Taiho Kambe, Hiroshi Narita, Yuko Yamaguchi-Iwai, Junko Hirose, Tatsuaki Amano, Naomi Sugiuira, Ryuzo Sasaki, Koshi Mori, Toshihiko Iwanaga and Masaya Nagao

J. Biol. Chem. 2002, 277:19049-19055.
doi: 10.1074/jbc.M200910200 originally published online March 19, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200910200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 17 of which can be accessed free at
http://www.jbc.org/content/277/21/19049.full.html#ref-list-1