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Intestinal and placental zinc transport pathways

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Mammalian members of the cation diffusion facilitator (CDF) and zrt-, irt-like protein (ZIP) families of Zn transporters, initially identified in *Saccharomyces cerevisiae* and *Arabidopsis thalania*, have been cloned during the last 8 years and have been classified as families SLC30 and SLC39 respectively. The cloning of human Zn transporters ZnT-like transporter 1 (hZTL1)/ZnT5 (SLC30A5) and hZIP4 (SLC39A4) were major advances in the understanding of the molecular mechanisms of dietary Zn absorption. Both transporters are localised at the enterocyte apical membrane and are, therefore, potentially of fundamental importance in dietary Zn uptake. hZTL1 mediates Zn uptake when expressed in *Xenopus laevis* oocytes and hZIP4 is mutated in most cases of the inherited Zn deficiency disease acrodermatitis enteropathica. Localisation of hZTL1/ZnT5 at the apical membrane of the placental syncytiotrophoblast indicates a fundamental role in the transfer of Zn to the foetus. Observations in rodent models indicate that in the intestine increased Zn availability increases expression of Slc30 Zn transporters. Human intestinal Caco-2 cells show a similar response to increasing the Zn^{2+} concentration of the nutrient medium in relation to the expression of mRNA corresponding to several Zn transporters and that of ZnT1 (SLC30A1) and hZTL1/ZnT5 proteins. In the human placental cell line JAR, however, expression at the mRNA level of a number of Zn transporters is not modified by Zn availability, whilst ZnT1 and hZTL1/ZnT5 proteins are reduced under Zn-supplemented conditions. These differences between Caco-2 and JAR cells in Zn transporter gene responses to Zn supply may reflect the different extracellular Zn concentrations encountered by the corresponding cell types *in vitro*.

Dietary zinc absorption: Placental zinc transport: SLC30 transporters: SLC39 transporters

The redox stability and ability of Zn^{2+} to form polyhedral coordination complexes with a variety of ligands confers properties that are exploited widely in biology, both structurally and catalytically. Zn finger and Zn cluster domains occur repeatedly in biological systems as structural motifs in the DNA-binding domains of proteins, including transcription factors and hormone receptor proteins (Coleman, 1992). Zn is found at the active site of enzymes from all six major classes, where ionisation, polarisation or replacement of Zn^{2+}-bound water is involved in the catalytic mechanism (McCall *et al*. 2000).

Zn is an essential dietary micronutrient, and reported UK intakes (10·2 and 7·4 mg/d for men and women respectively; Office for National Statistics, 2003) are close to the reference nutrient intake (9·5 and 7·0 mg/d for men and women respectively; Department of Health, 1991), with 4% of both men and women reporting daily intakes below the reference nutrient intake (Office for National Statistics, 2003). These levels of intake indicate that Zn deficiency is not a major health problem in the UK population. However, the lack of a reliable indicator of Zn status (Wood, 2000) makes subclinical deficiency

Abbreviations: CDF, cation diffusion facilitator; DMT1, divalent cation transporter 1; hZIP, human zrt-, irt-like protein; hZTL, human ZnT-like transporter; ZIP, Zip, zrt-, irt-like protein.

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difficult to assess. It is possible that certain groups of the population, particularly the elderly and possibly pregnant women, may suffer symptoms of marginal Zn deficiency, including compromised immune function (Rink & Gabriel, 2000) and poor outcome of pregnancy (Caulfield *et al*. 1998).

Measurement of zinc transport function in intestine and placenta

Functional studies on intestinal Zn transport and uptake using preparations such as everted rat gut sacs (Seal & Mathers, 1989), isolated vascularly-perfused rat intestine (Smith & Cousins, 1980; Hoadley *et al*. 1987) and intestinal brush-border-membrane vesicles from the small intestine of rat (Menard & Cousins, 1983) and pig (Tacnet *et al*. 1990) demonstrated saturable and non-saturable components of Zn transport, of which the saturable component was inhibited by ouabain, indicating an energy-dependent process (Seal & Mathers, 1989). Studies of Zn^{2+} transport by human intestinal Caco-2 cell monolayers have indicated saturable and non-saturable components, with a Zn concentration at half the maximum flux for the saturable component being in the order of 200 μM (Fleet *et al*. 1993). Although the majority of Zn absorption from an oral dose occurs in the proximal intestine (Lee *et al*. 1989), this phenomenon is probably a function of the increased level of Zn presentation to the epithelial cells in this region. The capacity of rat colon for Zn absorption is at least as high as that of more proximal regions (Seal & Mathers, 1989; Gisbert-Gonzalez & Torres-Molina, 1996; Condomina *et al*. 2002). Increased maximal rates of Zn absorption in rat intestinal preparations after dietary Zn depletion have been widely reported (Smith & Cousins, 1980; Menard & Cousins, 1983; Hoadley *et al*. 1987), in agreement with a stimulation in the transepithelial flux of Zn^{2+} by Caco-2 cell monolayers following treatment with 5 μM-Zn compared with 25 μM-Zn (Reeves *et al*. 2001). Functional studies of placental Zn transport using human placental microvillus border membranes (Aslam & McArdle, 1992; Page *et al*. 1992) and cultured human syncytiotrophoblasts (Bax & Bloxam, 1995) reveal the process to be saturable and temperature dependent.

Free and bound zinc

The high capacity of plasma albumin for binding Zn renders most plasma Zn (total concentration approximately 12 μM) protein bound. Similarly, free intestinal lumen Zn, in the presence of a complex digesta, is likely to represent only a very small proportion of the total. It is generally assumed, but has not been demonstrated, that Zn is transported across cell membranes following release from Zn-binding ligands at the cell surface. Within the cell total Zn concentrations are high, and have been estimated in baby hamster kidney cells by inductively-coupled plasma MS to be of the order of 200 μM (Palmiter & Findley, 1995). However, binding to intracellular protein again limits dramatically the concentration of free Zn. An estimation of the intracellular free Zn concentration based on the sensitivity of metallo-regulatory proteins that control Zn homeostasis in *Escherichia Coli* indicates that the free intracellular Zn concentration is in the femtomolar range, corresponding to less that one free Zn atom per cell (Outten & O'Halloran, 2001). The process by which Zn is transported across polarised cells for release across the basolateral membrane during dietary Zn absorption and placental Zn transfer is unknown. Furthermore, the role, if any, of the low-molecular-weight cystine-rich Zn-binding protein metallothionein in this process remains to be elucidated.

Cloned eukaryotic zinc transporters

The molecular identification of Zn transport proteins from *Saccharomyces cerevisiae*, *Arabidopsis thalania* and mammals led to the recognition of two major eukaryotic families, the cation diffusion facilitator (CDF) and zrt-, irt-like protein (ZIP) families. Members of both families have now been identified at all phylogenetic levels, including prokaryotes (Gaither & Eide, 2001*a*; Palmiter & Huang, 2003). Mammalian CDF proteins have been classified by the Human Genome Organisation Nomenclature Committee as family SLC30 and mammalian ZIP have been classified as family SLC39. The properties and localisation of CDF family members are generally consistent with a role in Zn efflux or intracellular sequestration in vesicles and vacuoles. Several genes in this diverse family, which is defined by a specific sequence motif (Paulsen & Saier, 1997), were cloned as a result of conferring resistance to transition metal toxicity. The CDF family includes ZRC1, which confers Zn resistance in yeast (Kamizono *et al*. 1989), and COT1, a yeast Co transporter (Conklin *et al*. 1992), plus the mammalian proteins Zn transporters (ZnT) 1–7 (SLC30A1–7; Palmiter & Findley, 1995; Palmiter *et al*. 1996*a*,*b*; Huang & Gitschier, 1997; Cragg *et al*. 2002; Huang *et al*. 2002; Kambe *et al*. 2002; Kirschke & Huang, 2003). Most CDF family members share the same predicted transmembrane topology, comprising six membrane-spanning domains with intracellular N- and C-termini and, in the case of eukaryotic members, a histidine-rich intracellular loop that represents a potential Zn-binding region (Paulsen & Saier, 1997; Fig. 1(a)). The ZIP family of transporters comprised initially the *Saccharomyces* proteins ZRT1 (Zhao & Eide, 1996*a*), ZRT2 (Zhao & Eide, 1996*b*) and the *Arabidopsis* Fe transporter IRT1 (Eide *et al*. 1996). The family is now known to include the cloned human ZIP (hZIP) 1, 2, 4, 6, 7 and 8 (SLC39A1, 2, 4, 6, 7 and 8; Gaither & Eide, 2000, 2001*b*; Begum *et al*. 2002; Kury *et al*. 2002; Wang *et al*. 2002; Eide, 2003) from a family of fourteen human SLC39 proteins revealed by analysis of the human genome (Eide, 2003). The ZIP family also includes the *Arabidopsis* transporters Zip1–4 (Grotz *et al*. 1998) from a family in *Arabidopsis thalania* with sixteen members, revealed by database analysis (Gaither & Eide, 2001*a*). Common to most members of this group of transporters appears to be the ability to mediate cellular Zn uptake or release of stored intracellular Zn. Most members of the ZIP family of proteins, from all organisms, share the same predicted topology, eight

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(a)
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hZTL1: 247 QIFYFLCLNLLFTFVELFYGVLTNSLGLISDGFHMLFDCSALVMGLFAALMS-RWKATRI 305
           ++   L L  +F  +E+    +T SL ++SD FHML D  ALV+ L A   + R  AT+
mZnT-1: 10 RLLCMLLLTFMFMVLEVVVSRVTASLAMLSDSFHMLSDVLALVVALVAERFARRTHATQK 69

hZTL1: 306 FSYGYGRIEILSGFINGLFLIVIAFFVFMESVARLIDPPELDTHMLT-PVSVGGLIVNLI 364
            ++G+ R E++   +N +FL  + F + +E+V R I+P E+   ++   V V GL+VN++
mZnT-1:70  NTFGWIRAEVMGALVNAIFLTGLCFAILLEAVERFIEPHEMQQPLVVLSVGVAGLLVNVL 129

hZTL1: 365 GI 367
           G+
mZnT-1:130 GL 131
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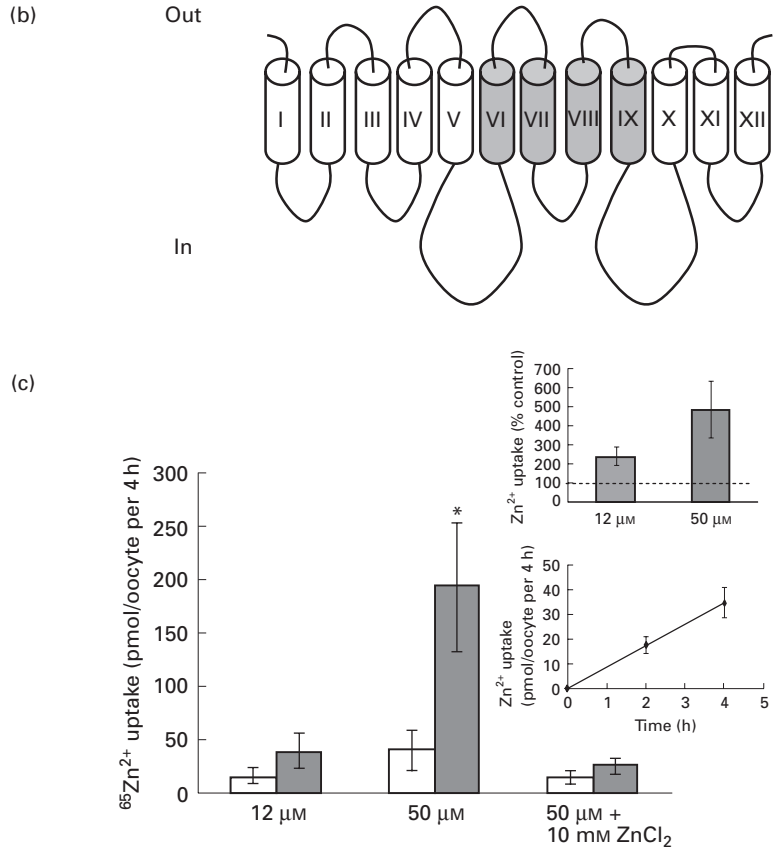

Fig. 1. Sequence and function of the human ZnT-like transporter 1 (hZTL1) (SLC30A5). (a) Alignment of the hZTL1 (SLC30A5) amino acid sequence with the sequence of mouse ZnT1 (Slc30A1). The cation diffusion facilitator signature sequence is underlined. (b) The predicted transmembrane topology of hZTL1. (▩), The region of homology with ZnT1. (c) Uptake of $^{65}Zn^{2+}$ from a solution containing 12 or 50 μM-zinc chloride over 4 h by control (water-injected) oocytes (□) compared with hZTL1-injected oocytes (▩). Values are means with their standard errors represented by vertical bars for pooled data for four experiments for 12 μM-zinc chloride and for three experiments for 50 μM-zinc chloride. Mean value was significantly different from that for the control (Student's *t* test): $*P < 0{\cdot}05$. In all individual experiments uptake by hZTL1-injected oocytes was significantly greater than that by control oocytes ($P < 0{\cdot}05$; Student's *t* test). The inserts show data expressed as a percentage of Zn^{2+} uptake by control oocytes and the time-course for hZTL1-mediated Zn^{2+} uptake by oocytes at 12 μM-zinc chloride. Uptake into water-injected controls has been subtracted. (From Cragg *et al.* 2002.)

membrane-spanning domains with extracytoplasmic N- and C-termini. Conserved histidine residues and polar or charged amino acids that lie close to these histidines in the predicted structure in transmembrane domains IV and V have been shown, in IRT1, to be essential for transport function (Rogers *et al.* 1999).

Zinc transporters involved in dietary zinc uptake

Apical localisation of human ZnT-like transporter 1 in the intestine

The cloning and localisation in the enterocyte of Zn transporters belonging to families SLC30 and SLC39 has

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led to the emergence of two proteins, one from each family (human ZnT-like transporter (hZTL1; SLC30A5) and hZIP4 (SLC39A4)), as having a potential role in the absorption of dietary Zn across the enterocyte apical membrane. Before the cloning of hZTL1 (on the basis of homology to ZnT1 (SLC30A1) at the level of the amino acid sequence; Cragg *et al*. 2002), there was no cloned Zn transporter known to be expressed in the mammalian enterocyte that was localised at this membrane. ZnT1 was shown by immunohistochemistry to be localised to the basolateral membrane of the rat enterocyte (McMahon & Cousins, 1998), a finding confirmed in the human intestinal cell line Caco-2 (RA Cragg and D Ford, unpublished results). Whilst expression of ZnT3 (SLC30A3) in rat is restricted to the brain and testis (Palmiter *et al*. 1996*b*), ZnT2 (SLC30A2) and ZnT4 (SLC30A4), also expressed in rodent intestine, were shown to be expressed at the membrane of intracellular vesicles. Localisation of a green fluorescent protein-tagged rat ZnT2 construct to vesicles in a Zn-sensitive baby hamster kidney cell line, revealed by lysotracker dye and acridine orange staining, indicated expression at the membrane of a lysosomal–endosomal compartment (Palmiter *et al*. 1996*a*). Immunolocalisation of ZnT4 in rat intestine revealed vesicular localisation, concentrated at the basal pole of the differentiated enterocyte, and a *myc*-tagged rat ZnT4 construct in Caco-2 cells was expressed in the membrane of vesicles in a pattern that overlapped partially with the localisation of the transferrin receptor and β-adaptin (Murgia *et al*. 1999). ZIP1 (SLC39A1) emerged initially as a potential candidate for mediating the uptake of Zn across the enterocyte apical membrane, on the basis of Zn uptake activity demonstrated by heterologous expression in K562 erythro-leukaemia cells coupled with plasma membrane localisation of the protein in these cells as demonstrated by expression of an haemagglutinin antigen-tagged construct (Gaither & Eide, 2001*b*). The suggestion that ZIP1 may play a role in Zn uptake across the enterocyte apical membrane was, however, refuted by a study that confirmed plasma membrane localisation in K562 cells, while demonstrating the expression of green fluorescent protein- and FLAG-tagged hZIP1 constructs at the endoplasmic reticulum in a variety of epithelial cell lines, including Caco-2 cells. Localisation in the endoplasmic reticulum was confirmed directly using an anti-hZIP1 antibody in PC-3 prostate cells (Milon *et al*. 2001). Early data indicated a potential role for the apically-localised broad-specificity divalent cation transporter 1 (DMT1) in Zn absorption (Gunshin *et al*. 1997). However, these findings have been subsequently and convincingly refuted by evidence demonstrating that in Caco-2 cells a reduction in the level of DMT1 expression has no effect on Zn uptake, Zn does not compete with Fe for DMT1-mediated uptake and Zn transport, unlike DMT1-mediated metal ion transport, is independent of membrane potential (Tandy *et al*. 2000).

Human ZnT-like transporter 1/ZnT5 structure, activity and subcellular distribution

SLC30A5 appears to exist as two major splice variants. The lower-molecular-weight transcript, hZTL1, was more abundant in all tissues examined (Kambe *et al*. 2002; RM Russi and D Ford, unpublished results) and codes for a protein of 523 amino acids with 34% identity to mouse ZnT1 over a stretch of 122 amino acids (Fig. 1(a)). Unusually for a member of the SLC30 family, the topology predicted by the algorithm TMpred (Hofmann & Stoffel, 1993) is twelve membrane-spanning domains with extracytoplasmic N- and C-termini (Cragg *et al*. 2002; Fig. 1(b)). Transient expression of the corresponding protein with a C-terminal *myc* epitope tag in polarised Caco-2 cells revealed localisation to the apical membrane (Cragg *et al*. 2002), which was subsequently confirmed in human small intestine and also in Caco-2 cells by direct immunohistochemistry (Cragg *et al*. 2003). Furthermore, when expressed in *Xenopus laevis* oocytes hZTL1 mediated the uptake of Zn (Fig. 1(c)) with an affinity in the range consistent with the estimated Zn concentration (100 μM) of the intestinal lumen contents post-prandially (Cragg *et al*. 2002) and with the reported concentration (226 μM) giving the half-maximal rate of saturable Zn^{2+} transport by Caco-2 cell monolayers (Fleet *et al*. 1993). hZTL1-mediated uptake of Zn^{2+} into oocytes was inhibited to a lesser extent than the endogenous oocyte Zn uptake mechanism at pH 5·5 compared with pH 7·6. This finding demonstrates direct action rather than stimulation by hZTL1 expression of endogenous oocyte Zn transport processes. Thus, experimental evidence is consistent with hZTL1 having a role in dietary Zn absorption.

The higher-molecular-weight splice variant of SLC30A5, ZnT5, predicted to comprise fifteen membrane-spanning domains, was found to be expressed in the Golgi complex when transfected into HeLa cells, and it drove increased uptake of $^{65}Zn^{2+}$ into Golgi-enriched vesicles isolated from these cells (Kambe *et al*. 2002). In human pancreatic β cells ZnT5 was associated with Zn-rich insulin-containing secretory granules, where it may play an important role in the import of Zn to store insulin in crystal forms (Kambe *et al*. 2002). Kambe *et al*. (2002) used a monoclonal antibody raised against a peptide that included regions shared by both of the SLC30A5 splice variants and also a region unique to the larger transcript. Cross reactivity with both splice variants has not been established, and there is the potential for the variant corresponding to the larger transcript (ZnT5) specifically to have been detected. The antibody used to demonstrate apical localisation of hZTL1 in human small intestine and Caco-2 cells was polyclonal (Cragg *et al*. 2003) and was raised against a peptide sequence shared by both of the SCL30A5 variants. However, no clear evidence of vesicular staining was observed in human intestine or placenta using this antibody, and the pattern of immunostaining in other tissues has not been investigated. Clarity with respect to the subcellular localisations of these two splice variants of SLC30A5 may be achieved by expressing both proteins as tagged constructs in a variety of cell types.

Human zrt-, irt-like protein 4 (SLC39A4) mutations in acrodermatitis enteropathica

A second gene product localising to the enterocyte apical membrane, hZIP4 (SLC39A4), was identified by two

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research groups (Kury *et al*. 2002; Wang *et al*. 2002) by virtue of its location on chromosome 8 in a region identified previously as being linked to the inherited Zn-deficiency disease acrodermatitis enteropathica, which is characterised by impaired absorption of Zn from the intestine. A variety of mutations in hZIP4 associated with acrodermatitis enteropathica have been reported, indicating that the gene product has an important role in the absorption of Zn from the intestine. Recently, it was reported that expression of the homologous mouse protein in HEK293 cells led to an increased rate of Zn accumulation, demonstrating functional activity consistent with this assumed role (Dufner-Beattie *et al*. 2003).

Other cloned zinc transporters potentially involved in dietary zinc absorption

Of the other Zn transporters known to be expressed in the intestine, the localisation of only ZnT1 (SLC30A1) suggests a role in the absorption of dietary Zn. Basolateral localisation of the protein (McMahon & Cousins, 1998), along with functional evidence for Zn efflux from cells mediated by plasma-membrane-associated ZnT1 (Palmiter & Findley, 1995), indicates that ZnT1 functions in the intestine to efflux absorbed dietary Zn from the enterocyte into the portal circulation. The cloning of additional members of the SLC30 family of Zn transporters has revealed no other potential candidates for Zn transport across the plasma membrane of the enterocyte. ZnT6 (SLC30A6) appears to be expressed at low levels in the mouse intestine, being detected by Northern blotting but not immunoblotting, and was detected by immunohistochemistry in a vesicular compartment plus the trans-Golgi network in cultured normal rat kidney cells (Huang *et al*. 2002). Similarly, ZnT7 (SLC30A7), although detectable in mouse intestine by immunoblotting, was revealed by immunohistochemisty and by expression of a *myc*-tagged construct to be restricted to Golgi and vesicular membranes in a variety of cell types, including normal rat kidney and Chinese hamster ovary cells (Kirschke & Huang, 2003). Of the fourteen SLC39 family members identified by analysis of the human genome sequence, only hZIP2 (SLC39A2), in addition to hZIP1 and 4 (SLC39A1 and 4), has been demonstrated to have functional activity, but appears to be expressed in only the prostate and the uterus (Gaither & Eide, 2000). Whilst hZIP6, 7 and 8 (SLC39A6, 7, and 8) have been cloned (Eide, 2003), it has not yet been demonstrated that any of them are expressed in the intestine, and whilst the subcellular localisation of ZIP6 and ZIP8 is unknown, mouse epitope-tagged Zip7 is expressed in the endoplasmic reticulum in transfected cells (Suzuki & Endo, 2002).

Zinc transport in the placenta

The expression profile of Zn transporters in the placenta is similar to that of the intestine, in that of the SLC30 family ZnT1, 2, 4 and 5 but not ZnT3 are detectable at the mRNA level in human tissue (RM Russi and D Ford, unpublished results). Expression of ZnT6 and 7 in the placenta remains to be examined. hZTL1/ZnT5 was detected by immunohistochemistry in mouse and human placenta (RM Russi and D Ford, unpublished results) at the apical membrane of the placental syncytiotrophoblast, consistent with a fundamental role in the transfer of Zn to the foetus. ZnT1 (SLC30A1)-specific immunoreactivity shows a similar but more punctate pattern in both mouse and human placenta (RM Russi and D Ford, unpublished results), consistent with the reported localisation in the mouse visceral yolk sac (Liuzzi *et al*. 2003), which plays a role in nutrient transfer to the foetus. The functional relevance of this pattern of expression, in the context of a transporter that appears to operate in an efflux mode, is unclear. In relation to the expression of human SLC39 family members, the intestine and placenta both express hZIP1 but there is no evidence for expression of hZIP2 (Gaither & Eide, 2000, 2001*b*). However, whilst ZIP4 is expressed in both human and mouse intestine (Wang *et al*. 2002; Dufner-Beattie *et al*. 2003), the gene is not expressed in human placenta (Wang *et al*. 2002) but is expressed in the mouse visceral yolk sac (Dufner-Beattie *et al*. 2003). The lack of expression in the placenta of hZIP4, which clearly has an important role in the absorption of dietary Zn across the intestine, may suggest that Zn transport by hZTL1/ZnT5 (SLC30A5) is relatively more important in foetal nutrition than in adult nutrition. As for the intestine, the expression of the less-well-characterised cloned members of the SLC39 family, ZIP6, 7 and 8, has not been investigated in the placenta.

The regulation of zinc transport in the intestine and placenta

Regulated intestinal absorption of dietary Zn is believed to be important in Zn homeostasis. Studies in the rat have demonstrated that in the small intestine there is up-regulation of the mRNA corresponding to ZnT1 (SLC30A1) when the Zn concentration of the diet is increased from 30 μg/g to 180 μg/g over 1 (McMahon & Cousins, 1998) and 2 (Liuzzi *et al*. 2001) weeks. The same studies report that the levels of ZnT1 mRNA in the small intestine are not affected when the Zn concentration of the diet is reduced from 30 μg/g to either 5 μg/g (McMahon & Cousins, 1998) or <1 μg/g (Liuzzi *et al*. 2001). In the small intestine the levels of the mRNA species corresponding to ZnT2 (SLC30A2), believed to function in the sequestration of Zn by intracellular vesicles (Palmiter *et al*. 1996*a*), were increased over 2 weeks of moderate Zn supplementation and reduced on severe Zn depletion (Liuzzi *et al*. 2001). In the mouse Zip4 (SLC39A4) mRNA expression was increased in the small intestine in response to dietary Zn deficiency (50 μg/g, reduced to 1 μg/g for 14 d) (Dufner-Beattie *et al*. 2003). The responses of ZIP4 and ZnT2 expression to Zn manipulation may offer molecular mechanistic explanations for functional data demonstrating increased Zn absorption by rat intestinal preparations (Smith & Cousins, 1980; Menard & Cousins, 1983; Hoadley *et al*. 1987) and Caco-2 cells (Reeves *et al*. 2001) following Zn depletion.

In the human intestinal cell line Caco-2 an increase in the Zn concentration of the nutrient medium from 3 μM to 100 μM over 7 d led to increased levels of mRNA species

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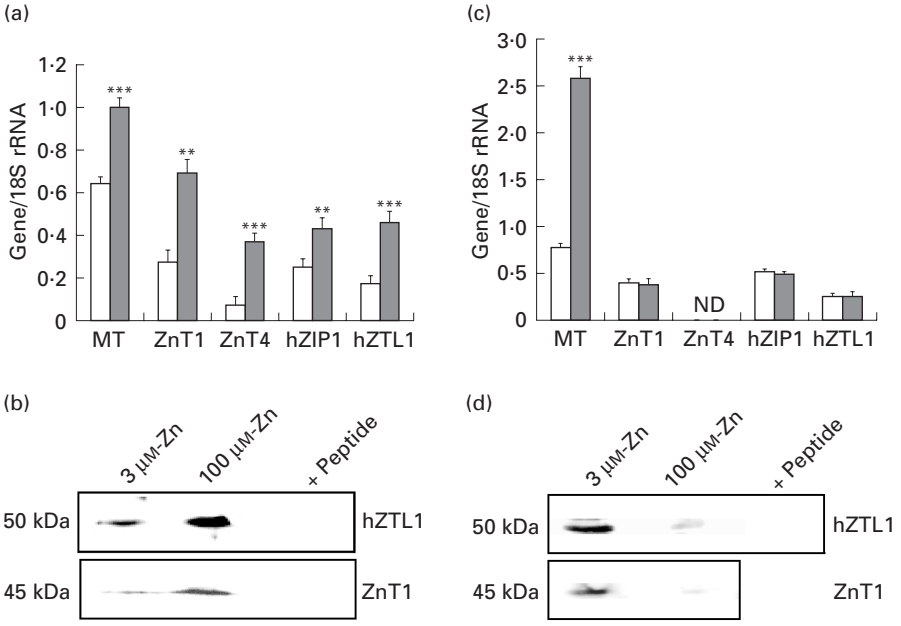

Fig. 2. Regulation of zinc transporter expression in cell line models by zinc. (a, c), Relative levels of transcripts (metallothionein (MT), zinc transporters (ZnT) 1 and 4, human zrt-, irt-like protein 1 (hZIP1), human ZnT-like transporter 1 (hZTL1)) in Caco-2 (a) and JAR (c) cells grown at 3 (□) or 100 μM-Zn (▩). Values, obtained by densitometric analysis of the products of semi-quantitative RT–PCR and normalised against the corresponding signal for 18S rRNA, are means with their standard errors represented by vertical bars for six determinations. Mean values were significantly different from those at 3 μM-Zn (Student's *t* test): $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$. (b, d), Analysis by immunoblotting, using an anti-peptide antibody, of the expression of hZTL1/ZnT5 and ZnT1 in Caco-2 (b) and JAR (d) cells grown at 3 or 100 μM-Zn. For each sample 7 (b) or 15 (d) μg protein, as determined by Bradford analysis, was resolved by SDS-PAGE before blotting.

corresponding to the Zn transporters hZTL1/ZnT5 (SLC30A5; Cragg *et al*. 2002; Fig. 2(a)), ZnT1 (SLC30A1), ZnT4 (SLC30A4) and hZIP1 (SLC39A1; Fig. 2(a)). A parallel change in the expression at the protein level for ZnT1 and hZTL1/ZnT5 has been confirmed in Caco-2 cells by Western blotting (Fig. 2(b)). Surprisingly, a different pattern of regulation of Zn transporter expression by Zn has emerged from studies in the human placental cell line JAR. Whilst an increase in the Zn concentration of the nutrient medium from 3 μM to 100 μM over 7 d resulted in no change in the level of any Zn transporter mRNA examined (Cragg *et al*. 2002; Fig. 2(c)), despite the expected increase in metallothionein mRNA being measured, a decrease in the expression of ZnT1 (SLC30A1) and hZTL1/ZnT5 (SLC30A5) at the higher Zn concentration was detected by Western blotting (Fig. 2(d)). Such a pattern of regulation, assuming a role for hZTL1/ZnT5 and ZnT1 in the placenta in the transfer of Zn to the foetus, may contribute to the maintenance of optimal foetal Zn nutrition despite variation in maternal Zn status. There is a paucity of knowledge about the mechanisms through which Zn might interact with the components of protein translation or degradation to explain this observed reduction in ZnT1 and hZTL1/ZnT5 protein in JAR cells following Zn supplementation. It has been demonstrated that Zn-stimulated degradation of the yeast plasma membrane Zn transporter ZRT1 is mediated through ubiquitination and subsequent endocytosis (Gitan & Eide, 2000). It can be speculated that such a mechanism might underlie the observed response to Zn supplementation of ZnT1 and hZTL1/ZnT5 in JAR cells, but there is no evidence to support this mechanism at present.

A hypothesis to explain the apparent difference in response of ZnT1 and hZTL1/ZnT5 to changes in Zn availability in intestinal and placental cells arises if consideration is given to the normal concentration of total extracellular Zn to which the two different tissues are exposed. The Zn concentration encountered by the placenta will be of the order of the plasma Zn concentration (12 μM), whereas the Zn concentration of the intestinal lumen following a meal is likely to be of the order of 100 μM (Cragg *et al*. 2002). It is possible, therefore, that expression of ZnT1 and hZTL1/ZnT5 in both tissues shows a similar pattern of response to Zn availability but over a different concentration range (Fig. 3). Both tissues may down regulate Zn transport mechanisms to avoid toxic effects at high Zn concentrations, yet require Zn at close to normal physiological levels to express Zn transporters at maximum levels, such that reduced Zn availability leads to reduced levels of expression. Such a hypothesis is consistent with data emerging from our laboratory on the regulation by Zn of these genes in the intestine and the placenta (RM Russi and D Ford, unpublished results). Regulatory mechanisms to explain the reduced expression

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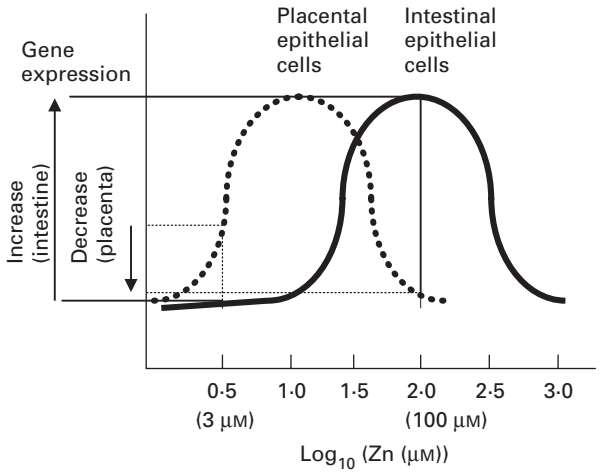

Fig. 3. An hypothesis to explain differential responses of zinc transporter genes in Caco-2 (intestinal; ——) and JAR (placental; ●●●●) cells to zinc concentration. Both intestinal and placental epithelial cells may respond to variation in zinc concentration by down regulating the expression of genes coding for zinc transporters, specifically hZTL1/ZnT5 and ZnT1, at both extremes. The response profile for intestinal cells, however, may be shifted towards higher zinc concentrations, reflecting the increased concentration of zinc that cells lining the gastrointestinal lumen would normally encounter. Thus, an increase in extracellular zinc concentration from 3 μM to 100 μM may result in reduced expression of zinc transporter genes in placental epithelial cells but increased expression in intestinal epithelial cells.

of the genes in response to abnormally high levels of Zn have not yet been described. The increase in transcription, specifically of genes coding for metallothionein and ZnT1 (SLC30A1), mediated by the transcription factor MRE-binding transcription factor 1, however, is a well-characterised response of gene expression to increased Zn availability (Heuchel *et al*. 1994; Langmade *et al*. 2000).

Future directions

Whilst the association of mutations in hZIP4 (SLC39A4) with acrodermatitis enteropathica clearly implicates a role for this protein in dietary Zn absorption, a role for the other apically-localised Zn transporter hZTL1 (SLC30A5) in dietary Zn uptake remains to be proven. Even with hZIP4 mutations leading to truncation of the protein that would undoubtedly render it inactive, patients with acrodermatitis enteropathica absorb a proportion of dietary Zn, which indicates that at least one other transporter must be involved in Zn uptake across the enterocyte apical membrane. Examination of the Zn transport function of Caco-2 monolayers from which the expression of each of these two transporters is separately eliminated will indicate the relative importance of each transporter in apical Zn uptake. Establishing the mode of regulation, subcellular localisation and function of SLC30A5 splice variants will make an important contribution to the understanding of intestinal and Zn transport mechanisms. Comparing the global response of the genome and proteome in Caco-2 and JAR cells to changes in Zn availability over a range of concentrations will establish whether both cell types have a similar, but offset, response profile in terms of the expression of Zn transporters, and will indicate whether any such shift in sensitivity to Zn concentration extends to other genes.

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