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Expression of the ZNT (SLC30) family members in the epithelium of the mouse prostate during sexual maturation

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Abstract A prostate contains ~10-fold higher zinc than other soft organs. The function of the prostate is to produce a zinc-enriched seminal fluid. To establish a protein expression profile for zinc transporters involved in zinc efflux and intracellular sequestration/storage in the mouse prostate during sexual maturation, ZNT expression were investigated by immunohistochemistry. Our study demonstrated that ZNT proteins were differentially expressed in the prostate during sexual maturation. ZNT1 was mainly detected on the lateral membrane of the epithelium. Other ZNTs examined resided intracellularly. Among differences were a staining of ZNT2/ZNT5 in the ER-rich area of the epithelium in the anterior lobe, a staining of ZNT2 along the lateral and apical membrane, a luminal border staining of ZNT4, a staining of ZNT5 in the Golgi area of the epithelium in the ventral lobe, a uniform expression of ZNT6 across the lobes and ages, and a staining of ZNT7 in all lobes across ages.

Keywords Zinc transporters · Slc30 family · ZNT · Mouse prostate · Male genital tract · Cellular zinc accumulation

Introduction

Zinc is an essential trace element for survival of mammalian species. Severe dietary deficiency leads to growth stunting, alopecia, dermatitis, and depressed immune competence (Black 1997). The level of zinc in the body is regulated through tight homeostatic controls. It is accomplished through both uptake and efflux systems in the cell by two solute-linked carrier gene families referred to as *Slc30* (*Znt*) and *Slc39* (*Zip*). Eight ZNT family members (ZNT1–8) (Chimienti et al. 2004; Cragg et al. 2002; Huang and Gitschier 1997; Huang et al. 2002; Kambe et al. 2002; Kirschke and Huang 2003; Palmiter et al. 1996a, b; Palmiter and Findley 1995) and eight ZIP (ZIP1–7, ZIP14) (Gaither and Eide 2000, 2001; Kelleher and Lonnerdal 2005; Taylor et al. 2004; Wang et al. 2002) family members have been characterized. Transmembrane domains are present in both families with the locations of N- and C-terminal regions of ZNT proteins on the intracellular side while ZIP protein's terminal regions are on the extracellular side (Eng et al. 1998; Palmiter and Huang 2004). ZNT proteins function to decrease the cytoplasmic zinc through sequestration of zinc into intracellular organelles of the cell or efflux of zinc to the extracellular space (Kirschke and Huang 2003; Liuzzi et al. 2001; Palmiter and Huang 2004). ZIP proteins increase the cytoplasmic zinc by zinc uptake at the cell surface or release of stored zinc from intracellular compartments within the cell (Dufner-Geattie et al. 2003a, b; Franklin et al. 2003; Huang et al. 2005).

Znt1 (*Slc30a1*) was discovered when a rat cDNA from kidney was able to rescue zinc-sensitive baby hamster kidney (BHK) cells in high zinc conditions (Palmiter and Findley 1995). ZNT1 was shown to reside on the plasma membrane along the basolateral membrane of cells

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(McMahon and Cousins 1998; Sekler et al. 2002; Yu et al. 2007). *Znt2* (*Slc30a2*) was discovered in the same manner as *Znt1* (Palmiter et al. 1996a). ZNT2 is localized to vesicles resembling late endosomes and lysosomes (Eng et al. 1998; Falcón-Pérez and Dell'Angelica 2007; Palmiter et al. 1996a). *Znt3* (*Slc30a*) was found using the rat *Znt2* cDNA to screen a mouse genomic λ library (Palmiter et al. 1996b). ZNT3 is predominantly expressed in brain and it is localized to the synaptic vesicles in neurons (Palmiter et al. 1996b). Over-expression of ZNT3 enhanced vesicular zinc accumulation as indicated by the increased zinquin fluorescence (Palmiter et al. 1996b). *Znt4* (*Slc30a4*) was discovered during positional cloning of the mouse mutant gene *pallid* (Huang and Gitschier 1997). ZNT4 is localized to intracellular vesicles and the *trans*-Golgi network in normal rat kidney cells, human breast epithelia, and human colon epithelia (Huang et al. 2002; Michalczyk et al. 2002; Murgia et al. 1999). In human fibroblastoid cells (M1), green fluorescent protein (GFP)-tagged ZNT2, ZNT3, and ZNT4 are localized to the late endosome and lysosome compartments as indicated by the overlapping expression with LAMP1 (Falcón-Pérez and Dell'Angelica 2007). *Znt5* (*Slc30a5*) was discovered in a EST database search for homologs/orthologs to *Znt1* and *zrc1*, a yeast ortholog of *Znt* genes (Cragg et al. 2002; Kambe et al. 2002). Human ZNT5 was found to have two splicing variants with different subcellular localizations. The variant A is localized to the Golgi apparatus and the variant B is localized throughout cell including plasma membrane when tagged with the GFP in Chinese hamster ovarian cells (CHO) (Cragg et al. 2002; Jackson et al. 2007). The ZNT5B variant is the first ZNT protein shown to be involved in raising the cytoplasmic zinc in the cell (Valentine et al. 2007). *Znt6* (*Slc30a6*) and *Znt7* (*Slc30a7*) were identified using the ZNT4 and ZNT1 amino acid sequences to search the EST database, respectively (Huang et al. 2002; Kirschke and Huang 2003). Both ZNT6 and ZNT7 are localized to the Golgi apparatus and vesicular compartments in cultured cells, such as normal rat kidney cells, rat intestinal epithelial cells, mouse choroid epithelial cells, and human lung fibroblasts (Chi et al. 2006; Huang et al. 2002; Kirschke and Huang 2003). The vesicular compartment staining of ZNT6 overlapped with that of transferrin receptor (Huang et al. 2002). Over-expression of ZNT7 in CHO cells increased zinc accumulation in the Golgi apparatus as evidenced by the enhanced zinquin fluorescence (Kirschke and Huang 2003). *Znt8* (*Slc30a8*) was identified by a search of the human EST database with the amino acid sequences of all known human ZNT proteins at the time (Chimienti et al. 2004). ZNT8 is mainly expressed in the pancreas and it is co-localized with insulin within the β -cell. In insulinoma cells (INS-1E cells), over-expression of a GFP-tagged ZNT8 led to zinc accumulation in

granules near the plasma membrane of the cell (Chimienti et al. 2004, 2006).

The prostate is an androgen-dependent secretory gland of the male genital tract. It produces a bulk of zinc-enriched seminal fluids. At sexual maturity, the mouse prostate gland consists of four histologically distinct lobes referred as the anterior or coagulating, ventral, dorsal, and lateral prostate (Marker et al. 2003). The latter two are usually referred to as dorsolateral prostate. These individual lobes are surrounded by a thin stromal layer consisting of spindle cells and collagen fibers. The glands have intervening nerve bundles and loose connective tissue. The human prostate consists of the peripheral zone, central zone, and transition zone surrounded by stroma and capsule to form a single lobe. Both mouse and human prostatic ducts consist of three major cell types: luminal secretory epithelial cells, basal epithelial cells, and stromal smooth muscle cells. Other less common cell types include neuroendocrine cells and stem cell candidates (Marker et al. 2003). Gene expression profiles have provided evidence that the mouse dorsolateral lobe mimics the human peripheral lobe (Berquin et al. 2005). This is also the region for the origin and pathogenesis of prostate cancer in humans and mouse prostate cancer models. Previous studies have also demonstrated that the mouse prostate and the peripheral zone of the human prostate contain 4–10 times more cellular zinc than any other tissue in the body (Costello and Franklin 1998; Kerr et al. 1960; Mawson and Fischer 1952). A persistent characteristic of prostate cancer is the decline of zinc levels in the luminal epithelial cells to that of other tissues in the body (Costello and Franklin 1998, 2000; Zaichick et al. 1997). Although zinc is an important factor of prostate biology and pathology, the exact roles of zinc and its regulation in the normal and malignant prostatic glands are not understood. Two zinc transporters have been implicated in the reduction of cellular zinc levels during pathogenesis of prostate cancer. ZIP1, a major zinc uptake protein in the prostate, and ZNT4 were shown to be down-regulated in the malignant epithelial cells leading to decrease of zinc and citrate levels in these cells (Beck et al. 2004; Franklin et al. 2005).

Given that (1) zinc is highly accumulated in the prostate, (2) the expression and regulation of zinc transporters are affected in human prostate cancer, and (3) mice are being utilized for human prostate cancer models, it was of the greatest interest to study the expression patterns of zinc transporters that are involved in cellular zinc accumulation in the normal secretory epithelium of the mouse prostate. Prostate branching morphogenesis in mice continues until 5 weeks of age at which time the prostate is considered developmentally mature but not sexually mature. The prostate continues to expand in both glandular and luminal diameters until 7 weeks (Singh et al. 1999). In the present

study, we examined the expression patterns and cellular localization of ZNT1, 2, 4, 5, 6, and 7 in the mouse prostate at the pre and middle of the puberty (4- and 6-week-old) and at the sexual maturity (8-week-old) using immunohistochemistry. We did not examine ZNT3 and ZNT8 expression in the prostate as the expression has been demonstrated being tissue-specific (Chimienti et al. 2004, 2006; Palmiter et al. 1996b). It is expected that our results will lay a foundation to the understanding of how a high level of zinc being achieved and maintained in the secretory epithelial cells of the prostate during sexual maturation.

Materials and methods

Mice

In house bred C57BL/6 mice were housed in a temperature-controlled room at 22–24°C with a 12 h light:dark cycle and fed a standard laboratory chow diet (picoLab[®] mouse diet 20) and double-distilled water ad libitum. Mice were killed at 4, 6, and 8 weeks by cardiac puncture while anesthetized by intraperitoneal injection of 250 mg/kg Trimbromoethanol (Avertin[®], Aldrich, St. Louis, MO). Animal experiments were conducted in accordance with NIH guidelines for the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee of the University of California at Davis.

Preparation of tissue

The entire complex consisting of bladder, urethra, prostate, and seminal vesicles was dissected out of mice and placed in a 4% paraformaldehyde solution made in 1× PBS, pH 7.4 overnight. Tissues were then rinsed in 1× PBS for 30 min at room temperature (RT) followed by 40 min in 80% FLEX (Richard-Allan Scientific, Kalamazoo, MI) and placed at 4°C until processed. Dehydration steps consisted of 95% FLEX for 20 min twice, 100% FLEX 20 min twice, and Clear-Rite 3 (Richard-Allan Scientific) 15 min twice. Tissues were infiltrated and embedded in Type 6 paraffin (Richard-Allan Scientific) at 58°C with 3 rotations and the last rotation was under vacuum pressure of 20 in.Hg for 30 min. Tissue sections in 5-μm thickness were cut, mounted on positive-charged slides, and dried first at 42°C for 30 min and then at 58°C for 45 min.

Immunohistochemistry

Tissue sections were deparaffinized with Xylene and rehydrated using FLEX alcohols. Tissue antigen retrieval

was accomplished in 0.1 M Tris-Cl, pH 10, at 95°C for 20 min. The endogenous peroxidase activity was eliminated by treating sections with 5% H₂O₂ at RT for 15 min. The non-specific binding of biotin/avidin was blocked using an Avidin–Biotin Blocking kit purchased from Vector Laboratories (Burlingame, CA). Tissues were then treated with 2% mouse and 2% goat serum in 1× PBS, pH 7.4, to block non-specific protein bindings. Affinity purified antibodies were diluted in 1× PBS, pH 7.4, containing 2% mouse and 2% goat sera as follows: ZNT1 1:500, ZNT2 1:250, ZNT4 1:5, ZNT5 1:75, ZNT6 1:25, ZNT7 1:900. Primary antibodies were applied to the sections and incubated overnight at 4°C. Immunoperoxidase staining was accomplished using a Vectastain ABC kit and a DAB substrate (Vector Laboratories). Sections were covered with coverslips using Permount mounting medium (Fisher, Pittsburgh, PA).

Antibodies

Rabbit anti-ZNT1, anti-ZNT4, anti-ZNT5, anti-ZNT6, and anti-ZNT7 polyclonal antibodies were raised against synthetic peptides and were affinity purified as described previously (Huang et al. 2002; Kirschke and Huang 2003; Yu et al. 2007). Rabbit anti-ZNT2 antibody was kindly given by Dr. Shannon L. Kelleher (the Pennsylvania State University, PA) (Chowanadisai et al. 2006).

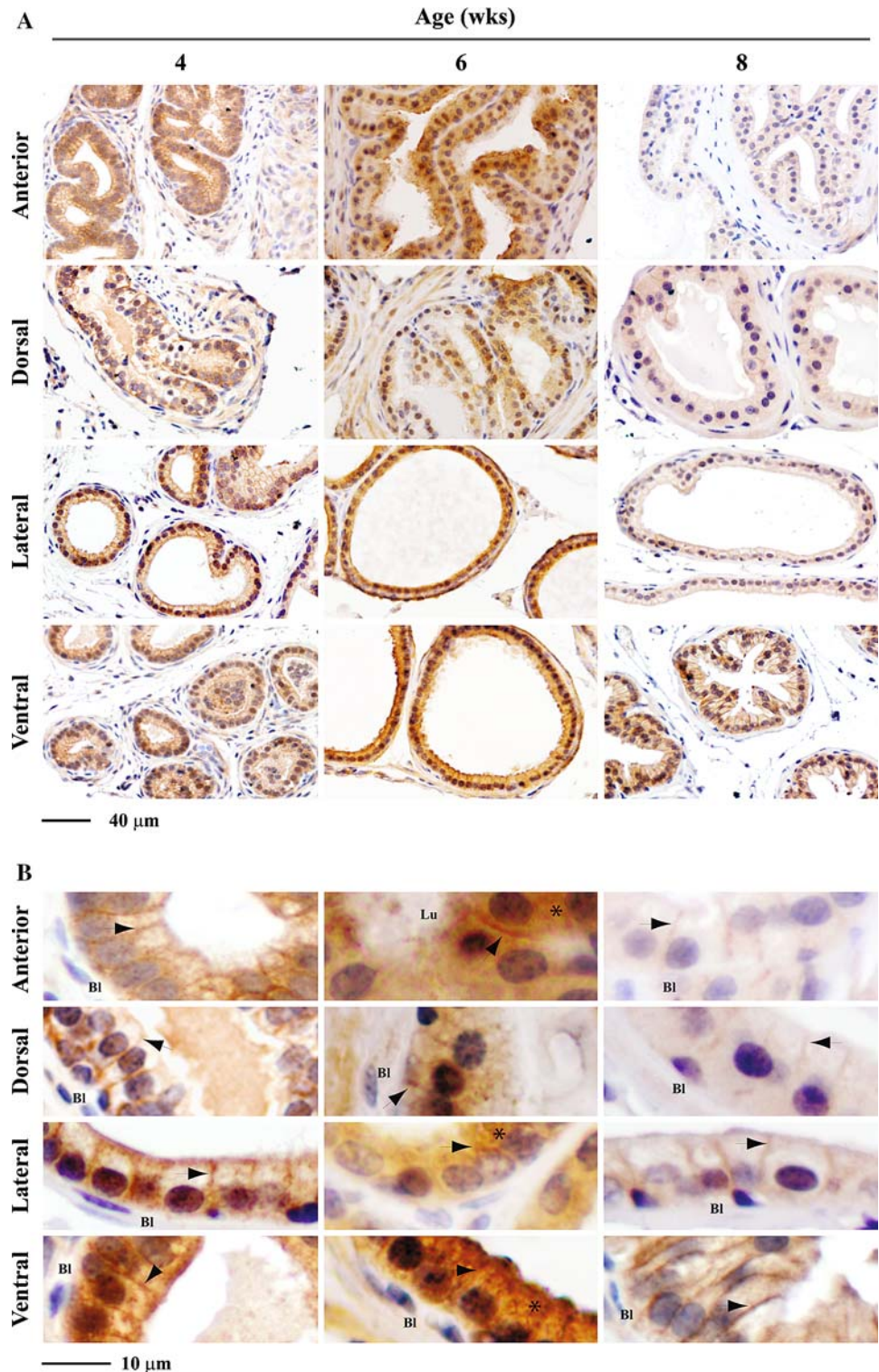
Results

To determine ZNT expression profiles in the epithelium of the anterior, dorsal, lateral, and ventral lobes of the prostate during sexual maturation of C57BL/6 mice, we performed immunohistochemical analysis in prostate tissues isolated from 4, 6, and 8-week-old mice. To control for inter-animal variations, three age-matched mice were analyzed for each time point. We did not observed obvious variations for the expression of the ZNT proteins examined for the same lobe at a specific age point across animals. Figures 1–6 of this report present the protein expression profiles from representative immunohistochemical results at different time points investigated. All control sections were free of immunostaining using the pre-immune sera isolated from the rabbits that the specific antibodies (ZNT1, 2, and 4–7) were raised from (data not shown).

ZNT1 expression

ZNT1 expression was detected in the epithelium across all lobes during the sexual maturation. However, the intensity of ZNT1 immunoreactivity reduced at 8 weeks, suggesting

Fig. 1 Immunohistochemical (IHC) localization of ZNT1 in the mouse prostate at 4–8 weeks. Immunoreactive ZNT1 was mainly observed in the lateral membrane of the epithelium of the prostate including anterior, dorsal, lateral, and ventral lobes (arrows). Expression of ZNT1 was also detected intracellularly in the epithelium of the prostate at 6 weeks of age (stars). Expression of ZNT1 decreased in the epithelium of prostate at 8 weeks when mice were sexually matured. Bl indicates the basal side of the epithelium. Lu indicates the luminal space of the prostatic duct. Bar = 40 and 10 μ m in the panels A and B, respectively

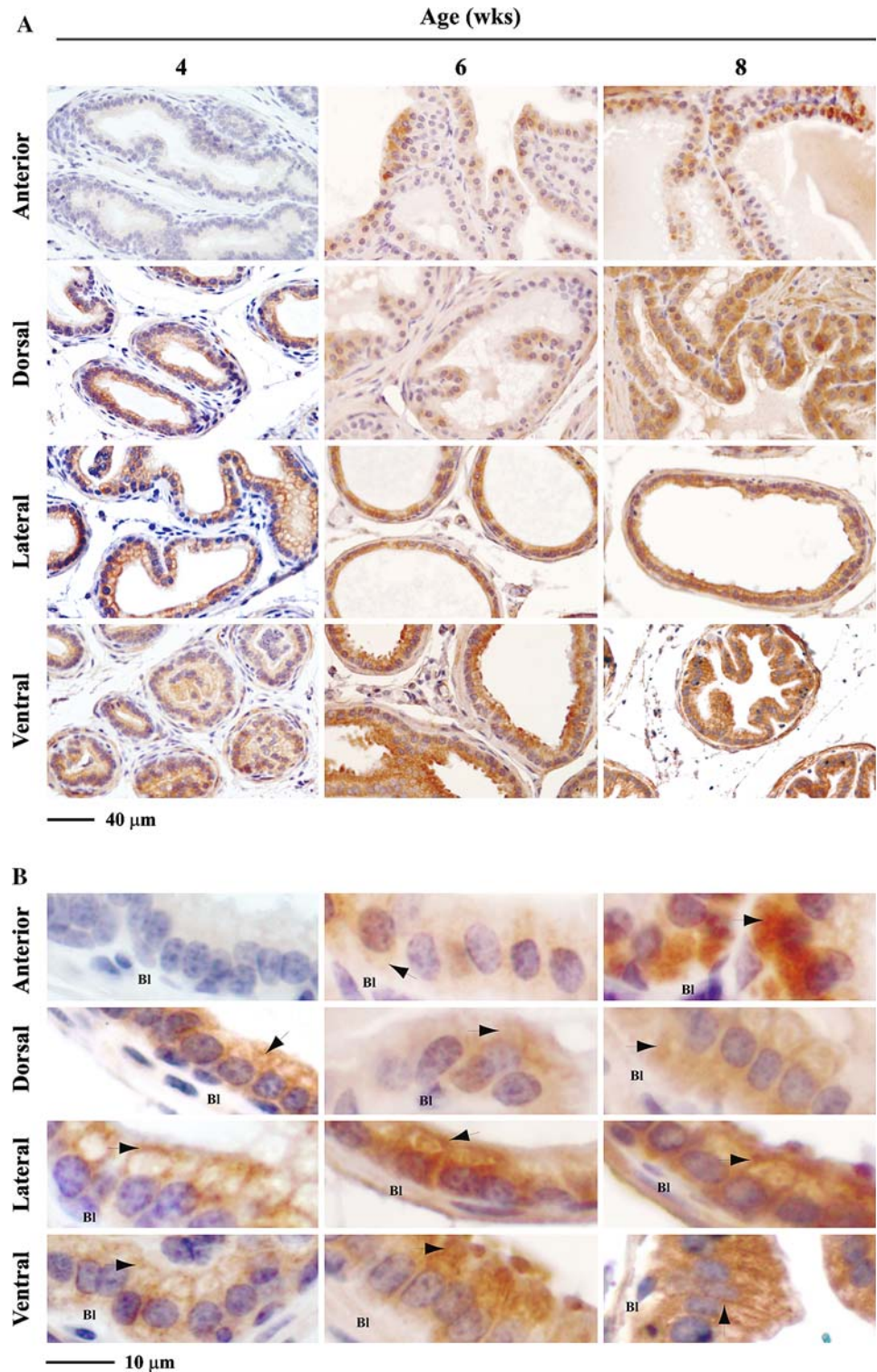


that ZNT1 might play a role in early prostate development in the epithelium of all prostatic lobes (Fig. 1). ZNT1 was mainly expressed on the lateral membrane of the epithelium of all lobes at 4, 6, and 8 weeks of age; but it was also seen in the cytoplasm of the epithelium of all prostatic lobes at 6 weeks (Fig. 1B).

ZNT2 expression

ZNT2 expression increased with age in the anterior lobe. As shown in Fig. 2, ZNT2 was completely absent from epithelial cells in the anterior lobe at 4 weeks. It was weakly stained in the perinuclear region of epithelial cells

Fig. 2 IHC localization of ZNT2 in the mouse prostate at 4–8 weeks. Expression of ZNT2 gradually increased from 4 to 8 weeks of age in the epithelium of the anterior prostate. Arrows indicate the immunoreactive ZNT2. The staining pattern of ZNT2 in the anterior prostate is different from that in the dorsal, lateral, and ventral lobes that display similar staining patterns. Bl indicates the basal side of the epithelium. Bar = 40 and 10 μ m in the panels A and B, respectively



in the anterior lobe at 6 weeks (Fig. 2B). However, at 8 weeks, strong ZNT2 immunoreactivity was detected in the cytoplasm of epithelial cells between the nucleus and basal membrane of the cell. On the other hand, expression of ZNT2 was constant in the dorsal, lateral, and ventral lobes across the ages examined (Fig. 2A). At a higher

magnification, ZNT2 immunoreactivity was observed along the lateral and apical membranes of the epithelium of the dorsal, lateral, and ventral prostates, which was more evident in the lobes at 4 weeks (Fig. 2B). A cytoplasmic staining of ZNT2 was also observed in the dorsal, lateral, and ventral lobes at 6 and 8 weeks. Moreover, a

homogenous ZNT2 staining was seen in the spheres arising from the apical cytoplasm of epithelial cells in the ventral prostate at 6 weeks.

ZNT4 expression

In the epithelium of the anterior and dorsal lobes, expression of ZNT4 increased with age. At 4 weeks, ZNT4 was stained as punctuate particles near the luminal border in the anterior lobe whereas ZNT4 was not detectable in the dorsal lobe (Fig. 3). At 6 and 8 weeks, ZNT4 staining was predominantly located in the region between the nucleus and luminal border of epithelial cells that corresponded with the location of the Golgi apparatus (Brandes and Portela 1960a) (Fig. 3B). In addition, ZNT4 was detected along the luminal border of the epithelium of the prostate (Fig. 3B). No obvious change in the intensity of ZNT4 immunoreactivity in the epithelium of the lateral and ventral lobes was observed (Fig. 3A). ZNT4 expression was mainly detected in the region between the nucleus and luminal border of epithelial cells; but it was also seen in the cytoplasm between the nucleus and basal membrane of the cell (Fig. 3B).

ZNT5 expression

In the epithelium of the anterior lobe, the immunoreactivity for ZNT5 was much weaker at 4 weeks than that at 6 and 8 weeks (Fig. 4). However, the staining pattern was similar at different ages in which the cytoplasmic localization was detected between the nucleus and basal membrane of the cell. In the epithelium of the dorsal lobe, the staining pattern of ZNT5 shifted as mice aged. At 4 weeks, ZNT5 was predominantly localized in the cytoplasm along the membrane in cell–cell contacts. At 6 weeks, ZNT5 immunoreactivity became more homogenous in epithelial cells. At 8 weeks, it mainly resided in the cytoplasm between the nucleus and basal membrane of the cell (Fig. 4B). In the lateral lobe, ZNT5 immunoreactivity was detected in the cytoplasm of epithelial cells except for the region where the Golgi apparatus located (Brandes and Portela 1960a) (Fig. 4B). In the ventral lobe, ZNT5 was predominantly distributed in the cytoplasm between the nucleus and luminal border of epithelial cells with enriched staining spots.

ZNT6 expression

ZNT6 was expressed in all prostatic lobes at all ages examined. However, its expression pattern was different among the lobes (Fig. 5). In the anterior lobe, ZNT6 was predominantly localized in the cytoplasm between the nucleus and luminal border (Fig. 5B). In the dorsal, lateral,

and ventral lobes, ZNT6 immunostaining was more ubiquitous in epithelial cells; but stronger staining was detected towards to the lateral cytoplasm of the cell (Fig. 5B). A Golgi complex staining pattern was also seen in epithelial cells, which was most obvious in epithelial cells at 4 weeks (Fig. 5B).

ZNT7 expression

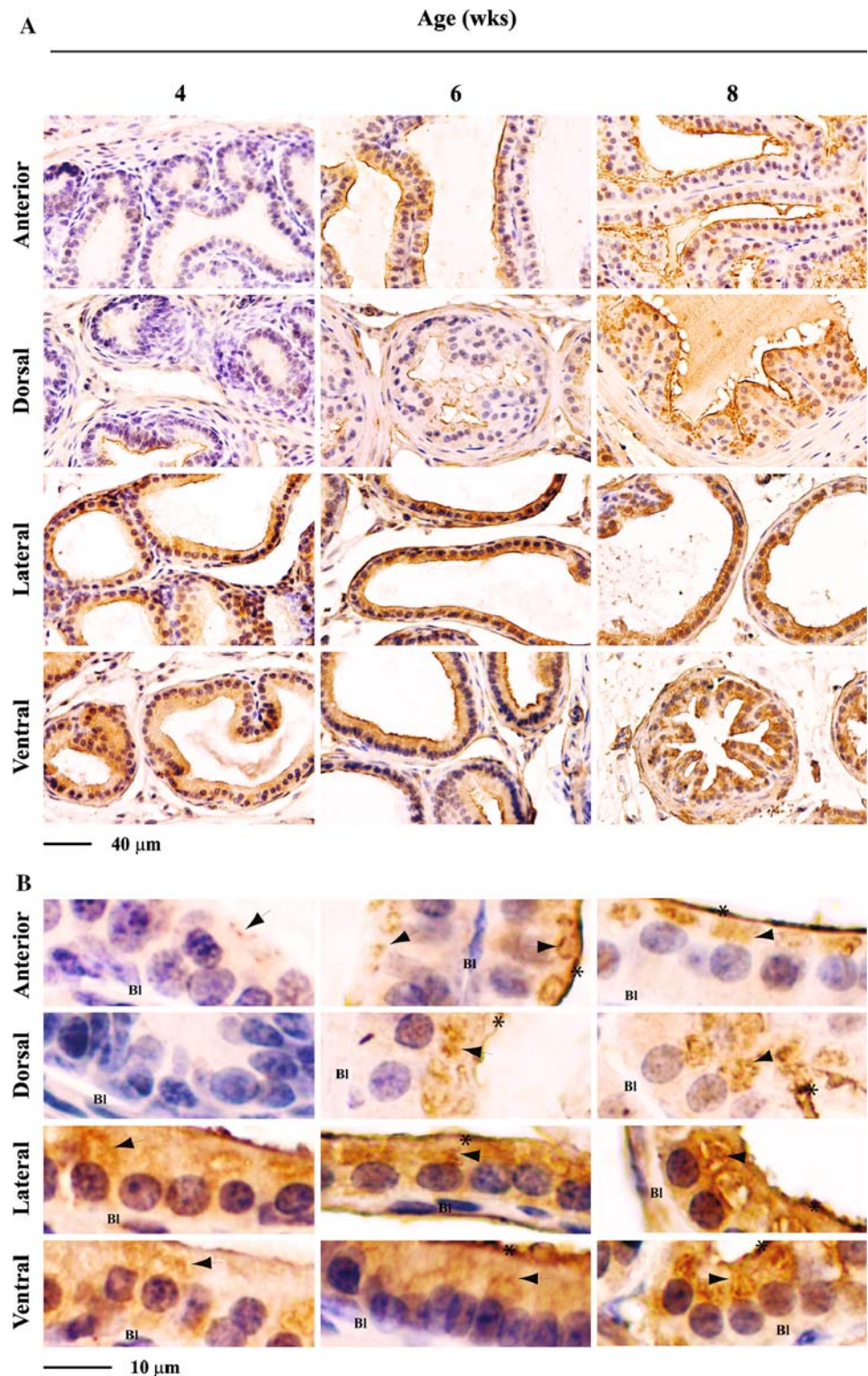
ZNT7 was mostly expressed in all lobes at 6 weeks and least expressed in these lobes at 4 weeks (Fig. 6). In the anterior, dorsal, and lateral lobes, ZNT7 was enriched in the apical cytoplasm of epithelial cells with a Golgi complex staining pattern (Fig. 6B). In the ventral lobe, ZNT7 was barely detected at 4 weeks. However, at 6 and 8 weeks, ZNT7 was strongly expressed in both apical and basal cytoplasm of epithelial cells. ZNT7-positive fragments pinching-off the apical cytoplasm were seen in the epithelium of the ventral lobe (Fig. 6B).

Discussion

It is known that normal prostatic epithelial cells accumulate almost 10-times more cellular zinc than other soft tissues, such as liver and kidney (Costello and Franklin 1998). Maintenance of a high zinc level in the prostatic epithelium and secretion of zinc into the prostatic fluid are essential functions of the prostatic secretory epithelial cells for male fertility (Canale et al. 1986; Kvist et al. 1987). In addition, loss of cellular zinc accumulation in the prostatic epithelial cells has been implicated in the development of prostate cancer (Costello and Franklin 2006; Huang et al. 2006). Members of the *Slc30* zinc transporter family are known to facilitate cellular zinc storage and export in mammalian cells (Palmiter and Huang 2004). Except for ZNT1, which has been shown residing on the plasma membrane of many mammalian cells (Liuzzi et al. 2001; Palmiter and Findley 1995; Yu et al. 2007), other ZNT proteins (ZNT2–8) are intracellularly localized and function to sequester the cytoplasmic zinc into intracellular organelles (Chimienti et al. 2004; Falcón-Pérez and Dell’Angelica 2007; Huang and Gitschier 1997; Huang et al. 2002; Kambe et al. 2002; Kirschke and Huang 2003; Palmiter et al. 1996a, b). The study reported herein describes the temporal and anatomical patterns of ZNT expression in the epithelium of the mouse prostate during sexual maturation.

ZNT1, a protein essential for mouse embryonic development (Andrews et al. 2004), was expressed in all lobes of the prostate during sexual maturation of mice and was mainly localized on the lateral membrane. However, the expression levels were reduced in the prostatic lobes

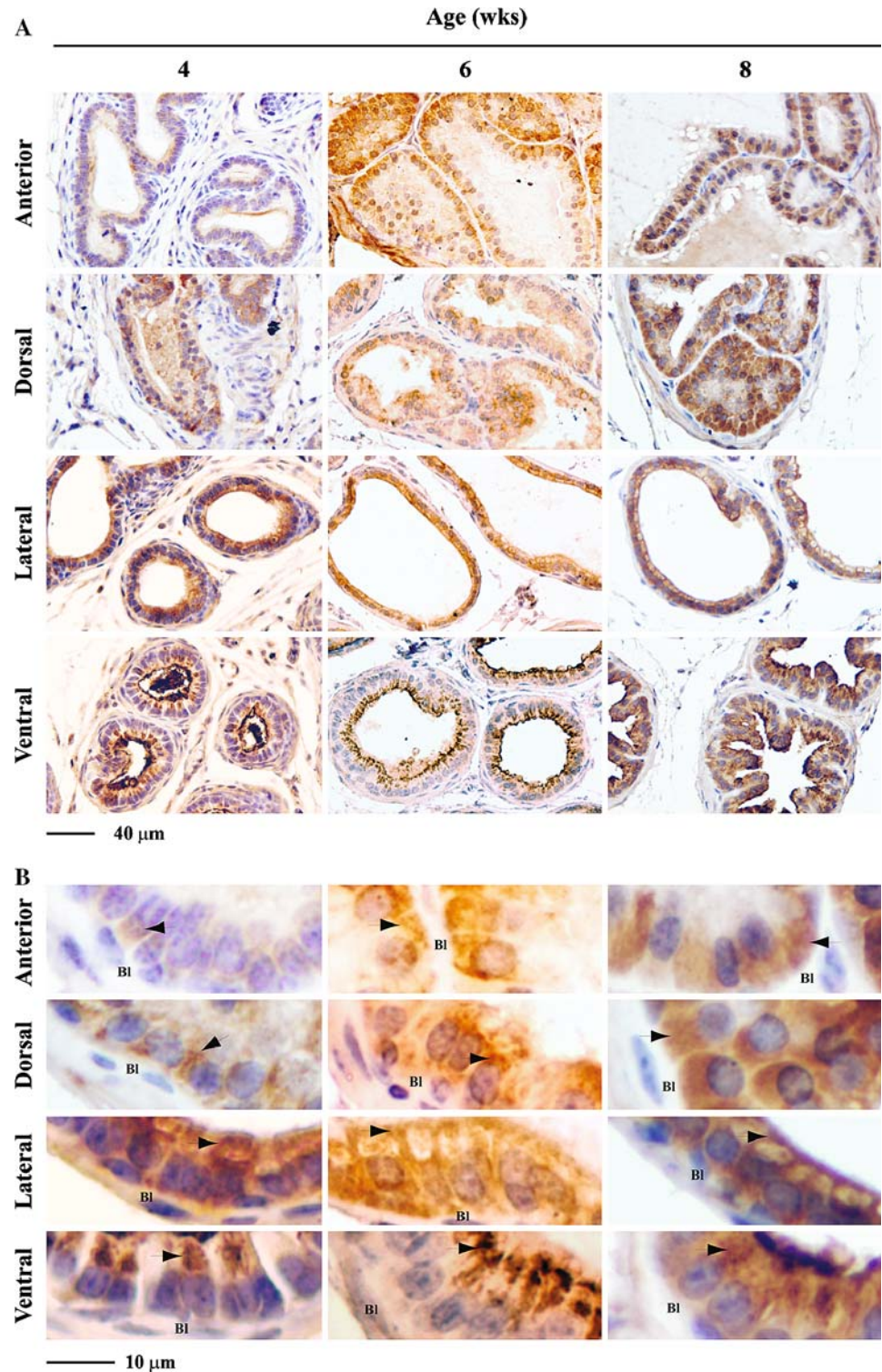
Fig. 3 IHC localization of ZNT4 in the mouse prostate at 4–8 weeks. Expression of ZNT4 gradually increased from 4 to 8 weeks of age in the epithelium of the anterior and dorsal lobes. Arrows indicate the immunoreactive ZNT4. Immunoreactive ZNT4 was also detected along the luminal border of the epithelium of the prostate (stars). Bl indicates the basal side of the epithelium. Bar = 40 and 10 μ m in the panels A and B, respectively



when mice reached sexual maturity at 8 weeks. This expression pattern is in a good agreement with the function of prostatic secretory epithelial cells, which are to accumulate zinc in cells and to secrete enormous amounts of zinc into the lumen of the prostate through the

secretory pathway as the prostate is sexually matured. The high expression of ZNT1 in the developing epithelium of the prostate suggests that ZNT1 may have an important role in promoting epithelial growth and/or morphogenesis during prostate development.

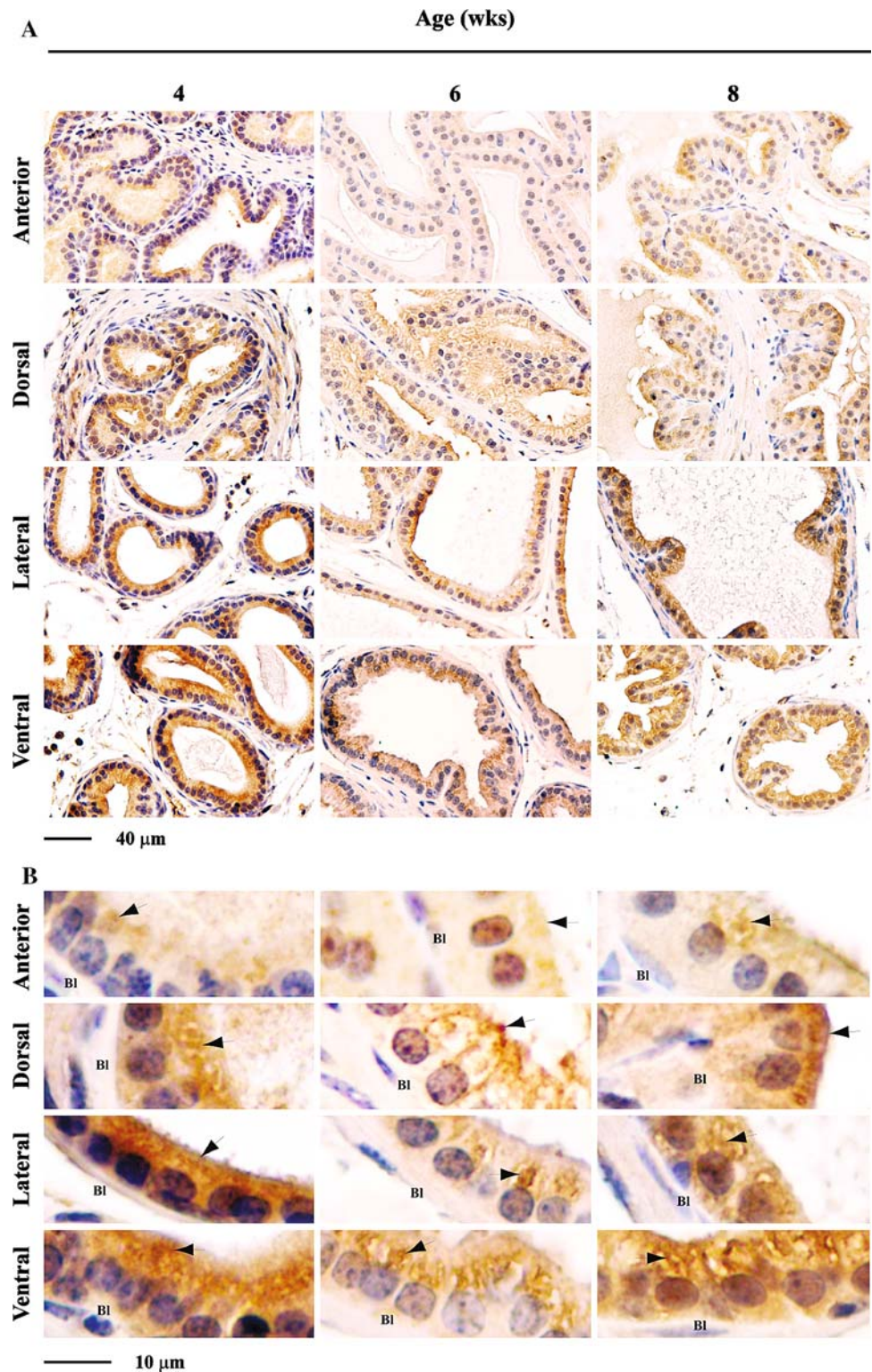
Fig. 4 IHC localization of ZNT5 in the mouse prostate at 4–8 weeks. Expression of ZNT5 gradually increased from 4 to 8 weeks of age in the epithelium of the anterior and dorsal lobes. Arrows indicate the immunoreactive ZNT5. The staining pattern of ZNT5 is different among the lobes. ZNT5 is predominantly localized in the basal part of the cytoplasm of epithelial cells in the anterior and dorsal lobes at 8 weeks whereas ZNT5 displays a staining throughout epithelial cells but the Golgi-rich area in the lateral lobe. In the ventral lobe of the prostate, ZNT5 immunoreactivity is largely detected in the Golgi-rich area of epithelial cells. Bl indicates the basal side of the epithelium. Bar = 40 and 10 μ m in the panels A and B, respectively



It has been proposed that both ZNT2 and ZNT4 are involved in zinc homeostasis of late endocytic organelles (late endosomes and lysosomes) in the cell (Falcón-Pérez and Dell'Angelica 2007; Huang and Gitschier 1997; Palmiter et al. 1996a). Therefore, it is anticipated that the expression of ZNT2 and ZNT4 would be somewhat unique

in the temporal expression pattern but overlapping in the cellular localization in a given tissue. In the mouse prostate, both ZNT2 and ZNT4 expression were increased as mice aged (4–8 weeks). However, their expressing locations in the cell were different among the prostatic lobes. Expression of ZNT2 appeared to be developmentally

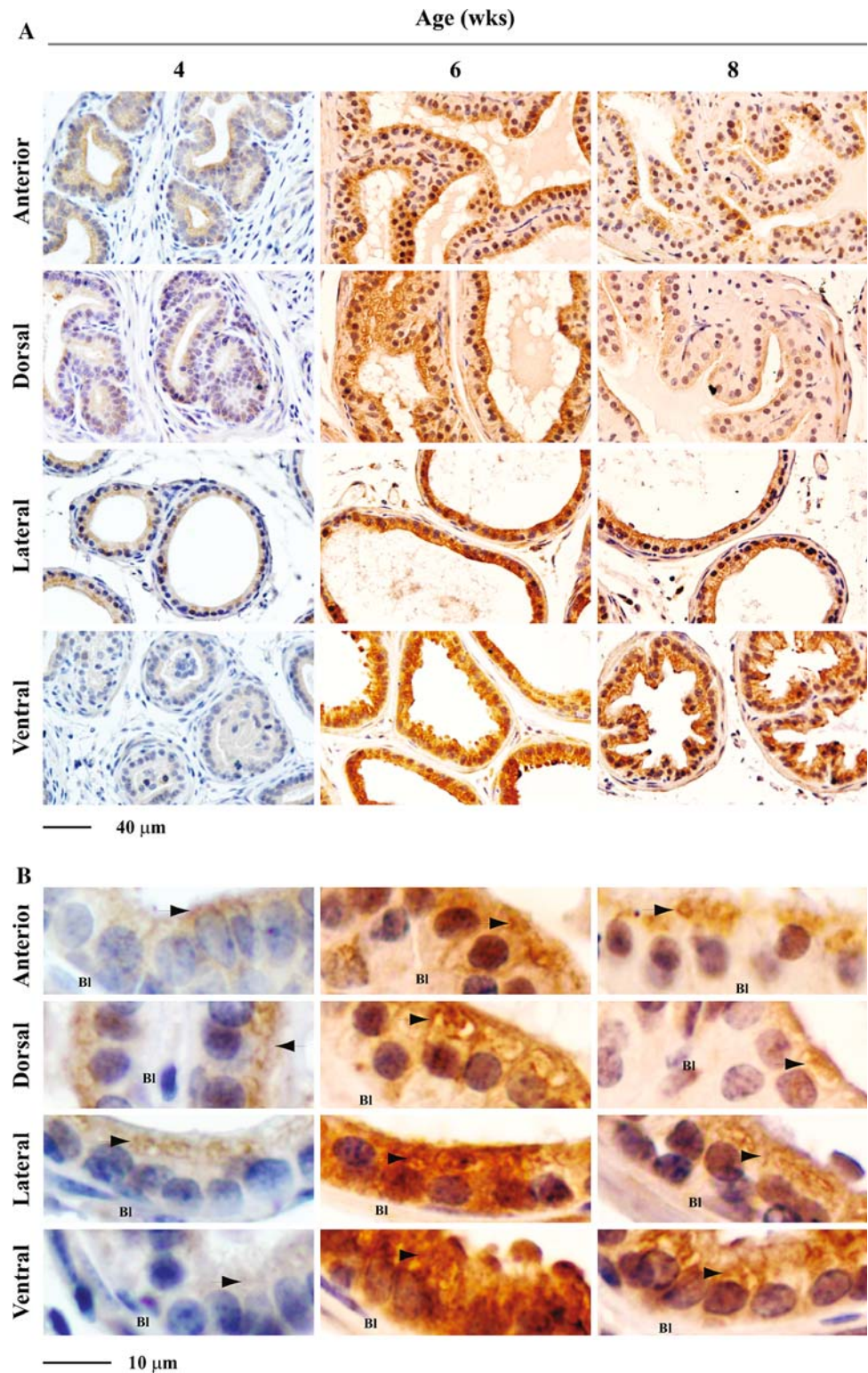
Fig. 5 IHC localization of ZNT6 in the mouse prostate at 4–8 weeks. Immunoreactivity of ZNT6 is observed in all lobes of the prostate from age 4 to 8 weeks. Arrows indicate the immunoreactive ZNT6. BI indicates the basal side of the epithelium. Bar = 40 μ m and 10 μ m in the panels A and B, respectively



regulated in the anterior lobe of the prostate. ZNT2 was not detectable at 4 weeks of age. But its expression became obvious at 8 weeks and it was mainly detected in the basal part of the epithelial cell where the most dilated cisternae of the endoplasmic reticulum resides (ER) (Brandes and

Portela 1960a). In the dorsal, lateral, and ventral epithelium of the prostate, ZNT2 was found in the cytoplasm near the lateral boundary and the apical pole of the cell where the flattened ER predominately resides (Brandes and Portela 1960b), suggesting the ER localization of ZNT2. On the

Fig. 6 IHC localization of ZNT7 in the mouse prostate at 4–8 weeks. The prostatic lobes at 6 weeks of age display the strongest immunoreactivity of ZNT7 whereas the lobes at 4 weeks of age show the weakest immunoreactivity. ZNT7 is mainly detected in the apical part of the cytoplasm of the epithelium. Arrows indicate the immunoreactive ZNT7. BI indicates the basal side of the epithelium. Bar = 40 μ m and 10 μ m in the panels **A** and **B**, respectively



other hand, expression of ZNT4 was primarily detected in the apical part of the epithelial cell just above the nucleus where the Golgi complex resides. ZNT4 was also found on the tip (the free border) of the epithelium in all lobes of the prostate. It is obvious, based on our protein expression

study, that ZNT2 and ZNT4 may play different roles in cellular zinc homeostasis in the mouse prostate during sexual maturation.

It has been demonstrated that two transcripts of human *ZNT5* that differ at both the 5' and 3' ends exist due to the

alternative splicing during transcription (Cragg et al. 2002; Jackson et al. 2007). The splicing variant A (ZNT5A) is localized to the Golgi complex and is believed to be involved in supplying zinc to secreted, zinc-requiring enzymes. The splicing variant B (ZNT5B) is localized on both the cell surface and Golgi complex, and is believed to play a role in intestinal zinc uptake (Jackson et al. 2007; Valentine et al. 2007). Similar to the human *ZNT5*, the mouse *Znt5* gene exists two transcripts that differ only at 5' end of the transcripts. The function and cellular localization of the mouse *ZNT5* variants have not been demonstrated. The *ZNT5* antibody that we used in this study could recognize both variants expressed in mouse tissues. Differential distribution of *ZNT5* was observed in the anterior and dorsal lobes of the prostate. Similar to *ZNT2*, expression of *ZNT5* was largely found in the basal part of epithelial cells in the anterior lobe of the prostate, suggesting the ER localization of *ZNT5*. In the dorsal and lateral lobes, *ZNT5* was expressed throughout epithelial cells that made it hard to define the location of the expression. Conversely, in the ventral lobe of the prostate, the staining pattern of *ZNT5* was reminiscent to that of *ZNT4*. The observation that *ZNT5* is expressed in different cellular compartments of epithelial cells in different lobes of the prostate may reflect the differential expression of *ZNT5a* and *ZNT5b* in these lobes.

Previous work has already documented the Golgi apparatus localization of *ZNT6* and *ZNT7* (Huang et al. 2002; Kirschke and Huang 2003). Again, in this study, we observed that both *ZNT6* and *ZNT7* were predominantly found in the apical part of the epithelium adjacent to the nucleus where the Golgi complex resides (Brandes and Portela 1960a, b) in all lobes of the prostate. We also observed an increased expression of *ZNT7* in the prostates isolated from 6- to 8-week-old mice, suggesting that expression of *ZNT7* may be regulated by male sex hormones in the prostate as during this time the plasma testosterone level rises rapidly as a result of the puberty.

In conclusion, we observed overlapping expression of *ZNT2*, 4, 5, 6, and 7 in the epithelium of developing and sexually matured mouse prostates. In vivo, *ZNT2* and *ZNT5* may locate in the ER-rich area of sexually matured epithelial cells of the anterior prostate, indicating roles of *ZNT2* and *ZNT5* in ER zinc homeostasis. The implication of overlapping expression of *ZNT* proteins in the mouse prostate is unclear. Perhaps it correlates to the physiological function of the secretory epithelial cells of the prostate in cellular zinc accumulation and secretion. Overlapping expression of *ZNT* proteins along the ER-Golgi secretory pathway may be an evolution of the prostate to keep prostate functional in the event of a loss-function-mutation in any of the *Znt* gene.

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References

- Andrews GK, Wang H, Dey SK, Palmiter RD (2004) Mouse zinc transporter 1 gene provides an essential function during early embryonic development. *Genesis* 40:74–81. doi:10.1002/gene.20067
- Beck FW, Prasad AS, Butler CE, Sakr WA, Kucuk O, Sarkar FH (2004) Differential expression of hZnT-4 in human prostate tissues. *Prostate* 58:374–381. doi:10.1002/pros.10344
- Berquin IM, Min Y, Wu R, Wu H, Chen YQ (2005) Expression signature of the mouse prostate. *J Biol Chem* 280:36442–36451. doi:10.1074/jbc.M504945200
- Black RE (1997) Zinc for child health. *Child Health Res Project Spec Rep* 1:1–24
- Brandes D, Portela A (1960a) The fine structure of the epithelial cells of the mouse prostate: I. Coagulating gland epithelium. *J Biophys Biochem Cytol* 7:505–509
- Brandes D, Portela A (1960b) The fine structure of the epithelial cells of the mouse prostate: II. Ventral lobe epithelium. *J Biophys Biochem Cytol* 7:511–514
- Canale D, Bartelloni M, Negroni A, Meschini P, Izzo PL, Bianchi B et al (1986) Zinc in human semen. *Int J Androl* 9:477–480
- Chi ZH, Wang ZY, Wang X, Gao HL, Dahlstrom A, Huang L (2006) Zinc transporter 7 is located in the cis-Golgi apparatus of mouse choroid epithelial cells. *Neuroreport* 17:1807–1811. doi:10.1097/01.wnr.0000239968.06438.c5
- Chimienti F, Devergnas S, Favier A, Seve M (2004) Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. *Diabetes* 53:2330–2337. doi:10.2337/diabetes.53.9.2330
- Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B et al (2006) In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. *J Cell Sci* 15:199–206
- Chowanadisai W, Lönnerdal B, Kelleher SL (2006) Identification of a mutation in SLC30A2 (ZnT-2) in women with low milk zinc concentration that results in transient neonatal zinc deficiency. *J Biol Chem* 281:39699–39707. doi:10.1074/jbc.M605821200
- Costello LC, Franklin RB (1998) Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. *Prostate* 35:285–296. doi:10.1002/(SICI)1097-0045(19980601)35:4<285::AID-PROS8>3.0.CO;2-F
- Costello L, Franklin RB (2000) The intermediary metabolism of the prostate: a key to understanding the pathogenesis and progression of prostate malignancy. *Oncology* 59:269–282. doi:10.1159/000012183
- Costello LC, Franklin RB (2006) The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots. *Mol Cancer* 5:17. doi:10.1186/1476-4598-5-17
- Cragg RA, Christie GR, Phillips SR, Russi RM, Küry S, Mathers JC et al (2002) A novel zinc-regulated human zinc transporter, hZTL1, is localized to the enterocyte apical membrane. *J Biol Chem* 277:22789–22797. doi:10.1074/jbc.M200577200
- Dufner-Geattie J, Langmade SJ, Wang F, Eide D, Andrews GK (2003a) Structure, function, and regulation of a subfamily of mouse zinc transporter genes. *J Biol Chem* 278:50142–50150. doi:10.1074/jbc.M304163200

- Dufner-Geattie J, Wang F, Kuo Y-M, Gitschier J, Eide D, Andrews GK (2003b) The acrodermatitis enteropathica gene *ZIP4* encodes a tissue-specific, zinc-regulated zinc transporter in mice. *J Biol Chem* 278:33474–33481. doi:[10.1074/jbc.M305000200](https://doi.org/10.1074/jbc.M305000200)
- Eng BH, Guerinot ML, Eide D, Saier MH Jr (1998) Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. *J Membr Biol* 166:1–7. doi:[10.1007/s002329900442](https://doi.org/10.1007/s002329900442)
- Falcón-Pérez JM, Dell'Angelica EC (2007) Zinc transporter 2 (SLC30A2) can suppress the vesicular zinc defect of adaptor protein 3-depleted fibroblasts by promoting zinc accumulation in lysosomes. *Exp Cell Res* 313:1473–1483. doi:[10.1016/j.yexcr.2007.02.006](https://doi.org/10.1016/j.yexcr.2007.02.006)
- Franklin RB, Ma J, Zou J, Guan Z, Kukoyi BI, Feng P et al (2003) Human ZIP1 is a major zinc uptake transporter for the accumulation of zinc in prostate cells. *J Inorg Biochem* 96:435–442. doi:[10.1016/S0162-0134\(03\)00249-6](https://doi.org/10.1016/S0162-0134(03)00249-6)
- Franklin R, Feng P, Milon B, Desouki MM, Singh KK, Kajdacsy-Balla A et al (2005) hZIP1 zinc uptake transporter down regulation and zinc depletion in prostate cancer. *Mol Cancer* 4:32. doi:[10.1186/1476-4598-4-32](https://doi.org/10.1186/1476-4598-4-32)
- Gaither LA, Eide DJ (2000) Functional expression of the human hZIP2 zinc transporter. *J Biol Chem* 275:5560–5564. doi:[10.1074/jbc.275.8.5560](https://doi.org/10.1074/jbc.275.8.5560)
- Gaither L, Eide DJ (2001) The human ZIP1 transporter mediates zinc uptake in human K562 erythroleukemia cells. *J Biol Chem* 276:22258–22264. doi:[10.1074/jbc.M101772200](https://doi.org/10.1074/jbc.M101772200)
- Huang L, Gitschier J (1997) A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nat Genet* 17:292–297. doi:[10.1038/ng1197-292](https://doi.org/10.1038/ng1197-292)
- Huang L, Kirschke CP, Gitschier J (2002) Functional characterization of a novel mammalian zinc transporter, ZnT6. *J Biol Chem* 277:26389–26395. doi:[10.1074/jbc.M200462200](https://doi.org/10.1074/jbc.M200462200)
- Huang L, Kirschke CP, Zhang Y, Yu Y-Y (2005) The ZIP7 gene (Slc39a7) encodes a zinc transporter involved in zinc homeostasis of the Golgi apparatus. *J Biol Chem* 280:15456–15463. doi:[10.1074/jbc.M412188200](https://doi.org/10.1074/jbc.M412188200)
- Huang L, Kirschke CP, Zhang Y (2006) Decreased intracellular zinc in human tumorigenic prostate epithelial cells: a possible role in prostate cancer progression. *Cancer Cell Int* 6:10. doi:[10.1186/1475-2867-6-10](https://doi.org/10.1186/1475-2867-6-10)
- Jackson KA, Helston RM, McKay JA, O'Neill ED, Mathers JC, Ford D (2007) Splice variants of the human zinc transporter ZnT5 (SLC30A5) are differentially localized and regulated by zinc through transcription and mRNA stability. *J Biol Chem* 282:10423–10431. doi:[10.1074/jbc.M610535200](https://doi.org/10.1074/jbc.M610535200)
- Kambe T, Narita H, Yamaguchi-Iwai Y, Hirose J, Amano T, Sugiura N et al (2002) Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic β cells. *J Biol Chem* 277:19049–19055. doi:[10.1074/jbc.M200910200](https://doi.org/10.1074/jbc.M200910200)
- Kelleher SL, Lonnerdal B (2005) Zip3 plays a major role in zinc uptake into mammary epithelial cells and is regulated by prolactin. *Am J Physiol Cell Physiol* 288:C1042–C1047. doi:[10.1152/ajpcell.00471.2004](https://doi.org/10.1152/ajpcell.00471.2004)
- Kerr WK, Keresteci AG, Mayoh H (1960) The distribution of zinc with the human prostate. *Cancer* 13:550–554. doi:[10.1002/1097-0142\(196005/06\)13:3<550::AID-CNCR2820130320>3.0.CO;2-X](https://doi.org/10.1002/1097-0142(196005/06)13:3<550::AID-CNCR2820130320>3.0.CO;2-X)
- Kirschke CP, Huang L (2003) ZnT7, a novel mammalian zinc transporter, accumulates zinc in the Golgi apparatus. *J Biol Chem* 278:4096–4102. doi:[10.1074/jbc.M207644200](https://doi.org/10.1074/jbc.M207644200)
- Kvist U, Björndahl L, Kjellberg S (1987) Sperm nuclear zinc, chromatin stability, and male fertility. *Scanning Microsc* 1:1241–1247
- Liuzzi JP, Blanchard RK, Cousins RJ (2001) Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. *J Nutr* 131:46–52
- Marker PC, Donjacoura AA, Dahiyab R, Cunha GR (2003) Hormonal, cellular, and molecular control of prostatic development. *Dev Biol* 253:165–174. doi:[10.1016/S0012-1606\(02\)00031-3](https://doi.org/10.1016/S0012-1606(02)00031-3)
- Mawson CA, Fischer MI (1952) The occurrence of zinc in the human prostate gland. *Can J Med Sci* 30:336–339
- McMahon RJ, Cousins RJ (1998) Regulation of the zinc transporter ZnT-1 by dietary zinc. *Proc Natl Acad Sci USA* 95:4841–4846. doi:[10.1073/pnas.95.9.4841](https://doi.org/10.1073/pnas.95.9.4841)
- Michalczyk AA, Allen J, Blomeley RC, Ackland ML (2002) Constitutive expression of hZnT4 zinc transporter in human breast epithelial cells. *Biochem J* 15:105–113
- Murgia C, Vespignani I, Cerase J, Nobilo F, Perozzi G (1999) Cloning, expression, and vesicular localization of zinc transporter Dri 27/ZnT4 in intestinal tissue and cells. *Am J Physiol* 277:G1231–G1239
- Palmiter RD, Findley SD (1995) Cloning and characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J* 14:639–649
- Palmiter R, Huang L (2004) Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. *Pflugers Arch Eur J Physiol* 447:744–751
- Palmiter R, Cole TB, Findley SD (1996a) ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *EMBO J* 15:1784–1791
- Palmiter R, Cole TB, Quaife CJ, Findley SD (1996b) ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc Natl Acad Sci USA* 93:14934–14939. doi:[10.1073/pnas.93.25.14934](https://doi.org/10.1073/pnas.93.25.14934)
- Sekler I, Moran A, Hershfinkel M, Dori A, Margulis A, Birenzweig N et al (2002) Distribution of the zinc transporter ZnT-1 in comparison with chelatable zinc in the mouse brain. *J Comp Neurol* 447:201–209. doi:[10.1002/cne.10224](https://doi.org/10.1002/cne.10224)
- Singh J, Zhu Q, Handelsman DJ (1999) Stereological evaluation of mouse prostate development. *J Androl* 20:251–258
- Taylor K, Morgan HE, Johnson A, Nicholson RI (2004) Structure-function analysis of HKE4, a member of the new LIV-1 subfamily of zinc transporters. *Biochem J* 377:131–139. doi:[10.1042/BJ20031183](https://doi.org/10.1042/BJ20031183)
- Valentine RA, Jackson KA, Christie GR, Mathers JC, Taylor PM, Ford D (2007) ZnT5 variant B is a bidirectional zinc transporter and mediates zinc uptake in human intestinal Caco-2 cells. *J Biol Chem* 282:14389–14393. doi:[10.1074/jbc.M701752200](https://doi.org/10.1074/jbc.M701752200)
- Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J (2002) A novel member of a zinc transporter family defective in acrodermatitis enteropathica. *Am J Hum Genet* 71:66–73. doi:[10.1086/341125](https://doi.org/10.1086/341125)
- Yu YY, Kirschke CP, Huang L (2007) Immunohistochemical analysis of ZnT1, 4, 5, 6, and 7 in the mouse gastrointestinal tract. *J Histochem Cytochem* 55:223–234. doi:[10.1369/jhc.6A7032.2006](https://doi.org/10.1369/jhc.6A7032.2006)
- Zaichick VY, Sviridova TV, Zaichick SV (1997) Zinc in the human prostate gland: normal, hyperplastic and cancerous. *Int Urol Nephrol* 29:565–574. doi:[10.1007/BF02552202](https://doi.org/10.1007/BF02552202)