Differential expression of divalent metal transporter DMT1 (Slc11a2) in the spermatogenic epithelium of the developing and adult rat testis

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Iron is required by all organisms and contributes to a wide range of biological functions, such as mitochondrial oxidation, DNA synthesis, oxygen transport, and nitrogen fixation (27, 29). In mammals, both iron excess and iron deficiency present as clinical conditions. As a result, body iron homeostasis is a tightly regulated process. Importantly, male fertility is affected by disruptions in iron balance. For example, hypogonadism and impaired testicular function are prevalent in patients with iron overload, exemplified by hereditary hemochromatosis (5).

The divalent metal transporter DMT1 (Slc11a2), also known as Nramp2 (13) and DCT1 (14), is a key component of the complex physiological process regulating body iron levels. The hydrophobic protein, with 12 predicted transmembrane domains and cytoplasmic NH2 and COOH termini, has broad selectivity, mediating transport of a spectrum of divalent cations. These include iron, copper, and some toxic metals such as cadmium (14). DMT1 is expressed in many tissues, including duodenum, placenta (11), brain (22), and kidney (2, 7). In duodenum, DMT1 is expressed on the apical membrane of enterocytes, where it is primarily responsible for the non-transferrin-mediated absorption of dietary iron. Its expression is regulated by dietary iron intake in a number of tissues (3, 7, 14, 28) and is particularly apparent in the mitotic phase of spermatogenesis (4, 17, 25). In the developing testis, DMT1 is localized to the phagosomal membranes of two Sertoli cell lines (18). As a first step in determining the role of DMT1 in testicular function, we have characterized alternative splicing of exons in the DMT1 gene to produce four distinct DMT1 mRNA. These differ at the 3’ end with respect to an iron-responsive element (IRE) present in the untranslated region (8, 20) and also at the 5’ end (16). As a result of alternative splicing, the encoded proteins differ at the NH2 and COOH termini but share a common central domain.

In the testis, the acquisition of iron is of particular importance because the spermatogenic cells, which acquire iron during development, are lost as mature sperm pass into the reproductive tract (26). The testis is divided into two compartments, the interstitia and the seminiferous tubules, in which spermatogenesis takes place. Here germ cells are present at various stages of development in the form of spermatogonia, spermatocytes, and spermatids, passing further toward the lumen of the tubule as they mature (3a). The tubular epithelium, like the brain, represents a compartment functionally separate from the systemic circulation by a barrier known as the blood-testis barrier. Consequently, the spermatogenic epithelium is unable to acquire iron directly from plasma transferrin (15). However, the somatic Sertoli cells, whose tight junctions form the blood-testis barrier, synthesize their own transferrin (31) to transport iron across the cell to the spermatocytes and spermatids (4, 17, 25). Elements of transferrin-mediated iron transport have been characterized in the rat testis (10, 23, 25, 26, 32) and are particularly apparent in the mitotic and meiotic germ cells (24, 34, 37). This suggests a role for iron in cell division, differentiation, and metabolism in the testis (30).

DMT1 mRNA is present in testis (14), and DMT1 protein has been localized to the phagosomal membranes of two Sertoli cell lines (18). As a first step in determining the role of DMT1 in testicular function, we have characterized DMT1 expression in both the immature and adult rat testis. We have shown that DMT1 is present in the testis and that the expression profile in the development of the rat testis is cell specific.

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and in the adult is highly coordinated with the spermatogenic cycle. This suggests an important role for DMT1 in spermatogenesis and implies that germ cells have a need for a precisely timed supply of iron. The stage-specific nature of expression indicates that DMT1 has a role in male fertility, and this knowledge may be useful when considering conditions of abnormal iron regulation.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats (Charles River, Margate, UK) were bred under controlled conditions in the University of Manchester Biological Services Unit. The day of delivery of pups was designated as the first day of age. All animals were housed with controlled temperature and lighting (12:12-h light-dark cycle) and access to food and water ad libitum. All procedures were performed within the regulations of the National Research Council as detailed in the Guide for Care and Use of Laboratory Animals.

Tissue preparation. Groups of male adult rats (mean body wt of 275 g, age ~60 days) and male rat pups 5, 15, 25, and 35 days of age were killed by concussion followed by cervical dislocation. After dissection, the left testes from three animals from each age group were fixed by immersion in Bouin’s fluid (71% picric acid, 24% formalin, and 5% glacial acetic acid) for 3–4 h. These tissues were dehydrated through a gradient of ethanol and stored in absolute ethanol for 18 h before being embedded in paraffin wax. All other testes from each group were pooled and frozen at −80°C.

RNA extraction and Northern blot analysis. Total RNA was extracted from frozen testes using the acid guanidinium isothiocyanate-phenol method, and Northern blot analysis was performed according to the method of Ferguson et al. (7) using dissected kidney cortex as a positive control. The double-stranded probe was randomly primed and labeled with 32P and corresponded to nucleotides 85–1,600 of rat DMT1, shared by both the IRE and non-IRE transcripts (GenBank accession no. AF029757). Briefly, 5 μg of kidney cortex mRNA and 3 μg of testis mRNA were separated on 1% agarose gels containing 2.2 M formaldehyde and blotted onto Hybond-N nylon membranes (Amersham Pharmacia Biotech, Little Chalfont, UK). Membranes were hybridized at 42°C overnight with 32P-labeled probe, and the hybridized membranes were washed at 65°C with 0.1% SSC-0.1% SDS and exposed to film (Kodak Biomax; Kodak, UK). Primer sets for IRE DMT1 (GenBank accession no. AF008439) and non-IRE DMT1 (GenBank accession no. AF029757) spanning introns were designed using Primer Express (Applied Biosystems, Warrington, UK). Both isoforms used the same forward primer: 5′-CAACCGGAATAGGCTGGAGGA-3′. The reverse primer sequences for IRE and non-IRE transcripts were 5′-GGCAGGAG-GATCTCTGTGAG-3′ and 5′-GGCACAAAGGCTTJAGAGA-3′, respectively. The amplification of IRE DMT1 cDNA was performed at 95°C for 30 s, at 55°C for 45 s, and at 72°C for 60 s for 35 cycles. The amplification of non-IRE DMT1 cDNA was performed at 95°C for 30 s, at 58°C for 30 s, and at 72°C for 30 s for 35 cycles. The PCR products were resolved on 1% agarose gels. Control reactions were performed with the primers in the absence of cDNA.

Peroxidase immunohistochemistry. Paraffin wax sections (5 μm) were cut using a Leica RM2135 rotary microtome (Leica Microsystems, Nussloch, Germany) and fixed onto Superfrost microscope slides (BDH, Poole, UK). Deparaffinized and rehydrated sections were incubated with 3% H2O2 in methanol for 20 min to quench endogenous peroxidase activity. Masked antigens were retrieved by microwaving the sections in Tris-EDTA-glycerol buffer (10 mM Tris, 1 mM EDTA, 0.1% Triton X-100, and 1 mM EGTA, pH 9.0) for 2 min at 800 W and 4 min at 400 W. Sections were allowed to cool for a minimum of 2 h before being treated with 50 μM NH4Cl in PBS for 30 min. Sections were blocked with 1% BSA in PBS and incubated overnight at 4°C in a humidified chamber with a previously characterized affinity-purified polyclonal anti-DMT1 antibody raised to an epitope common to all known DMT1 isoforms (6, 7, 37). Before use, the antibody was diluted 1:500 in PBS containing 0.1% BSA and 0.3% Triton X-100. Sections from each group were also incubated with the PBS-BSA-Triton X-100 solution alone for use as negative controls.

Slides were rinsed with PBS containing 0.1% BSA before being incubated with goat anti-rabbit immunoglobulin diluted 1:200 for 1 h at room temperature. After being rinsed with the PBS solution, the immunohistochemical reaction was visualized using 3,3′-diaminobenzidine-tetrahydrochloride. Sections were counterstained with Mayer’s hematoxylin, dehydrated, and mounted with Eukitt resin (Kindler, Freiburg, Germany). Immunostaining was examined under a Zeiss Axioshot light microscope (Carl Zeiss, Welwyn Garden City, UK), and photographs were taken using a Spot RT color digital camera (Diagnostic Instruments, Sterling Heights, MD) and analyzed with the accompanying Spot RT Advanced software, version 3.04.

Western blot analysis. Semiquantitative immunoblotting was performed according to the method described by Ferguson et al. (7). Crude membrane preparations from each group of frozen tissues were created using differential ultracentrifugation. Protein homogenates were spun at 2,500 g to remove nuclei and cell debris, and the postnuclear supernatants were centrifuged at 100,000 g to pellet the particulate proteins. These were then resolved using 8% SDS-PAGE.
and transferred to Biotrace nitrocellulose membranes (Paul Gelman Sciences, Northampton, UK). Adult rat kidney, proximal duodenum, and 35-day-old rat kidney were also prepared in this manner for use as positive controls. Immunoblotting was performed using the anti-DMT1 antibody diluted 1:5,000 in Tris-buffered saline-Tween 20 (TBST). The enhanced chemiluminescence system was used to visualize the signal (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Experiments were repeated in triplicate. Membranes incubated only in TBST or in antiserum preincubated with an excess of the immunizing peptide served as negative controls. In every case, staining of the gels with Coomassie blue and the membranes with Ponceau red S stain confirmed that equal amounts of proteins had been loaded and transferred to the membrane.

Protein deglycosylation. Particulate proteins were deglycosylated using N-glycosidase F (PNGase F; New England Biolabs) according to the manufacturer’s instructions. Briefly, particulate fractions were denatured at 65°C in buffer containing 0.5% SDS and 1% β-mercaptoethanol, then incubated at 37°C for 1 h in buffer supplemented with NP-40 (1% final) and PNGase F (25 U/mg of particulate protein). After deglycosylation, samples were solubilized using 5× Laemmli buffer and immunoblotted as before. The electrophoretic mobility of deglycosylated DMT1 was compared with that obtained from a sample processed identically but in the absence of PNGase F and also to a sample of particulate proteins stored on ice throughout.

RESULTS

Northern blot analysis. As previously reported, Northern blot analysis of testis polyadenylated RNA, using a probe corresponding to nucleotides common to both IRE DMT1 and non-IRE DMT1, detected transcripts with molecular masses of 2.4 and 4.4 kb in kidney cortex (Fig. 1; see also Ref. 7). The 4.4-kb species corresponded to the IRE transcript, while the 2.4-kb species related to the non-IRE transcript. Although weaker, these signals were also present in adult testis.

Reverse transcriptase-polymerase chain reaction. Primers specific for IRE DMT1 and non-IRE DMT1 were also used selectively to investigate the expression of these two isoforms in the immature (5-day-old) and adult testis. PCR products from the IRE and non-IRE primer pairs were detected as single

![Fig. 3. DMT1 immunoreactive species are present throughout development. Membrane extracts from the testes of 5-, 15-, 25-, and 35-day-old rats were prepared and separated by performing SDS-PAGE on an 8% acrylamide gel. Incubation with a DMT1 antibody common to all known DMT1 isoforms showed that immunoreactive species were present throughout development at the ages investigated. A: 2 sets of the testis protein homogenates. In the testes of all of the age groups tested, a 70-kDa protein was detected. A second, higher molecular mass signal between 75 and 90 kDa was detected that was particularly apparent at day 15. B: the size of the signal was especially obvious after overexposure of the signal. The 70-kDa protein was also detected in the kidney of the 35-day-old rat (K).](http://ajpcell.physiology.org/content/288/1/C178/F3)

![Fig. 4. In the adult rat, the predominant DMT1 isoform in the testis is the same as that in the duodenum. Membrane extracts from the kidney (K), testis, and duodenum (D) were prepared and separated by performing SDS-PAGE on an 8% acrylamide gel. Incubation with the DMT1 antibody allowed us to visualize broad immunoreactive bands in each of the 3 tissue types. In the testis, the main band was detected between 75 and 90 kDa. A band corresponding to this was observed in the duodenum. A second immunoreactive species was detected in the testis at ~70 kDa. A band of similar size was observed in the kidney. Multiple lower molecular mass bands between 30 and 60 kDa also were detected in the testes.](http://ajpcell.physiology.org/content/288/1/C178/F4)

![Fig. 5. Deglycosylation of DMT1 reveals that the main protein isoform in the testis is different from that in the kidney. Crude membrane extracts from the kidneys and testes of adult rats were prepared and deglycosylated with N-Glycosidase F. The deglycosylated samples (lanes 3 and 6) along with undigested protein homogenates were separated by performing SDS-PAGE on an 8% acrylamide gel. In the undigested testis samples, the main DMT1 isoform was detected between 75 and 90 kDa. The protein was detected at ~60 kDa after deglycosylation. In the undigested kidney samples, DMT1 was detected at ~70 kDa. The protein was detected at ~50 kDa after deglycosylation.](http://ajpcell.physiology.org/content/288/1/C178/F5)
bands of ~0.9 and 0.3 kb, respectively, at both ages (Fig. 2). This is consistent with the expected oligonucleotide lengths based on the primers used for each DMT1 isoform. Control reactions performed in the absence of cDNA did not give rise to PCR products. We conclude that IRE DMT1 and non-IRE DMT1 transcripts are present in immature and adult testis.

Semiquantitative immunoblotting. Semiquantitative Western blot analysis was used to profile DMT1 protein expression in the immature and adult rat testis. DMT1 was detected in the testis throughout the development of the immature rat (Fig. 3). The antibody detected an immunoreactive species of ~70 kDa in all of the age groups tested. This signal was strongest in the 5- and 15-day-old testis. A second, higher molecular mass species between 75 and 90 kDa was detected throughout development and was particularly apparent in the 15-day-old testis. The specificity of the DMT1 antibody was confirmed by the absence of any immunoreactive signal in the membranes incubated in TBST without the primary antibody or with antiserum preincubated with an excess of the immunizing peptide (data not shown).

Both of these immunoreactive species were detected in the testis of the adult rat (Fig. 4). The predominant DMT1 immu-
noreactive species had a molecular mass between 75 and 90 kDa. A band of similar size also was observed in the adult rat duodenum. The lower molecular mass species of ~70 kDa was comparable to that observed in the adult rat kidney. Several lower molecular mass bands between 30 and 60 kDa also were detected in the testis. These bands were ablated by incubation of the membranes with antisera preincubated with an excess of immunizing peptide. Whether these bands represent functional DMT1 isoforms is currently unknown.

To further characterize the DMT1 species expressed in the adult testis, we performed deglycosylation of testis and kidney protein homogenates with N-glycosidase F (Fig. 5). Treatment of kidney protein homogenates with N-glycosidase F reduced the molecular mass of the band to 50 kDa. In contrast, identical treatment of the testis protein reduced the predominant band from 75–90 to 60 kDa. This indicated that differences in molecular mass of the major proteins detected in testis and kidney were not due primarily to differences in the degree of glycosylation. However, these results do not rule out other forms of posttranslational modification such as phosphorylation.

**Immunohistochemistry of immature rat testis.** Immunohistochemistry was performed to investigate the ontogenic expression of DMT1 in the rat testis. DMT1 immunostaining was observed in the testes of all of the immature rats, regardless of age. Staining was confined to the seminiferous tubules and was absent from the interstitia (Fig. 6). Despite morphological changes due to the increasing number of germ cells with age, a distinct staining pattern was observed throughout the development of the testis. In 5-day-old animals, the seminiferous tubule lumen had not formed, and the pattern of staining did not vary between tubules. Higher magnification images showed staining resided in the cytoplasm of the Sertoli cells with minor variations in intensity toward the tubule membrane. In the 15-day-old rat, germ cells were more numerous, with many more types present. Zygote spermatocytes were observed in each of the tubules, while leptotene spermatocytes were present in a few select tubules. Despite the presence of more germ cells, the pattern of DMT1 localization matched that of the 5-day-old rat in that the Sertoli cell cytoplasm was immunopositive. In 25-day-old rats, the lumen of each tubule remained unstained, highlighting the stage-specific nature of DMT1 expression. In tubules at Stage VIII (Fig. 8, E and F) elongate spermatids were stained intensely. The residual bodies that accompany these mature germ cells also showed strong DMT1 staining. Pachytene spermatocytes were once again stained, while the pattern of immunostaining in the spermatids matched that in the Stage V tubule. At Stage X (Fig. 8, G and H), DMT1 was not detected in the elongating spermatids but was abundant in the pachytene spermatocytes. At Stage XII/XIII of the cycle (Figs. 8, I and J), the location and intensity of DMT1 expression was similar to that at Stage X but exhibited the occasional staining of zygote spermatocytes. Importantly, we did not detect plasma membrane staining of Sertoli cells at any stage, suggesting that in the adult rat, DMT1 is not the primary means of iron transport across the blood-testis barrier. The control slides of adult testis did not exhibit any immunopositive staining for DMT1.

**Immunohistochemistry of adult rat testis.** In the adult rat, with a fully developed spermatogenic cycle, the pattern of immunostaining (Fig. 7) was in marked contrast to that observed in 5-, 15-, and 25-day-old rats. First, both Sertoli cells and germ cells of the adult rat testis stained positively for DMT1. Second, while the germ cells exhibited mostly cytoplasmic staining, DMT1 could be localized not to all but to a select number of nuclei. Third, the Sertoli cell nuclei also were clearly stained throughout all of the stages, in contrast to the developing animal, in which staining was confined to the cytoplasm.

At Stage II/III of the cycle (Fig. 8, A and B), the pachyteme spermatocytes had abundant DMT1, while the cytoplasm of the elongate spermatids exhibited light immunopositive staining. At Stage V of the cycle (Fig. 8, C and D), the tubule was very darkly stained, which was attributable mainly to the tails of the elongate spermatids. The nuclei of the round spermatids exhibited faint staining, while the pachytene spermatocytes remained unstained, highlighting the stage-specific nature of DMT1 expression. In tubules at Stage VIII (Fig. 8, E and F) elongate spermatids were stained intensely. The residual bodies that accompany these mature germ cells also showed strong DMT1 staining. Pachytene spermatocytes were once again stained, while the pattern of immunostaining in the spermatids matched that in the Stage V tubule. At Stage X (Fig. 8, G and H), DMT1 was not detected in the elongating spermatids but was abundant in the pachytene spermatocytes. At Stage XII/XIII of the cycle (Figs. 8, I and J), the location and intensity of DMT1 expression was similar to that at Stage X but exhibited the occasional staining of zygote spermatocytes. Importantly, we did not detect plasma membrane staining of Sertoli cells at any stage, suggesting that in the adult rat, DMT1 is not the primary means of iron transport across the blood-testis barrier. The control slides of adult testis did not exhibit any immunopositive staining for DMT1.

**DISCUSSION**

It is well established that iron plays an important role in spermatogenesis and in the normal function of the testis (5). In other tissues, the mechanism of iron sequestration is becoming
Fig. 8. DMT1 expression is highly coordinated with the spermatogenic cycle in the adult rat. These images show immunohistochemical staining of DMT1 in 5-μm sections of adult rat testis at medium and high magnification. All scale bars, 100 μm. A: a tubule at Stage II/III of the cycle of the seminiferous epithelium. B: same tubule shown in A at higher magnification. The predominant staining is associated with the nuclei of pachytene (P) spermatocytes. There is also some faint staining associated with the cytoplasm of the elongating (E) spermatids. The Sertoli cell nuclei also stain positively for DMT1 (arrows). C and D: a Stage V tubule showing intense staining at the apical surface of the epithelium associated with the tails of the elongate (E) spermatids. The nuclei of round (R) spermatids are stained faintly. The nuclei of the Sertoli cells are again stained (arrows), while the pachytene (P) spermatocytes are unstained. E and F: a Stage VIII tubule showing intense immunostaining, which can be attributed to both the tails of the elongate (E) spermatids and their accompanying residual bodies (>). There is also positive staining of the round (R) spermatids, the pachytene (P) spermatocytes, and the Sertoli cell nuclei (arrows). G and H: a Stage X tubule showing no immunostaining of the characteristic elongating spermatids (Sp), while the pachytene (P) spermatocytes and Sertoli cell nuclei (arrows) remain heavily stained. I and J: a tubule at Stage XII/XIII of the cycle of the seminiferous epithelium. The elongated (E) spermatids and their associated cytoplasm are devoid of any staining for DMT1. However, the nuclei of the pachytene (P) spermatocytes and occasional zygotene spermatocytes (>) are strongly stained. Again, Sertoli cell nuclei are positively stained (arrows).
DMT1 has been localized to late endosomes in a number of primary routes of iron transport across the blood-testis barrier. That in the Sertoli cells of immature rat testis, DMT1 is not the cytoplasm of the Sertoli cells but absent from the plasma membrane of the Sertoli cell tight junctions, DMT1 was present in (3a). In the immature rat, both before and after the establishment of the epithelium, providing the factors needed for spermatogenesis including structural support of the germ cells embedded within the seminiferous tubules throughout the spermatogenic cycle but was absent at Stage V (hatched bars). The round spermatids expressed DMT1 between Stages V and VIII, while the elongate spermatids expressed DMT1 from Stage I through Stage VIII. The spermatogonia were devoid of any immunostaining throughout the cycle.

Immature testis. Using a combination of Northern blot analysis and RT-PCR, we detected both IRE and non-IRE DMT1 transcripts in the immature testis. The presence of the IRE transcript indicates that like other tissues, including kidney and duodenum, the testis can modulate iron translocation via DMT1 in response to cellular demands.

Semi-quantitative immunoblotting of immature rat testis revealed that two distinct DMT1 protein species were differentially expressed. The lower molecular mass isoform of ~70 kDa showed strong expression at days 5 and 15, but thereafter expression decreased. At 15 days, a 75- to 90-kDa protein was also particularly visible. This isoform has a molecular mass similar to that of the major DMT1 protein found in the adult testis (see below) and is analogous to the predominant isoform found in the duodenum (3). In contrast, the lower molecular mass protein expressed at days 5 and 15 is of the same molecular mass as the kidney isoform (7).

The pattern of DMT1 expression in the immature testis was found to be similar in all ages up to 35 days. Immunohistochemistry localized the DMT1 protein to the cytoplasm of the Sertoli cells. Despite the age-related changes in seminiferous tubule morphology that include an increase in tubule diameter and the number of germ cell types, no age-related changes in the cellular pattern of DMT1 expression were detected. In addition, the overall pattern of DMT1 immunoreactivity was unaffected by the changes in the levels of expression of the two isoforms as revealed by Western blot analysis. This may indicate changes in the required function of DMT1 by Sertoli cells over time.

In the 35-day-old rat testis, immunostaining was restricted to the cytoplasm of the Sertoli cells. As the number of germ cells increased, the overall staining of the tubules decreased. Also, at this age, the pattern of DMT1 expression was first observed to vary between some of the tubules in that some tubules exhibited intense regions of staining in the form of tracts running from the tubular membrane to the lumen. As the first wave of spermatogenesis approached completion, immunostaining began to appear more like that observed in the adult rat, indicating the onset of stage-specific DMT1 expression as seen in the adult testis. This pattern of expression was similar to that of transferrin mRNA, although differential tubular expression of transferrin was noted much earlier, at 14 days (21).

The Sertoli cell is responsible for the biochemical and structural support of the germ cells embedded within the epithelium, providing the factors needed for spermatogenesis (3a). In the immature rat, both before and after the establishment of the Sertoli cell tight junctions, DMT1 was present in the cytoplasm of the Sertoli cells but absent from the plasma membranes. Therefore, the results of the present study suggest that in the Sertoli cells of immature rat testis, DMT1 is not the primary route of iron transport across the blood-testis barrier. DMT1 has been localized to late endosomes in a number of transfected cell lines, including Chinese hamster ovary, RAW 264.7 (12), human embryonic kidney-293T (33), and Hep-2 cells (35), where it also has been observed in lysosomes. Without higher resolution images, which might be obtained using immunogold electron microscopy, it was not possible to resolve the intracellular location of DMT1 and therefore speculate on the regions of punctate staining observed. However, it is possible that DMT1 serves to transport iron between the intracellular compartments of the testis as it does in other cell types.

Adult testis. In the fully developed rat testis, Northern blot analysis and RT-PCR showed that both IRE and non-IRE DMT1 transcripts were present. This finding corroborates data reported by Gunshin et al. (13), who isolated a 4.4-kb cDNA encoding a 561-amino acid protein. On the basis of Northern blot analysis, Gunshin reported the expression of the 4.4-kb transcript in adult rat testis. This transcript encodes an iron-responsive DMT1 isoform expressed in the duodenum.

Western blot analysis showed that the predominant isoform of DMT1 in the adult testis has a molecular mass of 75–90 kDa, although proteins of lower molecular mass, such as those observed in the immature testis, were also present. Deglycosylation of protein isolated from the adult testis reduced the molecular mass from 75–90 to 60 kDa. This is consistent with the predicted molecular masses of 61 and 62 kDa of the IRE and non-IRE DMT1 isoforms, respectively. The predominant isoform in the adult testis had a molecular mass different from that expressed in the immature testis and kidney. The predicted difference in molecular mass between the IRE and non-IRE forms of DMT1 is ~1 kDa, whereas the difference between the molecular mass of the deglycosylated proteins was 10 kDa. Therefore, it is unlikely that expression of predominantly IRE or non-IRE proteins can explain these variations. It is more likely that the renal isoform and possibly that expressed in the immature testis are truncated splice variants or undergo post-translational modification other than glycosylation.

Fig. 9. Summary graph showing the stage-specific expression of DMT1 in the germ cells of the adult rat testis. DMT1 was located in the spermatocytes of the seminiferous tubules throughout the spermatogenic cycle but was absent at Stage V (hatched bars). The round spermatids expressed DMT1 between Stages V and VIII, while the elongate spermatids expressed DMT1 from Stage I through Stage VIII. The spermatogonia were devoid of any immunostaining throughout the cycle.
The data from the immature and adult testes together can be summarized as follows. Throughout development to maturation, the testis differentially expresses two predominant DMT1 isoforms: a 70-kDa protein that, based on molecular mass, is the same as the major DMT1 isoform expressed in the kidney, and a 75- to 90-kDa protein that, based on molecular mass, is the same as the predominant DMT1 isoform expressed in the duodenum.

Immunohistochemistry showed that, in contrast to the immature animal, DMT1 in the mature rat testis has a differential pattern of expression. In the adult, staining was both cytoplasmic and nuclear, and both Sertoli cells and germ cells were stained. DMT1 expression was cell specific; therefore, the pattern observed was dependent on the stage of the spermatogenic cycle. Figure 9 summarizes the stage-specific expression of DMT1 in the germ cells of the adult testes.

From the basement membrane to the lumen of the seminiferous tubules, germ cells are classified into three types: spermatogonia, spermatocytes, and spermatids, which are either round or elongate. Throughout the 14 stages of spermatogenesis, DMT1 was not observed in any of the spermatogonia. DMT1 was detected in all of the spermatocytes but was absent in these cells at Stage V of the cycle. DMT1 immunostaining was observed in the round spermatids between Stages V and VIII of the cycle and in the elongate spermatids between Stages I and VIII of the cycle. The most intense immunostaining was detected in the elongate spermatids, particularly between Stages V and VIII. This suggests that these cells have a particularly high requirement for iron, which may be of importance in the maturation and long-term survival of the sperm as it passes out of the seminiferous tubule. These data are consistent with those of Morales et al. (25), who reported that levels of radiolabeled transferrin were highest in the elongate spermatids and residual bodies of the testis.

DMT1 was clearly immunolocalized to a number of nuclei in the germ cells of the adult testis. The nuclear stain was not present in all of the spermatogenic cells of the testis, indicating that DMT1 is expressed specifically in these structures and that its presence is not an artifact of the methodology. DMT1 has been discovered in the nuclei of other cell types, and while its presence is not an artifact of the methodology, DMT1 expression implies an important role for DMT1 in spermato genesis and male fertility. This may be useful when considering conditions of abnormal iron regulation. The broad specificity of DMT1 may also implicate these findings in the transport of other divalent and toxic metals and could help to explain their testicular toxicity.

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