Cloning, expression and characterization of mouse spermatid specific thioredoxin-1 gene and protein*

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Thioredoxins are proteins that participate in different cellular processes via redox-mediated reactions. For humans, we have recently described two novel members of this family that display a male germ cell specific expression pattern, named spermatid specific thioredoxin (Sptrx-1 and Sptrx-2 respectively). We report here the cloning and characterization of the mouse Sptrx-1 gene and protein, which are similar to those described for the human orthologue. The mouse Sptrx-1 open reading frame encodes for a protein of 462 aa composed of an N-terminal repetitive domain of a 15 residue motif followed by a C-terminal domain typical of thioredoxins. The mouse Sptrx-1 gene sequence is interrupted by only one intron of 525 bp located in the 5'-UTR, and using fluorescence in-situ hybridization we have mapped its chromosomal location to 17E1.2–1.3. Northern blot analysis identified the testis as the only tissue expressing mouse Sptrx-1 mRNA, and by in-situ hybridization we found a strong labelling in the testicular seminiferous tubes, mostly in the round spermatids. Affinity purified antibodies against human Sptrx-1 crossreacted well with the mouse protein confirming its expression in seminiferous tubules at the later stages of spermiogenesis. Recombinant mouse Sptrx-1 displayed protein disulphide reducing activity in an enzymatic assay coupled to NADPH and thioredoxin reductase. The availability of the mouse Sptrx-1 gene sequence is the first step towards the generation of knock-out mice, whose characterization will provide significant information regarding the in-vivo function of Sptrx-1 and its possible implication in several sperm anomalies.

Key words: fibrous sheath/sperm/testis/thioredoxin

Introduction
Thioredoxins are a family of proteins that are conserved in all organisms through evolution and are characterized by the sequence of their highly conserved active site Cys-Gly-Pro-Cys (CGPC). Thioredoxins participate in different cellular mechanisms, mainly redox reactions, by the reversible oxidation of their active site from the dithiol form to disulphide (Arner and Holmgren, 2000; Powis and Montfort, 2001). To be active, thioredoxins must be in their reduced form, and this state is maintained by the flavoenzyme thioredoxin reductase at the expense of the reducing power of NADPH, thus forming the so-called thioredoxin system (Holmgren et al., 1994). In addition, thioredoxins reduce other metabolic enzymes such as PAPS (3'-phosphoadenosine 5'-phosphosulphate) reductase or methionine sulfoxide reductase, regulate transcription factor DNA binding activity, act as antioxidant molecules, modulate apoptosis and are also implicated in many pathological situations (Powis and Montfort, 2001).

All organisms so far investigated, from bacteria to mammals, contain several thioredoxin systems (Spyrou et al., 2001). During recent years, the thioredoxin field has experienced an important expansion as new members of the family have been described and new functions of known members of this family have been reported. The most recent breakthroughs in the field have been the characterization of a complete thioredoxin system in mitochondria of eukaryotic organisms (Miranda-Vizuete et al., 2000; Laloi et al., 2001); the discovery that, in Drosophila, thioredoxin reductase is able to reduce glutathione as this organism lacks a functional glutathione reductase (Kanzok et al., 2001); the characterization of a novel form of thioredoxin reductase with a N-terminal extension and displaying high homology to glutaredoxins (another redox enzyme closely related, both structurally and functionally, to thioredoxins) (Sun et al., 2001), and the identification of the first two members of the family with a tissue-specific expression pattern, exclusively located in the tail of human sperm, named spermatid specific thioredoxin (Sptrx-1 and Sptrx-2 respectively) (Miranda-Vizuete et al., 2001; Sadek et al., 2001).

The mammalian sperm tail is divided into three main regions running in a proximal to distal direction away from the sperm head: the mid-piece (attached to the sperm head by the neck or connecting piece), the principal piece and the end piece (Eddy and O’Brien, 1994; Curry and Watson, 1995; for a scheme on sperm tail organization). The
flagellum of the mammalian sperm is organized around the central axoneme which consists of 9+2 microtubule doublets, similar to those seen in the cilia and flagella of all eukaryotic cells, and extends through the full length of the flagellum (Eddy and O’Brien, 1994). In addition, mammalian sperm contain characteristic cytoskeletal elements associated with the axoneme, namely outer dense fibres and fibrous sheath (FS), that do not have any counterparts in any other cell types (Oko, 1998). The function of the outer dense fibres and FS is not fully elucidated, but it seems to be related to the control of flagellar motion (Curry and Watson, 1995) and protection against shearing forces during epididymal transit (Bultz et al., 1990). However, evidence for a more active role than merely structural role of the outer dense fibres and FS in sperm function is increasing, supported by the fact that among their constituent proteins there are several displaying either enzymatic or regulatory functions. For example in the FS, GAPDS (glyceraldehyde 3-phosphate dehydrogenase-S) encodes a novel glycolytic enzyme that is present only in condensing spermatids and produces ATP via the glycolytic pathway (Welch et al., 2000). This is critical for the transition to hyperactivated motility as well as capacitation (Williams and Ford, 2001). Moreover, the initiation and maintenance of sperm motility is regulated by a cascade of phosphorylation/dephosphorylation events (Tash and Bracho, 1994; review). In this respect A-kinase anchoring protein (AKAP), also located in the FS, tethers cAMP-dependent protein kinase A, directing and specifying the actions of the kinase in close proximity to the sperm’s axonemal machinery (Feliciello et al., 2001).

In rat, Sptrx-1 transiently associates to the longitudinal columns of the FS during sperm tail elongation, but it is not a structural FS component as it is discharged to the residual body and cytoplasmic elements associated with the axoneme, namely outer dense fibres and fibrous sheath of the FS (R.Oko and Vizuete, unpublished data). Sptrx-2 is also located in the sperm FS, proximity to the sperm kinase A, directing and specifying the actions of the kinase in close proximity to the sperm.

Expression and purification of mouse recombinant Sptrx-1

The ORF encoding mouse Sptrx-1 was cloned into the BamHI–EcoRI sites of the pGEX-4T-1 expression vector (Pharmacia) and used to transform E. coli BL21(DE3). A single positive colony was inoculated in 1 l of LB medium plus ampicillin and grown at 37°C until A600 = 0.5. The production of the fusion protein was induced by addition of 0.5 mmol/l isoprropyl-1-thio-β-D-galactopyranoside and growth was continued for another 3.5 h. Overexpressing cells were harvested by centrifugation and frozen until use.

The cell pellet was resuspended in 40 ml 20 mmol/l Tris–HCl, 1 mmol/l EDTA and 150 mmol/l NaCl plus protease inhibitor cocktail at the concentration recommended by the manufacturer (Sigma). Lysozyme was added to a final concentration of 0.5 mg/ml with stirring for 30 min on ice. Sarkosyl (1%) was added and the cells were disrupted by 10 min sonication. The supernatant was cleared by centrifugation at 15 000 g for 30 min and loaded onto a glutathione sepharose 4B column (Pharmacia). Binding to the matrix was allowed to occur for 2 h at room temperature. Thrombin (Sigma) 5 IU per mg fusion protein was used to remove GST by incubation overnight at 4°C. The resulting protein preparation was then subjected to ion exchange chromatography using a HiTrap Q column (Pharmacia) and mouse Sptrx-1 was eluted using a gradient of NaCl. Protein concentration was determined from the absorbance at 280 nm using a molar extinction coefficient of 11.290 mol−1 cm−1. A Western blot on mouse recombinant Sptrx-1 was performed as previously described for human Sptrx-1 using the same affinity purified antibodies (Miranda-Vizuete et al., 2001).

Enzymatic activity assays

Thioreredox reductase and NADPH were used as electron donors to determine the enzymatic activity of mouse Sptrx-1 and the assay was performed essentially as described previously (Spyrou et al., 1997). In brief, aliquots of recombinant mouse Sptrx-1 were added to 40 µl of a reaction mixture composed of 20 µl HEPES (1 mol/l), pH 7.6, 40 µl EDTA (0.2 mol/l), 40 µl NADPH (40 mg/ml) and 500 µl insulin (10 mg/ml). The reaction was initiated by the addition of 10 µl of thiorereductase from calf thymus (3.0 A412 unit), and incubation was continued for 20 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 6 mol/l guanidine–HCl, 1 mmol/l DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)], and the absorbance at 412 nm was measured. Human Trx-1 and Sptrx-1 were used as controls.

Immunohistochemistry and immunofluorescence analysis

CD mice were anaesthetized and the testes and epididymides were fixed by perfusion through the abdominal aorta and heart respectively in Bouin’s fixative. Fixed tissues were washed extensively in 75% alcohol before being completely dehydrated in ethanol and embedded in paraffin. For light microscopy immunocytochemistry, 5 µm paraffin sections were deparaffinized and hydrated through a graded series of ethanol concentrations before immunoperoxidase localization with human Sptrx-1 antibody by standard procedures (Oko, 1998). Staging of the cycle of the seminiferous epithelium and determining the steps of spermiogenesis was done according to the classifications of Leblond and Clermont (Leblond and Clermont, 1952).
Results

cDNA cloning, sequence analysis, genomic organization and chromosomal localization of the mouse Sptrx-1 gene

The complete sequence of the mouse Sptrx-1 cDNA consists of an ORF of 1386 bp, a 5'-UTR of 315 bp including one stop codon in frame, and a very short 3'-UTR of 17 bp before the poly(A) tail (Figure 1). Analysis of the mouse Sptrx-1 ORF identified five different potential start methionine residues (Figure 1). We propose methionine 4 as the putative start site based on the similarity to the human Sptrx-1 sequence. A potential polyadenylation signal is present within the ORF in close proximity to the stop codon, explaining the short 3'-UTR. Mouse Sptrx-1 ORF encodes a protein of 462 aa with an estimated molecular mass of 52 kDa and a pI of 5.24. It has an identical domain organization to that of the human protein: a N-terminal domain characterized by a unique arrangement organized as repeats of a 15 residue motif shortly after the start methionine, and a C-terminal thioredoxin domain. As shown in Figure 1, the repeated motif has a general pattern [PKSSEDIQ(S/P)KK(E/G)DR] which is highly conserved and rich in both basic (Lys) and acidic residues (Glu, Asp) which are responsible for the low pI and net charge at pH 7.0 of –17.28.

Other features previously described for the human Sptrx-1 protein (Miranda-Vizuete et al., 2001) also pertain to mouse Sptrx-1, including the presence of two additional cysteines (residues 443 and 450) in the thioredoxin domain at identical positions to that of mouse Trx-1 protein (Matsui et al., 1995) (which serves as module for the rest of the members of the thioredoxin family of proteins) and human Sptrx-1 (Figure 2). Also similar to human Sptrx-1, mouse Sptrx-1 contains several potential phosphorylation sites for different protein kinases and two highly scored PEST sequences for proteasome-dependent degradation centered at positions 12 and 315 (Figure 1). However, while we could not predict any coiled-coil domain within human Sptrx-1 protein, we found that mouse Sptrx-1 is predicted to organize as coiled-coil between residues 324 to 381 (we used the Lupa’s algorithm at http://psort.ims.u-tokyo.ac.jp/form2.html). A coiled-coil is a bundle of α-helices that are wound into a superhelix, which might be important for the maintenance of the oligomeric structure (Lupas, 1997). Interes-

Figure 1. Nucleotide and amino acid sequence of mouse Sptrx-1. Three 5'-UTR ATG in frame are in boldface and boxed. The upstream stop codon in frame is in boldface and underlined. The down arrow indicates the position of the intron within the 5'-UTR. The two PEST sequences are double underlined. The thioredoxin domain is shadowed and the WCGPC active site within the domain is boxed. The putative polyadenylation signal is in boldface and underlined. The stop codon is marked with asterisks.

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Figure 2. Alignment of the predicted amino acid sequence of human and mouse Sptrx-1. Identical residues are boxed. The arrowheads show the common cysteine residues in human and mouse Sptrx-1 proteins while the asterisks indicate the cysteine residues in the human protein not conserved in the mouse orthologue.

Interestingly, mouse Sptrx-1 lacks one of the repetitive domains (Figure 2) close to the C-terminal thioredoxin domain and this could be an explanation for the difference between the human and mouse protein structures.

Next, we used the mouse Sptrx-1 ORF to screen a mouse genomic library (genetic background ES129/SvJ) and identified seven BAC clones containing the mouse Sptrx-1 gene. We sequenced three of these clones in both directions and found that, similar to the human gene, the mouse Sptrx-1 gene contains only one intron of 525 bp located in the 5'-UTR (Figure 3A).

To determine the chromosomal localization of mouse Sptrx-1, we performed fluorescence in-situ hybridization analysis in mouse metaphase chromosomes using one of the above clones as probe. An initial experiment resulted in specific labelling of the middle region of a small chromosome which was believed to be chromosome 17 on the basis of DAPI staining. To confirm this point we conducted a second experiment with a probe specific for the telomeric region of mouse chromosome 17 which was co-hybridized with the mouse Sptrx-1 genomic clone. This experiment resulted in the specific labelling of the telomere and the middle portion of chromosome 17 (Figure 3B). Measurement of 10 specifically labelled chromosomes 17 demonstrated that the mouse genomic clone is located at a position which is 71% of the distance from the heterochromatic–euchromatic boundary to the telomere of chromosome 17, an area that corresponds to band 17E1.2–1.3 (Figure 3C). A total of 80 metaphase cells was analysed with 75 exhibiting specific labelling. We have confirmed this position in the Celera Mouse Database (http://www.celera.com) where mouse Sptrx-1 is flanked by the Vapa and Rip1 genes, both located in the middle portion of chromosome 17 (data not shown). Remarkably, Vapa protein contains a major sperm protein domain, which is found in proteins involved in sperm motility and are capable
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Figure 3. Genomic organization and chromosomal localization of the mouse Sptrx-1 gene. (A) The sequence of the 5'-UTR intron is shown with the conserved GT/AG dinucleotides at the intron junction in boldface. (B) Fine mapping of the mouse Sptrx-1 gene shown by FISH analysis. A telomeric probe for mouse chromosome 17 is pointed out by a white arrowhead, while the doublet signal for mouse Sptrx-1 is indicated by a red arrowhead, on a blue-painted full set of mouse chromosomes. (C) Ideogram of G-banded mouse chromosome 17 with the possible locus for mouse Sptrx-1 gene depicted by a red arrowhead.

of oligomerizing to form filaments (Skehel et al., 2000). Moreover, several genes in the close proximity to mouse Sptrx-1 also display a testis-specific expression pattern, such as Srrf1 (spermatid-specific RING zinc finger protein 1, also termed sperizin) or fert1 (fer testis tyrosine kinase) (Fischman et al., 1990; Fujii et al., 1999).

Tissue expression of mouse Sptrx-1 mRNA

First, we used multiple-tissue Northern blots to determine the size and tissue distribution of mouse Sptrx-1 mRNA (using the ORF as the probe), which was only detected in mouse testis as a single band of ~1.7 kb in good agreement with the size of the cloned cDNA (Figure 4A). To evaluate the possibility that mouse Sptrx-1 mRNA could be expressed in other tissues not present in these blots, we also screened an RNA dot blot containing poly(A)+ RNAs from 22 different mouse tissues. Among the tissues examined, a hybridization signal was observed only in testis mRNA (Figure 4B).

To further investigate the expression pattern of mouse Sptrx-1 mRNA, in-situ hybridization was performed in mouse testis sections.
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Expression pattern of mouse Sptrx-1 mRNA. (A) Mouse multiple tissue Northern blot. The mouse Sptrx-1 probe hybridized with one mRNA species at 1.7 kb only in testis. β-actin was used as control. (B) Mouse RNA master blot where Sptrx-1 probe hybridized only with testis mRNA (arrow). For a complete list of mouse tissues see CLONTECH homepage (http://www.clontech.com/archive/OCT97UPD/MasterBlot.shtml). (C) A strong signal for Sptrx-1 mRNA can be seen in a large number of seminiferous tubules in mouse testis section. Bar = 200 µm. (D) In dipped sections, signals for Sptrx-1 mRNA are seen in round spermatids (indicated by triangles), while pachytene spermatocytes (ps), elongating spermatids (es), Leydig cells (Lc) and other testicular cells are devoid of signal. Bar = 30 µm.

Figure 4. Expression pattern of mouse Sptrx-1 mRNA. (A) Mouse multiple tissue Northern blot. The mouse Sptrx-1 probe hybridized with one mRNA species at 1.7 kb only in testis. β-actin was used as control. (B) Mouse RNA master blot where Sptrx-1 probe hybridized only with testis mRNA (arrow). For a complete list of mouse tissues see CLONTECH homepage (http://www.clontech.com/archive/OCT97UPD/MasterBlot.shtml). (C) A strong signal for Sptrx-1 mRNA can be seen in a large number of seminiferous tubules in mouse testis section. Bar = 200 µm. (D) In dipped sections, signals for Sptrx-1 mRNA are seen in round spermatids (indicated by triangles), while pachytene spermatocytes (ps), elongating spermatids (es), Leydig cells (Lc) and other testicular cells are devoid of signal. Bar = 30 µm.

Sptrx-1 mRNA was identified in a vast majority of the seminiferous tubules (Figure 4C) and analysis of mouse testis sections at higher magnification clearly showed a strong labelling in round spermatids with no signal in the remainder of the testicular cells (Figure 4D). This expression pattern is consistent with the in-situ data reported for human Sptrx-1 mRNA (Miranda-Vizuete et al., 2001).

Expression and enzymatic activity of mouse Sptrx-1 protein

Recombinant human Sptrx-1 migrated in SDS–PAGE at 90 kDa although its theoretical size is 53 kDa (Miranda-Vizuete et al., 2001). This apparent discrepancy can be explained by the potential α-helical structure of the N-terminal repetitive domain. Using specific polyclonal antibodies directed to the repetitive N-terminal domain of human Sptrx-1, we were able to demonstrate the presence of a similar protein in extracts of murine testis (Miranda-Vizuete et al., 2001). To confirm this point we produced recombinant mouse Sptrx-1 and showed that it migrates in SDS gels at a similar size to its human orthologue, and it is readily recognized by the antibodies raised against the human protein (Figure 5A, inset). We also evaluated the reducing activity of mouse Sptrx-1 using NADPH and calf thymus thioredoxin reductase and found that mouse protein is able to reduce the disulphide bonds of insulin at a similar rate to that of human protein (Figure 5A).

Tissue expression and cellular localization of mouse Sptrx-1 protein

To investigate whether mouse Sptrx-1 protein distribution resembles that of the human counterpart (Miranda-Vizuete et al., 2001), we performed immunohistochemical analysis in mouse testis sections. As shown in Figure 5B, mouse seminiferous tubules immunostained mostly in the tail region of elongating spermatids and this staining was abolished when using the antibody previously pre-adsorbed with the mouse recombinant protein. A more detailed analysis showed that mouse Sptrx-1 is expressed at late steps of spermiogenesis, coincidental with the assembly of tail structures such as the FS or outer dense fibres (Figure 5C). This result is consistent with that obtained in rat testis sections where Sptrx-1 expression peaks at steps 14–16 (stages XIV–III) and transiently associates to the longitudinal columns of the FS (Y. Yu, R. Oko and A. Miranda-Vizuete, unpublished data). Other cellular types such as spermatogonia, spermatocytes, Leydig cells and Sertoli cells are devoid of signal (data not shown).
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Figure 5. Enzymatic activity, Western blot and immunohistochemical analysis of mouse Sptrx-1 expression in mouse testis. (A) Purified mouse Sptrx-1 was assayed for its ability to reduce insulin disulphide bonds in the presence of NADPH and calf thymus thioredoxin reductase. □, human Trx-1 reduced; ○, mouse Sptrx-1 oxidized; Δ, human Sptrx-1 oxidized. A representative experiment is shown. The inset shows the titration of mouse recombinant Sptrx-1 protein using the antibodies raised against the human orthologue. (B) Light microscopy micrograph of portions of paraformaldehyde-fixed mouse seminiferous tubules either incubated with anti Sptrx-1 antibody (left panel) or antibody pre-adsorbed with recombinant mouse Sptrx-1 (right panel). Only the former is immunoperoxidase reactive (black precipitate), indicating that the sperm tail immunoreactivity in the seminiferous tubular lumen is specific. Bar = 10 µm. (C) Stage identifiable sections through mouse seminiferous tubules immunoperoxidase-stained with affinity purified anti Sptrx-1 serum diluted 1/20. Staining (black precipitate) of the elongating sperm tails is especially evident in stages X (spermatid step 10), XI (step 11) and II (step 16) of the cycle, while faint immunostaining is first detected in stage IX (step 9). By stage V–VI (steps 17–18) immunostaining is practically absent from the luminal tails. Bar = 40 µm.

Discussion

Progress in the sequencing of mammalian genomes, including the human, has provided an invaluable tool for the discovery of new genes (Baltimore, 2001). Practically all protein families have expanded in numbers during recent years, and this is also the case for the thioredoxin family. Thioredoxins are a class of redox proteins that function as general protein disulphide reductases by the reversible oxidation of their conserved active site (Cys-Gly-Pro-Cys) (Holmgren and Björnstedt, 1995). In mammals, five proteins with the active site CGPC have been described to date: Trx-1, which is mostly cytosolic but can translocate into the nucleus upon certain stimuli and is also secreted (Powis and Montfort, 2001); Trx-2, a mitochondrial enzyme (Spyrou et al., 1997); Txl-1, an ubiquitous protein of unknown function (Miranda-Vizuete et al., 1998); and Sptrx-1 and Sptrx-2, the first two members of the family with a tissue-specific distribution in the flagellum of human sperm (Miranda-Vizuete et al., 2001; Sadek et al., 2001). In this context, we report here the cloning and characterization of the mouse Sptrx-1 gene and protein.
The mouse Sptrx-1 gene is identical in structure to its human orthologue (indicating a common ancestor originated before the rodent/primate radiation), with only one intron of ~0.5 kb that interrupts the 5′-UTR of the mRNA. Furthermore, both mouse and human Sptrx-1 mRNAs have an extremely short 3′-UTR with a potential polyadenylation signal within the ORF. However, while human mRNA contains two potential start methionine residues in frame, the mouse gene has five. Taken together, all these features point to the direction of an exquisite regulation of Sptrx-1 mRNA expression. Indeed, the synthesis of many sperm tail polypeptides is regulated at the translational level as their transcripts are synthesized and accumulated during the haploid phase of spermatogenesis while the spermatic nucleus is still transcriptionally active. Translation then occurs after condensation of the spermatic nucleus (Oko and Clermont, 1989; Catalano et al., 2001). Major translational regulatory mechanisms are, for instance, the presence of upstream AUG codons in the 5′-UTR prior to the main ORF (Gray and Wickens, 1998), introns harbouring regulatory sequences (mostly the first intron and located within the 5′-UTRs) that modulate the expression of their mRNA (Gray and Wickens, 1998) or control for the choice of the polyadenylation site (Zhao et al., 1999). All these mechanisms might account for Sptrx-1 regulation as its mRNA is mostly found in round spermatids while the protein expression starts later (at step 9) and peaks at step 15 of the rat spermiogenesis cycle (Y.Yu, R.Oko and A.Miranda-Vizuete, unpublished data).

Mouse and human Sptrx-1 proteins are quite similar in their overall domain structure with an N-terminal repetitive domain consisting of a 15 residue motif highly conserved among repetitions and a C-terminal domain typical of thioredoxins (Miranda-Vizuete et al., 2001). However, a detailed homology analysis between the two orthologues reveals a much higher amino acid identity at the thioredoxin domain (63%) than at the N-terminal repetitive domain (42%). This difference might reflect a more strict requirement for the Sptrx-1 dependency of thioredoxin activity while the higher divergence at the N-terminus might be a consequence of specific interactions with other FS components also with a high divergence between human and mouse sperm. This is further supported by the presence of a coiled-coil domain in the mouse protein, which might be important for the regulation of the oligomeric structure of the native mouse protein, whereas the human protein does not predict this domain. The identification of coiled-coil domains in other sperm proteins has been reported for outer dense fibre proteins, ODF2 and ODF3, thus suggesting that this coiled-coil organization might be required for some fibrillar proteins that conform the sperm tail (Petersen et al., 2002).

The mouse Sptrx-1 gene maps at chromosome 17, which plays a pivotal role in male fertility as it contains a naturally occurring variant of several genes, termed the h haplotypes, shown to influence male, but not female, fertility (Olds-Clarke, 1997). The variant alleles of genes at the h haplotypes are linked together in four inversions at the proximal part of chromosome 17 and males carrying two different h haplotypes are sterile because their sperm exhibit severe motility defects and also are unable to penetrate zona pelucida-free oocytes. This phenotype is reduced in sperm from mice carrying only one h haplotype, as they exhibit only mild motility and delayed penetration of zona pellucida-free oocytes (Olds-Clarke, 1997). Several gene candidates for sterility factors have been identified within the inversions of the h haplotypes, although their specific role in the infertility phenotype is still unknown (Olds-Clarke, 1997). Mouse Sptrx-1, a novel sperm-specific gene, maps at a more distal part of chromosome 17 and, therefore, outside the h haplotype interval. However, the Sptrx-1 locus at 17E1.2–1.3 also contains other genes with a testis-specific expression such as Vapa, Ssrz1 and fert1 (Fischman et al., 1990; Fujii et al., 1999; Skehel et al., 2000). Whether chance or selection has resulted in the accumulation of so many genes involved in sperm function on chromosome 17, the end result is an excellent model system for understanding the genetic basis of mammalian fertilization.

We have recently found that Sptrx-1 transiently associates to the longitudinal columns of the fibrous sheath during tail elongation in rat spermiogenesis (Y.Yu, R.Oko and A.Miranda-Vizuete, unpublished data). We show here that the mouse protein is also expressed during sperm tail assembly and therefore a conserved expression pattern in mammals pertains to Sptrx-1. Several pathologies, resulting in male sterility and affecting the correct assembly of the sperm tail have been described (Chemes, 2000); among these, dysplasia of the fibrous sheath (DFS) is the best characterized. The main features of DFSs are a marked hypertrophy and hyperplasia of random FS components that form thick rings or broad meshes without the orderly disposition in longitudinal columns and transversal ribs that characterize the normal FS (Chemes et al., 1998; Rawe et al., 2001). Although a genetic basis has been proposed to underlie this pathology, no genes have been thus far reported to be involved in this disease. Because of the transient association to the longitudinal columns of the FS, Sptrx-1 can be considered as a candidate gene for DFS as a defect in its expression or activity might result in abnormal sperm motility due to severe abnormalities of the FS. One way to explore this possibility is the generation of knock-out mice in Sptrx-1 and the data here reported are the first step towards this approach.

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