Reduced Body Weight and Increased Postimplantation Fetal Death in Tyrosylprotein Sulfotransferase-1-deficient Mice*

Ying-Bin Ouyang‡, James T. B. Crawley‡, Christopher E. Aston§, and Kevin L. Moore¶

From the ‡Cardiovascular Biology and ¶Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma Center for Medical Glycobiology, Oklahoma City, Oklahoma 73104

Tyrosine sulfation is mediated by one of two Golgi isoenzymes, called tyrosylprotein sulfotransferases (TPST-1 and TPST-2). A relatively small number of proteins are known to undergo tyrosine sulfation, including certain adhesion molecules, G-protein-coupled receptors, coagulation factors, serpins, extracellular matrix proteins, and hormones. As one approach to explore the role of these enzymes in vivo and how they might interact in biological systems, we have generated TPST-1-deficient mice by targeted disruption of the Tpst1 gene. Tpst1−/− mice appear normal and, when interbred, yield litters of normal size with a Mendelian genetic distribution and an equal sex distribution. Tpst1−/− mice appear healthy but have ~5% lower average body weight than Tpst1+/+ controls. In addition, we show that although fertility of Tpst1−/− males and females per se is normal, Tpst1−/− females have significantly smaller litters because of fetal death between 8.5 and 15.5 days postcoitum. These findings suggest that there are proteins involved in regulation of body weight and reproductive physiology, which require tyrosine sulfation for optimal function that are yet to be described. Our findings also strongly support the conclusion that TPST-1 and TPST-2 have distinct biological roles that may reflect differences in their macromolecular substrate specificity.

Tyrosine sulfation is mediated by one of two closely related and ubiquitously expressed Golgi isoenzymes, called tyrosylprotein sulfotransferases-1 and -2 (TPST-1 and TPST-2). TPSTs catalyze the transfer of sulfate from PAPS to the side chain hydroxyl of tyrosine residues within acidic motifs of proteins that transit the Golgi. Mouse and human cDNAs encoding TPST-1 were first isolated by Ouyang et al. (1) using the amino acid sequence obtained from a purified rat liver TPST preparation. Subsequently, a second member of the gene family, TPST-2, was identified based on its high degree of homology to TPST-1 (2, 3). There is no evidence for the existence of additional mouse or human TPST genes in the publicly accessible genomic or expressed sequence tag databases. Human and mouse TPST-1 are ~96% identical, and human and mouse TPST-2 have a similar degree of identity. TPST-1 is ~65% identical to TPST-2 in both mouse and man (4). Each enzyme is ~370 amino acids in length with type II transmembrane topology and a lumennally oriented catalytic domain. Experimental evidence shows that TPST-1 has two N-linked sugars and indicates that the luminal catalytic domain contains one or more disulfide bonds (1, 2).

Based on Northern blot analyses of a variety of human and mouse tissues, TPST-1 and TPST-2 transcripts are ubiquitously expressed, although there are some differences in the abundance of TPST transcripts in certain tissues (1, 2). Expressed sequence tags corresponding to both enzymes are represented in many cDNA libraries derived from a variety of normal tissues, including libraries derived from mouse embryos as early as 8 days postcoitum (dpc). We have detected TPST-1 and TPST-2 transcripts in several clonal cell lines including HL-60 (promyelocytic leukemia), HeLa S3 (cervical adenocarcinoma), K-562 (chronic myelogenous leukemia), MOLT-4 (T cell acute lymphoblastic leukemia), SW480 (colon adenocarcinoma), A549 (lung carcinoma), and G361 (melanoma) cells, as well as human umbilical endothelial cells. Taken together, these data strongly suggest that both enzymes are co-expressed in many and perhaps in all cell types.

An examination of the amino acid residues surrounding known tyrosine sulfation sites in proteins reveals that the major characteristic feature is the presence of several acidic amino acid residues within ±5 residues of the sulfotyrosine. However, there are no invariant residues in the window surrounding known sulfotyrosines. Based on this information coupled with in vitro studies using synthetic peptide acceptors and crude enzyme preparations, consensus features for tyrosine sulfation have been described (PROSITE accession number PS00003, www.expasy.ch/prosite) (5, 6). However, the positive predictive value of these features is unknown. To date, only a couple dozen tyrosine-sulfated proteins have been described in man, and substantially fewer have been documented in the mouse, but there are certainly many others yet to be described (4, 5, 7). Among the known tyrosine-sulfated proteins are many that play important roles in inflammation, hemostasis, immunity, and other processes. These include certain adhesion molecules (P-selectin glycoprotein ligand-1, platelet glycoprotein Ibα), G-protein-coupled receptors (CCCR5, CCR2B, CXXCR4, and C5α receptor), coagulation factors (factors V, VIII, and IX), serpins (α2-antiplasmin and heparin cofactor II), extracellular matrix proteins (vitronectin, fibronectin, laminin, type III col-
lagen, and microfibril-associated glycoprotein-1, hormones (gastrin and cholecystokinin), and others. Tyrosine sulfonation has been shown to be required for the optimal function of some proteins. For example, tyrosine sulfation of P-selectin glycoprotein ligand-1 is required for binding to P-selectin (8). Tyrosine sulfation of coagulation factors V and VIII is required for optimal thrombin-mediated activation (9, 10), and sulfation of cholecystokinin (CCK) is required for CCK-A receptor activation (11). However, in many of the known tyrosine-sulfated proteins, a functional role for tyrosine sulfation has not been established.

Little is known about the macromolecular substrate specificity of these two enzymes and whether their substrate repertoires are distinct or overlapping. However, the very limited data that have been published on this question suggest that the specificity of the two TPSTs toward small peptide acceptors differs (2).

This information, or lack thereof, makes it impossible to make cogent predictions regarding the phenotype(s) of TPST-deficient mice, although it would seem reasonable to expect that the phenotypic expression of TPST-1 or TPST-2 deficiency may be pleiotropic given the functional importance of tyrosine sulfation in the optimal function of several known and perhaps many unknown tyrosine-sulfated proteins. To gain a better understanding of TPST function in vivo we have developed TPST-1-deficient mice by targeted disruption of the Tpst1 gene. Herein we describe the details of our work on the production and initial characterization of these mice.

**EXPERIMENTAL PROCEDURES**

**Targeted Disruption of the Mouse Tpst1 Gene**—Mouse genomic clones were identified and plaque purified from a 129S6/SvEv spleen Lambda FIX II™ genomic library (Stratagene, La Jolla, CA) by high stringency hybridization with a full-length mouse Tpst1 cDNA probe. The genomic organization of the Tpst1 gene was partially characterized by a combination of Southern blotting, restriction mapping, and sequence analysis. Comparison of genomic and cDNA sequences showed that most of the Tpst1-1 open reading frame including the N-terminal 281 amino acids and the initiating methionine, all six lumenal cysteine residues, and both N-linked glycosylation sites (Fig. 1A). Most importantly this region contains both the 5'-phosphate-sulfate binding (PSB) motif and the 3'-phosphate binding (PB) motif described previously as being involved in binding to the 5'- and 3'-untranslated substrate analog PAP in the four known sulfotransferase crystal structures (12).

A construct was engineered for targeted disruption of the Tpst1 gene in which a 1.3-kb fragment spanning exon 3 was replaced by a 1.7-kb PGK neo cassette (Fig. 1B). A 4.5-kb SspI-XbaI genomic fragment was used as the left arm for homologous recombination and was cloned into the PGK neo vector upstream of the PGK neo cassette. The 1.1-kb right arm 3' to exon 3 was generated by PCR and cloned downstream of the PGK neo cassette. Sequencing of both strands confirmed that the nucleotide sequence was identical to the genomic template used to generate it. The construct was linearized with NotI and electroporated into AB2 embryonic stem cells, an ES cell line derived from male 129S6/SvEv mice (Lexicon Genetics Inc., Houston, TX). After G418 selection, five positive clones were identified from 900 G418-resistant colonies by PCR screening. Surviving clones were further analyzed by Southern blotting to confirm the correct homologous recombination event, and random integration of extra copies of the targeting construct was excluded by hybridization with a neomycin probe (not shown). ES clones were injected into blastocysts, and the blastocysts were implanted into pseudopregnant mice to generate chimeras. Chimeric males were bred with wild-type 129S6/SvEv females (Taconic Farms, Germantown, NY) to generate Tpst1+/− mice on a 129S6/SvEv inbred genetic background, and Tpst1−/− mice were interbred to generate mice with three Tpst1 genotypes (Tpst1−/−, Tpst1−/+ , Tpst1+/+) . Genotyping was performed by PCR using the following primers: 5′-GGACTGTCTGACCAAGTGGAA-3′ and 5′-GTTTGTCCTCGCTCCTCCCAATTGCTGCG-3′ for the 348-bp fragment from the wild-type allele, and 5′-CCCTGCTGTT-TACGGTATGGCCGC-3′ and 5′-GGTGGTCCCTGCCTCCCAATTGCTGCG-3′ for the 600-bp fragment from the mutant allele. All experiments reported here were performed on mice in the 129S6/SvEv background.

**Northern and Southern Blot Analysis**—Total RNA was isolated from tissues using Trizol Reagent (Invitrogen), separated on 1% denatured agarose, and transferred to nitrocellulose membrane. For Northern blotting a 1205-bp fragment corresponding to nucleotides 212–1416 of the full-length cDNA was used as a probe. For Southern blotting, tail DNA was isolated (Qiagen genomic DNA kit), exhaustively digested with XhoI, and then separated on 1% agarose gels and transferred to nitrocellulose membranes. A 793-bp TPST-1 DNA fragment downstream of the right arm (Probe B) was used as a probe. Filters were prehybridized with Hybrisol solution (Oncor) for 60 min at 42 °C and hybridized with 32P-labeled probes overnight at 42 °C. The blots were washed twice with 2× SSC, 0.1% SDS for 20 min at 22 °C and twice with 0.1× SSC, 0.1% SDS for 20 min at 55 °C. The membrane was exposed to a phosphorimaging screen at room temperature.

**Preparation of Mouse Liver Microsome Extracts and TPST Assays**—Mice were euthanized, and the livers were rapidly excised and immersed in cold homogenization buffer (10 mM Tris-HCl (pH 7.5), 1.5 mM MgCl2, 250 mM sucrose, 0.1 mM dithiothreitol, 0.5 mM phenylmethylsulfonfyl fluoride). Homogenates were centrifuged (100,000 × g, 10 min, 8000 × g), and the postnuclear supernatants were centrifuged (30 min, 16,000 × g). The microsomal pellets were suspended in 1.5 ml/g of liver in 2% Triton X-100, 20 mM TAPS (pH 9.0), 0.5 mM phenylmethylsulfonfyl fluoride, 10 μM leupeptin/antipain and stirred for 1 h. Microsomal extracts were then clarified by centrifugation (30 min, 16,000 × g), and the supernatants were centrifuged at 100,000 × g, and stored at 80 °C. TPST activity was determined by measuring the transfer of [35S]sulfate from [35S]PAPS to an immobilized peptide substrate (QATEYELDYLDFLPE) modeled on the N-terminal 15 residues of the mature P-selectin glycoprotein ligand-1 polypeptide as previously described (1).

**Chromosomal Localization**—Chromosomal mapping was performed using fluorescence in situ hybridization (FISH) signal mapping and 4',6-diamidino-2-phenylindole (DAPI) stain to assign chromosomal number by SeeDNA Biotech Inc. (Windsor, Canada). Lymphocytes were isolated from mouse spleen and cultured at 37 °C in RPMI 1640 supplemented with 15% fetal calf serum and 3 μg/ml concanavalin A, 10 μg/ml lipopolysaccharide, and 50 μM 2-mercaptoethanol. After 44 h the cells were treated with 0.18 mg/ml bromoethidine for an additional 14 h. Synchronized cells were washed and recultured at 37 °C for 4 h in α-minimal essential medium with 2.5 μg/ml thymidine, and chromosome slides were obtained.

A 5-kb mouse genomic DNA fragment spanning exon 3 was used for FISH mapping. The probe was biotinylated with dATP using the Invitrogen BioNick labeling kit (15 °C for 1 h). The procedure for FISH detection was performed according to Heng et al. (13) and Heng and Tuddenham. Briefly, slides were baked at 55 °C for 1 h, RNase-treated (70 °C for 2 × SSC, 2 min, 70 °C), then dehydrated with ethanol. Probes were denatured (75 °C, 5 min) in 50% formamide, 10% dextran sulfate, prehybridized for 15 min at 37 °C, and then hybridized overnight. FISH signals and DAPI banding patterns were captured using a CCD camera, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI-based chromosomes.

**RESULTS**

**Generation and Characterization of Tpst1 Knockout Mice**—To disrupt the Tpst1 gene we constructed a targeting vector in which exon 3 of the Tpst1 gene was replaced with a PGK neo cassette (Fig. 1B). Linearized targeting vector was used to electroporate ES cells, which were then subjected to positive selection. Homologous recombinants were detected using PCR and confirmed by Southern analysis of EcoNI-digested genomic DNA from the targeting vector. Genotyping of the targeting vector was performed by PCR using the following primers: 5′-GGACTGTCTGACCAAGTGGAA-3′ and 5′-GTTTGTCCTCGCTCCTCCCAATTGCTGCG-3′ for the 348-bp fragment from the wild-type allele, and 5′-CCCTGCTGTT-TACGGTATGGCCGC-3′ and 5′-GGTGGTCCCTGCCTCCCAATTGCTGCG-3′ for the 600-bp fragment from the mutant allele.
genomic DNA using probe A and XbaI-digested DNA using probe B (Fig. 1B). ES clones were injected into blastocysts, and the blastocysts were implanted into pseudopregnant mice using standard techniques. Male chimeras were mated with wild-type 129S6/SvEv females and heterozygotes were interbred to generate Tpst1+/− mice. Transmission of the targeted mutation was confirmed by PCR and genomic Southern blot analysis of tail DNA isolated from gene-targeted animals as described under “Experimental Procedures.” Homologous recombination at the Tpst1 locus resulted in the introduction of a XbaI site that results in a shorter XbaI genomic fragment (3.8 kb) than that from the wild-type allele (5 kb) that is detected by hybridization with Probe B (Fig. 1C).

Functional inactivation of the Tpst1 gene was confirmed by Northern blot analysis of total RNA isolated from Tpst1+/+, Tpst1+/−, and Tpst1−/− mouse organs (Fig. 1D). This analysis showed a ~50% reduction of full-length Tpst1 mRNA (~1.8 kb) in both spleen and testes of Tpst1−/− mice compared with wild-type and failed to detect full-length transcripts in Tpst1−/− even in testes, which expresses abundant levels of Tpst1 mRNA (1). A parallel analysis using a Tpst2 cDNA probe demonstrated that disruption of the Tpst1 gene had no detectable effect on expression of the Tpst2 gene in these tissues (Fig. 1D). In Tpst1−/− and Tpst1−/− mice, but not wild-type mice, a smaller mRNA species was detected in testes and was also apparent in the spleen when the blots were overexposed. To assess the structure of this transcript, Northern blots were reprobed with an exon 3-specific probe corresponding to nucleotides 212–740 of the full-length cDNA. As expected, this analysis detected the full-length message in Tpst1+/+, Tpst1+/−, and Tpst1−/− samples, thus confirming that this smaller transcript lacked exon 3 (not shown). Given that exon 3 encodes the first 281 of 370 amino acids of the mature protein, including the 5′-PB motif and the 3′-PB motif that are critical for catalysis, it is certain that this translation of this transcript could not result in a functional protein.

To determine the impact of TPST-1 deficiency on the total enzyme activity, assays of liver microsomal extracts were per-
formed as described under “Experimental Procedures” using a P-selectin glycoprotein ligand-1 acceptor peptide that is an efficient acceptor for both TPST-1 and TPST-2 (2). We found that TPST activity in liver extracts from Tpst1−/- mice was reduced to ~12% of that observed in wild-type mice (Fig. 1E). This suggests that TPST-1 is the predominant TPST in mouse liver. However, a firm conclusion cannot be made based on these data because we lack definitive knowledge regarding whether these enzymes differ with respect to their Km for PAPS and acceptor, and it is well established that PAPS is unstable in crude tissue extracts (15).

Growth and Development of Tpst1 Knockout Mice—In the 129S6/SvEv background, Tpst1−/- mice appeared normal. Male and female heterozygote mating pairs yielded litters of normal size (5.8 ± 2.1 (mean ± S.D., n = 57)) when compared with that reported for this strain by the supplier (Taconic Farms, Inc.). Among all offspring from Tpst1+/− × Tpst1+/− crosses, the ratio of the three Tpst1 genotypes was consistent with Mendelian inheritance, indicating that Tpst1−/- mice develop normally in utero and that the sex ratio in each Tpst1 genotype was ~50:50 (Table I).

To assess the growth of Tpst1−/- mice, a large cohort of mice were weighed weekly from age 2 to 10 weeks (Fig. 2). At 2 and 3 weeks of age, the mean weights of male and female Tpst1−/- mice were not statistically different from wild-type animals. However, after weaning, the mean body weights of male and female Tpst1−/- mice lagged behind that of wild-type animals. Beginning at 5–6 weeks of age, male and female Tpst1−/- mice weighed 0.8–1.3 and 0.6–1.0 g less than the wild-type cohort, respectively. Although modest, these differences in weight constitute ~5% of the body weight and were statistically significant beginning at 4–5 weeks (Fig. 2). The biological significance of this observation is substantially strengthened by the fact that these mice are on an inbred genetic background. The mean body weight of Tpst1+/− mice was not statistically different from wild-type animals (not shown). Otherwise, Tpst1+/− mice appeared healthy out to 12 months of age. Hematoxylin/eosin staining of tissues from 15-week-old mice showed no evidence of histological abnormalities in heart, liver, brain, spleen, kidney, lung, stomach, small or large bowel, testes, or ovary (not shown).

Reproductive Performance of Tpst1 Knockout Mice—Early in this study we noticed that litter sizes from Tpst1+/− females appeared somewhat low. Moreover, in a 1-year period, we observed that the mean size of litters at birth from Tpst1+/− × Tpst1+/−, Tpst1+/− × Tpst1−/-, and Tpst1−/- × Tpst1−/- crosses were 6.2 ± 2.0 (n = 55), 5.8 ± 2.1 (n = 57), and 3.8 ± 1.8 (n = 57), respectively. The lower mean litter size of Tpst1−/- × Tpst1−/- crosses compared with the other groups was highly statistically significant (p < 10^{-7}).

To examine the reproductive performance of Tpst1−/- mice in more detail, virgin female Tpst1+/− and Tpst1−/- mice from 8 to 20 weeks of age were pair-mated with either Tpst1+/− or Tpst1−/- males. The results of these matings are shown in Table II. To determine whether and when copulation occurred, females were examined for vaginal plugs each morning. Vaginal plugs were detected in all 20 Tpst1+/− females mated with Tpst1+/− or Tpst1−/- males and in 17 of the 20 Tpst1−/- females mated with Tpst1+/− or Tpst1−/- males. The time between the set up of these matings and the detection of vaginal plugs was not different in Tpst1+/− (2.6 ± 1.1 days, n = 20) and Tpst1−/- females (2.6 ± 0.9 days, n = 17). These data indicate that the copulatory behavior of both Tpst1−/- males and females is normal and that the estrous cycle length in Tpst1−/- females is normal.

The size of the litters from Tpst1+/− × Tpst1+/− crosses was indistinguishable from matings of Tpst1−/- males and Tpst1+/− females (group 1 versus 2, p = 0.59). This demonstrates that the fertility of Tpst1−/- males is normal. However, when wild-type males were mated with Tpst1−/- females, litter sizes were significantly reduced compared with the groups in which the females were wild-type irrespective of the genotype of the male (group 1 versus 3, p = 0.035; group 2 versus 3, p = 0.0099). When Tpst1−/- males were mated with Tpst1−/- females, litter sizes were similarly reduced (group 1 versus 4, p = 0.0099).

**Table II**

| Group | Male | Female | n | Copulations | Litters | Litter size* | Live births | Perinatal deaths | Surviving pups |
|-------|------|--------|---|-------------|---------|-------------|-------------|-----------------|---------------|
| 1     | +/-  | +/-    | 10| 10          | 9       | 5.9 ± 1.8   | 53          | 0               | 53            |
| 2     | +/-  | +/-    | 10| 10          | 9       | 6.3 ± 1.7   | 57          | 0               | 57            |
| 3     | +/-  | +/-    | 10| 10          | 9       | 4.1 ± 1.5   | 37          | 9 (24)          | 28            |
| 4     | +/-  | +/-    | 10| 8           | 6       | 3.2 ± 1.9   | 19          | 6 (32)          | 13            |

*Values are expressed as the mean ± S.D. of the indicated number of matings.
Animals were mated as described under “Experimental Procedures.”

| Group | Male  | Female | n    | Female age | Viable embryos | Resorbed embryos | Total implantations |
|-------|-------|--------|------|------------|----------------|------------------|-------------------|
| 1     | +/+   | +/+    | 21   | 78 ± 12    | 7.0 ± 1.6      | 0.7 ± 0.9        | 7.7 ± 1.4         |
| 2     | +/+   | −/−    | 18   | 85 ± 17    | 5.3 ± 1.4b     | 2.4 ± 1.7b       | 7.7 ± 1.7         |

*Values are expressed as the mean ± S.D. of the indicated number of matings.

0.0005; group 2 versus 4, p = 0.0015) but were not significantly different from crosses of wild-type males and Tpst1−/− females (group 3 versus 4, p = 0.30). These results show that the reduced litter size observed in Tpst1−/− females results exclusively from TPST-1 deficiency in the female and is independent of the genotype of the sire or the fetus. In addition, we observed that homoygosity in the female was associated with a striking incidence of perinatal mortality. In these experiments, 15 of 56 (28%) live births to Tpst1−/− females were found dead within 48 h of birth, whereas no death occurred in litters from wild-type females (Table II). Furthermore, over a 1-year period of observation, we observed a perinatal death rate of 3.9% in Tpst1−/− females (groups 1 and 2) as compared with wild-type female but that the total number of implantations was no different between the two groups (Table III).

To more precisely establish the timing of fetal loss a similar analysis was performed at 8.5 dpc. We observed that the total number of viable embryos in Tpst1−/− females at 8.5 dpc (7.5 ± 1.7, mean ± S.D., n = 12) was equivalent to the number of implantation sites observed in Tpst1−/− females at 15.5 dpc. The viability of embryos at 8.5 dpc was confirmed by histological examination of 4 embryos from uteri from five different Tpst1−/− females. These data establish that low litter sizes from Tpst1−/− females was because of postimplantation fetal loss between 8.5 and 15.5 dpc.

**Chromosomal Localization of the Tpst1 Gene**—Because the Tpst1 gene has not been mapped, fluorescence in situ hybridization was performed to determine the chromosomal localization of the Tpst1 gene as described under “Experimental Procedures.” Under the conditions used, positive hybridization signals were detected in 70 of 100 mitotic figures. Based on the DAPI banding pattern to identify specific chromosomes, the signals from the probe were localized to chromosome 5. Based on the further examination of 10 photos, the position of the Tpst1 gene was further refined to region F-G1 of mouse chromosome 5 (Fig. 4). The Tpst2 gene had been previously mapped on mouse chromosome 5 at 63 centimorgans (Mouse Genome Informatics accession ID: MGI: 1308519, www.informatics.jax.org). It is not clear how closely the Tpst1 and Tpst2 genes are linked.

**Low Litter Sizes from Tpst1−/− Females Is Because of Postimplantation Fetal Loss**—To evaluate the cause of the low litter sizes from Tpst1−/− females, timed matings between wild-type males and age-matched female wild-type or Tpst1−/− mice were set up. Females were examined for vaginal plugs each morning, plugged females were sacrificed at 15.5 dpc, and the uterine contents were examined. An embryo was considered viable if the size and morphology were consistent with 15.5 dpc. An embryo was considered to be resorbed if a placental remnant could be identified. The number of implantations was defined as the number of viable embryos plus the number of resorbed embryos. We observed that the frequency of fetal loss was three times higher in Tpst1−/− females when compared with wild-type female but that the total number of implantations was no different between the two groups (Table III).

**Discussion**

We have successfully generated Tpst1 null mice by targeted disruption of the Tpst1 gene. Our observations show that disruption of one allele at the Tpst1 locus has no discernible effect. Heterozygous Tpst1+/− mice appear normal and have normal fertility and normal growth curves. Furthermore, the frequency of Tpst1 genotypes among the offspring from Tpst1+/− mice was not statistically different.
× Tpst1−/− crosses was consistent with Mendelian inheritance and the sex ratio was 50:50 in each genotype. Thus, TPST-1 expression is not required for normal embryonic development. However, our analysis of Tpst1−/− mice revealed a constellation of pleiotropic effects.

A detailed analysis of postnatal growth showed a decrease of ~5% in mean body weight in both male and female Tpst1−/− mice. This weight difference appears 1–2 weeks after weaning and is apparent in Tpst1−/− mice from litters born to either Tpst1−/− or Tpst1−/− females. This would suggest that the reduced body weight is a direct effect of TPST-1 deficiency in the animal and not an indirect effect of the maternal genotype. The mechanism for this effect is not clear and is currently under investigation. One possible explanation could be a change in feeding behavior or digestion. CCK and gastrin are two gastrointestinal peptide hormones that are known to be tyrosine-sulfated (16). CCK stimulates exocrine pancreatic secretion and gallbladder contraction and can regulate feeding behavior. Tyrosine sulfation of CCK is required for high affinity binding to and activation of the CCK-A receptor (11, 17). Gastrin is the principal regulator of gastric acid secretion, tyrosine sulfation promotes proteolytic processing of progastrin (18), and sulfated gastrin is a more potent stimulator of acid secretion than unsulfated gastrin in humans (19). However, the CCK and gastrin genes and their cognate receptors, the CCK-A receptor and the CCK-B/gastrin receptor, respectively, have been knocked out in mice, and disruption of each of these genes individually has no discernible effect on body weight (20–23). Gastrin-deficient mice show a prominent decrease in parietal cell numbers, and CCK-B/gastrin receptor-deficient mice have severe gastric mucosal atrophy (22). In contrast, histological analysis of the stomach from Tpst1−/− mice revealed no abnormalities. It is not known if Tpst1−/− mice have abnormalities in CCK or gastrin sulfation. Nevertheless, in the first approximation the phenotype we observe is not consistent with major abnormalities in CCK and/or gastrin function. The low body weight may thus derive from deficient tyrosine sulfation of another yet unknown TPST-1 substrate(s).

Disruption of the Tpst1 gene also has a detrimental effect on reproductive performance. Our data show that litter sizes of Tpst1−/− mice at birth are ~35% lower than litters from either wild-type or Tpst1−/− females. In controlled experiments, we observed that when Tpst1−/− females were mated with either Tpst1−/− or Tpst1−/− males the size of firstborn litters was reduced by ~40% when compared with concurrent control matings of Tpst1+/+ or Tpst1−/− males with wild-type females (Table II). In addition, we observed that the reduced litter size in Tpst1−/− females was maintained throughout a subsequent 18-week period of continuous mating (Fig. 3). Taken together these observations demonstrate that the fertility of male Tpst1−/− mice is normal and that the reduced size of litters born to Tpst1−/− females appears to be due exclusively to maternal TPST-1 deficiency. Our investigation as to the cause of the reduced litter size in Tpst1−/− females clearly shows that it is not because of abnormal fertility. This conclusion is strongly supported by the observation that the number of implantation sites in Tpst1−/− females at 8.5 dpc was normal with no gross or histological evidence of fetal loss. At 15.5 dpc essentially identical numbers of implantation sites were present as at 8.5 dpc. However, at 15.5 dpc we observed a 3.3-fold higher number of resorption sites in Tpst1−/− females compared with a concurrent control group of Tpst1+/+ females. Furthermore, the number of viable fetuses in the uteri of a Tpst1−/− female at 15.5 dpc was no different from the number of live births in Tpst1−/− females, indicating that most, if not all, of the fetal loss occurs prior to 15.5 dpc. The mechanism of fetal loss in Tpst1−/− females is not known. At date there are no proteins involved in female reproductive physiology that are known to be tyrosine-sulfated or to require tyrosine sulfation for optimal function.

Other observations made during our studies are notable. First, we observed a ~4-fold higher incidence of perinatal mortality in litters born to Tpst1−/− females, compared with litters from either Tpst1+/+ or Tpst1+/− females. The higher frequency of perinatal mortality may reflect subtle defects in maternal behavior or physiology that impair survival. Another curious observation was that the timing of litters from Tpst1−/− females was dysynchronous over 18 weeks of observation, compared with wild-type females that dropped litters approximately every week (Fig. 3). One can safely infer that the loss of synchronicity was because of a tendency for the Tpst1−/− not to become pregnant during postpartum estrus. Further study will be required to further validate these findings and to explore potential mechanisms.

There are only two ubiquitously expressed TPST genes in mice and man that sulfate an unknown number of protein substrates. To gain a better understanding of TPST function in vivo we have developed TPST-1-deficient mice by targeted disruption of the Tpst1 gene. Our initial studies have revealed unexpected and pleiotropic phenotypic effects of disruption of the Tpst1 gene in mice, including effects on body weight, fecundity, and postnatal viability. Our results suggest that the phenotype(s) we have documented in Tpst1−/− mice may reflect differences in macromolecular substrate specificity of the two enzymes. This possibility is supported by in vitro data that show that TPST-1 and TPST-2 differ in their abilities to efficiently sulfate peptide substrates (2). The alternative possibility is that TPST-1 and TPST-2 have identical substrate specificities but that the level of the TPST-2 expression is simply insufficient to compensate for the loss of TPST-1. Although we have not yet directly linked these phenotypic effects to a deficiency of tyrosine sulfation of any known TPST substrate(s), these findings strongly support the conclusion that TPST-1 and TPST-2 have distinct biological roles. Furthermore, the diversity of phenotypes observed in the Tpst1−/− mice underscores the importance of tyrosine sulfation in general and of TPST-1 in particular in several different physiological processes in the mouse and highlights how little is known about the biology and substrate repertoire of this enzyme system.

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