IDENTIFICATION AND CHARACTERIZATION OF AN ALTERNATIVELY TRANSCRIBED FORM OF PEROXIREDOXIN IV THAT IS SPECIFICALLY EXPRESSED IN SPERMATIDS OF THE POSTPUBERTAL MOUSE TESTIS

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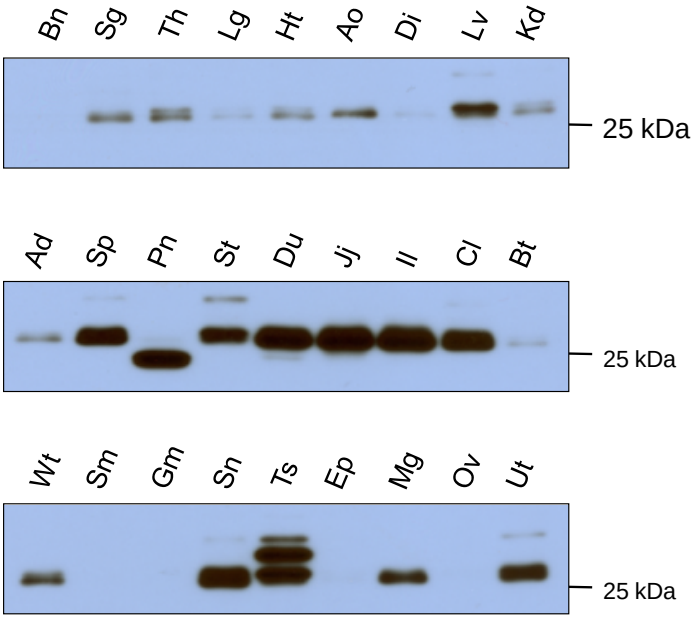
SUPPLEMENTAL DATA

Computational analyses - To identify transcription initiation sites and promoter regions for both mouse Prx IV genes, we extracted a 5000-bp genomic sequence (Fig. 2A) containing the upstream region of exon 1B (>gi|149272536: 14188586–14193585 *Mus musculus* strain C57BL/6J chromosome X genomic contig, MGSCv37 C57BL/6J) and analyzed it with Web-based programs. Promoter prediction with BDGP (http://www.fruitfly.org/seq_tools/promoter.html) identified two transcription start sites at positions -306 bp → -356 bp and -2486 bp → -2536 bp in the analyzed 5000-bp genomic sequence, which are correspond to the transcription start sites of Prx IV-S and a position –90 bp relative to Prx IV-L. The Promoter 2.0 prediction program (http://www.cbs.dtu.dk/services/promoter) identified the position –204 bp upstream of exon 1B as a possible transcription promoter site with the highest prediction score (1.191) in the region analyzed. Overall, the computational prediction of transcription start sites identified two independent such sites. We further analyzed Prdx4-001 (ENSMUST00000026328, Prx IV-S) and Prdx4-003 (ENSMUST00000130349, Prx IV-L). A 400-bp region upstream of exon 1A and a 400-bp region upstream of exon 1B were analyzed for potential transcription factor binding sites with the use of TFSEARCH (http://molsun1.cbrc.aist.go.jp/research/db/tfsearch.html). In the immediate upstream sequence of exon 1B, three USF binding sites, two sites each for AP-1, MZF1, Nkx-2, N-Myc, and Sp1, as well as single sites for C/EBPα, E2F, FATA-1, FATA-2, GATA-X, Ik-2, SRY, and Tst-1 were identified. Analysis of the upstream sequence of exon 1A revealed nine sites for CdxA, two sites each for deltaE and SRY, and one site each for AML-1α, Ik-2, MZF1, Oct-1, SREBP, TATA, Th1/E4, and v-Myb. Analysis of promoter signals with BIMAS (http://www-bimas.cit.nih.gov/cgi-bin/molbio/signal) identified 16 binding sites for Sp1 as well as sites for α-CB, ATF, CACCC-binding factor, CBF-B, CP1, GR, and NF-1 in the immediate upstream region of exon 1B; NF-1, NF-E, NF-E2, and NF-Y binding sites were identified multiple times in the upstream region of exon 1A. These data thus suggest that the two alternative promoters for the Prx IV gene locus are regulated independently by specific transcription factors.

A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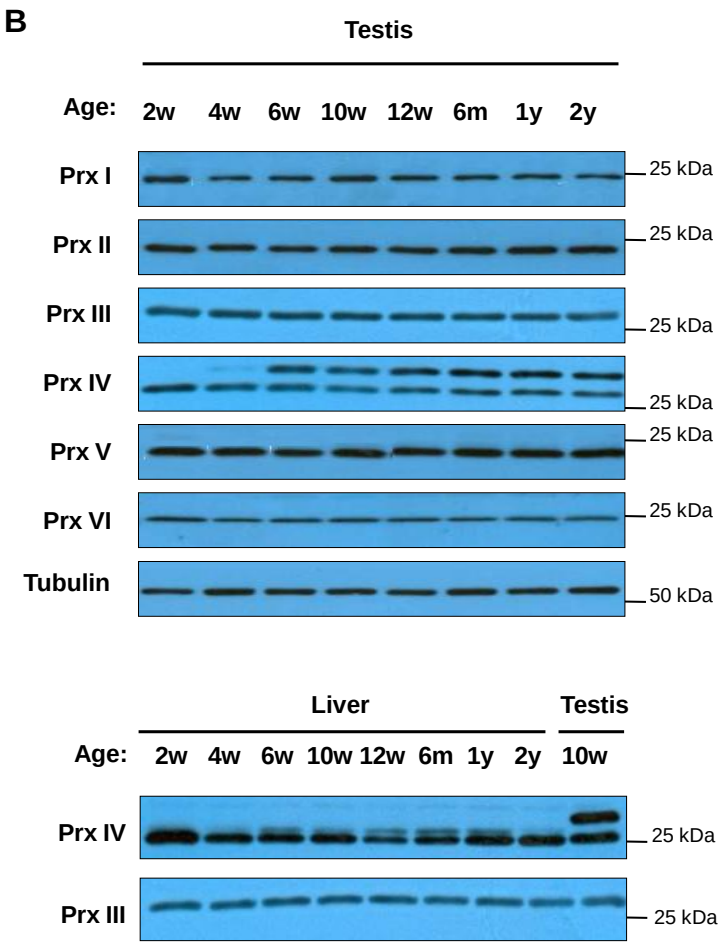
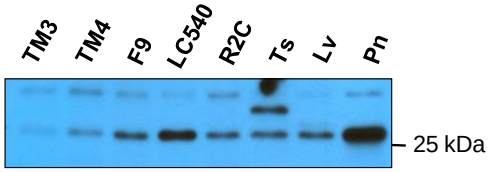
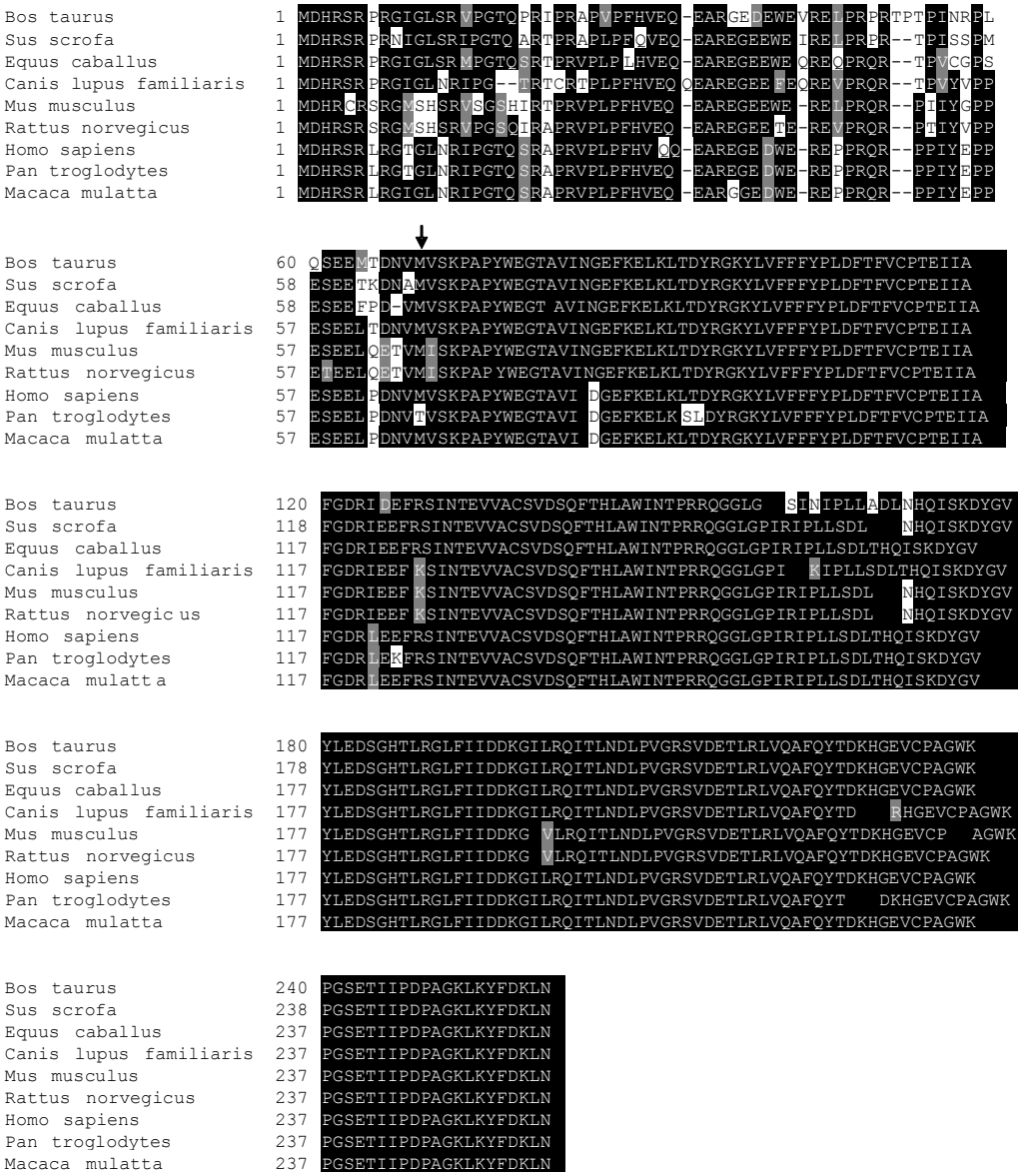


Supplemental Figure S1. Expression of Prx IV in mouse tissues. *A*. Immunoblot analysis of Prx IV in various tissues from 12-week-old C56BL/6J mice. Proteins were separated by SDS-PAGE on a 14% gel and probed with polyclonal antibodies specific for the COOH-terminal region of Prx IV. The testis exhibited two pronounced immunoreactive proteins of 29.5 and 27 kDa. Abbreviations: Bn, brain; Sg, salivary gland; Th, thymus; Lg, lung; Ht, heart; Ao, aorta; Di, diaphragm; Lv, liver; Kd, kidney; Ad, adrenal gland; Sp, spleen; Pn, pancreas; St, stomach; Du, duodenum; Jj, jejunum; Il, ileum; Cl, colon; Bt, brown adipose tissue; Wt, white adipose tissue; Sm, soleus muscle; Gm, gastrocnemius muscle; Sn, skin; Ts, testis; Ep, epididymis; Mg, mammary gland; Ov, ovary; Ut, uterus. The faster moving band in the pancreas tissue is likely due to proteolyzed Prx IV. ***B*.** Immunoblot analysis of Prx isoforms in the testis of C57BL/6J mice of the indicated ages in weeks (w), months (m), and years (y) is shown in the upper panels. α-Tubulin served as a loading control. Immunoblot analysis of Prx III and Prx IV in the liver of the same animals as well as in the testis sample of the 10-week-old mouse is shown in the lower panels.



Supplemental Figure S2. Expression of Prx IV-L and Prx IV-S in testis-derived cell lines. Lysates of TM3 (neonatal mouse Leydig), TM4 (neonatal mouse Sertoli), F9 (mouse embryonic testis carcinoma), LC540 (adult rat Leydig), and R2C (adult rat Leydig cell tumor) cells were fractionated by SDS-PAGE on a 14% gel and probed with antibodies to the COOH-terminal sequence of Prx IV. Homogenates of the testis (Ts), liver (Lv), and pancreas (Pn) of adult C57BL/6J mice were also analyzed as positive controls.



Supplemental Figure S3. Multiple alignment of Prx IV-L protein sequences. The last residue of the first exon is indicated with arrow. Residues shown in white on black or on gray are identical or conserved, respectively, among homologs.