Supporting Methods and Materials

***In Silico* Subtraction and Genomic Database Mining.** ESTs (49,064) from three libraries [Lib.6786 (round spermatids), Lib.2549 (adult testis), and Lib.2511 (adult testis)] were downloaded from the mouse Unigene database (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Mm) at the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). The BLAST program (www.ncbi.nlm.nih.gov/BLAST) was used to search the nonredundant and EST database. ESTs that were present in testis or male germ cell libraries, but were absent in libraries from other tissues, were analyzed by using multitissue RT-PCR as described (1). To identify the human HILS1 ortholog, we used the mouse *Hils1* cDNA sequence to search the human genome database at the Sanger Center by using a BLAST program (www.ensembl.org/Homo_sapiens/blastview).

Protein Tertiary Structure Prediction. The target sequence was submitted to the 3D-PSSM web server (www.sbg.bio.ic.ac.uk/servers/3dpssm). The program MODELLER (www.salilab.org/modeller/modeller/html) was applied to model the structure of target sequence based on the PDB coordinates of homolog 1GHC. Simulation was implemented to release the modeled structure, and to allow a more energy favorable conformation. All simulation work was performed by using the CHARMM (Chemistry at Harvard Macromolecular Mechanics) program.

RNA and Protein Analyses. Northern blot, RT-PCR, and *in situ* hybridization analyses was performed as described (1). A pair of mouse *Hils1*-specific primers (5'-TCCCAAGCCAGAGTGAGAGT-3' and 5'-CACCCTACGGACTCCTTTGA-3'), and a pair of human *HILS1*-specific primers (5'-ATGGCCTTGGACTTGAACAT-3' and 5'-GCAAAGTTAGCTTGCCGATG-3') were used for RT-PCR. Mouse *Hprt* and human *ACTIN* were amplified as described (1). Twenty-two (for mouse *Hprt*), 19 (for human *ACTIN*), and 35 (for both mouse *Hils1* and human *HILS1*) cycles were used to ensure that the PCR was in the exponential phase. The SMART RACE cDNA amplification kit

(BD Biosciences, Palo Alto, CA) was used to isolate the 5' and 3' ends of the human *HILS1* cDNA according to the manufacturer's instructions.

The pET protein production system (Novagen) was employed to produce mouse HILS1 and histone H1a. Protein induction and purification were performed according to the manufacturer's instructions. Histone H1a was used for binding assays described below. A 19-kDa fusion protein consisting of the full-length mouse HILS1 protein, an N-terminal T7 tag, and a C-terminal histidine tag was used to immunize rabbits to produce polyclonal antibodies (Cocalico Biologicals, Reamstown, PA). Western blot, immunohistochemistry, and immunofluorescent microscopy were performed as described (1). The rabbit anti-HILS1 polyclonal antisera was used at a 1:1,000 dilution. For Western blot and immunohistochemistry analyses, the antibodies were diluted as follows: rabbit anti-TNP1, 1:1,000; rabbit anti-TNP2, 1:800; mouse anti-protamine 1, 1:500; and mouse anti-protamine 2, 1:500.

Preparation and Analysis of Nuclear Proteins. Testes from three adult male mice were used for preparation of SRS, and nuclei were isolated as described (2, 3). Nuclei were lysed with 1× NuPAGE LDS sample buffer (Invitrogen), followed by heating at 96°C for 10 min in the presence of 10 mM DTT for Western blot analysis of total nuclear protein, or subjected to chromatin preparation (4). Chromatin was prepared by lysis of nuclei in 0.5 ml of high-salt buffer (0.35 M KCL/10mM Tris, pH 7.2/5mM $MgCl_2$/0.5 mM phenylmethanesulfonyl fluoride), followed by centrifugation at 15,000 rpm in an Eppendorf 5417C microcentrifuge (Brinkman Instruments, Westbury, NY). The chromatin pellet was lysed with 1× NuPAGE LDS sample buffer (Invitrogen), followed by heating at 96°C for 10 min in the presence of 10 mM DTT for Western blot analyses. Total BNPs were isolated from whole testis or sonication-resistant spermatids, and were separated by using acid-urea 15% polyacrylamide gels, followed by Coomassie blue staining or electrotransfer, by using 0.7% acetic acid as described (2, 3).

Linker Histone Binding Assay. A pMMTV-CAT plasmid was digested with *Sac*I/*Bam*HI to generate a 210-bp DNA fragment containing a partial MMTV LTR

promoter for mononucleosomal reconstitution. A 1.3-kb fragment liberated from the same plasmid with *Bam*HI/*Hin*dIII was used for preparing polynucleosomes. Core histone preparation, mononucleosome and polynucleosome reconstitution, and binding assays were performed as reported (5), except that all DNA fragments were labeled by using digoxigenin-11-ddUTP (Roche Diagnostics, Indianapolis) as described (6).

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