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Disruption of Ledgf/Psip1 Results in Perinatal Mortality and Homeotic Skeletal Transformations

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PC4- and SF2-interacting protein 1 (Psip1)—also known as lens epithelium-derived growth factor (Ledgf)—is a chromatin-associated protein that has been implicated in transcriptional regulation, mRNA splicing, and cell survival in vitro, but its biological function in vivo is unknown. We identified an embryonic stem cell clone with disrupted Psip1 in a gene trap screen. The resulting Psip1-fgeo fusion protein retains chromatin-binding activity and the PWWP and AT hook domains of the wild-type protein but is missing the highly conserved C terminus. The majority of mice homozygous for the disrupted Psip1 gene died perinatally, but some survived to adulthood and displayed a range of phenotypic abnormalities, including low fertility, an absence of epididymal fat pads, and a tendency to develop blepharitis. However, contrary to expectations, the lens epithelium was normal. The mutant mice also exhibited motor and/or behavioral defects such as hind limb clenching, reduced grip strength, and reduced locomotor activity. Finally, both Psip1−/− neonates and surviving adults had craniofacial and skeletal abnormalities. They had brachycephaly, small rib cages, and homeotic skeletal transformations with incomplete penetrance. The latter phenotypes suggest a role for Psip1 in the control of Hox expression and may also explain why PSIP1 (LEDGF) is found as a fusion partner with NUP98 in myeloid leukemias.

PC4- and SF2-interacting protein 1 (Psip1) has been isolated in a number of independent experimental settings and has been assigned a variety of names and putative functions. Psip1 encodes two protein isoforms with molecular masses of 52 and 75 kDa. p52 and p75 were identified as interacting with transcription factor PC4 and shown to act as transcriptional coactivators (8). p75 is also referred to as lens epithelium-derived growth factor (LEDGF) since it was identified as a cell survival factor under a variety of conditions of environmental stress, and it has been implicated as a transcriptional regulator of stress-related genes (24). Psip1 contains a PWWP domain (Fig. 1B), and some of these have been implicated in DNA binding in vitro (36). Targeting the protein to chromatin and chromosomes (2, 10), and the Psip1 PWWP domain also affects the interaction of the protein with chromatin in vivo (36). Psip1 also contains AT hook-like motifs (Fig. 1B), and some of these have been implicated in DNA binding in vitro (36).

Apart from its suggested role as a transcriptional regulator (21, 32), an integrase-binding domain (IBD) in p75/PSIP1 tethers human immunodeficiency virus type 1 integrase to host chromosomes and so prevents integrase degradation (3, 4, 23, 37) (Fig. 1B). This is important for retroviral integration (5) and replication (6), but the normal cellular role of the IBD is unknown.

Most assays for normal Psip1 function have involved overexpression of the protein in cultured cells. Apart from the role in tethering viral integrase, depletion of PSIP1 from cells in culture by RNA interference reveals no obvious cellular phenotype (5, 6, 22); therefore, the biological function of PSIP1 remains unknown.

We originally identified Psip1 as a chromosomally associated protein during the course of a gene trap screen to identify novel murine nuclear and chromosomal proteins (35). Two independent gene-trapped clones were obtained, one in mouse F9 embryonal carcinoma cells and the other in mouse embryonic stem (ES) cells (Fig. 1). Here we have used the disrupted Psip1 (Ledgf) gene from the trapped ES cell clone to generate mutant mice and to investigate the resulting biological phenotype. While the majority of mice with disrupted Psip1 died perinatally, some survived to adulthood and displayed complex phenotypic abnormalities, including fertility and motor defects. However, significantly, given the association of Psip1
(LEDGF) with growth of the lens epithelium in vitro, Psip1−/− mice had a normal lens epithelium. Both Psip1+/− neonates and surviving adults also had brachycephaly, small rib cages, and homeotic skeletal transformations similar to those seen in mice with mutations in Hox genes or components of the PRC1 polycomb complex. Therefore, proteins encoded by Psip1 may function in the control of Hox gene expression. This would also be consistent with the finding of human PSIP1 (LEDGF) as a fusion partner with NUP98 in myeloid leukemias (11, 15). The other recurrent leukemia-associated fusion partners of NUP98 are encoded by Hox genes themselves (1).

MATERIALS AND METHODS

Cell culture and immunostaining. The F9 and ES cell Psip1 gene trap lines (WF9/3G5 and ES149) were generated, and immunostained with an antibody against β-galactosidase (β-gal; Europa) as previously described (35).

Generation of p75 antibodies. A fragment of human p75/Psip1 from amino acid 326 to amino acid 529 encompassing the whole of the p75-specific region (Fig. 1B) was generated by PCR with primers 5′ ACTGGATCCAGCAGATTTAAAGATGAAGG 3′ and 5′ TAGTAAGCTGATCTAGTGTAGAATCTCAG 3′, fused in frame in pET-32a, and expressed in Escherichia coli [BL21-CodonPlus(DE3)-RP; Stratagene] to produce a fusion protein with thioredoxin and a His tag. Fusion protein was purified on a nickel-agarose column and used to immunize sheep (Scottish Diagnostics). For antibody affinity purification, the same fragment of p75 was fused in frame with glutathione S-transferase in pGEX 4T-1, purified on glutathione-agarose, and then immobilized on a cyanogen bromide-activated Sepharose 4B column (Pharmacia). Serum from the fourth bleed was diluted in phosphate-buffered saline, filtered, and put over the column three times. The column was then washed with 20 bed volumes of high-salt wash buffer (10 mM Tris-Cl [pH 7.5], 500 mM NaCl), followed by 50 bed volumes of phosphate-buffered saline. Antibody was eluted with 100 mM glycine (pH 2.7)–150 mM NaCl–10% glycerol into 1 M Tris-HCl (pH 8.8) for neutralization. Protein-containing fractions were pooled and concentrated with a Centricon 30 column (Millipore), the concentration was estimated from the A280, and the antibody was stored at −80°C in 20% glycerol.

Generation and genotyping of Psip1 mutant mice. The ES149 cell line was a gene trap of E14 ES cells (35), which are derived from the mouse substrain 129/Ola. ES149 cells were passaged the day before injection into blastocysts collected at 3.5 days postcoitum (dpc) from superovulated C57BL/6 females and transferred into pseudopregnant recipient females. Chimeric pups were identified by their agouti coat color and, when mature, were mated to both MF1 outbred and C57BL/6 mice. Two male chimeras yielded germ line transmission, and heterozygotes for the Psip1 mutation were identified by Southern blot analysis for presence of the lacZ portion of the gene trap vector. Heterozygotes obtained from crosses with either MF1 or C57BL/6 mice (termed first backcross) were backcrossed with wild-type (WT) mice from each respective strain at least three more times before matings between heterozygotes were set up.

DNA for genotyping of mice was obtained from tail tips or ear punches. To detect the presence of the gene trap vector (SA-β-gal construct) by Southern blotting, genomic DNA was digested with EcoRI and hybridized with a dCTP-p32-labeled probe 3-kb BamHI fragment encompassing lacZ. More routinely, mice were genotyped by testing for lacZ by PCR with primers 5′ GTTGCGCA GCGTGAATGCAG 3′ and 5′ GCCGTCACTCCAAGCAAGCA 3′, which generate a 432-bp PCR fragment. To genotype offspring from Psip1 heterozygous (+/−) crosses, HindIII-digested genomic DNA was analyzed by Southern blot-
ting with a 232-bp PCR product generated from exon 9 of Psip1 (5′ GTTGTATGATAGATAGATAAGG 3′ and 5′ TTCACCTCCTGTGATCTCTC 3′). Alternatively, genomic DNA was analyzed by PCR for the presence of both the WT locus, but no product is obtained from the gene-trapped locus.

Analysis of Psip1 expression in mice generated from the ES149 gene trap line. Embryos obtained at 14.5 dpc from a cross between male and female Psip1+/− mice were stored at −80°C after a biopsy was taken for genotyping each individual. For analyzing mRNA, half of an embryo for each genotype was homogenized in Trizol Reagent (Sigma) and the RNA was extracted according to the manufacturer’s instructions. After DNase I treatment of the RNA, reverse transcription (RT)-PCRs were performed with primers for Psip1 (5′ AGATGCA TAA GGCCCTGATG 3′ and 5′ ACATCTGAGCTGCCAGCTCTAG 3′), which generate a 499-bp product from the endogenous Psip1 transcript in the transcript, and Snap3 (5′ TGGAGCTACATAGAAAAGACCTC 3′ and 5′ AGGTCTCTGGTCTAACATAAGG 3′), which generate a 511-bp product—to check the transcript from the opposite strand, and a 70-bp product of p75 that detects the transcript from the opposite strand, and a 70-bp product of Snap3 (5′ TGGAGCTACATAGAAAAGACCTC 3′ and 5′ AGGTCTCTGGTCTAACATAAGG 3′) as described elsewhere (26).

Pathology and histochemistry. Neonatal mice were humanely killed by decapitation, and thoracoabdominal organs and heads were fixed in 10% neutral buffered formalin and processed for histopathology. Thoracoabdominal organ blocks were serially sectioned parallel to the sagittal plane at 4 μm. Fifty evenly spaced sections spanning the entire block were stained with hematoxylin and eosin (H&E). Heads were serially sectioned transversely, and 25 evenly spaced sections spanning the entire block were stained with H&E. Adult mice were humanely killed with CO2 gas. The initial group (two mice) received detailed gross and histopathological examination of all organ systems. A second group (two Psip1+/−, and four Psip1−/− mice) received detailed gross and histopathological examination of the adrenal gland, kidney, eyelid, and stomach. Tissues were fixed in 10% neutral buffered formalin and wax embedded, and 4-μm sections were stained with H&E. Alizarin red-alcan blue staining of adult and newborn mice was performed as described previously (29). Briefly, animals were skinned, eviscerated, and fixed in 95% ethanol. They were then transferred to acetone for 2 days and then stained with alizarin red-alcan blue (Sigma) for 3 days at room temperature, subsequently cleared with 1% KOH, and finally stored in glycerol.

Behavioral and motor function studies. In hind limb extension tests, mice were suspended by their tails and the extent of hind limb extension was measured. If both hind limbs showed the extension reflex, including splayed toes, the mouse was considered normal. If the toes and one or two hind limbs were clenched to the body, the mouse was considered to fail the test. In grip strength tests, mice were suspended from a pencil with their forepaws. A mouse that was not able to suspend itself for any amount of time was considered to fail this test. In open-field tests, mice were placed in an open-field box (60 by 60 cm) marked off into 25 equal squares. Tests were videotaped or captured by a computer tracking program (Limeight/Acmetrics) to allow full analysis. The outer row of squares adjacent to the walls of the field are considered less anxiogenic than the inner squares. For 5 min, the number of crossings, time, and distance (movement of all four legs into a new square) into each square was noted together with other behavioral parameters such as the numbers of rearing and fecal bolii. Total movement in the field reflects general activity, and relative movement into the inner zone correlates with the anxiety state of the mouse.

RESULTS

Gene-trapped forms of Psip1 are missing conserved domains of the protein but still retain chromatin-binding activity. We previously performed a gene trap screening of mouse cells to identify nuclear proteins and characterize their localization within the nucleus (35). We obtained a number of clones in which the resulting βgeo fusion protein was found to associate with chromosomes. In two independent clones obtained (one in F9 embryonal carcinoma cells and the other in E14 ES cells), the gene that had been trapped was identified as Psip1. The sites of integration of the gene trap vector in the F9/3G5 and ES149 cell lines are shown in Fig. 1A. In F9/3G5, the gene trap vector was integrated into the intron between exons 6 and 7 of Psip1, whereas in ES149 insertion occurred between exons 8 and 9. This results in fusion proteins that encode the N-terminal 152 or 208 amino acids of Psip1, respectively, fused to the βgeo reporter (Fig. 1B). These proteins are expressed under the control of the endogenous Psip1 promoter but are missing C-terminal functional protein domains (Fig. 1B). For example, one or two of the treacle motifs have been removed by the ES149 and F9/3G5 integrations, respectively. These are highly polar motifs that feature in the nucleolar protein treacle, which is mutated in Treacher Collins syndrome, an autosomal dominant disorder of craniofacial development (16). The IBD is also removed by both gene traps.

Psip1 is highly conserved among vertebrates over the known protein motifs shared by both the p52 and p75 isoforms but also the IBD and other p75-specific sequences in the C-terminal end of the protein that are lost in the F9/3G5 and ES149 gene traps. Hence, it is likely that the gene-trapped proteins have perturbed function.

With an antibody against β-gal that detects the βgeo portion of the gene-trapped proteins, we found that the Psip1 fusion proteins in both gene trap clones localize to interphase nuclei and to mitotic chromosomes (Fig. 2). In ES149 cells, the fusion protein could sometimes be seen to colocalize with the brightly 4',6'-diamidino-2-phenylindole (DAPI)-stained foci of pericentric heterochromatin that are easily observed in mouse cells (35). This was not seen in F9/3G5 cells and may reflect the loss of additional AT hook domains in this case (Fig. 1B). AT
hooks are known to bind preferentially to AT-rich sequences, such as those found in pericentric heterochromatin, and they have been shown to directly affect the interaction of another protein, HmgA1a, with heterochromatin (12). In that case, two functional AT hooks have been shown to be necessary for proper chromatin binding, and this would be consistent with the differences in subnuclear localization between the Psip1 fusion proteins in F9/3G5 and ES149 cells.

**Generation of mice with disrupted Psip1.** To investigate the phenotype of Psip1 mutation, we generated chimeras from the ES149 cell line by blastocyst injection. Of the three chimeras obtained, two transmitted the transgene through the germ line. Heterozygous Psip1 gene trap mouse lines from each were established in both inbred C57BL/6 and outbred MF1 backgrounds, and in most subsequent experiments mice backcrossed at least five times into each respective background were used.

The presence of the gene trap vector in genomic DNA from these mice was detected by Southern blotting or by PCR with lacZ-specific primers that amplify a 432-bp fragment (Fig. 3A). The endogenous Psip1 locus was detected by amplification of an 860-bp PCR product from introns 8 and 9, which is where the gene trap construct was inserted. No amplification product was obtained from the gene-trapped locus (Fig. 3A). Southern blotting of HindIII-digested DNA with a PCR product generated from exon 9 of Psip1 was used to confirm the genotype of homozygous gene-trapped animals. The WT locus gave a 4-kb band, while the gene-trapped locus generated a band of 8 kb (Fig. 3B).

**Expression of Psip1 during development.** As gene-trapped Psip1-βgeo is under the control of the endogenous promoter, we analyzed its expression during embryonic development by X-Gal staining. While no expression was detected in preimplantation embryos (0 or 2.5 dpc), ubiquitous expression was observed in heterozygous embryos at 7.5 dpc and then throughout gestation (Fig. 4A). Previous studies have shown by Northern blotting that both splice forms of Psip1 are expressed ubiquitously in adults, although both the levels and ratios of isoforms can differ between different tissues (5).

Alternative splicing around gene trap integrations can sometimes result in the production of WT mRNAs from the trapped gene (25). We confirmed that full-length p75/Psip1 mRNA is not produced from the gene-trapped locus by RT-PCR with primers downstream of the gene trap site in RNA prepared
from 14.5-dpc embryos of each genotype. p75/Psip1 mRNA is detected in RNA from +/+ or +/- embryos but absent from –/– embryos (Fig. 4B).

A recent analysis of the mouse transcriptome revealed that a large proportion of mouse genes have antisense transcripts (19). Indeed, Psip1 partially overlaps the Snapc3 gene on the opposite strand and the ES149 gene trap insertion site is within an intron in the 3’ untranslated region of Snapc3 (Fig. 1A). Snapc3 encodes a component of the snRNA-activating protein complex that recognizes snRNA promoters (13). The gene trap insertion should be spliced out of the Snapc3 mRNA, but to ensure that the gene trap integration was not interfering with Snapc3 expression we performed RT-PCR with primers specific for this gene. Snapc3 mRNA was detected in both +/+ and –/– embryos, so we concluded that the gene trap integration does not prevent Snapc3 expression (Fig. 4B).

Psip1 expression was also assessed in protein extracts prepared from 14.5-dpc embryos. The p75 isoform was detected in extracts from +/+ and +/- embryos but not in samples from Psip1-/- embryos (Fig. 4C). Since the p75 isoform of Psip1 is functionally deleted in our homozygous gene-trapped mice but expression of the overlapping Snapc3 gene does not appear to be affected, we can attribute any resultant phenotypes in the mutant mice to the loss of Psip1 function alone.

Perinatal lethality of homozygous Psip1 mutant mice in a C57BL/6 background. In the outbred background, Psip1 +/- mice were physically indistinguishable from WT littermates and were obtained at the expected frequency (Table 1). However, in an inbred (C57BL/6) background there was a small but significant deficit of heterozygous animals (x^2 = 4.33, df = 1) (Table 1).

In crosses between heterozygotes, there was no deficit of Psip1-/- embryos at various stages of development (Table 2) and they were indistinguishable from +/+ and +/- embryos. Psip1-/- pups were also present immediately after birth, in numbers compatible with normal Mendelian ratios (Table 2). However, we noticed that many pups from both C57BL/6 and MF1 breeding pairs died just after birth. The majority of these were Psip1-/- (x^2 = 7.74, df = 2) (Table 2), suggesting that there is significant perinatal lethality of Psip1-/- mice.

There were no external abnormalities in these Psip1-/- pups. Histopathology of serial sections of three -/- neonates revealed no structural abnormalities in heads, brain, thoracic organs, or abdominal organs. All had empty stomachs and

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<th>TABLE 1. Genotypes of offspring from Psip1+/+ × Psip1-/- crosses at weaning</th>
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<tr>
<td>Background</td>
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<tr>
<td>MF1</td>
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<td>C57BL/6</td>
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<th>TABLE 2. Genotypes of embryos from timed matings of Psip1+/+ × Psip1-/- crosses</th>
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<tr>
<td>Background and age</td>
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<tr>
<td></td>
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<tr>
<td>C57BL/6 12.5 dpc</td>
</tr>
<tr>
<td>16.5 dpc</td>
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<tr>
<td>Birth</td>
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<td>MF1 + C57BL/6 Birth (dead)^a</td>
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<th>TABLE 3. Genotypes of offspring from Psip1+/+ × Psip1-/- crosses at weaning</th>
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<tr>
<td>Background</td>
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<tr>
<td></td>
</tr>
<tr>
<td>C57BL/6, 1st backcross</td>
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<tr>
<td>C57BL/6, &gt;3 backcrosses</td>
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<td>MF1</td>
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^a One male and four females.  
^b One male.  
^c Twenty-four males and three females.

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<th>TABLE 4. Phenotypic differences found in Psip1 mutant mice in an MF1 background</th>
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<td>Phenotype</td>
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<td></td>
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<tr>
<td>Infertility^a</td>
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<tr>
<td>Size of testes^c (mean % ± SEM)</td>
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<tr>
<td>Epididymal fat pads^d</td>
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<tr>
<td>Chronic ulcerative blepharitis^e</td>
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<tr>
<td>Hind limb extension^f</td>
</tr>
<tr>
<td>Normal grip strength</td>
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<tr>
<td>Brachycephaly^g</td>
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^a Unless otherwise indicated, the data are number of mice that display the observed phenotype/number of mice specifically studied for phenotype. Rare indicates that the number was not determined but the phenotype was rarely observed, if at all, during breeding of >500 offspring.  
^b Fertility of Psip1-/- mice was tested by setting up crosses between Psip1-/- males and at least two WT MF1 females. Mice were considered infertile if no offspring were obtained over a period of approximately 3 months.  
^c Size of testes was determined as the weight of both testes expressed as a percentage of body weight.  
^d See Fig. 5A.  
^e See Fig. 5D and E.  
^f See Fig. 5F.  
^g See Fig. 5G and H and 6A.  
^h See Fig. 6C.  
^i See Fig. 6D.  
^j See Fig. 6E.  
^k See Fig. 6F.
FIG. 5. Phenotypic analysis of surviving adult Psip1−/− mutant mice in the MF1 background. (A) Psip1−/− mutant mice have a reduction of intraabdominal fat, in particular, an absence of epididymal fat pads (fp), compared to their +/+ littermates. (B) H&E staining of seminiferous tubules from heterozygous (+/-) and Psip1−/− mutant mice. Both show normal-appearing basilar spermatogonia, several layers of differentiating...
intestinal tracts, atrophic spleens, and scattered petechiae in the wall of the bladder near the dome. Stomachs and intestinal tracts were full in two Psip1+/− and two WT littermates which had normal spleens and no periurachal hemorrhage. The empty gastrointestinal tracts of the three null mice suggested a failure to nurse. Atrophy of the spleens was interpreted as stress related. Periurachal hemorrhage was mild and its significance questionable. On the rare occasion that litters were actually observed being born, a couple of pups (which when subsequently genotyped were Psip1−/−) appeared to gasp, suggesting that they had trouble breathing, but the majority did not die immediately after birth but survived for up to the first day. As histological examination had revealed minimal structural abnormalities (including normal lungs, diaphragms, and palates) but empty stomachs, death of Psip1−/− neonates appears to be mainly the result of a failure to nurse. Inanition could be due to an inability of mutants to compete with littermates for teats, abnormal or absent nursing behavior, or rejection by the mother because of behavioral abnormalities.

Survival of a subset of homozygous mutants in a 129/Ola-C57BL/6 hybrid or outbred MF1 background. Only one homozygous animal in the C57BL/6 background survived to weaning at 4 weeks (Table 3). From Psip1+/− intercrosses of mice from either the first backcross into the C57BL/6 background or in the outbred MF1 background, the majority of homozygous mutant offspring also died just after birth and showed a failure to nurse. However, of those that were present after weaning (χ² = 21.3 and 121.8) (Table 3), the majority then survived for >6 months, although a number of subtle differences between Psip1−/− adults and their WT and heterozygous littermates were detected. Psip1−/− mice were very lean, with reduced intraabdominal fat and a complete absence of epididymal fat pads (Table 4 and Fig. 5A). Most (24 out of 27) of the surviving homozygotes in the MF1 background were male. These had low fertility; the majority of those tested failed to produce any offspring, and those that did only produced one or two litters. They had small testes compared to their +/+ and −/− littermates, even when corrected for body weight (Table 4). Testicular histopathology did not reveal any differences in the appearance of seminiferous tubules or the Leydig cell-containing interstitium between Psip1−/− and WT mice. All stages of spermiogenesis were present (Fig. 5B).

Eye phenotypes in homozygous mutant mice. Psip1 (LEDGF) was originally described as a growth factor produced by lens epithelial cells and in cells of the cornea, sclera, uvea, and retina (data not shown). However, after H&E staining, the lens epithelium appeared to be normal in Psip1−/− mice, suggesting that, in vivo, Psip1 (Ledgf) does not function to promote the survival or growth of the lens epithelium (Fig. 5C).

The majority of Psip1−/− mice did develop persistent inflammation of the eyelids of one or both orbits (Fig. 5D), which was rarely observed in Psip1+/− or WT littermates. This varied in severity but worsened with age. Histopathology confirmed this to be chronic ulcerative blepharitis with sparing of the adjacent conjunctiva. The epidermis was ulcerated and covered with adherent fibrin plaque. Chronic mixed inflammatory cells infiltrated the dermis. There were no structural abnormalities to explain this proclivity (Fig. 5E).

Motor and behavioral abnormalities in Psip1 mutants. Survivor Psip1−/− mice and their +/+ and −/− littermates were tested for basic motor functions. Hind limb extension and spaying of toes are natural reflexes for a mouse suspended by its tail, but Psip1−/− mice had a tendency to clench their toes and their hind limbs to their bodies (Table 4 and Fig. 5F). This became more pronounced with age. Psip1−/− mice also had reduced grip strength compared to littermates (Table 4).

In an open-field test, the total number of squares visited by Psip1−/− mice was significantly reduced (Table 5). A one-way analysis of variance [(F2,32) = 10.62 and P = 0.003] demonstrated a highly significant difference between the genotypes. Post hoc analysis by Dunnett’s test showed that the significance lies in homozygous mutant mice being significantly different (P < 0.05) from both WT and heterozygous mice. There was no difference in these parameters between +/+ and −/− mice. Decreased movement by Psip1−/− mice could be because they find it difficult or painful to move or because they are anxious or inhibited. The latter explanation is less likely, as the proportions of crossings in the more anxiogenic inner zones are similar among all of the genotypes (Table 5). Furthermore, rearing on the hind legs is also significantly reduced, which may reflect the general problems observed with movement in Psip1−/− mice.

The motor abnormalities of Psip1−/− mice will affect behavior and could explain why the majority of mutants fail to nurse. They may either have difficulty in, or be uninterested in, feed-

| Table 5. Performance of WT and Psip1 mutant mice in an open-field test |
|-----------------------------|-----------------|-----------------|-----------------|
| Genotype | No. of animals | Total no. of squares visited | % of inner-square visits | No. of rearings |
| +/+ | 10 | 208 (30.7) | 16.9 (4.3) | 15.5 (3.5) |
| +/− | 16 | 172.3 (19.5) | 11.5 (2.6) | 16.5 (1.7) |
| −/− | 9 | 49 (18.7) | 14.7 (4.6) | 2.7 (1.7) |

*Mean (standard error of the mean).

P < 0.05 compared to +/+ and −/− mice by Dunnett’s multiple comparison.
ing, or they may be rejected by their mothers because of their abnormal movement or behavior.

**Brachycephaly and skeletal homeotic transformations in Psip1 mutants.** Surviving Psip1−/− mice tend to have broad, shortened faces and jaws and often a slightly hunched appearance (Fig. 5G). Alizarin red-alcian blue staining of skulls showed no obvious differences in the sutures or in the composition of the skull bones but that the cranial bones and nasal process are broader and shorter in the homozygous mutants compared to WT and heterozygous mice (Fig. 5H). In particular, the occipital bone at the back of the skull appears to be flatter and broader. Some mutants had highly domed cranial bones. Craniofacial abnormalities characterized by a domed skull and a reduction in the extent of the interparietal, exoccipital, and supraoccipital bones were also seen in −/− newborns (Fig. 6A). The skulls of Psip1−/− newborns were often more fragile than those of WT littermates during staining, suggesting that the bones of the roof of the skull may be thinner. No difference compared with WT animals was detected in the bones of the base of the skull.

Skeletal abnormalities were also found in Psip1−/− newborns and adults (Fig. 6), although the penetrance was incomplete and variable (Table 4). For example, the spine was less curved and the rib cage smaller, with the sternum sitting closer to the backbone (Fig. 6B). This is due to a short sternum and short and fewer ribs (6 connected to the sternebrae, compared to the usual 7, resulting in 12 in total, compared to the usual 13) (Fig. 6C). Furthermore, the ribs are attached asymmetrically and the alternating pattern of cartilage and bone up the sternebrae is disorganized.

Some −/− animals exhibited skeletal alterations along the anterior-posterior axis that were consistent with homeotic transformations. Figure 6D highlights the abnormally shaped first and second cervical vertebrae (C1 and C2) of Psip1−/− pups. As seen in some of the surviving Psip1−/− adult mice, the seventh cervical vertebra (C7) had an incomplete ectopic rib
that fused with the cartilage of the first thoracic rib (T1), suggesting a posterior homeotic transformation of C7 to T1. A prominent spinous process, characteristic for the second thoracic vertebra (T2) was incorrectly associated with the third thoracic vertebra (T3) in one adult mutant, suggesting an anterior shift in the identity of T3 to T2 (Fig. 6E).

Most Psp1<sup>−/−</sup> mice also show homeotic transformations in the lumbar region, with only five lumbar vertebrae (Fig. 6F, inset), indicating a posterior transformation of L6 to sacral vertebra 1 (S1).

**Evidence for upregulation of Hox genes in the absence of Psp1 (LEDGF).** Posterior transformation of cervical vertebra C7 to thoracic vertebra T1 is a phenotype also seen in mice mutant for Hoxa4 (14), Hoxa5 (18), and Hoxa6 (20). Therefore, Psp1 may be involved either in the control of Hox gene expression or as a downstream effector of Hox function.

To find evidence for Hox gene deregulation in the absence of Psp1, mouse embryonic fibroblasts (MEFs) were derived from the bodies of genotyped embryos at 11.5 dpc, a stage when there appears to be widespread Psp1 expression (Fig. 4A). RNAs prepared from passage 5 WT and Psp1<sup>−/−</sup> MEFs were analyzed for the expression of Hox genes by RT-PCR. No expression of Hoxa9, Hoxa13, Hoxb1, Hoxb8, or Hoxb13 was detected in either WT or mutant MEFs. Expression of Hoxa6, Hoxb3, Hoxb5, Hoxb6, Hoxb7, and Hoxb9 was detected, but there was no obvious difference between the levels of expression of these genes in WT and mutant MEFs (data not shown).

However, analysis of the results of transcriptional profiling of human embryonic kidney (HEK) 293 cells subjected to small interfering (siRNA) for p75/PSIP1 does support the hypothesis that Psp1 can be involved in the regulation of HOX gene expression (5). Compared to control cells (with scrambled siRNAs), global levels of gene expression were unaltered in knockdown cells (mean ratio of p75/PSIP1 siRNA expression to scrambled control expression = 0.96) (http://www.ncbi.nlm.nih.gov/geo; accession no. GSE3485). For each probe’s signal deemed “present” in this analysis, we calculated the mean value and standard deviation across the four knockdown and the four control arrays. Probes significantly (P < 0.01) up- or down-regulated in the knockdown were then identified with an unpaired t test. This gave 358 significantly up-regulated and 479 down-regulated probes, mapping to 268 and 342 Ensembl genes, respectively. Among these up-regulated genes were Hoxa5, Hoxa6, Hoxa9, Hoxa10, and Hoxa13. Genes from other Hox clusters were generally found not to be expressed in HEK 293 cells, but Hoxd8 was found to be down-regulated in the knockdown cells. Although established by adenovirus transformation of primary HEK cells, subsequent microarray analysis has indicated that HEK 293 cells are of neuronal origin (31). These data therefore indicate that loss of Psp1 can lead to dysregulation of Hox genes in some cell types.

**DISCUSSION**

We have used gene trap mutagenesis to investigate the biological function of Psp1 in the mouse. Psp1 is a ubiquitous chromatin-associated protein, isoforms of which have been variously implicated in transcriptional regulation, mRNA splicing, and cell survival in vitro (7, 8, 32). Gene trap insertion into Psp1 abrogates expression of both isoforms of the normal Psp1 protein (Fig. 1 and 4), so we cannot attribute mutant phenotypes to one or other of the isoforms. Psp1 is expressed throughout mouse embryonic development, and even though the gene-trapped Psp1-βgeo fusion protein lacks important functional and conserved domains (Fig. 1), the mutant phenotype is only manifested after birth. Therefore, full-length Psp1 is not essential for the survival of cells or for their proliferation.

Psp1 (LEDGF) was originally described as a growth factor produced by lens epithelial cells and has been reported to function in the survival of lens epithelial cells in vitro (34). It has also been suggested to play a protective role against stress in corneal keratinocytes (21). We found that p75/Psp1 is expressed in nuclei of lens epithelial cells and in cells of the cornea, sclera, uvea, and retina. However, the lens epithelium and cornea appear to be normal in Psp1<sup>−/−</sup> mutant mice (Fig. 5C), suggesting that, in vivo, Psp1 does not function to promote the survival or growth of the lens epithelium or cornea.

The most dramatic aspect of the Psp1<sup>−/−</sup> phenotype is that the majority of pups died during the first day after birth. However, a subset of Psp1<sup>−/−</sup> mutant mice survived and these displayed skeletal abnormalities reminiscent of homeotic transformations. The ectopic ribs seen on cervical vertebra C7 are consistent with a posterior homeotic transformation to thoracic vertebra T1 (Fig. 6D and E), and this is also seen in mice with Hoxa4 (14), Hoxa5 (18), and Hoxa6 (20) mutations. Hoxc4 mutant animals also have an abnormal C7 vertebra, but in this case only rib heads are formed (30). Also, similar to Hoxc4 mutants, the surviving Psp1<sup>−/−</sup> mutant animals have a process on T3, which is normally associated with T2, suggestive of an anterior T3-T2 transformation (Fig. 6E).

We therefore considered the possibility that Psp1 is involved either in the control of Hox gene expression or as a downstream effector of Hox function. Although we found no evidence for misregulation of Hox gene expression in MEFs derived from Psp1<sup>−/−</sup> embryos, we did find it in a microarray data set of transcriptional profiling of HEK 293 cells subjected to siRNA for p75/PSIP1 (5). Almost 2% of the genes most significantly (P < 0.01) up-regulated in the knockdown cells were genes from the 5′ end of the HOXA cluster (HOXa5, HOXa6 HOXa9, HOXa10, and HOXa13). The upregulation of these “posterior” members of the HOXa cluster in cultured cells deficient in p75/PSIP1 would be consistent with the posterior homeotic transformations in our Psp1<sup>−/−</sup> mice.

As well as a role in transcriptional regulation, the p52 isoform of Psp1 has been shown to interact with the splicing factor SF2 and to modulate its activity (9). It is therefore intriguing that animals heterozygous for mutation in an essential splicing factor, SF3b1, a component of the U2 snRNP, showed posterior homeotic transformations similar to those seen in Psp1<sup>−/−</sup> mutant mice, including ectopic ribs on C7 and L6-to-S1 transformation (17).

We suggest that Psp1 may have an unexpected function in the transcriptional repression of homeotic genes and the specification of identity along the axial skeleton. In this regard, it is interesting that human PSIP1 (LEDGF) is found as a translocation-induced fusion partner with NUP98 in acute and chronic myeloid leukemias (11, 15). In these cases, the part of PSIP1 that is retained in the fusion protein (beyond exon 7) is the part that is missing in our mutant mice. The other recur-
rent leukemia-associated fusion partners with NUP98 are encoded by the HOX genes themselves (1). Further investigation of the interactions between HOX genes and Psp1 may help to elucidate the molecular etiology of these leukemias. Lastly, knocking inigenes for the different Psp1 isoforms back into our mutant mice may help to dissect the different functions of the alternatively spliced isoforms of Psp1.

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