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The Multi-Copy Mouse Gene *Sycp3*-Like Y-Linked (*Sly*) Encodes an Abundant Spermatid Protein That Interacts with a Histone Acetyltransferase and an Acrosomal Protein[1]

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ABSTRACT

Deletion analysis has established that genes on the Y chromosome are essential for normal sperm production in humans, mice, and *Drosophila*. In mice, long-arm deletions have an impact on spermiogenesis, with the most extensive deletions resulting in severe sperm head malformations and infertility. Intriguingly, smaller deletions are compatible with fertility but result in a distorted sex ratio in favor of females, and recently it was found that Y long-arm deletions are also associated with a marked upregulation of several X-encoded and Y-encoded spermatid-expressed genes. The mouse Y long arm encodes a number of distinct transcripts, each of which derives from multiple gene copies. Of these multicopy genes, the recently described *Sly* has been favored as the gene underlying the spermiogenic defects associated with Y long-arm deletions. To assess the candidacy of *Sly*, the expression of this gene was examined in the testis at the transcript and protein levels. *Sly* is transcribed after the first meiotic division in secondary spermatocytes and round spermatids and encodes two transcript variants, *Sly_v1* and *Sly_v2* (proteins referred to as SLY1 and SLY2). We raised an antibody against SLY1 which detected the protein in round and early elongating spermatids, where it is predominantly cytoplasmic. Yeast two-hybrid and coimmunoprecipitation studies demonstrated that SLY1 interacts with the acrosomal protein DKKL1, the histone acetyltransferase KAT5 (also known as TIP60), and the microtubule-associated protein APPBP2. Together, these data suggest SLY1 may be involved in multiple processes during spermiogenesis, including the control of gene expression and the development or function of the acrosome.

APPBP2/PAT1, DKKL1/SOGGY1, KAT5/TIP60, mouse, multicopy Y gene, SLY, spermatid, spermatogenesis, testis, Y gene functions

INTRODUCTION

The mouse Y chromosome encodes a limited number of functions that appear to be largely restricted to testis determination, spermatogenesis, and male fertility. Partial deletion or absence of the Y chromosome long arm (MSYq) is associated with defects in spermiogenesis, including increased incidence of malformed sperm heads; abnormal acrosome development, structure, and content; upregulation of X- and Yp-linked genes expressed during spermiogenesis; offspring sex ratio distortion; and reduced or absent fertilizing ability [1–8]. The severity of the spermatogenic phenotypes associated with deletion of the MSYq correlates with deletion size, suggesting that deficiency/loss of a multicopy, MSYq-encoded element—the "spermiogenesis factor"—is responsible [1]. However, the identity of this factor is currently unknown.

Of the four known multicopy genes on the mouse MSYq, the most promising candidate for the spermiogenesis factor is *Sly* (SYCP3-like, Y-linked). *Sly* is a member of the X and Y chromosome-encoded *Xlr* superfamily and was apparently created by the fusion of the 5′ region of the spermatid-specific *Slx* gene with the 3′ portion of the *Xlr* gene, followed by an internal duplication of two *Slx*-derived exons [9]. It is only expressed in the testis during spermatogenesis and decreases in proportion to the extent of the MSYq deletion [10]. *Sly* is the most highly transcribed of all known MSYq-encoded genes and is present in at least 65 copies on the MSYq (given the incompleteness of Yq sequencing, there may be more copies), more than half of which retain an open reading frame (ORF) encoding a protein that is clearly related to SLX and XLR [10]. The putative SLY protein (along with other XLR family members) contains a conserved COR1 domain implicated in chromatin binding [3, 10–13]; however, SLY shares the highest amino acid identity with SLX, a spermatid-specific cytoplasmic protein of unknown function [14]. Nevertheless, the COR1 domain of the predicted SLY protein shares 79% identity with that of the chromatin-associated protein XLR [10], and it has been proposed that SLY may have a role in sperm development by regulating the gene expression and chromatin conformation of the sex chromosomes during spermiogenesis [3]. Based on this hypothesis, it was suggested that the increased prevalence of abnormal spermatozoa and offspring sex ratio distortion in the MSYq deletion models may be a consequence of *Sly* deficiency triggering the upregulation of sex-linked genes involved in sperm head development and meiotic drive. To better assess the candidacy of *Sly* as the multicopy, MSYq-encoded spermiogenesis factor, the expression and function of *Sly* during murine spermatogenesis were investigated.

MATERIALS AND METHODS

Mice

Animal procedures were in accordance with the United Kingdom Animal Scientific Procedures Act 1986 and were subject to local ethics review. All mice were produced at the Medical Research Council National Institute for

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Medical Research on an MF1 random-bred background. XY mice carry an RIII strain of Y chromosome. 2/3MSYq$^-$ males have an RIII strain of Y chromosome with a deletion removing approximately two thirds of the MSYq and were derived from a stock originating from the mice described by Conway et al. [2]. The 9/10MSYq$^-$ mice have a 129 strain Y chromosome with a deletion removing approximately nine tenths of MSYq and also a small deletion removing the testis determinant *Sry* from the short arm, which is complemented by an autosomally located *Sry* transgene [10]. Mice lacking all of the MSYq (X*Sxr*^{a}Y*^{X} mice [1], referred to here as MSYq$^-$) were produced by mating XY*^{X} females [15, 16] with XY*Sxr*^{a} males [17, 18]. *Sly* transgenic mice were produced by pronuclear injection of the *Sly*-containing RP24-402P5 BAC.

RNA Isolation and RT-PCR

Total testis RNA was isolated, treated with DNase I, and reverse transcribed as described previously [14]. One microliter of cDNA was then used in a 25-μl PCR reaction with amplification for 35 cycles and an annealing temperature of 60°C, using primers for *Sly* [3] and *Hprt* [19] or using primers *Sly*-ORF-F (5′-ATGGCTCTTAAGAAATTGAAGG-3′) and *Sly*-ORF-R (5′-TTAG TTCTTGGTCCCCAAGTTCATC-3′), which encompass the *Sly_v1* and *Sly_v2* ORFs with product sizes of 659 and 557 bp, respectively (Fig. 1A).

RNA FISH, DNA Fluorescence In Situ Hybridization, and Chromosome Painting

RNA fluorescence in situ hybridization (FISH) was performed on surface spread spermatogenic cells from 28.5 days postpartum (dpp) and adult XY testes, as described previously [14]. Probes used for RNA FISH were: 1) the Y chromosomal BAC RP24-502P5 containing one copy of the *Sly* locus (BACPAC Resources) and 2) a long-range PCR product spanning intron 4 to exon 10 of *Sly* that was amplified using the primers 5′-CCCAGCTTGG GATCCTTTAGACTTTGTG-3′ and 5′-TGAGGTACATCTGAGGTCA AATTCCCATT-3′ and using the conditions described previously [20]. The long-range probe specifically does not cross-react with *Orly*, which is known to contain some *Sly*-derived sequences [9]. Identification of testicular cells was based on 4′,6′-diamidino-2-phenylindole (DAPI) fluorescence microscopy together with either RNA FISH for the Sertoli cell-specific gene *Wt1*, immunostaining for the meiotic marker γH2AFX, or immunostaining of the heterochromatin-associated protein CBX1. DNA FISH and chromosome painting were performed after RNA FISH using digoxigenin-labeled probes or fluorescein isothiocyanate StarFISH mouse X or Y chromosome paint (Cambio), respectively, as described previously [14].

Antibodies

The SLY1-specific peptide PAIGKDENISPQVK was used to immunize two rabbits, followed by booster injections at 2, 4, and 8 wk (standard peptide immunization package; Eurogentec). The primary antibodies used for immunofluorescence in this study were mouse monoclonal anti-γH2AFX (1:150; 05–636; Millipore), rat anti-CBX5 (1:200) [21], mouse anti-Flag m5 (1:100; F4042; Sigma), goat anti-DKKL1 (1:200; R & D Research), and rabbit polyclonal anti-KAT5/TIP60 chromodomain (1:100) [22]. Secondary antibodies were Alexa 488 chicken anti-mouse immunoglobulin G (IgG), Alexa 488 chicken anti-rabbit IgG, Alexa 594 chicken anti-mouse IgG, Alexa 594 chicken anti-rabbit IgG, Alexa 568 donkey anti-goat IgG, and Alexa 594 anti-rat IgG (all from Molecular Probes).

Western Blot Analysis

Protein extraction from tissues and Western blotting were performed as described previously [14] using the following primary antibodies: rabbit polyclonal anti-SLY1 SK97 (1:5000), rabbit polyclonal anti-SLY1 SK98 (1:5000), mouse anti-ACTIN (1:2000; sc-8432; Santa Cruz Biotechnology), anti-KAT5/TIP60 (1:500), mouse anti-APPBP2/PAT1 (1:50) [23], and anti-DKKL1 (1:1000). After incubating with the appropriate secondary antibody (anti-mouse IgG, anti-rabbit IgG, or anti-goat IgG) coupled to peroxidase (DAKO), the signal was revealed by chemiluminescence (SuperSignal; Pierce) and recorded on x-ray film.

Immunohistochemistry

Immunohistochemistry of paraffin-embedded sections was performed as described previously [14]. For SLY1 staining of testis cryosections, freshly dissected mouse testes were fixed in 4% paraformaldehyde (PFA) diluted in PBS overnight at room temperature, rinsed twice in PBS, and placed in 30% sucrose solution at 4°C. Testes then were embedded in OCT (VWR International, Ltd.) in Dispomoulds (Raymond A. Lamb, Ltd.), and 10-μm sections were cut with a Cryostat (Leica) and transferred onto Superfrost Plus slides (BDH). Slides were rinsed twice in PBS with 0.1% Triton X-100 to remove the OCT, fixed in 4% PFA, and rinsed twice more in PBS with 0.1% Triton X-100. Slides then were blocked (PBS with 0.1% Tween-20 and 0.15% bovine serum albumin) and were incubated with primary and then secondary antibodies. For immunostaining of COS7 cells transfected with Flag-*Sly_v1*, cells were grown on coverslips and fixed with 4% PFA before being blocked. DAPI (Dabco) was used to stain the nuclei.

Yeast Two-Hybrid Screening

Yeast two-hybrid screening of a pretransformed mouse adult testis Matchmaker cDNA library was performed using the Matchmaker Two-Hybrid System 3 (Clontech) according to the manufacturer's instructions. Briefly, the SLY1 ORF was subcloned in-frame into pGBKT7 (Clontech) and was expressed as a GAL4 DNA-binding domain fusion protein. The yeast reporter strain AH109 containing the pGBKT7-SLY1 bait construct was mated with the library yeast strain Y187. After mating, yeast cells were plated on selective media lacking Leu, Trp, Ade, and His in the presence of 5-bromo-4-chloro-3-indolyl α-D-galactopyranoside (X-α-Gal; Glycosynth); blue yeast colonies able to grow under these conditions were considered to contain cDNA coding for an SLY1-interacting protein. Library plasmids from selected positive colonies were retransformed into AH109 with either pGBKT7-SLY1 or pGBKT7 vector to confirm the interaction before being sequenced.

Coimmunoprecipitation

Testis material was homogenized on dry ice, and 10% w/v of either native cell lysis buffer (300 mM NaCl; 10 mM Tris, pH 7.4; 5 mM ethylenediaminetetraacetic acid; 1% Triton X-100; 0.2 mM PMSF; and 0.2 mM sodium vanadate) or denaturing lysis buffer (1% SDS; 10 mM Tris, pH 7.4; and 1 mM sodium vanadate) was added. Native samples were vortexed and incubated at 4°C for 30 min, and denatured samples were boiled at 95°C. Testis samples were then centrifuged at 4°C, and the cell lysate supernatant was collected. The lysates then were passed through a small-gauge needle several times and precleared by incubating with protein A sepharose beads. Protein A sepharose beads (50 μl; GE Healthcare) were washed in binding buffer (PBS with 0.5% Nonidet P-40) and resuspended in binding buffer with 5 μg of antibody overnight at 4°C to allow the antibodies to bind. Beads were washed in 0.2 M sodium borate (pH 9.0), incubated in 30 mM dimethyl pimelimidate for 30 min to covalently couple the antibody to the beads, and washed twice in 0.2 M ethanolamine (pH 8.0), followed by washing in binding buffer. Antibody-coupled beads were incubated at 4°C overnight with 500 μl of native or denatured testis protein lysates, washed three times in binding buffer at 4°C, and resuspended in 40 μl of binding buffer and 20 μl of 5× Laemmli sample buffer. The beads were boiled for 5 min at 100°C to elute the proteins, and the protein supernatant was removed and visualized by SDS-PAGE and Western blotting.

RESULTS

Transcriptional Analysis of Sly During Spermatogenesis

The testicular expression of *Sly* was investigated by RT-PCR analysis on total testis RNA from mice at different postnatal ages (Fig. 1B). In prepubertal mice, successive germ cell subtypes populate the seminiferous epithelium at defined time points, allowing the correlation of gene expression with appearance of a specific cell type. Using primers that give the same-sized product for both *Sly* transcript variants, *Sly* mRNA was weakly detectable at 18.5 dpp, which corresponds to the meiosis-spermatid transition, with transcription increasing by 21.5 dpp and remaining high thereafter. Similar results were obtained with primers designed to amplify the entire *Sly* ORF, which give different product sizes for *Sly1* and *Sly2* transcript variants. The control *Hprt* RT-PCR showed similar transcript levels at all time points assayed.

To precisely determine which spermatogenic cell types actively transcribe the *Sly* loci, gene-specific RNA FISH was performed on spermatogenic cells from 28.5-dpp and adult XY testes using an *Sly*-containing BAC as a probe (Fig. 1C). This

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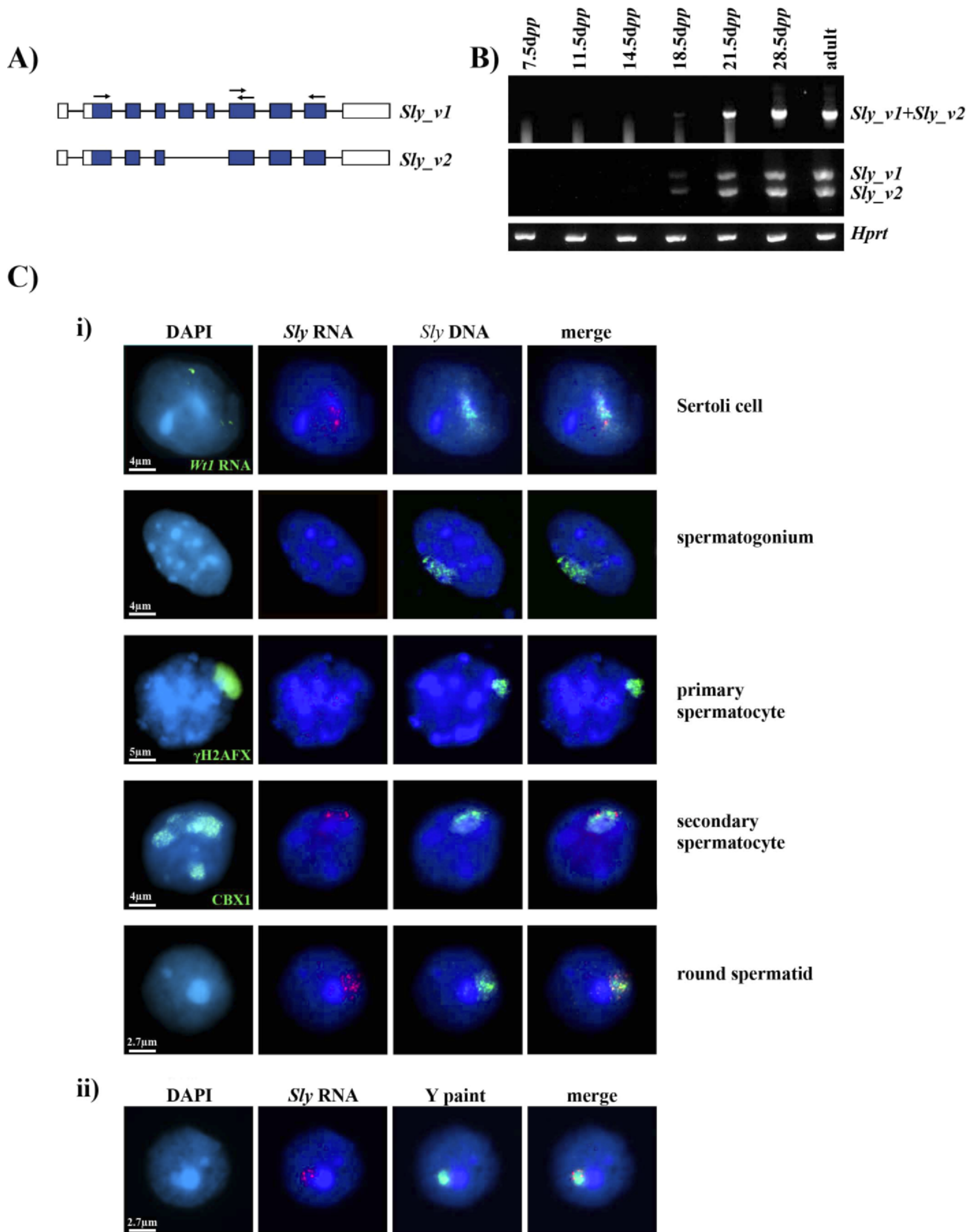

FIG. 1. Transcriptional analysis of *Sly*. **A**) Schematic diagram of *Sly_v1* and *SLY_v2* transcript variants indicating the positions of the RT-PCR primers used. Exons are denoted by boxes and introns by lines. Filled boxes represent exons encoding the *Sly_v1* and *Sly_v2* ORFs. **B**) An RT-PCR analysis of testis RNA samples taken at time points during the first spermatogenic wave using *Sly*-specific primers designed to sequences within exon 7 (top), as well as primers designed to amplify the *Sly_v1* and *Sly_v2* ORFs (middle). The products corresponding to *Sly_v1* and *Sly_v2* mRNA are present from 18.5 dpp. An RT-PCR

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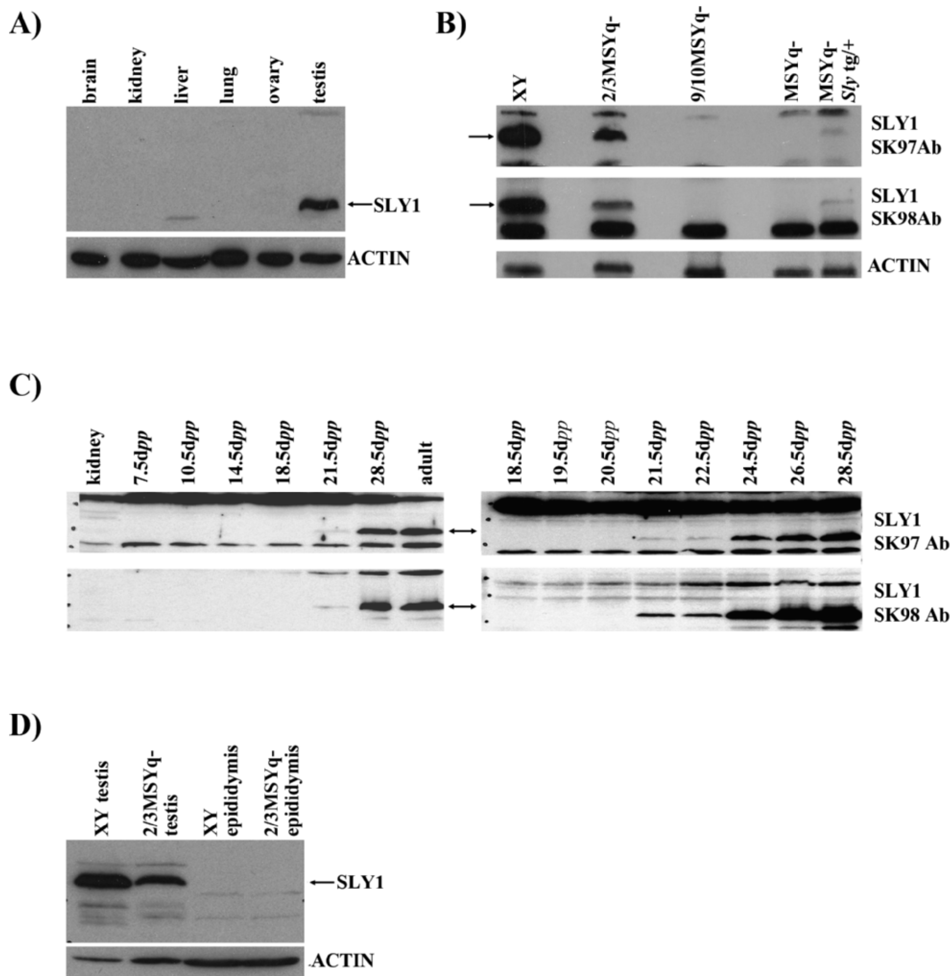

FIG. 2. Western blot analysis of SLY1. **A**) Western blot analysis of multiple tissues from an adult mouse. The anti-SLY1 antibody detects a testis-specific protein of approximately 40 kDa (arrow) that is presumed to be SLY1. An antibody against ACTIN was used as a loading control. **B**) Analysis of testis protein lysates from XY, 2/3MSYq$^-$, 9/10MSYq$^-$, MSYq$^-$, and MSYq$^-$*Sly* hemizygous transgenic males. Blots were probed with the anti-SLY1 SK97 antibody (top), the anti-SLY1 SK98 antibody (middle), and an anti-ACTIN antibody as a loading control (bottom). The bands corresponding to SLY1 are shown with arrows. **C**) Analysis of XY testis samples taken at time points during the first spermatogenic wave, from 7.5 dpp to adult (left) and 18.5 dpp to 28.5 dpp (right). The anti-SLY1 SK97 (top) and anti-SLY1 SK98 (bottom) antibodies detect a band corresponding to SLY1 (arrows) from 21.5 dpp. **D**) Western blot of testis and epididymal protein samples from XY and 2/3MSYq$^-$ males. SLY1 (arrow) is present in the testis but not the epididymis. ACTIN was used as a loading control.

probe detects nascent *Sly* transcripts in the nucleus before they are processed and exported to the cytoplasm. *Sly*-specific RNA FISH signals were seen in the nuclei of adult Sertoli cells (11 of 31) but not immature Sertoli cells from 28.5-dpp testis, secondary spermatocytes (16 of 44), and round spermatids. Approximately one half (1199 of 2422 [49.5%]) of round spermatids studied had RNA FISH signals, and these were multiple in nature, as expected from the multicopy nature of *Sly*. *Sly* RNA was not detected in spermatogonia (0 of 112) or primary spermatocytes (0 of 143). The same pattern of expression was observed using the long-range *Sly* probe. DNA FISH was performed using the BAC probe; RNA and DNA FISH signals colocalized in Sertoli cells, secondary spermatocytes, and round spermatids, confirming that these transcripts originated from *Sly*. Y chromosome paint also showed 100% concordance with the presence of the RNA FISH signal, confirming that the signal originates from the Y chromosome. Together, these data show that *Sly* is transcribed primarily in germ cells after the first meiotic division.

Sly *Encodes a 40-kDa Testis-Specific Protein*

Of the 65 copies of *Sly* identified based on predicted DNA sequence, 34 have retained protein coding potential [10]. To further characterize the function of *Sly* in spermatogenesis, antibodies were raised against the putative SLY proteins. A ClustalW alignment of XLR, SLX, and SYCP3 with the predicted SLY1 and SLY2 amino acid sequences (see Supplemental Fig. S1A; all Supplemental Figures are available online at www.biolreprod.org) identified a 14-amino acid, putatively SLY1-specific peptide, although a related peptide was present in SLY2. This SLY1 peptide was used to generate two polyclonal rabbit anti-SLY antibodies. Both antibodies are able to detect the SLY1 protein in HEK293 cells transfected with the *Sly1* ORF fused to a FLAG tag. Unfortunately, we were unable to resolve whether these antibodies would cross-react with the related SLY2 peptide, because the Flag-*Sly_v2* construct was not translated in transfected cells (Supplemental Fig. S1B).

for *Hprt* was used as a control (bottom). **C**) Gene-specific RNA FISH for *Sly* nascent transcripts. **i**) RNA FISH on surface-spread spermatogenic cells. Left column shows staining of cell nuclei with DAPI (blue) combined with other markers used for cell identification (Sertoli cells are positive for *Wt1* transcripts [green], primary spermatocytes for the histone variant γH2AFX [green], and secondary spermatocytes for the heterochromatin protein CBX1 [green]); second column from the left, shows *Sly* RNA FISH signals (red) and DAPI (blue); second column from the right, *Sly* DNA FISH signals (green) and DAPI (blue); and right column, overlay of *Sly* RNA and DNA FISH signals, with the nuclei stained for DAPI (blue). Weak *Sly* RNA FISH signals were seen in Sertoli cells, and stronger signals were seen in secondary spermatocytes and round spermatids. **ii**) An *Sly*-expressing round spermatid nucleus with Y chromosome paint. Left column shows spermatid nucleus stained with DAPI (blue); second column from the left, *Sly* RNA FISH (red); second column from the right, Y chromosome paint (green); and right column, overlay of RNA FISH and chromosome paint signals.

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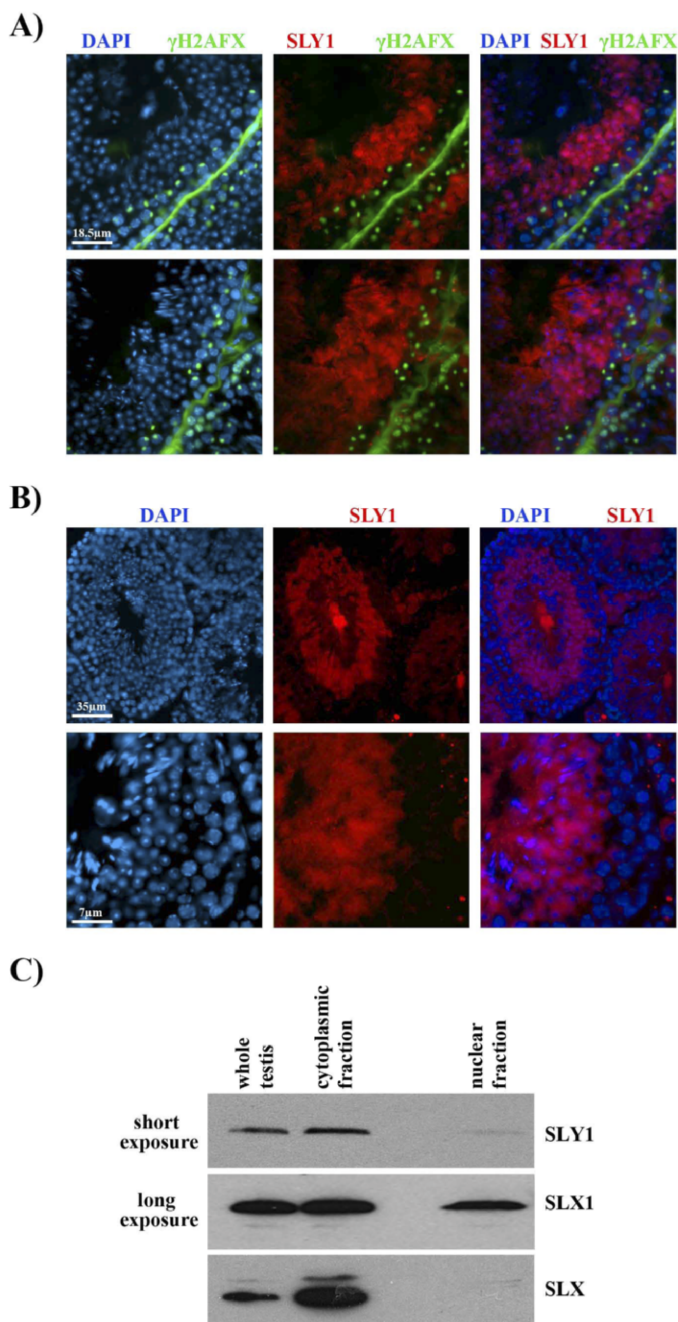

FIG. 3. Localization of SLY1 in the testis. **A**) Costaining of adult testis sections for the primary spermatocyte marker γH2AFX (green) and DAPI (blue) with SLY1 (red) using the SLY1 SK97 antibody (top) and the SLY1 SK98 antibody (bottom). SLY1 shows no overlap with γH2AFX-positive spermatocytes. The anti-mouse secondary antibody (green) also stains the basal lamina of the seminiferous tubules. **B**) Antibody staining of XY seminiferous tubules with the anti-SLY SK97 antibody (original magnifications, ×40 [upper row] and ×100 [lower row]). Left column shows DAPI staining of cell nuclei (blue); middle column, anti-SLY SK97 antibody (red); right column, overlay. SLY1 localizes to the cytoplasm and nucleus of round and early elongating spermatids. **C**) Western blot analysis of cytoplasmic and nuclear fractions from adult testis with the anti-SLY1 SK97. SLY1 is present in the cytoplasmic and nuclear fractions. A whole-testis sample is included a positive control, and an SLX antibody was used as a negative control for the nuclear fraction.

Next, Western blot analysis was carried out using protein lysates from multiple adult mouse tissues using the two anti-SLY antibodies. These antibodies recognized a protein of approximately 40 kDa that was present only in the testis (Fig. 2A), in agreement with previous studies identifying *Sly* as a testis-specific transcript [10]. This protein was not detected by the preimmune serum (Supplemental Fig. S2A) or after preabsorption of the antibody with the peptide to which it was raised (Supplemental Fig. S2B). If this antigen is the putative SLY1 protein, it should be reduced or absent when copies of *Sly* are deleted in the three MSYq deletion models. These models lack varying lengths of the Y chromosome long arm but produce near-normal levels of sperm. Western blot analysis indicates the 40-kDa protein is reduced in the 2/3MSYq$^-$ testis and is not present in the 9/10MSYq$^-$ or MSYq$^-$ samples (Fig. 2B) but is present in MSYq$^-$ mice carrying the *Sly* transgene, confirming that the 40-kDa band is SLY1. These findings imply that *Sly1* mRNA is effectively translated in the testis.

To determine the developmental time course of SLY1 expression, Western blot analysis of testis protein samples at different time points during the first spermatogenic wave was performed. SLY1 was present at low levels from 21.5 dpp, increased as spermatogenesis proceeded, and reached adult levels by 28.5 dpp, suggesting that the protein is restricted to spermatids (Fig. 2C). However, SLY1 was not present in epididymal protein lysates (Fig. 2D), indicating that this protein is not maintained in mature sperm but is lost during spermiogenesis or spermiation.

SLY1 Is a Predominantly Cytoplasmic Protein

To confirm that SLY1 is expressed exclusively in spermatids, immunostaining of adult testis cryosections was performed using both SLY1 antibodies. Antibody staining was seen in round and early elongating spermatids from stage II to XI seminiferous tubules (Fig. 3, Supplemental Fig. S3, and data not shown). No staining was observed with the preimmune serum or after the antibody had been preincubated with the peptide to which it had been raised (Supplemental Fig. S3). No colocalization was seen between SLY1 and a γH2AFX antibody, which labels the nucleus of leptotene and zygotene cells and the sex body domain of the nucleus in pachytene cells [24], confirming that SLY1 is not present during meiotic prophase (Fig. 3A). Closer analysis of SLY1 immunostaining revealed that SLY was predominantly located in the spermatid cytoplasm (Fig. 3B). This observation was confirmed by Western blot analysis of cytoplasmic and nuclear testis fractions; SLY1 was present at higher levels in the cytoplasmic fraction compared with the nuclear fraction (Fig. 3C).

SLY1 Interacts with Components of the Acrosome and Chromatin Modifiers

To investigate the function of SLY1 during spermiogenesis, potentially interacting proteins were identified. A yeast two-hybrid analysis was carried out using a pretransformed *Saccharomyces cerevisiae* testis cDNA library as prey. Approximately 1.5 × 10^8 library clones were screened, and 14 proteins were identified that were able to interact with SLY1, at least in a two-hybrid assay. To confirm these interactions in vivo, coimmunoprecipitation experiments were performed using adult testis extracts incubated with either the two anti-SLY antibodies or preimmune serum. Dickkopf-like 1 (DKKL1, also known as SOGGY1), the histone H4 lysine acetyl transferase (KAT5, also known as TIP60), and beta-amyloid precursor protein (cytoplasmic tail)-binding protein 2

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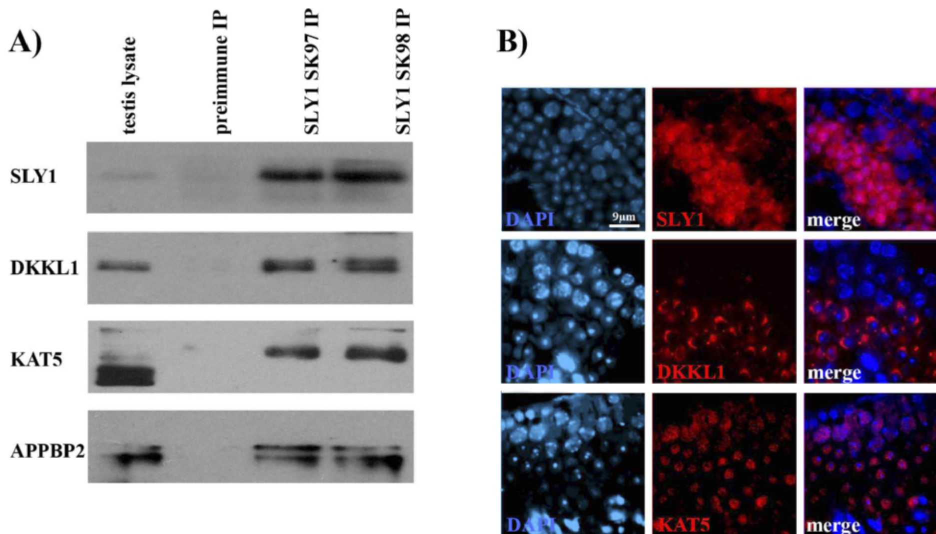

FIG. 4. Confirmation of SLY1-interacting proteins in vivo. **A**) Coimmunoprecipitation of DKKL1, KAT5/TIP60, and APPBP2 with SLY1. Protein lysates from adult XY testis (input; lane 1) were immunoprecipitated (IP) with the anti-SLY1 preimmune serum antibody (lane 2), the anti-SLY1 SK97 antibody (lane 3), and anti-SLY1 SK98 antibody (lane 4), followed by Western blot analysis. KAT5 is found at low levels in the testis, so it was not detected in the input lane. The immunoprecipitated proteins were all of the expected size. **B**) SLY1, DKKL1, and KAT5/TIP60 antibody staining of XY seminiferous tubules. Left column shows nuclei stained with DAPI (blue); middle column, antibody stain (red); right column, overlay. All three proteins are present in round spermatids.

(APPBP2, also known as PAT1) coimmunoprecipitated with both SLY antibodies and were of the expected size but were not present in the preimmune serum control (Fig. 4A), confirming that these proteins interact with SLY1 in the testis. DKKL1 is related to the Dickkopf gene family of secreted antagonists of WNT signal transduction and is a component of the mouse spermatid acrosome (Fig. 4B and Kohn et al. [25]). KAT5 belongs to the MYST family of histone acetyltransferases and is involved in DNA repair and apoptosis [26]. *Kat5* is transcribed in the testis at high levels from stages IX to XII [27], and the encoded protein is present in the nucleus of late spermatocytes and round spermatids (Fig. 4B). APPBP2 is a microtubule-binding protein involved in intracellular trafficking and processing of proteins, including the beta-amyloid precursor proteins [23, 28].

DISCUSSION

In this study, we have analyzed the expression of the multicopy MSYq-linked *Sly* during murine spermatogenesis. Transcription analysis demonstrated that *Sly* is expressed after the first meiotic division in secondary spermatocytes and round spermatids, validating previous reports that *Sly* is transcribed during spermiogenesis [3, 10]. There are two *Sly* transcript variants expressed in the testis, and the full-length *Sly_v1* isoform encodes a 40-kDa protein present in the cytoplasm and nucleus of round and early elongating spermatids. To gain insight into the function of SLY1, a testis cDNA library was screened by yeast two-hybrid analysis. This led to the identification of three proteins that interact with SLY1 in the testis: the acrosomal protein DKKL1, the histone acetyltransferase KAT5, and the microtubule-associated protein APPBP2.

The SLY1-interacting protein DKKL1 localizes to the developing acrosome in spermatids and the acrosome of mature sperm. The function of DKKL1 in the testis is unknown, but several possibilities have been suggested, including facilitating acrosome formation, preventing premature spermiation, or guiding the mature sperm to the oocyte [25], and defects in these processes have been reported in mice with MSYq deletions [5–8, 29, 30]. Several acrosomal abnormalities have been found in sperm from B10.BR-Y^{del} mice that lack approximately two thirds of the MSYq, including distortion of the acrosome shape, lack of the proacrosomal granule in round spermatids, decreased levels of the acrosomal protease acrosin, and increased numbers of epididymal sperm with damaged or absent acrosomes [5, 6, 29]. The aberrant acrosome formation and content observed in the B10.BRY^{del} sperm led Styrna et al. [5] to conclude that an MSYq-linked factor is involved in controlling acrosomal development by coordinating the various synthetic and morphological processes involved. *Sly* is a very good candidate for this "acrosomal factor" in light of the interaction between the acrosomal protein DKKL1 and SLY1, which advocates a role for SLY1 in development or function of the acrosome. Because SLY1 is not present in condensing spermatids or epididymal sperm, the putative acrosomal function of SLY may be restricted to the initial stages of acrosomal development.

As well as having a role in acrosomal development, SLY1 may, as previously hypothesized, also be involved in regulating the chromatin conformation and gene transcription of the sex chromosomes during spermiogenesis through its interaction with KAT5 and APPBP2. The KAT5/TIP60 complex is involved in chromatin remodeling, including the dephosphorylation of nucleosomal γH2AFX [31], exchange of phosphorylated H2AFV for unmodified H2AFV in regions of DNA damage [32, 33], chromatin incorporation of the histone variant H2AFZ [34], and the maintenance of chromatin modifications linked to embryonic stem cell identity [35]. KAT5 interacts with a variety of transcription factors and chromatin-remodeling complexes to regulate gene transcription by repressing or stimulating activity of specific promoters [36–38]. KAT5 can also affect transcription through altering the local chromatin conformation, and the KAT5/TIP60 complex has been shown to promote the generation of silent heterochromatin in *Drosophila* [39]. Thus, it is possible that SLY1 forms a complex with KAT5 to regulate the expression of specific X- and Y-linked genes during spermiogenesis by binding to and repressing the activity of their promoters. Alternatively, KAT5 may be involved in the remodeling of the sex chromosomes during the meiosis-spermatid transition, with SLY1 required for the maintenance of the spermatid sex chromosome conformation. With both hypotheses, loss of SLY1 in mice with MSYq deletions could lead to upregulation of X and Y genes observed in these mice by affecting the function of the KAT5/TIP60 complex. KAT5 is also a tumor suppressor that responds to ultraviolet- and ionizing radiation-induced DNA damage by stimulating phosphorylation and dephosphorylation of H2AFX [31, 40, 41], and acetylating p53 and the kinase ATM, leading to the transcriptional activation of proapoptotic genes [42, 43]. Accordingly, SLY1 may have a role in the response to DNA damage in spermatids, and consistent with

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this, transcription of *Sly* is known to be regulated by the heat shock response transcription factor HSF2 [44].

APPBP2 is a microtubule-associated trafficking protein that binds to the basolateral sorting signal of the amyloid precursor protein (APP). As well as being involved in the transport of APP [23], APPBP2 also promotes the processing of APP by gamma-secretase to yield two peptides: the beta-amyloid protein implicated in Alzheimer disease and the APP intracellular domain (AICD) [28]. AICD is involved in the regulation of apoptosis, calcium flux, and gene transcription, and it forms a trimeric transcription activator complex with Fe65 and KAT5. The AICD-Fe65-KAT5 complex has a role in AICD signaling [45] and activates target genes that include p53 [46]. The interaction between SLY1 and APPBP2 suggests that SLY1 may have a role in intracellular protein transport during the early stages of spermiogenesis. In addition, SLY1 could potentially be involved in AICD signaling within spermatids by interacting with APPBP2 to control APP processing and affecting the function of the AICD-Fe65-KAT5 trimer in modulating gene expression.

Aside from an increase in sperm head defects, mice with deletions of two thirds of the MSYq (the B10.BR-Y^{del} and 2/3$MSYq^{-}$ mice) generate offspring with a distorted sex ratio in favor of females [2, 47]. Ellis et al. [3] proposed that this is a consequence of a reduction in MSYq-encoded transcripts resulting from the deletion, which causes a disruption in the equilibrium between an X-linked meiotic driver and a suppressor gene located on the MSYq. Mild overexpression of an X-linked distorter is expected to lead to sex ratio distortion if the distorter is usually suppressed in a dosage-dependent manner, as observed in 2/3$MSYq^{-}$ mice. Furthermore, gross overexpression of the X-linked distorter may be the cause of sterility in the 9/10$MSYq^{-}$ and $MSYq^{-}$ mice, because unrepressed drivers (e.g., *Stellate* in *Drosophila melanogaster*) can lead to sterility rather than drive [48]. The *Sly*/*Slx* conflict hypothesis proposes that *Sly* and its multicopy, X-linked homologue *Slx* may be important players in this genomic conflict, perhaps because of a mutually antagonistic action of their encoded proteins on the transcriptionally repressed X and Y chromatin domains of spermatids [3, 10]. However, SLX is a cytoplasmic protein, and thus it is unlikely to have a direct effect on sex chromosome gene expression [14]. Instead, SLX could negatively regulate the function of SLY1 by competition for interacting proteins, and it would be interesting to determine whether SLX interacts with DKKL1, APPBP2, and KAT5. Unfortunately, our attempts to test this using a yeast two-hybrid screen were thwarted by the toxicity of the SLX protein when expressed in yeast. This was in marked contrast to SLY, and points to divergent functionalities of these proteins.

In conclusion, our findings indicate SLY1 may have a role in the development of the acrosome and the regulation of spermatid gene expression, potentially explaining the spermiogenic defects associated with deletion of the MSYq. However, further work is needed to confirm that *Sly* is the multicopy, MSYq-encoded spermiogenesis factor. We are currently examining whether specific loss of *Sly* recapitulates the phenotypes resulting from MSYq deletions and whether the spermiogenic problems in the deletion mice can be rescued by an exogenous *Sly* transgene.

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