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Deficiency in the Multicopy Sycep3-Like X-Linked Genes Slx and Slxl1 Causes Major Defects in Spermatid Differentiation

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The human and mouse sex chromosomes are enriched in multicopy genes required for postmeiotic differentiation of round spermatids into sperm. The gene Sly is present in multiple copies on the mouse Y chromosome and encodes a protein that is required for the epigenetic regulation of postmeiotic sex chromosome expression. The X chromosome carries two multicopy genes related to Sly: Slx and Slxl1. Here we investigate the role of Slx/Slxl1 using transgenically-delivered small interfering RNAs to disrupt their function. We show that Slx and Slxl1 are important for normal sperm differentiation and male fertility. Slx/Slxl1 deficiency leads to delay in spermatid elongation and sperm release. A high proportion of delayed spermatids are eliminated via apoptosis, with a consequent reduced sperm count. The remaining spermatids are abnormal with impaired motility and fertilizing abilities. Microarray analyses reveal that Slx/Slxl1 deficiency affects the metabolic processes occurring in the spermatid cytoplasm but does not lead to a global perturbation of sex chromosome expression; this is in contrast with the effect of Sly deficiency which leads to an up-regulation of X and Y chromosome genes. This difference may be due to the fact that SLX/SLXL1 are cytoplasmic while SLY is found in the nucleus and cytoplasm of spermatids.

INTRODUCTION

Spermatogenesis is the process during which spermatogonial stem cells multiply and generate spermatocytes, which through two meiotic divisions form haploid spermatids that differentiate into spermatzoa. The differentiation of haploid round spermatids into spermatzoa (spermiogenesis) involves major alteration of cell structure and function as the nucleus is restructured via chromatin remodeling and compaction to form the sperm head, and sperm-specific structures, such as the acrosome and the flagellum, are formed (Russell et al., 1990). The X and Y chromosomes are enriched in genes presumed to be important for sperm differentiation (Burgoyne and Mitchell, 2007; Mueller et al., 2008) but functional studies remain rare (for review, see Stouffs et al., 2009). The sex chromosomal complement of spermatid-expressed genes in man and mouse is enriched for multicopy genes (Skaltskaya et al., 2003; Toure et al., 2005; Mueller et al., 2008), and this has hindered gene function studies as a classical targeting strategy is not applicable (Burgoyne and Mitchell, 2007). Using an RNA interference–based strategy to disrupt the function of the multiple copies (>100) of the Y-encoded Sly (Sycp3 like Y-linked) gene, we found that its protein is crucial for the epigenetic regulation of sex chromosome expression after meiosis and for sperm differentiation (Cocquet et al., 2009). The X chromosome carries two multicopy genes related to Sly: Slx (formerly known as Xmr; ~43 copies) and Slx-like1 (Slxl1, formerly known as AK015913 or 4930527E24Rik; ~16 copies) (Reynard et al., 2007; Scavetta and Tautz, 2010). Like Sly, Slx and Slxl1 are specifically expressed in male postmeiotic germ cells. Slx encodes a cytoplasmic protein of unknown function, while for Slxl1 it has been unclear whether transcripts are translated in the testis (Reynard et al., 2007). In the case of Sly deficiency, Slx and Slxl1 genes are up-regulated, along with other sex-chromosome genes, and thus are candidates to explain the aberrant sperm differentiation phenotypes and the near sterility of Sly-deficient males (Ellis et al., 2005; Cocquet et al., 2009). Intriguingly, genomically and genetic evidence suggests the existence of an...
ongoing postmeiotic intragenomic conflict between multicopy X and Y genes (Partridge and Hurst, 1998; Ellis and Affara, 2006), and Slx and Sly genes have been hypothesized to be key mediators of this ‘competition’ between X-bearing and Y-bearing gametes (Ellis et al., 2005).

In the present study, we sought to determine the function of Slx and Sxl1 and to see whether, like their Y-encoded counterpart Sly, they have a critical role in the control of sex chromosome expression during spermiogenesis. For this, we produced Sxl/Sx1L1-deficient mice using transgenically-delivered small interfering RNAs (siRNA). We show that the Slx gene family is important for normal mouse sperm differentiation (and thus for male fertility), but in contrast to Sly this is not a consequence of a global perturbation of sex chromosome expression.

**MATERIALS AND METHODS**

**Plasmid Construction, Generation, and Breeding of Transgenic Mice**

To generate the U6shSLX constructs, we used a PCR-based approach similar to that described in Harper et al., 2005, using primers designed to generate the short hairpin SLX sequences (Harper et al., 2005) (Supplementary Table 1). The PCR products were cloned into the pcRI2.1 vector (TOPO TA Cloning, Invitrogen, Life technologies, Carlsbad, CA). An insulator element from the chicken β-globin domain (Chung et al., 1993) and a genotype tag (GTag) were then added to each construct between Sall and BamHI restriction enzyme sites. These genotyping tags were inserted to enable discrimination of the different shSLX constructs by PCR (see Supplementary Table 1 for primer sequences). All constructs were sequenced before testing their specificity and efficiency.

Before injection, the plasmids were linearized at PvuI and BamHI sites and on-column purified from agarose gels (Gel Extract II kit, Macherey Nagel, Germany). Fertilized eggs from CBA/Ca × C57BL/10 matings were microinjected with the construct, using standard protocols. Transgenic founders carrying a shSLX construct were identified by PCR and crossed with random-bred MF1 (National Institute for Medical Research colony) mice. Two founders (one male shSLX1 and one female shSLX2) transmitted the transgenic to their offspring. The lines have been maintained by backcrossing shSLX transgenic females to MF1 XY male (i.e., with a Y chromosome originating from the XLI strain maintained on the random-bred MF1-NIMR background). Two-month-old MF1 XY wild type males with (tsgic) and without (neg -) the transgene were processed for all the analyses presented here except IVF, sperm motility, and electron microscopy for which males with a mixed B6D2F1 (C57BL/6 x DBA/2)/MF1 background were used. Animal procedures were in accordance with the guidelines of the Laboratory Animal Research of the National Academy of Science, Bethesda, MD, 1996.

**Elution of Spermatozoids**

Fractions enriched in round spermatozoids (>90%) were obtained using an adapted protocol of Meistrich (Meistrich, 1977), as described previously (Cocquet et al., 2009).

**Western Blot and Immunofluorescence**

Western blot analyses were performed as described previously (Reynard et al., 2007). Briefly, 10–15 micrograms of testis or spermatid fraction protein extracts were run on a 12% SDS/polyacrylamide gel or a 4–12% gradient Bis/Tris gel (NuPage, Invitrogen). After transfer and blocking, membranes were incubated overnight with either anti-SLX/SXL1I antibody (Reynard et al., 2007) or anti-Acrv1 (an autosomal spermatid specific gene) was included on every plate as a loading control. The difference in PCP cycles with respect to Acrv1 (ΔCp) for a given experimental sample was subtracted from the mean ΔCt of the reference samples (negative samples, (ΔΔCt). For quantitation on purified round spermatid samples, β-actin was used as the loading control. Primer sequences are available in Supplementary Table 1.

Microarray analyses were performed on RNA from purified round spermatid fractions as previously described (Cocquet et al., 2009) using two groups (a pool of 2–4 individuals) of shSLX1 transgenic mice and four groups of WT mice. Results were compared with those obtained with purified round spermatid fractions from Sly-deficient males (Cocquet et al., 2009). Differentially expressed genes were classified according to their likely biological function in Onto-Express.

**Statistical Analysis**

For comparisons of the incidence of sperm head abnormalities, differences between genotypes were assessed by ANOVA after angular transformation of percentages, using the Generalized Linear Model provided by NCSST statistical data analysis software. The same test was applied to the frequency of abnormal head-tail connections, the proportion of oocytes fertilized in vitro, tubules with sperm shedding delay, TUNEL+ elongating spermatids, abnormal H4K12Ac staining, or sperm motility. Student’s t test was used to compare the data obtained for fecundity, sperm number, testis weight, Western blot quantification, and real-time PCR (performed on the ΔΔCt values). For microarray analysis, quantile normalization of all expression data was performed using BeadStudio (Illumina Inc., San Diego, CA). Data for the shSLX1/sLx control spermatids was compared in BeadStudio, using the Illumina custom error model. Significant up- or down-regulation was defined as a false discovery rate-corrected p value of ≤0.05 and a fold change of ≥1.5.

**RESULTS**

**Specific Knockdown of Slx and Sxl1 Transcripts by Transgenically-Delivered shRNAs**

To study the function of the Slx multicopy gene family, we used a strategy similar to the one previously developed for Sly (Cocquet et al., 2009), i.e., the transgenic delivery of short hairpin RNAs (shRNAs) that generate small interfering RNAs (siRNAs). These siRNAs mediate gene-specific knockdown via RNA interference. In our strategy, the shRNAs are...
expressed under the control of the strong and ubiquitous U6 promoter (Figure 1).

Four shRNA sequences expected to target Slx and Slx-like 1 (SlxII) transcripts were carefully selected (to avoid cross-reaction with related sequences such as Sly and Xlr) and used to design shRNA constructs. The efficiency and specificity of these shRNA constructs were tested in cell culture by cotransfection (Supplementary Figure 1). Based on these results, two efficient and specific shRNA constructs (shSLX1 and shSLX2) were used to produce transgenic mice via pronuclear injection. Each transgene was stably inserted in the genome and was transmitted to the progeny. The resulting transgenic lines (designated shSLX1 and shSLX2) showed a dramatic knockdown of Slx transcripts: ~62% decrease in shSLX1 testes and ~97% decrease in shSLX2 testes, as quantified by real-time PCR (Figure 1). The shSLX2 transgene did not have a significant effect on SlxII expression; however, shSLX1 testes displayed a ~68% decrease in SlxI transcript level. These results are in agreement with predictions from cotransfection experiments (cf. Supplementary Figure 1). Males carrying both shSLX1 and shSLX2 transgenes (shSLX1,2 transgenic mice) had decreased levels in both Slx and SlxII transcripts (~97% and ~69% decrease, respectively). When using primers designed to amplify together Slx and SlxI transcripts (Slx-all), global knockdown was estimated as ~68% for shSLX1, ~59% for shSLX2, and ~83% for shSLX1,2.

SLX and SLXL1 Protein Levels Are Reduced in shSLX Testes

Two antibodies had been developed for the study of SLX (Reynard et al., 2007). In cell lines transiently expressing an Slx or SlxII transgene, one antibody (anti-SLX aka anti-SLX69-81) detected specifically SLX protein, while the other (anti-SLX/SLXL1 aka anti-SLX96-106) detected both SLX and SLXL1 protein. In testes, SLX protein was detected by both antibodies but it remained unclear whether SlxII transcripts were translated (Reynard et al., 2007). Making use of our two shSLX mouse lines (shSLX1 targeting Slx and SlxII transcripts, and shSLX2 only targeting Slx) we decided to reinvestigate SlxII translation. SLX protein is predicted to be 25 kDa, while SLXL1 is expected at 18 kDa. After separation on a 12% polyacrylamide gel, Western blot detection with anti-SLX specific antibody showed a fainter band in shSLX1 testis protein samples compared with wild-type testes (i.e., nontransgenic siblings). In shSLX2 testes, the band was barely visible (Figure 2A) in agreement with transcript quantifications. Detection with the anti-SLX/SLXL1 antibody showed a double band of reduced intensity in shSLX1 and shSLX2 testes (Figure 2A). After separation on a 4–12% gradient gel, it became clear that the upper band (indicated by the gray arrowhead) corresponds to SLX protein (reduced in shSLX1 and in shSLX2 testes) while the bottom band (indicated by the black arrowhead) corresponds to SLXL1 protein (reduced in shSLX1 but almost unaffected in shSLX2 testes). As expected, double transgenic shSLX1,2 mice showed a deficiency in both types of proteins. Similar results were obtained on round spermatid protein extracts (Figure 2B). Immunofluorescence on testis section using anti-SLX/SLXL1 antibody confirmed a dramatic decrease in SLX/SLXL1 protein levels in round spermatids (Supplementary Figure 2).

All in all, we have produced mouse models deficient for Slx/Slxl1 genes. We proceeded to analyze their phenotypes to elucidate the roles of these genes in spermiogenesis and the extent to which Slx/Slxl1 depletion mimics Sly deficiency.

**SLx/Slxl1 Deficiency Leads to Impaired Male Fertility**

The first phenotype of Slx/Slxl1-deficient males we observed was poor fertility. Over a breeding period of 6½ months with young WT (MF1) females, shSLX1 and shSLX2 males had a considerable reduction in the number of litters and in litter sizes compared with WT males (nontransgenic siblings) (Table 1). Double transgenic shSLX1,2 males were sterile. Transgenic females did not have obvious breeding problems (data not shown). In vitro, the fertilizing ability of Slx/Slxl1-deficient epididymal sperm was also severely impaired relative to controls (Table 1). Testis weight for shSLX1 and shSLX2 males did not differ from controls but shSLX double transgenic testes were slightly smaller (90 mg vs. 102 mg in WT) (Table 1).
Deficiency Leads to Abnormal Spermatid Elongation

We sought to determine the origin of the fertility defect observed in shSLX males. Histology of all types of Slx/Slxl1-deficient males did not differ from WT until post-meiotic stages. This is in agreement with the observation that Slx and Slxl1 expression is restricted to the postmeiotic stage of male germ cells (i.e., spermatids) (Reynard et al., 2007). There was no detectable delay of round spermatid development; however, Slx/Slxl1-deficient testes had a defect in spermatid elongation (from stage IX and onwards). For instance, in PAS-stained stage XII tubules of Slx/Slxl1-deficient mice, elongating spermatids are visibly less developed (with the presence of step10 spermatids instead of step12) than in controls (Figure 3, A and C). Histone H4 hyperacetylation is a good marker of the spermatid elongation stages, as it occurs in early elongating spermatids before histone replacement by transition proteins (Hazzouri et al., 2000; Gaucher et al., 2009). Using antibodies directed against acetylated lysine 8 and 12 of histone H4 (H4K8Ac and H4K12Ac) as markers for spermatid elongation, we observed a delay in the development of elongating spermatids in shSLX1 and shSLX2 mice (Figure 3, B).

Table 1. Analysis of the reproductive parameters of Slx/Slxl1-deficient males and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WT</th>
<th>shSLX1</th>
<th>shSLX2</th>
<th>shSLX1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of litters per male*</td>
<td>6</td>
<td>1.83*</td>
<td>3.33*</td>
<td>0*</td>
</tr>
<tr>
<td>Total number of offspring per male*</td>
<td>70.17</td>
<td>9.83*</td>
<td>18.83*</td>
<td>0*</td>
</tr>
<tr>
<td>Average litter size*</td>
<td>11.8</td>
<td>3.92*</td>
<td>4.26*</td>
<td>0*</td>
</tr>
<tr>
<td>Oocytes fertilized in vitro (%)</td>
<td>54.5</td>
<td>4.6**</td>
<td>1.0**</td>
<td>N/D</td>
</tr>
<tr>
<td>Testis weight (mean value in mg ± SEs)</td>
<td>102.3 ± 2.5</td>
<td>99.8 ± 3.0</td>
<td>104.1 ± 3.1</td>
<td>90.1* ± 3.4</td>
</tr>
<tr>
<td>Total motile sperm (%)</td>
<td>46.3</td>
<td>25.4**</td>
<td>22.9**</td>
<td>N/D</td>
</tr>
<tr>
<td>Sperm number/cauda (mean value × 10^-6 ± SEs)</td>
<td>13.6 ± 0.8</td>
<td>2.0* ± 0.8</td>
<td>6.6* ± 1.2</td>
<td>0.2* ± 1</td>
</tr>
<tr>
<td>MF1</td>
<td>7.0 ± 1.8</td>
<td>5.9 ± 1.9</td>
<td>5.2 ± 2.0</td>
<td>N/D</td>
</tr>
<tr>
<td>B6D2F1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Six males of each genotype were mated with MF1 females over a period of six and a half months.
** Significantly different from WT (p < 0.05; t test).
* Significantly different from WT (p < 0.05; ANOVA).
Slx/Slxl1 Deficiency Leads to Delay in Sperm Shedding and Reduction of Sperm Count

We then observed that mature (step 16) sperm were retained in most stage IX to XI tubules, instead of being normally shed at stage VIII (Figure 3, B and D). The occurrence of sperm shedding delay was significantly higher in Slx/Slxl1-deficient males compared with WT (95 and 77% of stage IX to XI tubules with retained sperm in shSLX1 and shSLX2 males respectively vs. 12% in WT; ANOVA, \( p < 0.0005 \)). Interestingly, a delay in sperm shedding has previously been observed in Sly-deficient mice and MSYq-deficient mice (Touré et al., 2004; Cocquet et al., 2009), but in these cases the unshed sperm were located adjacent to, and were eventually released into, the lumen; in Slx/Slxl1-deficient tubules, unshed sperm were often located near the basal lamina or form a group of dying cells (Figure 3, E and F).

Apoptotic (TUNEL-positive) elongating spermatids were found in all types of tubule stages of the seminiferous epithelium cycle (Figure 3, K–N, and Supplementary Figure 4). The proportion of testicular tubules containing TUNEL-positive elongating/condensing spermatids was determined and found to be significantly higher in Slx/Slxl1-deficient testes compared with controls (~70% in shSLX1 and shSLX1,2 testes, ~25% in shSLX2 testes vs. ~3% in WT; ANOVA, \( p < 0.0005 \)). The substantial loss of these spermatids was associated with reduced sperm counts in cauda epididymis from Slx/Slxl1-deficient males (Table 1).

Slx/Slxl1-Deficient Sperm Are Abnormally Formed and Exhibit Reduced Motility

Analysis of Slx/Slxl1-deficient epididymis revealed a significant increase in the incidence of abnormal sperm with ~49% of spermhead abnormalities in shSLX1 and ~36% in shSLX2 (vs. ~8% in WT; ANOVA, \( p < 0.0005 \)) (Figure 4, A and B; Supplementary Figure 3). In addition to spermhead malformations, shSLX1 and shSLX2 spermatozoa displayed abnormal head to tail connections (values significantly different from WT; ANOVA, \( p < 0.005 \)) (Figure 4, C and D). Using electron microscopy, we observed epididymal sperm with partially detached heads or abnormally-oriented tails: either aligned parallel to the head, curved or looped (Figure 4, E–J). Abnormal connection between the spermhead and its tail could have an effect on sperm motility, and, indeed, sperm motility was impaired as shown by the decrease in the proportion of motile sperm (Table 1).

Transcriptome Analyses Show that Slx/Slxl1 Deficiency Alters Metabolic Processes Occurring in the Spermatids

To better understand the consequences of Slx/Slxl1 deficiency, we performed microarray analyses on purified round spermatids (the site of Slx and Slxl1 expression) from shSLX1 mice and controls. First, this analysis confirmed the knockdown of Slx and Slxl1 transcripts. Second, in Slx/Slxl1-deficient round spermatids, 101 genes were found to be significantly up-regulated compared with controls. Of these 101 genes, 77 are annotated and, of these, 54% (42/77) encode proteins involved in various metabolic processes, among which are the serine proteases belonging to Kallikrein 1 family (kkk1, kkk1b26, kkk1b5, kkk1b9, kkk1b4, kkk1b27) and Ela2a; several enzymes implicated in lipid metabolism (Acs5, Cyp2a12, St8sia5, Agpat2, Ppap2b) and energy metabolism (Nqo2, Atp6v1h, 4933437F05Rik); and proteins of the ubiquitin pathway (Usp3, Ubc2q2). Several genes coding for proteins of the cytoskeleton and the extracellular matrix (classified in category ’cellular component organization’) were also affected by Slx/Slxl1 deficiency such as Tmod4.
(Tropomodulin 4), Myl2 (myosin light chain 2), Col20a1 (colla-
gen, type XX, alpha 1), Pstpip1 (proline-serine-threonine phos-
phatase interacting protein 1), which is a member of the actin-
asociated protein family (Spencer et al., 1997), and
LOC100045019 (similar to Tubulin, gamma 2) (Figure 5A).
Interestingly, several genes involved in positive or negative
regulation of apoptosis were also found affected (such as
Hipk2, Tnfsf13b, Jund1). Complete results can be found in
Supplementary Figure 5.

Slx/Slxl1 Deficiency Does Not Lead to Global
Perturbation of Sex Chromosome Gene Expression

Slx and Slxl1 genes are related to the Y-encoded Sly multi-
copy gene. SLY protein has been shown to repress sex chro-
mosome genes in spermatids and, as a result, X- and
Y-encoded transcripts are up-regulated in the case of Sly
deficiency (Cocquet et al., 2009). Here, in round spermatids
from Slx/Slxl1-deficient males, none of the genes found up-
regulated were located on the sex chromosomes (Figure 5B).
Only one X-encoded transcript (apart from Slx and Slxl1
transcripts and cross-hybridization with an ‘Sly’ probe),
Wdr13, was found significantly down-regulated in Slx/Slxl1-
deficient round spermatids. Real-time PCR analysis on Slx/
Slxl1- and Sly-deficient round spermatid fractions confirmed
that Wdr13 is differentially regulated by SLY and SLX/
SLXL1 (Figure 5C).

DISCUSSION

Using an siRNA approach to produce mice with markedly
reduced transcript levels for the multicopy genes Slx and
Slxl1, we have demonstrated that the Slx gene family is
important for normal mouse sperm development. Slx/Slxl1
deficiency considerably delays spermatid elongation and
sperm release in the lumen. These perturbations of sperm
differentiation are associated with the elimination of many
elongating/condensing spermatids via apoptosis, with a
consequent reduced sperm count. The remaining spermato-
zoa are abnormally shaped and have decreased motility and
fertilizing abilities. All these factors are likely to contribute
together to the near sterility observed in Slx/Slxl1-deficient
males.

Microarray analyses revealed that Slx/Slxl1 deficiency
does not lead to global up-regulation of X- and Y-linked
genes after meiosis; this is in striking contrast with what we
had observed in the case of Sly deficiency, where the vast
majority of the genes found up-regulated were encoded
either by the X or the Y chromosome (Cocquet et al., 2009).
This difference may stem from the fact that i) SLX/SLXL1
proteins are restricted to the cytoplasm while SLY is found
in both the nucleus and cytoplasm of spermatids (Reynard et
al., 2007; Cocquet et al., 2009); ii) SLX/SLXL1 only share
~43% overall homology with SLY (while SLX and SLXL1 share ~66% identity).
transcript levels are likely to contribute to the spermio-
deficient males, such as spermatid elongation delay
and abnormal head-tail connections. The increase of
Slx spermiogenic phenotype(s) observed in
determine the contribution of these candidate genes to the
gain or loss of function approaches) will be required to
Slxl1 deficient males.

miogenic genes are up-regulated, among which are
(Singh pressed in male germ cells, but its function is yet unknown
et al. et al. and
have been implicated in spermatid–Sertoli cell
adhesion (Obholz et al. 2003). Interestingly, kilikrein 1–related
transcripts are also found up-regulated in mouse models
with deletions of the Y long arm (which represents a non
level of the X-encoded
Klk1 on chromosome 7. This is due to the presence of the
Kkl1 genes that are affected by the loss of Slx/Slxl1. (C) Quantification of the transcript
level of the X-encoded Wdr13 gene in purified round spermatids of shSLY, SLX1, shSLX2, and WT males by real-time RT-PCR. Values were
normalized to β-actin. One star indicates significant difference from WT value (p < 0.05; t test). Wdr13 transcript level is differentially affected by
Slx/Slxl1 or Sly deficiency.

Only a few genes (mainly kallikrein 1-related genes) were
found up-regulated in both models (Slx/Slxl1- and Sly-deficient
males). Kallikrein-related peptidases are serine proteases with a variety of physiological roles; they have notably
been reported to be involved in semen liquefaction and consequently in sperm motility but through their production
by the prostate (Emami et al., 2009). Their biological role in
the testis remains to be defined, but they could act (as they
do in other contexts) as signaling molecules (for review, see
Sotiropoulou et al., 2009). Interestingly, kilikrein 1–related
transcripts are also found up-regulated in mouse models
with deletions of the Y long arm (which represents a non
siRNA model of Sly deficiency) (Cocquet et al., 2009).

Among the few genes that were found differentially af-
fected by Slx/Slxl1 and Sly deficiency (i.e., up-regulated in
one model and down-regulated in the other) are Fndc3 and
Wdr13. Fndc3 has been implicated in spermatid–Sertoli cell
adhesion (Obholz et al., 2006). Wdr13 is predominantly ex-
pressed in male germ cells, but its function is yet unknown
(Singh et al., 2003; Suresh et al., 2005). Future work (such as
gain or loss of function approaches) will be required to
determine the contribution of these candidate genes to the
spermiogenic phenotype(s) observed in Slx/Slxl1- and Sly-
deficient males.

In the case of Sly deficiency, ~100 X and Y-linked sper-
miogenic genes are up-regulated, among which are Srx and
Slxl1 (Cocquet et al., 2009). Both Slx/Slxl1-deficient males and
Sly-deficient males present sperm differentiation defects,
though some of these defects appear to be specific to Slx/
Slxl1 deficient males, such as spermatid elongation delay
and abnormal head-tail connections. The increase of Srx and
Slxl1 transcript levels are likely to contribute to the spermiogenic
defects observed in Sly-deficient males. Srx and Sly
genes have been hypothesized to be involved in an intragenomic conflict occurring between X-bearing and
Y-bearing gametes (Ellis et al., 2005). The production of
double transgenic mice deficient for both Slx/Slxl1 and Sly
would address this question.

The structural changes, such as acrosome and flagellum
formation, that lead to the transformation of a round cell
into a highly specialized sperm cell start soon after meiosis
in the round spermatid (Russell et al., 1990). Many of the
genes found altered in our microarray analysis of Slx/Slxl1-
deficient round spermatids encode enzymes associated with
specific cellular organelles and involved in various meta-
bolic processes (such as energy production, ubiquitin-medi-
ad degradation, etc.). Several proteins of the cytoskeleton
were also affected. These results suggest that a ‘cytoplasmic’
defect underlies the above-mentioned sperm differentiation
abnormalities. The mechanism by which SLX/SLXL1 pro-
teins regulate these processes remains mysterious as no
functional domain (apart from the Cor1 domain) is known
and previous attempts to identify potential protein partners
have failed (L. Reynard and P Burgoyne, unpublished data).
Because SLX/SLXL1 proteins are restricted to the spermatid
cytoplasm, it is unlikely that the genes found altered in our
microarray analysis are direct transcriptional targets of SLX/
SLXL1. One attractive hypothesis is that a yet unidentified
partner of SLX/SLXL1 is at the basis of the transcriptional
changes that are necessary for normal spermatid elongation.

Finally, before the elimination of elongating spermatids
by apoptosis, several ‘cell death’ genes were found altered in
round spermatids deficient for Slx/Slxl1. Hipk2, a potentiator
of p53-mediated transcriptional activation of proapoptotic
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