SLY regulates genes involved in chromatin remodeling and interacts with TBL1XR1 during sperm differentiation

Charlotte Moretti1,2,3,8, Maria-Elisabetta Serrentino1,2,3,8, Côme Ialy-Radio1,2,3, Marion Delessard1,2,3, Tatiana A Soboleva4, Frederic Tores5, Marjorie Leduc6, Patrick Nitschké5, Joel R Drevet7, David J Tremethick4, Daniel Vaiman1,2,3, Ayhan Kocer7 and Julie Cocquet1,2,3

Sperm differentiation requires unique transcriptional regulation and chromatin remodeling after meiosis to ensure proper compaction and protection of the paternal genome. Abnormal sperm chromatin remodeling can induce sperm DNA damage, embryo lethality and male infertility, yet, little is known about the factors which regulate this process. Deficiency in Sly, a mouse Y chromosome-encoded gene expressed only in postmeiotic male germ cells, has been shown to result in the deregulation of hundreds of sex chromosome-encoded genes associated with multiple sperm differentiation defects and subsequent male infertility. The underlying mechanism remained, to date, unknown. Here, we show that SLY binds to the promoter of sex chromosome-encoded and autosomal genes highly expressed postmeiotically and involved in chromatin regulation. Specifically, we demonstrate that SLY knockdown directly induces the deregulation of sex chromosome-encoded H2A variants and of the H3K79 methyltransferase DOT1L. The modifications prompted by loss of SLY alter the postmeiotic chromatin structure and ultimately result in abnormal sperm chromatin remodeling with negative consequences on the sperm genome integrity. Altogether our results show that SLY is a regulator of sperm chromatin remodeling. Finally we identified that SMRT/N-CoR repressor complex is involved in gene regulation during sperm differentiation since members of this complex, in particular TBL1XR1, interact with SLY in postmeiotic male germ cells.

Cell Death and Differentiation (2017) 24, 1029–1044; doi:10.1038/cdd.2017.32; published online 5 May 2017

The postmeiotic phase of spermatogenesis is a fascinating process in terms of transcriptional regulation and chromatin re-organization. Indeed, after meiosis, during which the genetic material is recombined and then partitioned in haploid cells, round spermatids experience a differentiation program characterized by profound morphological changes: elongation, nucleus condensation and acquisition of new structures such as the acrosome and the flagellum. In many organisms including mammals, this process involves transcriptional regulation by master genes, and expression of thousands of genes in round and early elongating spermatids, before the spermatid chromatin is compacted and transcription is progressively shut down.1–6

Chromatin compaction is achieved by a transition from a nucleosome-based organization to a unique genome-packaging structure based on non-histone proteins, called protamines. The replacement of histones by protamines starts with incorporation of spermatid-enriched histone variants and post translational modifications of histone residues, the most predominant of which is histone H4 hyperacetylation. These steps are thought to open the chromatin to facilitate the action of topoisomerases and the removal of histones; then, transition proteins are incorporated and finally replaced by protamines (for review, see7). In mice, haploinsufficiency of genes coding for protamines (i.e., Prm1 or Prm2) is sufficient to result in male infertility and leads to sperm DNA damage and embryo lethality.8,9 Protamines are therefore essential to mammalian fertility with a function in compaction, as well as protection of the paternal genome until after fertilization. Little is known about the factors and molecular mechanisms which regulate chromatin remodeling during sperm differentiation. Studies of mouse genetic models have identified a few nuclear factors, histone variants and chromatin remodelers required for histone-to-protamine transition, such as BRDT (Bromodomain testis specific) protein,10 histone H2B variant TH2B,11 and the chromodomain helicase DNA-binding protein 5.12

We and others have also shown that the mouse Y chromosome long arm (MSYq) encodes genetic information required for normal chromatin compaction during sperm differentiation: males with deletions of MSYq have severe sperm differentiation defects and produce deformed spermatozoa with poorly compacted chromatin, which are unable to
fertilize oocytes in vivo and in vitro. Sly, a multicopy gene of MSYq only expressed in postmeiotic cells, largely contributes to these phenotypes since males with Sly specifically knocked down (Sly-KD males) also present abnormal sperm differentiation, including abnormal chromatin compaction and increased sperm DNA damage. It has been shown that

---

**Sly** controls sperm chromatin re-organization

C Moretti et al

---

**Figure a**

- Genome
- SLY ChiP Seq

**Figure b**

- Percentage of genes with SLY at their TSS

**Figure c**

- SLY ChiP-qPCR

**Figure d**

- SLY enrichment at TSS

**Figure e**

- Percentage of expressed vs. not expressed genes

**Figure f**

- SLY_CHIP

**Figure g**

- Comparison of SLY ChiP-Seq with other ChiP-Seq

**Figure h**

- Biological process
- Cellular component
- Molecular function
 Sly knockdown leads to the upregulation of ~100 sex chromosome-encoded genes in round spermatids. At the protein level, SLY lacks any conserved domain except for a COR1 region identified in SYCP3, a protein involved in the meiotic synaptonemal complex. Therefore, the mechanism by which SLY controls gene expression and the origin of the sperm differentiation defects observed in its absence remain unclear.

In the present study, we investigated the molecular function of SLY by performing chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) and by co-immunoprecipitation followed by mass spectrometry. We discovered that SLY associates with the transcriptional start sites of thousands of genes expressed postmeiotically, many of which are involved in gene regulation or chromatin remodeling. We focused on SLY-target genes relevant to the chromatin defects observed in SLY-deficient sperm, and found that SLY controls the expression of genes coding for spermatid-specific histone variants and for chromatin regulators such as the H3K79 methyltransferase, DOT1L. Overall, we show that SLY deficiency leads to changes in the chromatin composition just prior to histone removal, which impact on histone-to-protamine exchanges and, ultimately, on spermatozoa chromatin content and function, as well as on their genome integrity. Finally, we found that SLY is part of the SMRT/N-CoR complex and interacts with TBL1XR1.

Altogether our data identify for the first time the molecular role of SLY and link the sperm chromatin compaction phenotype observed in SLY-deficient males to molecular pathways important for chromatin remodeling during sperm differentiation, in particular, the regulation of H3K79 methylation.

### Results

**SLY marks the sperm differentiation genetic program.** To investigate the molecular mechanism by which SLY controls postmeiotic gene expression, we performed ChIP-Seq analyses on purified round spermatids from wild-type (WT) males with anti-SLY antibody. We found that SLY protein preferentially binds to the start of genes, in the 1 kb region surrounding the transcription start site (TSS) and, overall, occupies the TSS of ~16% of mouse genes (6,381 genes with SLY at TSS, 7,280 genes with SLY at ±1 kb of TSS) (Figure 1a, Supplementary Figure 1A and Table 1). No particular bias towards the sex chromosomes was observed (Figure 1b and Supplementary Figure 1B). ChIP followed by real-time PCR (ChIP-qPCR) confirmed the ChIP-Seq results (Figure 1c). Strikingly, comparison with published RNAseq data showed a strong correlation between SLY-genomic targets and genes expressed in round spermatids (~89% versus 41% of all mouse genes, χ², P < 0.0001; Figures 1d and e), and more specifically with a high expression level (91% of SLY-associated genes are among the 50% most expressed genes, χ², P < 0.0001; 36% among the 10% most expressed genes, χ², P < 0.0001)). Interestingly, SLY was found to bind to the TSS of master genes of spermatid transcriptional regulation such as Crem, Creb1, Crebbp, Kif17, Tatf11, Tbp11, Papolb, Piwil1 or Brd4. SLY presence appears as an excellent marker of genes essential for spermatogenesis.

**Figure 1** SLY marks the sperm differentiation program and co-localizes with active epigenetic marks. (a) Annotation of SLY-enriched genomic regions (right) compared to whole genome (left), using cis-regulatory element annotation system (CEAS). (b) Graphic representation of the percentage of genes found occupied by SLY protein by ChIP-Seq (present study) and were found deregulated in Sly-KD vs WT samples by micro-array and/or RT-qPCR analyses. 13 The TSS of H2ab3, Jmjd1tc and Dot11 were found enriched in SLY by ChIP-Seq (present study) and were found deregulated in Sly-KD vs WT round spermatids by RT-qPCR (present study, cf. Figure 2b). Sex chromosome-encoded genes are represented in black and autosomal genes, in gray. The Y-axis represents the mean enrichment (%IP/INPUT) ± S.E.M. normalized to a negative control region (NC) located at ~170 kb from a TSS. All regions shown were found significantly enriched in SLY compared to the negative control region (Htest, P < 0.05; n = 3–6 samples). (c) Graphic representation of SLY ChIP-Seq profile showing the average enrichment of SLY around the TSS of genes expressed (in red) and not expressed (in black) in round spermatids. (d) Graphic representation of the percentage of genes that are expressed (in red) and not expressed (in black) in round spermatids, among all mouse genes (mm10 genome version) or among SLY-associated genes. SLY is significantly enriched at the start (i.e., TSS ± 1 kb) of genes expressed in round spermatids (χ², P < 0.0001). (e) Representation of SLY ChIP-Seq and input profiles at SLY gene locus. (f) Graphic representation of the comparison between SLY ChIP-Seq data set and ChIP-Seq data sets from chromatin marks (Kcr, H3K4me3, H3K9ac, H4ac, H3K27ac, H3K27me3, H3K9me3) and from BRD4, in round spermatids. In red is represented the percentage of the SLY-enriched genomic regions which overlaps with that of the chromatin mark/factor-enriched genomic regions; in black is represented the percentage of the genome covered by the chromatin mark/factor. A star indicates a significant enrichment at SLY-covered regions compared to global genomic coverage (χ², P < 0.03). (h) Table recapitulating the results of gene ontology analyses of SLY ChIP-Seq genes using EnrichR. See also Supplementary Figure 1C.
to the spermatid differentiation program. This was confirmed by gene ontology analyses in which one of the most significant hits was ‘spermatids’ (Supplementary Figure 1C). Of note, SLY promoter itself is occupied by SLY protein (Figure 1f).

Next, we compared SLY ChIP-Seq with published ChIP-Seq of chromatin marks performed in round spermatids.19-22 SLY profile was found to be very similar to that of active marks (Figure 1g, Supplementary Figure 2A), i.e., chromatin marks associated with the promoter of expressed genes,19,21,23,24 such as H3K4me3 (trimethylation of histone H3 lysine 4), Kcr (histone lysine crotonylation), H3K9ac (acetylation of histone H3 lysine 9), H3K27ac (acetylation of histone H3 lysine 27) and H4ac (acetylation of histone H4). Interestingly, the overlap of SLY with active marks is not restricted to gene TSS and proximal promoters. Indeed, approximately half of SLY-genomic targets are located at gene TSS and proximal promoters (Table 1) while ~2/3rd of SLY-genomic targets correlate with Kcr and H3K9ac (Figure 1g). This suggests that SLY overlaps with active marks not only at gene TSS/proximal promoter but also at distal promoters and enhancer regions, since active chromatin marks are known to be enriched in these regions. SLY also correlates with BRD4 genomic localization, which has been shown to be enriched at spermatogenesis-specific genes.19 On the other hand, it differed significantly from chromatin marks associated with transcriptional repression such as the repressive marks H3K27me3 (trimethylation of histone H3 lysine 27) and H3K9me3 (trimethylation of histone H3 lysine 9) (Figure 1g, Supplementary Figure 2A). Benayoun et al. have described that broad H3K4me3 regions correlate with a specific cell identity.25 Here, of the broadest H3K4me3 domains of round spermatids, 74% intersect with SLY domains, confirming that SLY is a good marker of sperm differentiation program/spermatid identity (Figure 1g).

**Table 2 Microarray analyses of Sly-KD versus WT round spermatids**

| Nb of upregulated genes in Sly-KD round spermatids | Nb of downregulated genes in Sly-KD round spermatids |
|---------------------------------------------------|-----------------------------------------------------|
| Total Nb of genes | XY (%) | Autosomal (%) | XY (%) | Autosomal (%) |
| Minimal list > 1.5 × deregulation | 413 | 276 (66.8%) | 96 (23.2%) | 0 (0%) | 41 (10%) |
| List extended to close paralogs > 1.5 × deregulation | 752 | 485 (61.8%) | 232 (30.9%) | 0 (0%) | 55 (7.3%) |
| All deregulated genes (P < 0.05) | 1171 | 433 (37%) | 353 (30%) | 0 (0%) | 385 (33%) |

The most recent version of the mouse genome (GRCm38/mm10) since it is more complete in term of sequence length and annotation, especially of the Y chromosome, than the previous version GRCm37/mm9 (i.e., in mm9 version, only 15% of the Y chromosome was assembled). Over 400 genes were found deregulated more than 1.5-fold (P < 0.05), a majority of which are encoded by the sex chromosomes, in agreement with our previous observations.15 Since many of those genes are present in multiple copies, we included closely related paralogous genes and obtained a total of 752 deregulated genes, again with a strong bias towards X- and Y-encoded upregulated genes (Table 2). Comparison with SLY-associated genes (i.e., the 7280 SLY ChIP-Seq genes) showed a higher proportion of upregulated genes versus downregulated genes ($\chi^2$, P = 0.005) (Table 2, Supplementary Figure 2B). When including all 1171 significantly deregulated genes (no threshold, P < 0.05), a higher proportion of autosomal upregulated and downregulated genes was found and, this time, there were more downregulated than upregulated genes among the genes enriched in SLY at their TSS ($\chi^2$, P = 0.012) (Table 2, Supplementary Figure 2B).

Next, we investigated why some autosomal genes had SLY at their TSS and yet were not found deregulated in SLY-KD round spermatids by microarray. Since real-time PCR is a more sensitive technique than expression microarrays,26 we re-examined by quantitative real-time PCR (RT-qPCR) the expression level of those autosomal genes, focusing on those with the highest SLY enrichment at their gene start (10% of genes with highest SLY peak, Supplementary Figure 3). In this gene list are several members of the *Speer* gene cluster, multicopy genes of chromosome 14 with yet unknown functions and, interestingly, genes encoding proteins with a known role in chromatin regulation, such as Dot1l which encodes an H3K79 methyltransferase (Figure 2a, Supplementary Figure 3). By RT-qPCR we found several of those autosomal genes significantly deregulated (up or downregulated) in SLY-KD spermatids compared to WT spermatids (Figure 2b). With the same approach, we identified additional sex chromosome-encoded genes significantly upregulated in SLY-KD spermatids such as Spin2d, Gmc11l, Ube2a, Kdm5c and genes encoding spermatid-specific histone variants, such as H2aAb3, H2al1 (aka 1700012L04Rik) or H1fnt (Figures 2b and c).27-30 Their closely related paralog H2aAb1 encoded by an autosome, is not regulated by SLY (Figures 2b and c). Since SLY knockdown leads to increased transcription of H2aAb3, we checked whether it affects H2 A.B3 incorporation in the spermatid chromatin by ChIP-qPCR and found that H2A.B3 level is higher at the TSS of...
expressed genes in Sly-KD compared to WT round spermatids (Figure 2d).

Overall our data show that, when considering genes expressed in spermatids, all sex chromosome-encoded genes are upregulated in Sly-KD spermatids while autosomal genes are either upregulated, downregulated or unchanged.

**Sly-dependent deregulation of the H3K79 histone methyltransferase DOT1L impairs H3K79 methylation prior to histone-to-protamine exchange.** One of the genes with highest enrichment of SLY at its start is **Dot1l** (Figure 2a) which encodes the only known H3K79 histone methyltransferase. It is presumed to be important for chromatin remodeling during sperm differentiation, because high levels of H3K79 methylation precede histone removal during spermatid elongation.\(^{31,32}\) Besides, analyses of published RNASeq and microarray data show that **Dot1l** is particularly expressed after meiosis in mice (Figure 3a) and humans (Figure 3b). Immunofluorescence experiments on WT mouse testicular samples show that DOT1L protein is enriched at the sex chromatin in round spermatids and appears as nuclear punctuated signals in step 9–11 elongating spermatids (n=3 samples per genotype). The Y-axis represents the mean enrichment (ΔIP/input) normalized to the corresponding WT value. A significant increase in H2A.B3 level at the TSS was found in Sly-KD compared to WT samples when considering all tested genes (t-test, P<0.05)
in Sly-KD spermatids (Figure 2b, Supplementary Figure 5), we looked at H3K79me2 expression: by immunofluorescence, there was a notable decrease in H3K79me2 levels in step 10–12 elongating spermatids from Sly-KD males compared to WT elongating spermatids (Figures 4a, b and Supplementary Figure 6). We next purified elongating/condensing spermatid fractions from WT and Sly-KD testes by elutriation.33 Despite an elevated intra-genotype variability we did not observe major differences in the proportion of elongating/condensing spermatids between WT and Sly-KD fractions; those fractions can therefore be compared. We quantified H3K79me2 levels by ChIP-qPCR and western blot and confirmed that H3K79me2 is reduced in Sly-KD compared to WT elongating/condensing spermatids (Figure 4c, Supplementary Figure 7A and C). Finally, we observed by immunofluorescence that H3K79me2 persists in spermatozoa (Figure 4d and Supplementary Figure 8). Quantification of H3K79me2 revealed that the decrease observed in Sly-KD elongating/condensing spermatids persists in spermatozoa (when normalized to histone H3 level, see below, Supplementary Figure 7B and D).

Sly deficiency leads to reduced histone H4 acetylation prior to histone-to-protamine exchange. Extensive acetylation of histone H4 (acH4) is a hallmark of chromatin remodeling during spermatid differentiation and is detected at the same postmeiotic stages than H3K79 dimethylation, just prior to nucleosome eviction.31,32 It has recently been demonstrated that DOT1L-mediated H3K79me2 facilitates acH4.34 We therefore tested whether acH4 was impacted by
Sly deficiency and found reduced level of acH4 in Sly-KD versus WT step 10–12 elongating spermatids by immunofluorescence (Figures 5a, b and Supplementary Figure 9). This was confirmed by ChIP-qPCR analyses in all tested sites (Figure 5c). Together, these data show that Sly deficiency affects chromatin remodeling during spermatid differentiation. Defects in postmeiotic chromatin remodeling lead to a higher proportion of residual histones and increased DNA oxidation in Sly-deficient spermatozoa. Approximately 1–5% of histones remain in WT mouse spermatozoa. To determine whether abnormal chromatin marks in elongating spermatids have consequences on chromatin content in spermatozoa, we next compared the quantity of remaining histones in sperm and observed a ~2.5-fold increase in histone H3 and a ~2.3-fold increase in TH2B in Sly-KD compared to WT spermatozoa (Figures 6a and b). Using antibody against protamine 2 (Hup2B) we also detected a small (~20%) but significant decrease in the quantity of protamine 2 in Sly-KD compared to WT sperm (Figure 6c). No significant difference was observed for protamine 1 (data not shown).

Finally, we tested whether abnormal chromatin content could alter spermatozoa genome integrity by measuring the proportion of spermatozoa showing oxidation of their DNA (measurement of oxidized deoxyguanosine, 8-oxo-dG). We found an average of ~34% of WT spermatozoa with 8-oxo-dG staining, as described in other studies, and a significant increase to ~53% of 8-oxo-dG positive spermatozoa in Sly-KD epididymis (Figure 6d).

SLY interacts with TBL1XR1 and other members of the SMRT/N-Cor complex. To understand how SLY controls gene expression during sperm differentiation, we searched for its protein partners by co-immunoprecipitation followed by mass spectrometry. We used FLAG antibody to immunoprecipitate SLY and its partners on two types of materials: (i) testicular cells from a transgenic mouse model expressing SLY protein fused to a FLAG-tag (Supplementary Figure 10) and (ii) a spermatogonia cell line (GC1) transfected with a FLAG-SLY construct. WT testes and GC1 cells transfected with an empty vector were used as negative controls. Immunoprecipitated proteins were analyzed by liquid chromatography coupled to tandem mass spectrometry.
Two complementary approaches (MASCOT and label-free quantification (LFQ) Maxquant analysis) were used to analyze LC-MS/MS data. They showed that TBL1XR1 and several other members of the SMRT/N-CoR repressive complex (TBL1X, NCOR1 and HDAC3) were specifically immunoprecipitated with SL Y (Table 3). Interaction of SL Y and TBL1XR1 was confirmed in WT and FLAG-SL Y transgenic testes by western blot following immunoprecipitation using anti-SL Y, anti-FLAG or anti-TBL1XR1 antibody (Figure 7).

**Discussion**

In the present paper, we investigated the molecular role of SL Y and the consequences of its absence on chromatin structure; based on acquired data we identified novel regulators of postmeiotic gene expression and of chromatin remodeling during sperm differentiation.

**SL Y and the regulation of postmeiotic gene expression.**

First, we found that SL Y protein is present at the TSS of thousands of genes relevant to postmeiotic cell identity, with a known role during postmeiotic differentiation and/or significantly upregulated postmeiotically. We also showed that SL Y overlaps with active chromatin marks, such as H3K4me3 or Kcr. Comprehensive transcriptome analyses of Sly-deficient postmeiotic germ cells demonstrated that Sly deficiency does not switch on genes normally silent in postmeiotic cells but rather modulates the expression level of >1000 genes expressed postmeiotically. Sly deficiency chiefly induces upregulation of XY genes (37% of all deregulated genes) with no XY gene found downregulated, while hundreds of autosomal genes are either upregulated or downregulated (respectively 30% and 33% of all deregulated genes). The consequences of Sly deficiency on postmeiotic gene expression are therefore different for XY genes compared to autosomal genes, suggesting distinct regulatory mechanisms, probably due to a different chromatin environment. Indeed, postmeiotically, the XY chromatin significantly differs from that of autosomes as a consequence of the meiotic silencing of sex chromosomes.37,38

How does SL Y control gene expression? Despite the fact that SL Y is related to SYCP3, a protein of the meiotic synaptonemal complex which has been proven to bind double-stranded DNA,14,17,39 the mechanism by which SL Y regulates genes remained elusive. Indeed, SL Y-SYCP3 conservation is relatively low (28% of identity) and SL Y, contrary to SYCP3, is very acidic (with an isoelectric point ~4.8); this most likely precludes direct DNA binding and rather
suggests that SLY is part of a protein complex able to recruit regulators of gene expression, including proteins with DNA-binding domains. In that respect, our findings that SLY interacts with TBLX1R1 and other members of the SMRT/N-CoR complex provide an interesting model. This complex, expressed in many tissues, contains five proteins (NCOR1, HDAC3, GPS2, TBL1XR1 and TBL1X) and has been shown to interact with nuclear hormone receptors, transcription factors or chromatin modifying enzymes. The recruitment/release of SMRT/N-CoR complex on repressed gene promoters is a dynamic process on which SLY could act to control gene expression. Further studies will be required to...
characterize the role of SMRT/N-Cor complex in the context of sperm differentiation.

By gene ontology analyses, we showed that many of SLY targets are involved in gene regulation and chromatin modification. Focusing on those genes, we found that SLY regulates XY-encoded H2A variants and DOT1L, a promising candidate for the chromatin remodeling defects observed in Sly-KD spermatids (see below).

The sex chromosomes encode several H2A variants, all of which are particularly, if not specifically, expressed in spermatids. We found that all of them are upregulated in Sly-KD round spermatids and that H2A.B3, known to be enriched at the start of active genes, is more incorporated in Sly-KD than in WT spermatid chromatin. Several other genes encoding histones are also SLY-targets (both enriched in SLY at their TSS and deregulated when Sly is knocked down), such as Histh3 and Histh4 clusters, and so on. Their deregulation could also contribute to the gene deregulation and abnormal chromatin remodeling observed in Sly-deficient spermatids.

It is quite intriguing that SLY controls the expression of essential and evolutionary conserved genes but is itself not conserved throughout evolution. Indeed, Sly has been shown to be involved, together with its X-linked homolog Ssx, in an intragenomic conflict in which an unbalanced number of Sly versus Ssx gene copies leads to transmission distortion. The fact that SLY regulates chromatin components fits with the observation that transmission distorters are often involved in gene/chromatin regulation processes.

SLY and chromatin remodeling during sperm differentiation.

We particularly investigated Dot1l and the consequence of its downregulation, as it is an interesting candidate gene involved in spermatid chromatin remodeling and histone-to-protamine replacement. DOT1L is the principal H3K79 methyltransferase identified to date and is ubiquitously expressed and conserved throughout evolution. During spermatogenesis, it has been shown to be expressed in spermatocytes and spermatids. Here, we show that Dot1l is actually expressed at a higher level in spermatids compared to spermatocytes or spermatogonia. In elongating spermatids, high H3K79me2 coincides with histone H4 hyperacetylation, just prior to histone removal. Using our mouse model, we showed that postmeiotic downregulation of Dot1l induced by Sly deficiency leads to reduction in H3K79me2 and in acH4 levels in elongating spermatids.
the end of the differentiation process, Sly-KD spermatozoa display a moderate but significant alteration in spermatozoa chromatin content, with more residual histones, and less protamination (~20% reduction). We propose this is a consequence of the abnormal spermatid chromatin composition resulting from the deregulation of genes essential to spermatid chromatin remodeling, in particular of DOT1L (Figure 8). It has recently been shown that DOT1L-mediated H3K79me2 facilitates histone H4 acetylation in the context of MLL leukemia,34 and that BRD4-mediated acetylation promotes chromatin decompaction and nucleosome eviction.52 We propose that, in the context of sperm differentiation, DOT1L and H3K79me2 play a critical role in H4 acetylation which itself is required for an open chromatin state enabling nucleosome removal prior to protamine incorporation.

Finally, we show that abnormal chromatin content and compaction of Sly-KD spermatozoa is associated with increased susceptibility to oxidative stress. This could also explain the increase in sperm DNA breaks that was previously observed.16 A modest reduction in protamine levels (~33%) in mice haploinsufficient for Prm1 or Prm2 gene results in reduced sperm compaction, increased DNA damage and embryo lethality.9 Experiments in which Sly-KD sperm were directly injected into the oocytes (i.e., intracytoplasmic sperm injection, ICSI) have shown that Sly deficiency does not dramatically impair the early post-fertilization events and does not lead to gross paternal chromosome breaks in the zygotes.16 But, in light of the high incidence of sperm with oxidized DNA in Sly-KD males that we reported here, ICSI with Sly-KD sperm may produce offspring with increased mutational load. If not properly repaired, DNA lesions such as oxidized deoxy-guanosine (8-oxo-dG) can indeed lead to mutations. Despite the existence of DNA repair strategies in the mammalian zygote, studies have shown deleterious consequences on genome integrity and embryo development when incidence of DNA lesions is too high.13,36,53 Oxidative damage to sperm DNA resulting from age, environmental or lifestyle factors (such as smoking), has been shown to be associated with increased incidence of diseases (such as cancers, neurological disorders, etc.) in the progeny.54 Oxidative stress associated with impaired chromatin remodeling in case of male infertility could similarly have negative consequences on the health of children conceived using ICSI to bypass the father’s infertility. Further studies using relevant models will be needed to address this question.

Material and Methods

ChIP-Seq analyses. SLY ChIP-Seq was performed by Active Motif ChIP-Sequencing service, as follow: ~10 million of FACS-sorted enriched fractions of round spermatids (with a purity >90%) were fixed with 1% formaldehyde for 15 min then quenched with 0.125 M glycine. Chromatin was isolated by adding lysis buffer,
followed by disruption with a Dounce homogenizer. Lysates were sonicated and the DNA sheared to an average length of 300–500 bp. Genomic DNA (input) was prepared by treating aliquots of chromatin with RNase, proteinase K and heat for de-crosslinking, followed by ethanol precipitation. Pellets were resuspended and the resulting DNA was quantified on a NanoDrop spectrophotometer. Extrapolation to an original chromatin volume allowed quantitation of the total chromatin yield. An aliquot of chromatin (30 μg) was precleared with protein A agarose beads (Life technologies, Carlsbad, CA, USA). Genomic DNA regions of interest were isolated from GSE42629, GSE32663 and GSE56526.19 ChIP-Seq data sets for H3K4me3, H3K27me3, H3K9me3 and H3K9me3 were obtained available at the Galaxy website https://mississippi.snv.jussieu.fr/. Round spermatids –ndeficient spermatozoa were obtained using the last version of the mouse genome (GRCm38/mm10). From Illumina microarray WG6 v2 of WT round spermatids, 15 all genes >1.5× fold deregulated (i.e., Log2 ratio <−0.58 or >0.58) with a P-value <0.05 were converted to ENSEMBL ID using Biomart (http://www.ensembl.org/biomart) and the last version of the mouse genome (GRCm38/mm10). Close paralogs (>70% identity) were added to >1.5× extended to close paralog list (752 genes in total). All genes significantly

Bioinformatics. SLY ChIP and input sequences (50-nt reads, single end) were aligned to the mouse genome (GRCm38/mm10) using BWA algorithm55 and filtered by mapping quality using Samtools –q0.56 For reads with multiple good alignments, one alignment was reported at random. Alignments were extended in silico at their 3′-ends to a length of 200 bp, which is the average genomic fragment size in the selected-library, and assigned to 32-nt bins along the genome. The resulting histograms (genomic ‘signal maps’) were stored in BAR and bigWig files. Data sets have been submitted to SRA http://www.ncbi.nlm.nih.gov/sra with accession number SRP055115. Sly peak locations were determined using the MACS algorithm (v1.4.2)57 with a cutoff of P = 1e −7. Annotation of SLY-enriched genomic regions (Figure 1a and Supplementary Figure 1A) was performed using Cis-regulatory Element Annotation System available at http://llulab.dfc.harvard.edu/CEAS/index.html. P-values for the significance of the relative enrichment with respect to the background were calculated using one-sided binomial test. BED files containing SLY ChIP-Seq regions (WT and Sly-KD) were intersected with Ensembl80 gene coordinates (gene start, or gene start ±1 kb). Graphical representation of enrichment around the start of genes (expressed and not expressed in round spermatids) was done using Profiler and ComputeMatrix tools58 available at the Galaxy website https://mississippi.snv.jussieu.fr/. Round spermatids ChIP-Seq data sets for H3K4me3, H3K27me3, Kcr and H3K9me3 were obtained from GSE46229, GSE32663 and GSE56526.19–21 All data sets were re-analyzed using the last version of the mouse genome (GRCm38/mm10) as described in Moretti et al.59 Overlap comparison was achieved by intersecting the intervals of two ChIP-Seq data sets. Graphic representations of ChIP-Seq data were performed using IGV (Integrative Genomics Viewer, https://www.broadinstitute.org/igv/). Gene ontology analyses were performed using Genomatix (https://www.genomatix.de/), GSEA (http://www.broadinstitute.org/gsea)55 and EnrichR (http://amp.pharm.mssm.edu/Enrichr).60

**Figure 8** Model presenting the mechanism by which SLY controls gene expression and chromatin remodeling during sperm differentiation. In WT round spermatids (left panel), SLY (in blue) interacts with the SMRT/N-CoR complex (which comprises TBL1XR1, TBL1X, NCOR1 and HDAC3) and is located at the start of genes involved in gene regulation, chromatin regulation and the ubiquitin pathway. In particular, SLY directly controls the expression of X-chromosome-encoded genes coding for H2.A variants (such as H2A.B3) and of the H3K79 methyltransferase DOT1L. In elongating spermatids, there is a wave of H3K79 dimethylation (orange circles) and of histone H4 acetylation (green circles); those modifications are expected to be a prerequisite to the efficient removal of nucleosomes (light pink oval) and replacement by protamines (purple oval), a process which is required to achieve optimal compaction of the spermatozoa nucleus. When SLY is knocked down (right panel), X-encoded H2.A variants are upregulated and more incorporated in the spermatid chromatin, while DOT1L is downregulated. DOT1L downregulation leads to a decrease in dimethylated H3K79 and acetylated histone H4 in elongating spermatids. Alterations in the spermatid chromatin structure affect the replacement of nucleosomes by protamines and lead to a higher proportion of nucleosomes and a decreased proportion of protamines. As a result, Sly-deficient spermatozoa are abnormally shaped, less compact and present a higher susceptibility to DNA damage than WT spermatids.

**Microarray and RNA-Seq analyses.** From Illumina microarray WG6 v2 of SLY-KD versus WT round spermatids,15 all genes >1.5× fold deregulated (i.e., Log2 ratio < −0.58 or > 0.58) with a P-value ≤0.05 were converted to ENSEMBL ID using Biomart (http://www.ensembl.org/biomart) and the last version of the mouse genome (GRCm38/mm10). Close paralogs (>70% identity) were added to >1.5× extended to close paralog list (752 genes in total). All genes significantly
deregulated with a P-value < 0.05 were converted to ENSEMBL ID using Biomart and mm10 version of the mouse genome. This gave a list of 1171 deregulated genes. Gene expression data from human testicular biopsies were obtained from published microarray data set (ArrayExpress: E-TABM-234). Gene expression data of mouse purified germ cells were obtained from RNA-Seq data sets GSE35005 and GSE43717, and analyzed by GenoSplice (http://www.genosplice.com) using the following parameters: reads were aligned onto the mouse genome (Ensembl 75, mm10) using STAR v2.3.0, with an exon-exon junction database built using annotations from Ensembl version 75. For each gene present in ENSEMBL, reads aligning on constitutive regions (that are not prone to alternative splicing) were counted. Based on these read counts, normalization and differential gene expression were performed using DESeq (v1.12.0 on R v3.0.0).

**Mice.** All animals used in the present study were on > 90% C57BL/6 background and processed at adult age (between 2- and 6-month old males). Sly-KD mice were obtained as described in Coquet et al. FLAG-SLY1 transgenic mice were produced by pronuclear micro-injection of a linearized construct containing SLY open reading frame (i.e., SLY long and main isoform, see Riel et al. Fused with Flag sequence, under the control of the spermatid-specific promoter SP-10 (aka ACRY1)1,2,3,4,5 Fertilized eggs from CBA/Ca x C57BL/10 mating were microinjected with the construct, using standard protocols. Transgenic founders carrying the SP10-FLAG-SLY1 construct were identified by PCR using the following primers (35 cycles, annealing temperature 60 °C): Flag-F primer: 5'-GGA CT A CAA GGAC GA CGA TGA CAA-3' and Flag-R primer: 5'-GCA GCC TGC ACC TGA GGA GT-3' (711 bp). Several founders were obtained and crossed with XYRIII males on a random-bred MF1 albino (National Institute for Medical Research colony) background. One line (called FLAG-SLY) was established from a male founder which transmitted the transgene with a – similar expression of FLAG-SLY1 compared to endogenous SLY1 protein. The line was maintained by further backcrossing transgenic males and females to B6/N mice and generate XYRIII males with (tsgic) and without (neg sib) the transgene. RTPCR and Western blot experiments showed a ~2-fold increase in SLY transcript and protein level in FLAG-SLY compared to WT testes. By immuno-fluorescence on FLAG-SLY and WT testicular tubules it was observed that FLAG-SLY transgene was only expressed in round spermatids (Supplementary Figure 10).

For all experiments, WT controls were of same age and background (i.e., non-transgenic siblings from the same matings). Animal procedures were subjected to local ethical review (Comité d’Éthique pour l’Expérimentation Animale, Université Paris Descartes; registration number CEEA34.JC.114.12).

**Germ cell purification by FACs.** Testicular cells were isolated from one adult male per experiment following a protocol adapted from with some modifications previously described in Comptour et al. Cell purity was assessed for each collected fraction by microscopy observation following DAPI (4,6-diamidino-2-phenylindole) staining (VECTASHIELD Mounting Medium with DAPI, Vectorlab, Burlingame, CA, USA) of cells spread onto glass slides and fixed with 4% buffered paraformaldehyde. Round spermatids and elongating spermatids were collected with a purity >90%.

**Germ cell purification by elutriation.** Enriched fractions of round spermatids and elongating/condensing spermatids were obtained using two to three mice per experiment (i.e., 4–6 testes) by centrifugal elutriation as described previously. Cell purity was assessed for each collected fraction as described above. Only fractions with > 95% purity were used for ChIP-qPCR analyses. It has previously been shown that Sly-KD and WT testicular tubules contain the same proportion of each type of round spermatids, elutriated Sly-KD and WT round spermatid fractions can therefore be compared.

**Collection of epididymal spermatozoa.** Cauda epididymides were dissected out from 2- to 6-month-old males, freed of connective tissues and fat, and transferred to a small petri dish containing 1 ml of M2 medium (Sigma-Aldrich, Saint-Louis, MO, USA). Using small pipette tips, spermatozoa cells were very gently squeezed out of cauda epididymides, to limit contamination with somatic cells. Following 5 min of incubation at 37 °C, sperm suspensions were collected and counted using a Malassez hemocytometer. Sperm cell purity was assessed for each sample by microscope observation following DAPI (4,6-diamidino-2-phenylindole) staining (VECTASHIELD Mounting Medium with DAPI, Vectorlab) of sperm cells spread onto glass slides and fixed with 4% buffered paraformaldehyde. All samples used in our analyses contained >99% of spermatozoa. Samples were aliquoted to five millions of spermatozoa per tube then centrifuged (600 g for 5 min) and washed once in 1 x PBS (Life technologies). Pellets were flash-frozen in liquid nitrogen.

**SLY ChIP prior to qPCR.** About 5 x 10⁶ elutriated round spermatids (collected from two to three mice per sample) were crosslinked for 10 min at room temperature in 1 x PBS containing 1% PFA. Reaction was stopped by adding 125 mM Glycine and incubating for 5 min at room temperature. Cells were washed twice with ice-cold PBS and centrifuged at 500 g, 4 °C for 10 min. Cells were resuspended in 600 μl of Lysis buffer (50 mM Tris pH 7.4, 300 mM NaCl, 0.1% NP-40, 0.1% DOC, 1 mM DTT) with Complete Protease Inhibitor Cocktail Tablets EDTA-free (Roche, Basle, Switzerland), 1 mM PMSF 1 mM and 10 mM Aprotinin added extemperaneously, and incubated on ice for 30 min. The suspension was then sonicated using PICO-Diagenode to obtain fragments of approximate size of 500 bp (as verified by 2100 Bioanalyzer, Agilent, Santa Clara, CA, USA). The lysate was centrifuged at 10 000 g at 4 °C for 5 min. Twenty microfstor of supernatant were taken as input and the remaining supernatant was incubated on a rotating wheel at 4 °C overnight with Protein G Dynabeads (Life Technologies) which were previously coupled with antibody against SLY1 as recommended by the manufacturer. The following day beads were washed subsequently in Lysis buffer, wash buffer no. 2 (50 mM Hepes pH 7.4, 0.5 mM NaCl, 5 mM EDTA, 1% Triton, 0.1% NaDeoxycholate), wash buffer no. 3 (10 mM Tris-HCl pH 8.0, 0.25 M LiCl, 0.5% NP-40, 0.5% NaDeoxycholate, 5 mM EDTA pH 8) and in TE buffer. Supernatant was completely removed and beads were resuspended in Elution buffer containing 1% SDS. Input was defrosted on ice and diluted in Elution buffer. Samples were incubated for 15 min at 65 °C with gentle mix every 2 min. IP and input were reverse crossinkled overnight at 65 °C. Samples were then treated with Proteasine K and incubated for 1 h at 37 °C. IP and input DNAs were purified using NucleoSpin Kit (Macherey-Nagel, Hoerdt, France) and eluted in 50 μl of distilled water. Experiments were repeated on independent pools of elutriated round spermatids (each representing 2–3 mice) three to six times. T-test was used for statistical analyses.

**H2A.B3 ChIP prior to qPCR.** Monoclonal antibodies were prepared from elutriated round spermatids (> 90% purity) as previously described in Montellier et al. with some modifications. Briefly, about 5 x 10⁷ round spermatids purified by elutriation were lysed by incubation with 300 μl of lysis buffer (KCl 60 mM, NaCl 15 mM, Tris HCl pH 7.4 15 mM, Saccharose 0.34 M, EDTA 2 mM, EGTA 0.5 mM, Spermidine 0.65 mM, DTT 1 mM, Triton 0.03%, Glyceral 1%, Complete Protease Inhibitor Cocktail Tablets Roche EDTA-free, PMSF 1 mM, Aprotinin 10 mM) for 20 min on ice, followed by centrifugation at 500 g at 4 °C for 10 min. The pellet was gently resuspended in 200 μl of wash buffer (KCl 60 mM, NaCl 15 mM, Tris HCl pH 7.4 15 mM, Saccharose 0.34 M, spermidine 0.65 mM, DTT 1 mM, Complete Protease Inhibitor Cocktail Tablets Roche EDTA-free, PMSF 1 mM, aprotinin 10 mM) and centrifuged again. Nuclei were resuspended in 200 μl of MNase buffer (Tris HCl pH 7.10 15 mM, KCl 10 mM, CaCl₂ 2 mM) and 5U of Micrococcal Nuclease (ThermoFisher, Waltham, MA, USA) were added. Samples were immediately incubated in a waterbath at 37 °C for 10 min. MNase reaction was stopped by adding EDTA to 5 mM final concentration. The nucleosome fraction was isolated by centrifugation at 10 000 g, 4 °C, for 5 min. ChIP were performed by adding LSD250 (glycerol 20%, Hepes 50 mM, MgCl₂ 3 mM, KCl 250 mM, Complete Protease Inhibitor Cocktail Tablets Roche, PMSF 1 mM, Aprotinin 10 mM) to the nucleaseo fraction to a final volume of 500 μl. Twenty microfstor of this suspension were kept as input and immediately frozen at − 20 °C and the remaining volume was incubated on a rotating wheel at 4 °C overnight with the beads (Dynabeads protein G, Life technologies) previously coupled with 5 μl of anti-H2A.B3. Samples were processed as described above. Experiments were repeated twice on independent pools of elutriated round spermatids (each representing 2–3 mice) and gave similar results.

**ChIP of chromatin marks on elongating/condensing spermatids.** Elongating/condensing spermatids were collected by centrifugal elutriation. Aliquots of about 1 x 10⁷ elongating/condensing spermatids were used for chromatin preparation as described previously by Montellier et al. with some modifications. Briefly, aliquots of 10⁶ were suspended in 75 μl of lysis buffer (Tris pH 7.4 50 mM, NaCl 300 mM, NP-40 0.1%, DOC 0.1%, DTT 1 mM) Complete Protease Inhibitor Cocktail Tablets EDTA-free Roche, PMSF 1 mM, aprotinin 10 mM, sodium butyrate 5 mM) for 15 min on ice with gentle shaking every 3 min. Spermatids were centrifuged at 10 000 g at 4 °C for 10 min. Supernatant was kept on ice and the pellet was resuspended in 100 μl of Lysis buffer and sonicated on PICO-Diagenode for 4 min.
(30 sec ON–30 sec OFF) to allow the suspension of larger chromatin fragment. Spermatozids were centrifuged again at 10,000 g at 4 °C for 10 min and supernatant was pooled with the first supernatant for MNAse digestion. Seventy-five microliters of MNAse Buffer (Tris, pH 7.5 10 mM, KCl 10 mM, and CaCl2 1 mM) were added and MNAse digestion was performed by adding 10 U of MNAse. Digestion was performed for exactly 10 min in a waterbath at 37 °C. Digestion was stopped by adding 5 mM EDTA. Five microliter were kept to check MNAse digestion on Agilent’s 2100 Bioanalyzer. The remaining volume was diluted with Lysis buffer and 20 μl were kept as input. The remaining volume was incubated on a rotating wheel, 4 °C overnight with magnetic beads (Dynabeads Protein G, Life technologies) previously coupled with the appropriate antibody (5 μl of anti-H3K79me2 (ab-3594 from Abcam, Cambridge, UK), or 9 μl of anti-H4pAcytEl antibody (06-866 from Millipore, Billerica, MA, USA). IP and input were further processed as described above. Experiments were repeated three to four times on independent pools of elutriated elongating/condensing spermatozids (each representing 2–3 mice). T-test was used for statistical analyses.

**Real-time quantitative PCR.** Real-time PCR was performed using Roche LightCycler 480 and SYBRgreen Mastermix (Roche). Chip-qPCR were performed on purified round spermatozoa or on elongating/condensing spermatozids obtained by elutriation. Each sample represents a pool of 2–3 animals. Primers were designed to amplify regions across the TSS of indicated genes, except for NC which represents a negative control region (used to normalize) located 170 kb away from any TSS. The sequences and qPCR condition of Akrac4 and Actr1 Chip primers can be found in Soboleva et al.12,13 for other primers see Supplementary Figure 11.

RT-qPCR were performed on RNA extracted from WT and Sly-KD elutriated round spermatozoa and reversed-transcribed as described in Cocquet et al.14 using primers listed in Supplementary Figure 11. The sequences and qPCR condition of Jmjd1c primers can be found in Kuroki et al.66 of -α-actin primers in Cocquet et al.15 of Dot1l H9LAN primers in Dottermus-Heidel et al.67 of H2a2b3 primers in Soboleva et al.68 of H2a2id H2a2a2 H2a2a2 and H2a2a1 (aka H2a2id) primers in Ellis et al.69 For the quantification of Flag-Sly transgene expression, RT-qPCR was performed on whole testes as described in Cocquet et al.15 using Sly global primers (which amplify Sly1 and other Sly isoforms), Sly1 primers and Actr1 primers.10,15 Student’s t-Test was used for all qPCR statistical analyses.

**Immunofluorescence.** Immunofluorescence on sections and on surface-spread testicular cells were performed as previously described.15,65 Antibody against DOT1L (ab-64077 from Abcam), Hist979me2 (ab-3594 from Abcam) or AcH4 (06-866 from Millipore) were diluted 1:100 to 1:200. Pictures were taken with an Olympus BX63 microscope. Quantification was performed on pictures obtained from six samples per genotype using ImageJ 1.48v (http://image.nih.gov/ij/). T-tests were performed with GraphPad Prism 5.02.

**Quantification of DNA oxidation.** Detection of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) was carried out on spermatozoa from cauda epididymis. Detection of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) was carried out on spermatozoa from cauda epididymis. 8-oxo-dG immunostaining were performed as previously described by Noblanc et al.46 After centrifugation at 800 g for 5 min at room temperature, spermatozoa were extracted using ice-cold lysis buffer (150 mM NaCl, 20 mM Tris/HCl pH 8.0, 5 mM EDTA, 0.5% lgepal CA-630 (Sigma-aldrich), 1 mM phenylmethylsulfonyl fluoride and 1 mM sodium orthovanadate). After homogenization and incubation at 4 °C for 30 min, tissue lysates were centrifuged at 13,000 g at 4 °C for 10 min. The supernatant was collected and immediately used for the immunoprecipitation assay.

**Protein extraction from testes.** Nuclear and cytosolic fractions were obtained from adult testes as described in Cocquet et al.12,13 Protein extraction prior to immunoprecipitation was performed as follow: flash-frozen testes were ground and resuspended in 1:9 w/v ice-cold extraction buffer (150 mM NaCl, 20 mM Tris/ HCl pH 7.5, 5 mM EDTA, 0.5% lgepal CA-630 (Sigma-aldrich), 1 × protease inhibitor cocktail (Sigma-Aldrich), 1 mM phenylmethylsulfonyl fluoride and 1 mM sodium orthovanadate). After homogenization and incubation at 4 °C for 30 min, tissue lysates were centrifuged at 13,000 g at 4 °C for 10 min. The supernatant was collected and immediately used for the immunoprecipitation assays.

**Co-immunoprecipitation.** For immunoprecipitation assays in GC1 cells, 60 μl of anti-FLAG M2 magnetic beads (Sigma-aldrich) were washed three times in 1 × TBS (150 mM NaCl, 50 mM Tris, pH 7.4) and incubated with 600 μl of protein extract from transiently transfected cells for 2 h at 4 °C. The magnetic beads were then washed twice in 500 μl TBS with 0.5% Tween-20 and then incubated for a long wash for 5 min at room temperature in TBS only. For non-denaturing elution, beads were resuspended in 150 μl elution buffer (0.1 M glycine/HC1, pH 3.0), and incubated at room temperature for 5 min. The pH was neutralized using triethyrammonium bicarbonate buffer (pH 8.4).

For immunoprecipitation assays in whole-testicular extracts, 40 μl of Bio-Adembeads PAG (Ademetech, Pessac, France) or were washed two times in 80 μl of PBS 0.65% Tween 20 and resuspended with 4 μg of anti-SL1 antibody, anti-TBL1X11 antibody (ab 24550, Abcam) and purified rabbit IgG (O2-6102, Invitrogen) for 30 min at room temperature to allow binding of the antibody. Beads were washed twice in PBS 0.65% Tween 20, incubated with 200 μl of 20 mM of dimethylpimelimidate to covalently couple the antibodies to the beads for 30 min at room temperature and the reaction was stopped by resuspended the beads with 40 μl of 50 mM Tris pH 7.5 for 15 min. Bio-Adembeads PAG beads and 40 μl of anti-FLAG M2 magnetic beads (Sigma-aldrich) were incubated with 400 μl of protein extract for 2 h at 4 °C, and washed twice in PBS 0.65% Tween 20 and then incubated for a long wash for 5 min in PBS only. For non-denaturing elution, Bio-Adembeads PAG beads were resuspended in 30 μl elution buffer and anti-FLAG M2 magnetic beads were resuspended in 100 μl elution buffer, and incubated at room temperature for 5 min. The pH was neutralized using triethyrammonium bicarbonate buffer (pH 8.4).

Alternatively, Dynabeads Protein G (Life Technologies) were used as instructed by the manufacturer and resuspended in 800 μl binding buffer with anti-SL1 antibody or purified rabbit IgG (Life technologies), and incubated overnight at 4 °C to allow binding of the antibody. Beads were washed twice 0.2 M sodium borate (pH 9.0), incubated in 30 mM dimethylpimelimidate for 30 min at room temperature to covalently couple the antibodies to the beads, washed three times in 0.2 M ethanolamine (pH 8.0), and then washed twice in 1 ml binding buffer. Antibody-coupled beads were incubated with whole-testis extracts for 1 h, and washed three times in extraction buffer and once in 1 x TBS with 0.05% Triton X-100 for 5 min. Elution was performed using 0.1 M glycine/HC1, pH 3.0. The pH was neutralized using triethyrammonium bicarbonate buffer (pH 8.4).

**Western blot.** Protein extraction and western blot experiments on whole testes and elutriated spermatozids were performed as described in Comport et al.26 In brief, 15 μl of each immunoprecipitated sample and a volume of input corresponding to 10% of IP sample were denatured using 4 x NuPAGE LDS sample buffer (Life Technologies) with 10% β-mercaptoethanol, boiled for 10 min at 95 °C and loaded. Extraction of sperm proteins was as follow: five millions of spermatozoids (purity > 99%) were resuspended in 200 μl of 4 x NuPAGE LDS sample buffer (Life Technologies) and boiled for 10 min at 95 °C. Ten microliter of sample were loaded per lane. Antibody against SL1 was diluted 1/3000, antibody against SLX/SXL1,26 1/6000, anti-H3K79me2 (ab-3594 from Abcam), anti-TBL1X11 (ab 24550 from Abcam), anti-Tubulin antibody (T-9026 from Sigma–Aldrich), anti-parkH3 antibody (O5-928 from Abcam), anti-FLAG (MS from Sigma–Aldrich) and anti-Hup2B antibody (Briar Patch Biosciences, Grass Valley, CA, USA), 1/1000.

**Sample digestion and LC-MS/MS.** Co-immunoprecipitated samples were subjected to a bottom-up analysis at the 3PS university platform. After, cytoines reduction with dithiothreitol and alkylation with chloroacetamide, proteins were...
digested using trypsin and analyzed by LC-MS/MS using method described in Lahouasse et al.170

Spectra processing, peptide identification. The software Proteome Discoverer 1.3 was used to generate.mgf files. The threshold of signal to noise for extraction values was 3. MS/MS spectra were submitted to MASCOT version 2.5.1 and MaxQuant version 1.5.2.8. The database used was a concatenation of Mouse sequence of NCBI database. Oxidation of methionine was permitted partially, whereas carbamidomethylation of cysteine was considered complete. LFQ option without match between runs was used in Maxquant. Two anti-FLAG co-immunoprecipitation assays were performed respectively on whole testis and on transiently transfected GC1 cells. For whole-tests experiment, duplicate mass spectrometry analyses were performed and were independently analyzed with MASCOT; Table 3 shows the results of MASCOT analysis of the second experiment. The results of the two duplicate experiments were pooled for Maxquant analysis. In MASCOT analyses, the protein score is the sum of the highest MS/MS ions score for each distinct peptide sequence. So the highest the protein score, the more it is represented in the sample. However, for an identical mole of protein, a larger protein can give more peptides than a smaller one. EmPAI (Exponentially Modified Protein Abundance Index) value provides a complementary information as it depends on the number of detected peptides compared to the total number of detectable peptides. The higher the EmPAI, the more abundant the protein. In Table 3, only Maxquant LFQ is more accurate than EmPAI because it reflects the total LC-MS intensity of peptides for the proteins.

Conflict of Interest
The authors declare no conflict of interest.

Acknowledgements. We thank Monika Ward for helpful discussions, Aine Rattigan and Anne-Marie Lachages for help with elicitation settings, Sophie Wood and other members of the NIMR transgenesis facility for pronuclear injections. We also thank Matthieu Benard and other staff members of the Cochin Institute Mouse House Facility, Antoine Gueraud for DNA extraction, Franck Letourneur, Florent Dumont and Juliana Pipoli from the Genomic Facility, Evangeline Bennanna and François Guillonneau from the 3P5 proteomic facility, as well as the Histology, Immunostaining and Laser Microdissection Facility, the Cellular Imaging Facility and François Guillonneau from the 3P5 proteomic facility, as well as the Histology, Immunostaining and Laser Microdissection Facility, the Cellular Imaging Facility and the Cytometry and Immunobiology Facility of the Cochin Institute (INSERM U1016, CNRS UMR1014, Université Paris Descartes). This work was supported by Inserr (Institut National de la Sante et de la Recherche Medicale), the Agence Nationale de CNRS UMR8104, Université Paris Descartes). This work was supported by Inserr (Institut National de la Sante et de la Recherche Medicale), the Agence Nationale de CNRS UMR8104, Université Paris Descartes). This work was supported by Inserr (Institut National de la Sante et de la Recherche Medicale), the Agence Nationale de

1. Yan W. Male infertility caused by spermiogenic defects: lessons from gene knockouts. Mol Cell Endocrinol 2009; 306: 24–32.
2. White-Cooper H, Davidson I. Unique aspects of transcription regulation in male germ cells. Cold Spring Harb Perspect Biol 2011; 3: a002626.
3. Schutz N, Hamra FK, Garbers DL. A multitude of genes expressed solely in meiotic or spermiogenesis. Mol Cell 2011; 42: 2179–2186.
4. Benneker BE, Kamal M, Lindbo-Tob K, Bekiranov S, Bailey DK, Huebert DJ et al. Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 2005; 120: 169–181.

References
10. Gaucher J, Boussouar F, Montellier E, Curtet S, Buchou T, Bertrand S et al. Chromatin dynamics during spermatozoa transition is controlled by the histone H3B variant TH0B. Genes Dev 2013; 27: 1680–1692.
11. Montellier E, Boussouf F, Rousseaux S, Zhang K, Buchou T, Fenaille F et al. Chromatin-to-nucleopro胎me transition is controlled by the histone H2B variant TH0B. Genes Dev 2013; 27: 1680–1692.
12. Li W, Wu J, Kim SY, Zhao M, Hearn SA, Zhang MQ et al. Chd5 orchestrates chromatin remodeling during sperm development. Nat Commun 2014; 5: 5812.
13. Yamachi Y, Reil JM, Stoytcheva Z, Brygosyne P, Ward MA. Deficiency of mouse Y chromosome long arm gene complement is associated with sperm DNA damage. Genome Biol 2010; 11: R66.
14. Reynard LN, Cocquet J, Brygosyne P. The multi-copy mouse gene SycP3-like Yinkled (Sly) encodes an abundant spermatid protein that interacts with a histone acetyltransferase and an acrosomal protein. Biol Reprod 2000; 61: 250–257.
15. Cocquet J, Ellis PJ, Yamachi Y, Mahadevaiah SK, Affara NA, Ward MA et al. The multicopy gene Sly represses the sex chromosomes in the male germ cell meiosis after PloS Biol 2005; 7: e1000244.
16. Reil JM, Yamachi Y, Sugawasa A, Li HY, Ruthig V, Stoytcheva Z et al. Deficiency of the mouse Y chromosome long arm gene complement is associated with sperm DNA damage and abnormal chromatin packaging. J Cell Sci 2013; 126: 803–813.
17. Yuan L, Liu G-Z, Zhao J, Brundell E, Daneholt B, Höög C. The murine SCPD gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol Cell 2006; 23: 73–83.
18. Gan H, Wen L, Liao S, Lin X, Ma T, Liu J et al. Dynamics of 5-hydroxymethylcytosine during mouse spermato genesis. Nat Commun 2013; 4: 1995.
19. Bryant JM, Donahue G, Wang X, Meyer-Ficca M, Luense LJ, Weller AH et al. Characterization of BRD4 during mammalian postmeiotic sperm development. Mol Cell Biol 2015; 35: 1433–1448.
20. Erkik S, Hisano M, Liang CY, Gill M, Murr R, Dieker J et al. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. J Struct Mol Biol 2013; 20: 868–875.
21. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 2011; 146: 1016–1028.
22. Hammond SS, Low DH, Yi C, Carrell DT, Guccione E, Cairns BR. Chromatin and transcription transitions of mammalian adult germine stem cells and spermatogenesis. Cell Stem Cell 2014; 15: 239–253.
23. Bernstein BE, Kamal M, Lindbo-Tob K, Bekiranov S, Bailey DK, Huebert DJ et al. Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 2005; 120: 169–181.
24. Chrygmont MP, Cheng AW, Welstead GD, Kociostra T, Carey BW, Steine JH et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci USA 2010; 107: 21931–21936.
25. Benavoy UA, Polina EA, Ucar D, Mahmoudi S, Karr K, Wong ED et al. H3K4me3read is linked to cell identity and transcriptional consistency. Cell 2014; 158: 673–688.
26. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. Biotechniques 2005; 39: 75–85.
27. Solana T, Neves A, Palha A, Williams R, Huttley GA. CineMaghic D. A unique H2A histone variant occupies the transcriptional start site of active genes. Nat Struct Mol Biol 2011; 19: 25–30.
28. Govin J, Escotter F, Rousseaux S, Kuhn L, Ferro M, Thevenon J et al. Pericentric heterochromatin reprogramming by new histone variants during mouse spermatogenesis. J Cell Biol 2017; 283: 294–305.
29. Ferguson L, Ellis PJ, Affara NA. Two novel mouse genes mapped to chromosome Yp are expressed specifically in spermatsids. Mamm Genome 2009; 20: 193–206.
30. Martsovan I, Bransonsi S, Catena R, Gansmuller A, Kotaja N, Parvinen M et al. Polar nuclear localization of H17a, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. Proc Natl Acad Sci USA 2005; 102: 2808–2813.
31. Dottermusch-Heidel C, Gerlach SM, Tegeder I, Ruthig C, Barkmann B, Barkum MN et al. H3K9 methylation directly precedes the histone-to-proteome transition in mammalian spermatids and is sensitive to bacterial infections. Andrology 2014; 2: 655–665.
32. Cocquet J, Ellis PJ, Mahadevaiah SK, Affara NA, Vaiman D, Brygosyne P. A genetic basis for a postmeiotic X versus Y chromosome intragenic conflict in the mouse. PLoS Genet 2012; 8: e1002900.
33. Gian O, Lam EY, Becker L, Dugan L, Cannizzaro E, Jorgrey B et al. Functional interdependence of BRD4 and DOT1L in MLL leukemias. Nat Struct Mol Biol 2016; 23: 673–681.
34. Brykcyznska U, Hisano M, Erkik E, Saramo L, Oakeley EJ, Rollot TC et al. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nat Struct Mol Biol 2017; 24: 678–687.
35. Grabore B, Damon C, Lenior A, Kaulstein G, Kern H, Zevnik B et al. Epidymis seleno-independent glutathione peroxidase 5 maintains sperm DNA integrity in mice. J Clin Invest 2009; 119: 2074–2085.
36. Sin HS, Kartoshov AV, Hasegawa K, Barski A, Namekawa SH. Posed chromatin and bivalent domains facilitate the mitosis-to-meiosis transition in the male germline. BMC Biol 2015; 13: 53.
37. Moretti C, Vaiman D, Tores F, Cocquet J. Expression and epigenomic landscape of the sex chromosomes in mouse post-meiotic male germ cells. Epigenetics 2016; 8: 47.
54. Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health.

51. Ontoso D, Kauppi L, Keeney S, San-Segundo P A. Dynamics of DOT1L localization and silencing in mouse spermatocytes. Bioinformatic 2010; 1: 540–548.

49. Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K et al. Syntenin, a novel N-CoR-interacting protein, associates with the N-CoR-HDAC3 nuclear receptor corepressor complex inhibiting the INK pathway through the integral subunit GPS2. Mol Cell 2002; 9: 611–623.

41. Zhang J, Kalkum M, Chait BT, Roeder RG. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the INK pathway through the integral subunit GPS2. Mol Cell 2002; 9: 611–623.

40. Li J, Wang J, Wang J, Nawaz Z, Liu JM, Qin J et al. Oxidative stress and male reproductive health.

39. Syrjanen JL, Pellegrini L, Davies OR. A molecular model for the role of SYCP3 in meiotic chromosome segregation. EMBO J 2004; 23: 1052–1064.

38. Hajkova P, van Steensel B, Ptacek J, Cao Q, Kuan E Y et al. DNA methylation in mammals. Curr Biol 2002; 12: 723–734.

37. Zhang D, Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J et al. JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2). EMBO J 2002; 21: 3264–3275.

36. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N et al. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the INK pathway through the integral subunit GPS2. Mol Cell 2002; 9: 611–623.

35. Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J et al. JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2). EMBO J 2002; 21: 3264–3275.

34. Zhang W, Hayashizaki Y, Kone BC. Structure and regulation of the mdot1 gene, a mouse histone H3 methylesterase. Biochem J 2004; 377: 641–651.

33. Ontoso D, Kauppi L, Keeney S, San-Segundo P A. Dynamics of DOT1L localization and H3K79 methylation during meiotic prophase I in mouse spermatocytes. Chromosoma 2014; 123: 147–164.

32. Davaia BN, Case-Borden C, Gagonne G, Hsu C H, Chen Q, Meerzaman D et al. Brd4 is a histone acetyltransferase that evicts nucleosomes from chromatin. Nat Struct Mol Biol 2010; 17: 329–335.

31. Hajkova P, van Steensel B, Ptacek J, Cao Q, Kuan E Y et al. DNA methylation in mammals. Curr Biol 2002; 12: 723–734.

30. Zhang W, Hayashizaki Y, Kone BC. Structure and regulation of the mdot1 gene, a mouse histone H3 methylesterase. Biochem J 2004; 377: 641–651.

29. Ontoso D, Kauppi L, Keeney S, San-Segundo P A. Dynamics of DOT1L localization and H3K79 methylation during meiotic prophase I in mouse spermatocytes. Chromosoma 2014; 123: 147–164.

28. Davaia BN, Case-Borden C, Gagonne G, Hsu C H, Chen Q, Meerzaman D et al. Brd4 is a histone acetyltransferase that evicts nucleosomes from chromatin. Nat Struct Mol Biol 2010; 17: 329–335.

27. Lord T, Atken RJ. Fertilization stimulates 8-hydroxy-2'-deoxyguanosine repair and antioxidant activity to prevent mutagenesis in the embryo. Dev Biol 2015; 406: 1–13.

26. Atken RJ, Smith TB, Joling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. Asian J Androl 2014; 16: 31–38.

25. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 2010; 26: 589–595.

24. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009; 25: 2078–2079.

23. Zhang Y, Li T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol 2008; 9: R137.

22. Ramirez F, Dunbar F, Diehl S, Gruning BA, Marke T, deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res 2014; 42: W187–W191.

21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005; 102: 15545–15550.

20. Joulin M, Chalet BT, Roeder RG. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the INK pathway through the integral subunit GPS2. Mol Cell 2002; 9: 611–623.

19. Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J et al. JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2). EMBO J 2002; 21: 3264–3275.

18. Hajkova P, van Steensel B, Ptacek J, Cao Q, Kuan E Y et al. DNA methylation in mammals. Curr Biol 2002; 12: 723–734.

17. Zhang W, Hayashizaki Y, Kone BC. Structure and regulation of the mdot1 gene, a mouse histone H3 methylesterase. Biochem J 2004; 377: 641–651.

16. Ontoso D, Kauppi L, Keeney S, San-Segundo P A. Dynamics of DOT1L localization and H3K79 methylation during meiotic prophase I in mouse spermatocytes. Chromosoma 2014; 123: 147–164.

15. Lord T, Atken RJ. Fertilization stimulates 8-hydroxy-2'-deoxyguanosine repair and antioxidant activity to prevent mutagenesis in the embryo. Dev Biol 2015; 406: 1–13.

14. Atken RJ, Smith TB, Joling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. Asian J Androl 2014; 16: 31–38.

13. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 2010; 26: 589–595.

12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009; 25: 2078–2079.

11. Zhang Y, Li T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol 2008; 9: R137.

10. Ramirez F, Dunbar F, Diehl S, Gruning BA, Marke T, deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res 2014; 42: W187–W191.

9. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005; 102: 15545–15550.

8. Joulin M, Chalet BT, Roeder RG. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the INK pathway through the integral subunit GPS2. Mol Cell 2002; 9: 611–623.

7. Zhang W, Hayashizaki Y, Kone BC. Structure and regulation of the mdot1 gene, a mouse histone H3 methylesterase. Biochem J 2004; 377: 641–651.

6. Ontoso D, Kauppi L, Keeney S, San-Segundo P A. Dynamics of DOT1L localization and H3K79 methylation during meiotic prophase I in mouse spermatocytes. Chromosoma 2014; 123: 147–164.

5. Lord T, Atken RJ. Fertilization stimulates 8-hydroxy-2'-deoxyguanosine repair and antioxidant activity to prevent mutagenesis in the embryo. Dev Biol 2015; 406: 1–13.

4. Atken RJ, Smith TB, Joling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. Asian J Androl 2014; 16: 31–38.

3. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 2010; 26: 589–595.