Super-enhancer switching drives a burst in gene expression at the mitosis-to-meiosis transition

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Owing to bursts in the expression of thousands of germline-specific genes, the testis has the most diverse and complex transcriptome of all organs. By analyzing the male germline of mice, we demonstrate that the genome-wide reorganization of super-enhancers (SEs) drives bursts in germline gene expression after the mitosis-to-meiosis transition. SE reorganization is regulated by two molecular events: the establishment of meiosis-specific SEs via A-MYB (MYBL1), a key transcription factor for germline genes, and the resolution of SEs in mitotically proliferating cells via SCML2, a germline-specific Polycomb protein required for spermatogenesis-specific gene expression. Before entry into meiosis, meiotic SEs are preprogrammed in mitotic spermatogonia to ensure the unidirectional differentiation of spermatogenesis. We identify key regulatory factors for both mitotic and meiotic enhancers, revealing a molecular logic for the concurrent activation of mitotic enhancers and suppression of meiotic enhancers in the somatic and/or mitotic proliferation phases.

Meiosis is an essential step in the preparation of haploid gametes, and the transition from mitotic proliferation to meiosis is a fundamental event in the maturation of germ cells. In the male germline, this mitosis-to-meiosis transition coincides with a fundamental alteration to the transcriptome: a dynamic and massive change in genome-wide gene expression2–3. Because of bursts in the expression of thousands of germline genes, the testis has the most diverse, complex and rapidly evolved transcriptome of all organs4–5. During spermatogenesis, the mitosis-to-meiosis transition accompanies the dynamic reorganization of epigenetic modifications, accessible chromatin and three-dimensional (3D) chromatin conformation—all in preparation for the next generation of life6–11. However, it remains largely unknown how DNA regulatory elements underlie massive, dynamic transcriptional changes in the mammalian male germline.

Enhancers play key roles in the control of cell type-specific gene expression programs through the binding of transcription factors (TFs) and interactions with promoters12–14. Some of these enhancers aggregate to regulate the expression of genes important for establishing cellular identity15–19; such aggregates have been termed ‘super-enhancers’ (SEs)20. SEs are prevalent in various cell and tissue types and are also found in cancer cells, where they direct the expression of key tumor pathogenesis genes21. For the most part, the characterization of SEs has been limited to somatic and/or mitotically proliferating cells. Given the massive scale of the mitosis-to-meiosis transcriptome alteration, there are compelling questions as to the detailed profiles of active enhancers in spermatogenesis as well as the existence of meiosis-specific SEs. Here, we determine the profiles of active enhancers in representative stages of mouse spermatogenesis. We identify a meiotic type of SE, and we demonstrate that the dynamic transcriptome at the mitosis-to-meiosis transition is driven by a switch from mitotic to meiotic SEs. Through functional and systematic analyses, we identify key regulatory factors for both mitotic and meiotic enhancers. Our analyses expose the molecular logic for SE switching at the mitosis-to-meiosis transition, revealing activation mechanisms for mitotic enhancers that are concurrent with suppression mechanisms for meiotic enhancers in the somatic and/or mitotic proliferation phases.

Results
The landscape of active enhancers during spermatogenesis. To determine the landscape of active enhancers in spermatogenesis, we performed chromatin immunoprecipitation with sequencing (ChIP-seq) for the histone modification H3K27ac, a marker of active enhancers22. We analyzed four representative stages of wild-type spermatogenesis: THY1+ undifferentiated spermatogonia (a population that contains both spermatogonial stem cells and progenitor cells), KIT+ differentiating spermatogonia, pachytene spermatocytes (PSs) in meiotic prophase and postmeiotic round spermatids (RSs) (Fig. 1a). We carried out H3K27ac crosslinking ChIP-seq with ChiPmentation23 for two independent biological replicates and obtained reproducible, detailed profiles of putative active enhancers during spermatogenesis (see Methods, Fig. 1b and Extended Data Fig. 1a). Consistent with the massive, dynamic transcriptional changes that occur at the mitosis-to-meiosis transition, we observed...
three types of H3K27ac peak: (1) those in mitotically proliferating spermatogonia (that is, the ‘mitotic phase’; blue shadow); (2) those that appeared in meiotic spermatocytes and continued to grow in postmeiotic spermatids (red shadow); (3) constitutive peaks (gray shadow; Fig. 1b).

For the quantitative comparison of putative active enhancers during spermatogenesis, we analyzed H3K27ac ChIP-seq peaks ±1 kb outside transcription start sites (TSSs; ‘distal peaks’, see Methods). We detected 11,433 distal H3K27ac ChIP-seq peaks that were present in at least one stage of spermatogenesis (Supplementary Data 1). Through k-means clustering, the distal peaks were categorized into three classes (10 clusters) as follows (Fig. 1c): (1) the first class (705 peaks) represents constitutive active enhancers, that is, those observed throughout spermatogenesis; (2) the second class (2,524 peaks) represents enhancers that are active in the mitotic phase but are inactive in meiotic and postmeiotic phases; (3) the third class (8,204 peaks) consists of enhancers that are largely inactive in the mitotic phase yet are highly active in meiotic and postmeiotic stages. In contrast to a previous view that enhancer activation may not be limited to the meiotic stages, these results demonstrate how massive, dynamic transcriptional change is stimulated at the mitosis-to-meiosis transition (that is, between KIT+ spermatogonia and PSs). These results suggest that a majority of active enhancers in KIT+ spermatogonia disappear prior to meiosis, and an extensive de novo formation of active enhancers takes place in meiotic prophase. Additionally, the level of H3K27ac is augmented at the promoters in the KIT+ to PS transition (Extended Data Fig. 1b). The continued alteration of active enhancers occurred from meiotic PSs to postmeiotic RSs, and we observed an additional de novo activation of enhancers in RSs (Fig. 1d).

Robust expression of key spermatogenesis genes is facilitated by de novo establishment of meiotic SEs. To elucidate how massive, dynamic transcriptional change is stimulated at the mitosis-to-meiosis transition, we sought to test the following hypothesis: the transcriptional change of the mitosis-to-meiosis transition is associated with the establishment of SEs. SEs have been defined as large chromatin domains enriched with H3K27ac and/or other active enhancer marks. Drawing on this definition, we identified SEs based on elevated H3K27ac enrichment in spermatogenesis using the same criteria and algorithm as previously described. We found that SEs are established in the course of spermatogenesis, with a common SE profile (Fig. 1b) that appears in meiotic spermatocytes and continues to grow in postmeiotic spermatids; promoters) were largely common at each transition, and THY1+ and KIT+ spermatogonia share a common active-enhancer profile (distal H3K27ac peaks) (Fig. 1d). By contrast, there is a dynamic change in the distribution of active enhancers at the mitosis-to-meiosis transition (that is, between KIT+ spermatogonia and PSs). These results suggest that a majority of active enhancers in KIT+ spermatogonia disappear prior to meiosis, and an extensive de novo formation of active enhancers takes place in meiotic prophase. Additionally, the level of H3K27ac is augmented at the promoters in the KIT+ to PS transition (Extended Data Fig. 1b). The continued alteration of active enhancers occurred from meiotic PSs to postmeiotic RSs, and we observed an additional de novo activation of enhancers in RSs (Fig. 1d).

![Fig. 1](image-url) | The landscape of active enhancers during spermatogenesis. a. Schematic of mouse spermatogenesis and the four representative stages analyzed in this study: THY1+, undifferentiated spermatogonia; KIT+, differentiating spermatogonia; PS, pachytenes spermatocytes; RS, round spermatids. b. Track views of H3K27ac ChIP-seq enrichment with biological replicates for representative stages of spermatogenesis. ESCs, embryonic stem cells. c. k-means clustered heatmap of distal H3K27ac peaks (detected by MACS2) in spermatogenesis. d. MAAnorm analysis for H3K27ac peaks at each transition of spermatogenesis. The genomic distribution for each peak is shown with colored bars. H3K27ac peak enrichment for each genomic entity (intergenic, intronic, and so on) was compared to randomly selected genomic regions. ∗P < 0.00001, chi-squared test with Yates’s correction. Data for c and d are available as source data.
Fig. 2 | The identification of SEs during spermatogenesis. a, Identification of SEs in representative stages of spermatogenesis. H3K27ac signal (as defined by the ROSE algorithm) enrichment represents, for each enhancer region, density × length. b, SE overlap at the stage transitions of spermatogenesis. c, Track views of H3K27ac ChIP-seq enrichment for representative SEs in spermatogenesis. d, Average tag densities and heatmaps for H3K27ac ChIP-seq enrichment at SEs. H3K27ac enrichment values are shown in reads per million (RPM). e, Gene ontology (GO) analysis of genes adjacent to meiotic SEs (that is, genes within 20 kb upstream to 50 kb downstream of SEs). f, RNA-seq profiles for 101 ‘spermatogenesis’ genes out of SE-adjacent genes. g, Box-and-whisker plots show the distributions of RNA-seq reads per kilobase of transcript per million mapped reads (RPKM) values for late spermatogenesis genes. Among 2,623 ‘late spermatogenesis’ genes (that is, genes that are not highly expressed in spermatogonia but are highly expressed in PSs and/or RSs; a gene list is included in Supplementary Data 4), 652 genes are located adjacent to meiotic SEs (genes within 20 kb upstream to 50 kb downstream of SEs). Central bars represent medians, the boxes encompass 50% of data points, and the whiskers indicate 90% of the data points. ***P < 0.0001, Mann-Whitney U-test. Data for f and g are available as source data.
in mitotically dividing THY1+ and KIT+ spermatogonia. However, among the 182 SEs in KIT+ spermatogonia, only 32% (59/182) are common to SEs in PSs (Fig. 2b). These data reveal the dynamic, de novo formation of SEs at the mitosis-to-meiosis transition. After the mitosis-to-meiosis transition, 57% (278/487) of SEs in PSs were common to RSs; we observed the establishment of 836 new SEs in RSs (Fig. 2b).

We identified distinct characteristics for SEs common to the mitotic stages (that is, between THY1+ and KIT+ spermatogonia) as well as distinct characteristics for SEs common to the meiotic (that is, PS) and postmeiotic (that is, RS) stages. First, with respect to SEs common to mitotic stages (THY1+ and KIT+ spermatogonia: termed ‘mitotic SEs’), H3K27ac was decreased in PSs but tended to persist throughout spermatogenesis into at least as late as the RS stage (Fig. 2c,d). On the other hand, with respect to PS and RS SEs (termed ‘meiotic SEs’), H3K27ac was largely absent from corresponding genomic loci in THY1+ and KIT+ spermatogonia and was robustly established during the mitosis-to-meiosis transition (Fig. 2c,d). Intriguingly, meiotic SEs tend to consist of large and broad H3K27ac peaks, while mitotic SEs tend to comprise clusters of distinct, narrow H3K27ac peaks (Fig. 2c,d). Importantly, we found that meiotic SEs overlap pachytene piRNA clusters, which produce pachytene piRNAs (Fig. 2b, right and Fig. 3f). Among pachytene piRNA clusters, we found that BTB18-dependent piRNA loci are highly likely to overlap meiotic SEs (Fig. 3f); BTB18 is an essential factor for pachytene piRNA production by way of transcriptional elongation4. A-MYB is essential for the production of pachytene piRNA4, and the numbers of A-MYB peaks are in proportion to the numbers of meiotic SEs on piRNA clusters (Fig. 3f). Thus, A-MYB and meiotic SEs may comprise, in part or whole, a potential mechanism for the production of pachytene piRNA.

To understand the functions of A-MYB in spermatogenesis, we examined A-myb mutant (Mybl1mut1) mice, which exhibit complete meiotic arrest in the mid pachytene stage and subsequent cell death (Extended Data Fig. 3)4. In normal meiosis, H3K27ac was present throughout the nucleus with enrichment on the X chromosome domain in the pachytene stage (Fig. 4a)35. Yet in A-myb mutants, meiotic nuclei were largely devoid of H3K27ac and, when present, H3K27ac was clustered in large, ectopic puncta (arrowheads, Fig. 4a) and along the axis of chromosome X (magnified panel, Fig. 4a). These observations indicate a global alteration of H3K27ac deposition in A-myb mutants.

To evaluate the role of A-MYB in the establishment of meiotic SEs, we performed ultra-low-input native ChIP-seq4 for H3K27ac in A-myb mutant PSs due to their limited availability. H3K27ac deposition was significantly reduced at sites of meiotic SEs in A-myb mutant PSs compared to wild-type controls (Fig. 4b,c). Consistent with this result, we observed a significant overlap of meiotic SE–adjacent genes and genes differentially expressed in A-myb mutants at postnatal day 14 (P14)19, and many of the differentially expressed genes were found to be downregulated in A-myb mutants. Meanwhile, there is no significant correlation between A-MYB enrichment on the promoters and the degree of gene deregulation of these SE target genes in the A-myb mutants (Extended Data Fig. 3d), further supporting the function of meiotic SEs in the regulation of adjacent genes. To confirm the activation of meiotic genes by adjacent meiotic SEs, we performed CRISPR activation (CRISPRa) experiments using embryonic stem cells (ESCs), in which meiotic SEs and germline genes are not active. Using doxycycline (Dox)-inducible CRISPRa ESCs (J1 ESCs harboring a Dox-inducible dCas9-VPR transgene), the germline gene Zfp37, which is adjacent to a meiotic SE, was activated upon introduction of four guide RNAs (gRNAs) targeting its adjacent meiotic SE (Fig. 4e). Induction of A-MYB expression further stimulated CRISPRa-mediated activation (Fig. 4e). Taken together, we conclude that A-MYB establishes meiotic SEs that activate adjacent germline genes (Fig. 4f).
SCML2 facilitates the resolution of mitotic SEs during meiosis. Next, we sought to determine a mechanism underlying the resolution of mitotic SEs at the mitosis-to-meiosis transition. We focused on the function of SCML2, a germline-specific Polycomb protein that is responsible for suppression of somatic/progenitor genes. We observed an increase in H3K27ac signal intensity at promoters of both classes of bivalent genes in Scml2−KO mice (Extended Data Fig. 4c). We presume that this is the consequence—at least in part—of an antagonistic relationship between H3K27me3 and H3K27ac at promoters, because both post-translational modifications occupy the same amino acid residue (K27) of the histone H3 tail.

Next, we sought to evaluate the binding of SCML2 to mitotic SEs and the establishment of H3K27me3 upon the resolution of mitotic SEs. We found that SCML2 peaks overlap with H3K27ac peaks at mitotic SEs (Extended Data Fig. 5a). Although the majority of SCML2 peaks appeared on promoters, SCML2 peaks are present...
Fig. 4 | A-MYB establishes meiotic SEs for the targeted activation of germline genes. a, Chromosome spreads of wild-type (WT) and A-myb mutant (A-myb Mut) PSs immunostained with antibodies raised against SYCP3 and H3K27ac. Dashed squares indicate sex chromosomes, which are magnified in the panels at the bottom. White arrowheads indicate ectopic clusters of H3K27ac signal. Immunostaining intensity (in arbitrary units, a.u.) is quantified by densitometry across the indicated path (‘ to ’). Scale bars, 10 μm (top) and 5 μm (bottom). b, Track views of H3K27ac ChIP-seq enrichment for representative meiotic SEs in WT and A-myb Mut PSs. c, Average tag densities and heatmaps for H3K27ac ChIP-seq enrichment at SEs in WT and A-myb Mut PSs. H3K27ac enrichment values: RPM. d, RNA-seq analysis of A-myb Mut versus A-myb heterozygous control testes at postnatal day 14 (P14). The 1,705 genes exhibiting significant changes in expression (FDR < 0.01, binomial test with Benjamini–Hochberg correction) in A-myb mutants are represented by blue circles. ***P = 1.0 × 10−7, hypergeometric probability test. 211 dysregulated meiotic SE-adjacent genes (red circles) divided by 652 meiotic SE-adjacent genes; 1,705 dysregulated differentially expressed genes (blue circles) divided by 22,661 NCBI RefSeq genes. e, Activation of a representative meiotic SE via CRISPR activation (CRISPRa). Top: schematic for CRISPRa experiments. Four guide RNAs (gRNAs) were designed to target adjacent regions (less than 1kb) from two A-MYB binding sites (two gRNAs for each A-MYB binding site). Bottom: CRISPRa-dependent expression of the gene Zfp37 as measured by quantitative PCR with reverse transcription (qRT-PCR). Data are presented as mean ± s.e.m.: *P < 0.05, **P < 0.01, unpaired t-tests. Three biological replicates were examined. f, A model for the A-MYB-dependent establishment of meiotic SEs. Data for a, d, and e are available as source data.

on mitotic SEs and other enhancers in spermatogonia (Extended Data Fig. 5b,c). H3K27me3 was established at SCML2-containing mitotic SEs after the resolution of mitotic SEs (Extended Data Fig. 5d). These data suggest SCML2 may bind a portion of mitotic SEs to establish H3K27me3 (Fig. 5c).

We also observed a decrease in H3K27ac intensity at meiotic SEs in Scml2-KO PSs and RSs (Fig. 5d)—observations that are consistent with the downregulation of late spermatogenesis genes in Scml2-KO PSs and RSs1. Because SCML2 is a suppressor, we suspected that meiotic SEs were indirectly impaired in Scml2-KO PSs and RSs. Of note, six out of nine of the candidate regulators of meiotic enhancers (identified in Extended Data Fig. 2b and including A-MYB) were downregulated in Scml2-KO PSs and RSs. Therefore, our results suggest meiotic SEs are indirectly downregulated in Scml2-KO PSs and RSs.

SCML2 is required for the formation of SEs on the X chromosome during meiosis. Switching our focus to meiosis, we...
performed analyses to elucidate mechanisms governing the activation of enhancers on the male sex chromosomes. During male meiosis, the sex chromosomes undergo regulation distinct from autosomes due to meiotic sex chromosomes inactivation (MSCI)\(^4\). MSCI engages a DNA damage response (DDR) pathway to regulate gene silencing on the sex chromosomes, independent of its functions on autosomes\(^4\). Although we observed a paucity of distal H3K27ac peaks on the sex chromosomes of THY1\(^+\) and KIT\(^+\) spermatogonia, we identified the de novo establishment of H3K27ac peaks in the mitosis-to-meiosis transition (Fig. 6a).

To dissect the regulatory mechanisms underlying this process, we focused on SCML2, which has a critical regulatory function on the sex chromosomes, independent of its functions on autosomes\(^1\). SCML2 functions downstream of the DDR pathway that initiates MSCI, where it cooperates with RNF8 to establish H3K27ac\(^35\). On the sex chromosomes of PSs and RSs, a large portion of distal H3K27ac peaks (particularly those in intergenic and intronic regions) depend on SCML2 (Fig. 6b). Interestingly, ATAC-seq peaks appeared specifically on the sex chromosomes of PSs in an SCML2-dependent fashion too\(^1\). Thus, SCML2 is a key regulatory factor for chromatin accessibility and H3K27ac deposition on the meiotic sex chromosomes. Accordingly, 26 SEs are established on the X chromosomes (Fig. 6c), and these largely depend on SCML2 (Fig. 6d). This is unlike SCML2’s function to resolve mitotic SEs (Fig. 5); as
such, we observed increased numbers of SEs on autosomes of Scml2-KO PSs (Fig. 6d). These results demonstrate autosome- and sex chromosome-specific functions for SCML2 in the regulation of enhancers in spermatogenesis.

**Meiotic SEs on autosomes are poised in undifferentiated spermatogonia.** Because SEs on the sex chromosomes are established downstream of the DDR pathway in meiosis, we suspected that SEs on the autosomes are regulated by a distinct mechanism. Thus, to determine the mechanism by which autosomal SEs are established, we examined the epigenetic status of meiotic SEs in progenitor cells, specifically the active marks H3K4me2 and H3K4me3, which were previously reported to be associated with poised gene promoters during spermatogenesis. Notably, prior to the establishment of H3K27ac, H3K4me2 was present on autosomal meiotic SEs in THY1+ spermatogonia (Fig. 6e,f). Additionally, H3K4me3 is also enriched on autosomal meiotic SEs in THY1+ spermatogonia (Fig. 6f). These features were unique to meiotic SEs; other meiotic enhancers detected through analyses of distal H3K27ac peaks did not exhibit these features (Extended Data Fig. 6a). These results suggest that meiotic SEs are poised as early as the THY1+ spermatogonia phase to prepare for the expression of key spermatogenesis genes after the mitosis-to-meiosis transition. Such features were not observed on meiotic SEs associated with the X chromosome (Fig. 6g), lending further support for the distinct regulation of meiotic SEs between autosomes and chromosome X. We also found that the TSSs of late spermatogenesis genes are broadly poised for activation in spermatogonia (Extended Data Fig. 6b) and that the TSSs

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**Fig. 6 | The distinct regulation of meiotic SEs on autosomes versus the sex chromosomes.**

**a.** MA norm analysis for H3K27ac peaks on the sex chromosomes in THY1+ and KIT+ spermatogonia, and in KIT+ spermatogonia and PSs. **b.** MA norm analysis for H3K27ac peaks on the sex chromosomes in PSs and RSs in WT and Scml2-KO. **c.** Numbers of SEs on the X chromosome in each stage of spermatogenesis. **d.** Numbers of SEs on the X chromosome and autosomes in PSs. **e.** Track views of H3K27ac ChIP-seq enrichment at a representative meiotic SE in spermatogenesis. **f-g.** Box-and-whisker plots showing the distributions of ChIP-seq enrichment around autosomal meiotic SEs (f) and X-linked meiotic SEs (g). Central bars represent medians, the boxes encompass 50% of the data points, and the whiskers indicate 90% of the data points. *****P < 0.0001, Mann-Whitney U-test. **h.** Model showing that poised meiotic SEs in THY1+ spermatogonia facilitate the establishment of active meiotic SEs in pachytene spermatocytes. Data for **a, b, f, and g** are available as source data.
of genes adjacent to these SEs retain similar epigenetic patterns in each stage (Extended Data Fig. 7). Together, these data indicate that SE-associated late spermatogenesis genes on autosomes are poised for activation in two layers: SEs and TSSs (Fig. 6c). We propose that this form of epigenomic programming ensures the unidirectional differentiation of spermatogenesis.

Next, we sought to identify mechanisms for the expression of postmeiotic spermatid-specific genes. On autosomes, a proportion of distal H3K27ac peaks around RS-specific genes are poised with H3K4me2 in THY1+ spermatogonia (Extended Data Fig. 6c). On the PS X chromosome, at distal H3K27ac peaks around RS-specific genes, H3K27ac and H3K4me2 became temporarily enriched (Extended Data Fig. 6d).

These results underscore distinct gene activation mechanisms for autosomes versus the sex chromosomes in haploid RSs.

Identification of key regulatory factors for both mitotic and meiotic enhancers. Finally, we took advantage of our new data sets to infer general mechanisms underlying the regulation of mitotic and meiotic enhancers. Because the meiotic gene program is largely repressed in cell types that undergo mitotic divisions, we sought to identify putative TFs that meet one of two counteractive conditions: (1) those that can operate on and/or promote the activity of mitotic enhancers and (2) those that can suppress meiotic enhancers. To this end, we used our recently published regulatory element locus intersection (RELI) algorithm to compare the genomic locations of our H3K27ac peak data with a large collection of publicly available ChIP-seq data. Taking the genomic location information for H3K27ac peaks detected to be mitotic enhancers in KIT+ spermatogonia, we analyzed the intersections between these data and publicly available ChIP-seq datasets for many TFs in many contexts.

Because the overwhelming majority of public ChIP-seq data are from somatic cells that undergo mitotic divisions in between cell cycles, this informs (at least) one interpretation for such an experiment: the enrichment of intersections between mitotic enhancers and TFs could be indicative of general mechanisms that operate on mitotic enhancers. In addition to TFs that were previously associated with spermatogonia, such as STAT3, TCP3, MAZ, and ETS1, we identified additional factors with enriched ChIP-seq peaks at distal H3K27ac peaks in KIT+ spermatogonia: SRF, TCF12, GATA4, BCL6, CEBPB and MAX (Fig. 7a and Supplementary Data 6). When we applied the same analysis to mitotic SEs, we identified UTF1, RBP1, CHD1, ZFX and KLF4 as specific factors that may be involved in their regulation (Fig. 7b and Supplementary Data 7).

Among them, ZFX was previously implicated in spermatogenesis. Next, we applied this strategy to identify factors that suppress meiotic enhancers in the mitotic phase. Compellingly, at sites of meiotic enhancers as determined by the loci of distal H3K27ac peaks in PSs, we revealed high enrichment for factors that comprise, in part, transcriptional silencing machinery, including REST, TRIM28, ROR2, SIN3A and YY1 (Fig. 7c and Supplementary Data 8). Of note, when we applied this analysis to mitotic SEs, we identified UTF1, RBP1, CHD1, ZFX and KLF4 as specific factors that may be involved in their regulation (Fig. 7b and Supplementary Data 7).

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Discussion

In this study, we determined the profiles of putative active enhancers in representative stages of spermatogenesis and demonstrated that SE switching underlies the dynamic transcriptome change of the mitosis-to-meiosis transition. Using an improved H3K27ac...
ChIP-seq method, we were able to profile active enhancers at high resolution and with high signal-to-noise ratios. Because SEs regulate the gene expression that underlies cellular identity\textsuperscript{15,16}, the A-MYB-dependent regulation of meiotic SEs becomes a conceivable mechanism for gene expression that defines the cellular identity of germ cells in late spermatogenesis. Our analyses revealed SE-adjacent genes that are critical for late spermatogenesis. In particular, Pitx1 and Tdrd1 are involved in the regulation of piRNA\textsuperscript{25,26}. Thus, meiotic SEs may be central to the robust production of pachytene piRNA that, in turn, determines cellular identity. Interestingly, the A-MYB-dependent activation of germline genes is an ancient mechanism also found in rooster testes\textsuperscript{33}. Given the robust and evolutionarily conserved nature of germline gene activation via SEs, such a mechanism stands in stark contrast to a concomitant mechanism whereby rapidly evolved enhancers, driven by endogenous retroviruses, activate species-specific germline genes (Sakashita et al.)\textsuperscript{46}.

In tumor cells, SEs are regulated by BRD4, a member of the bromodomain and extraterminal (BET) subfamily of proteins\textsuperscript{47}. In spermatogenesis, a testis-specific member of the BET family, BRDT, is required for the meiotic gene expression program\textsuperscript{48-50}. Given the molecular similarities between BRD4 and BRDT, it is possible that BRDT could be a binding protein for meiotic SEs, and loss of function of BRDT could represent loss of function of meiotic SEs. Curiously, another protein containing bromodomains, BRWD1, can also recognize acetylated lysine residues and is required for postmeiotic transcription in spermatids\textsuperscript{51}. Likewise, BRD4 is also associated with gene expression in spermatids\textsuperscript{52}. Thus, BRWD1 and BRD4 may be involved in the increased numbers of active enhancers established in the transition from PSs to RSs. Of note, many of the highly expressed genes in RSs are initially transcribed in PSs\textsuperscript{53}, and SEs that have been identified in RSs were initially enriched with H3K27ac in PSs (Fig. 2d). Hence, it is likely that, in PSs, A-MYB may also initiate RS SE formation to facilitate RS gene expression.

Our study has shown that SCML2 mediates distinct forms of regulation for active enhancers on autosomes versus active enhancers on sex chromosomes. On the autosomes, SCML2, a highly expressed protein in undifferentiated spermatagonia,\textsuperscript{54} is involved in the resolution of mitotic SEs after the mitosis-to-meiosis transition (Fig. 5), while meiotic SEs are already poised with H3K4me2 in undifferentiated spermatagonia (Fig. 6). Therefore, it is conceivable that dual mechanisms preprogram meiotic gene expression in progenitor cells, defining unidirectional differentiation of spermatogenesis. On the other hand, our results indicate that active enhancers and postmeiotic gene expression are directly downstream of a DDR pathway specific to the sex chromosomes.

Finally, through genome-wide analyses, we revealed TFs that might bind mitotic and meiotic enhancers, as well as mitotic and meiotic SEs (Fig. 7). Among the factors we identified, the transcriptional repressor KDM5A (also known as RBP2 or JARID1A), which evinced the highest enrichment value for meiotic SEs, is of particular interest because KDM5A was originally implicated in tumorigenesis\textsuperscript{55}. Because many germline genes are expressed in many cancer types—so-called cancer/testis genes\textsuperscript{56}—it is interesting to consider that the regulation of meiotic SEs could, in turn, drive or otherwise regulate germline gene expression in various cancers.

In summary, our current study provides a framework to understand the regulation of gene expression during spermatogenesis. Because our study focuses on representative stages, it will be important to further dissect the complex and well-coordinated nature of spermatogenesis. Recent studies using single-cell analyses have revealed new details for the transcription factors of progressive cells types in human and mouse spermatogenesis\textsuperscript{57,58}. Such dynamism is achievable through the functional interplay of TFs and enhancers, as well as other regulatory elements. Indeed, more than a thousand TFs are differentially expressed in spermatogenesis\textsuperscript{59}. Of note, the testis has the largest number of specifically expressed TFs of all organs\textsuperscript{60}.

The systematic determination of germline cis-regulatory elements makes for a compelling future research direction. Furthermore, given the evolutionary divergent nature of piRNA loci in modern humans\textsuperscript{61}, investigation of the evolutionary aspect of meiotic SEs makes for a promising research direction too.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41594-020-0488-3.

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Methods

Animals. Mice were maintained and used according to the guidelines of the Institutional Animal Care and Use Committee (protocol no. IACUC/2018-0040) at Cincinnati Children's Hospital Medical Center. A-myb mutant (Mybl1repro9) mice, which carry A-myb null allele, were crossed with wild-type serumosum area (ESNU)–induced on the C57BL/6 background, have been reported previously. Scml2-KO mice on the C57BL/6 background have been reported previously.

Cell lines. Wild-type 11 male ESCs derived from male agouti 129S4/SvJae embryos have been described previously. The C10TRPSa ESC cell line was generated as described in ref. 41. Because these cells were easily distinguished based on colony morphologies, cell lines were authenticated by microscopical inspection. None of the cell lines have been used for mycoplasma contamination.

Cell culture. ESCs were cultured in ESC medium (15% FBS, 25 mM HEPES, 1× GlutaMAX, 1× MEM non-essential amino acids solution, 1x penicillin/streptomycin and 0.05 mM β-mercaptoethanol in DMEM high glucose (4.5 g l−1)) containing 2i (1 μM PD0325901 and 3 μM CHIR99021, both LC Laboratories) and LIF (1,300 U ml−1, in-house) on cell culture plates coated with 0.2% gelatin under feeder-free conditions. The expanded ESC colonies were dissociated using 0.25% trypsin–EDTA solution for passaging.

Germ cell fractionation. WT and Scml2-KO male mice on the C57BL/6 background (at least 12 independent mice at 90–120 days of age) were used for isolation of germ cells for ChIP-seq experiments.

PSs and RSs were isolated via BSA gravity sedimentation as previously described. Purity was confirmed by nuclear staining with Hoechst 33342 using fluorescence microscopy. In keeping with previous studies from the Namekawa laboratory, only fractions with a mean purity of ≥90% were used to generate next-generation sequencing datasets.

Spermatocytes were isolated as described previously and collected from C57BL/6 wild-type or Scml2-KO male mice aged 6–8 days. Testes were collected in a 24-well plate in DMEM supplemented with Glutamax (Thermo Fisher Scientific), non-essential amino acids (NEAA; Thermo Fisher Scientific) and penicillin/streptomycin (Thermo Fisher Scientific). After removing the tunica albuginea membrane, testes were digested with collagenase (1 mg ml−1) at 34 °C for 20 min to remove interstitial cells, then centrifuged at 188g for 5 min. Tubules were washed with the medium and then digested with trypsin (2.5 mg ml−1) at 34 °C for 20 min to obtain a single cell suspension. Cells were filtered with a 40-μm strainer to remove Sertoli cells, and the cell suspension was plated in a 24-well plate for 1 h in medium supplemented with 10% FBS, which promotes adhesion of remaining somatic cells. Cells were washed with magnetic cell-sorting (MACS) buffer (PBS supplemented with 0.5% BSA and 5 mM EDTA) and incubated with CD117 (KIT) MicroBeads (Miltenyi Biotec) on ice for 20 min. Cells were washed and resuspended with MACS buffer and filtered with a 40-μm strainer. Cells were separated by an autoMACS Pro Separator (Miltenyi Biotec) with the program ‘posse.’ Cells in the flow-through fraction were washed with MACS buffer and incubated with CD90.2 (THY1) MicroBeads (Miltenyi Biotec) on ice for 20 min. Cells were washed and resuspended with MACS buffer and filtered with a 40-μm strainer. Cells were separated by an autoMACS Pro Separator (Miltenyi Biotec) with the program ‘posse.’ Purity was confirmed by immunostaining.

Histology and immunofluorescence analyses. WT and A-myb mutant male mice on the C57BL/6 background (three independent mice, at 90–120 days of age) were used for histological analysis. For preparation of testicular paraffin blocks, testes were fixed with 4% paraformaldehyde overnight at 4 °C with gentle inverting. testes were fixed with 4% paraformaldehyde overnight at 4 °C with gentle inverting. One Histo (Nacalai) for 1 h at room temperature (RT) and then incubated with 5-µm sections. testes were fixed with 4% paraformaldehyde overnight at 4 °C with gentle inverting. The remaining testes were excised from mice and detunicated in 1× PBS for 5 min. Tubules were washed with MACS buffer and filtered with a 40-μm strainer. Cells were separated by an autoMACS Pro Separator (Miltenyi Biotec) with the program ‘posse.’ Cells in the flow-through fraction were washed with MACS buffer and incubated with CD90.2 (THY1) MicroBeads (Miltenyi Biotec) on ice for 20 min. Cells were washed and resuspended with MACS buffer and filtered with a 40-μm strainer. Cells were separated by an autoMACS Pro Separator (Miltenyi Biotec) with the program ‘posse.’ Purity was confirmed by immunostaining.

Preparation and immunofluorescence analysis of meiotic chromosome spreads. Meiotic chromosome spreads from testes were prepared as described in ref. 41. Briefly, testes were excised from mice and detunicated in 1× PBS. Seminiferous tubules were dissociated from whole testes, and approximately one-quarter of the tubules were transferred into 1 ml culture containing 1 ml hypotonic chromosome spread buffer (375 mM NaCl, 0.3% SDS, 0.1 μg ml−1 proteinase K) at 34 °C for 30 min, and then incubated at 65 °C for another 5 h to reverse crosslinking. DNA was purified with the MinElute Reaction Cleanup Kit (Qiagen) and amplified with NEBNext High-Fidelity 2× PCR Master Mix (NEB). Amplified DNA was purified by Agencourt AMPure XP (Beckman Coulter). Afterwards, DNA fragments in the 250– to 500-bp size range were selected by gel purification. DNA libraries were prepared using small numbers of A-myb mutant PSs and littermate WT PSs and described by Sakashita et al. (ref. 46).

Dna libraries were prepared through the ChIPmentation method. Briefly, beads were resuspended in 30 μl of the tagmentation reaction buffer (10 mM Tris-HCl pH 8.0 and 5 mM MgCl2) containing 1 μl Tagment DNA enzyme from the Nextera DNA Sample Prep Kit (Illumina) and incubated at 37 °C for 10 min in a thermal cycler. The beads were washed twice with 150 μl cold wash buffer 1, incubated with elution buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1× SDS, 0.1 M NaCl, 1% Triton X-100, 2 μl of 25% Chelex) for 10 min, washed with 3 M sodium acetate (pH 5.0), 1 volume of isopropanol, and centrifuged at 14,000 g for 1 min. The supernatant was aspirated, and the DNA was resuspended in 10 μl of 10 mM Tris-HCl pH 8.0 and sequenced with an Illumina HiSeq 2500 system.

Preparation and immunofluorescence analysis of meiotic chromosome spreads. Meiotic chromosome spreads from testes were prepared as described in ref. 41. Briefly, testes were excised from mice and detunicated in 1× PBS. Seminiferous tubules were dissociated from whole testes, and approximately one-quarter of the tubules were transferred into 1 ml culture containing 1 ml hypotonic chromosome spread buffer (375 mM NaCl, 0.3% SDS, 0.1 μg ml−1 proteinase K) at 34 °C for 30 min, and then incubated at 65 °C for another 5 h to reverse crosslinking. DNA was purified with the MinElute Reaction Cleanup Kit (Qiagen) and amplified with NEBNext High-Fidelity 2× PCR Master Mix (NEB). Amplified DNA was purified by Agencourt AMPure XP (Beckman Coulter). Afterwards, DNA fragments in the 250– to 500-bp size range were selected by gel purification. DNA libraries were prepared using small numbers of A-myb mutant PSs and littermate WT PSs and described by Sakashita et al. (ref. 46).

CRISPR activation. We performed functional evaluations of a representative SE locus adjacent to the Zif37 gene locus using the CRISPRa Rna-PolII activator system. We chose the Zif37 gene locus because A-MYB binding was not observed in the Zif37 promoter. To that end, we used CRISPOR tools to design four gRNAs for the locus at flanking regions of two A-MYB binding sites at the SE locus. The four gRNA sequences are as follows: Zif37-se_gRNA1, Zif37-se_gRNA2, Zif37-se_gRNA3, and Zif37-se_gRNA4.
GAU CGG UCG GGU CUG UGC; Zifp37-se_sgrNA_2; GCC GAC CCA GUC GUG UGC; Zifp37-se_sgrNA_3; UCC ACG UUU GGC UGC CC; Zifp37-se_sgrNA_4; ACU AAC AUU AGU GGA GCU GA. The gRNAs were used at 200 ng of A-MYB expression vector (PGK-A-MYB plasmid; established in ref. 1) was transfected with Lipofectamine 3000 transfection reagent (Thermo Fisher) following the manufacturer's instructions. A 240 ng sample of equimolar pooled sgrRNA was used. At day 2, 500 ng of A-MYB expression vector (PGK-A-MYB plasmid; established in ref. 1) was transfected with Lipofectamine 3000 transfection reagent (Thermo Fisher) following the manufacturer's instructions. The culture medium was exchanged with ESC medium without 2i and LIF, containing defined as ‘unique’ by the MAnorm algorithm; (2) defined as ‘common’ by the MAnorm algorithm and (2) raw read criteria: (1) absolute peak values

The total amounts of tag values in promoter or enhancer regions were calculated for 4-kb windows, promoter regions of genes (±2 kb surrounding TSSs) and enhancer regions. To normalize the tag value, read counts were multiplied with PBS. At day 4, the adherent cells in each well were lysed for RNA extraction. RNA extraction and RT-qPCR. Total RNA was isolated using an RNeasy Plus Mini Kit (Qiagen). First-strand cDNA synthesis was performed using 200 ng of total RNA with the SuperScript IV reverse transcriptase and oligo-dT (20) primer (Thermo Fisher) according to the manufacturer's instructions. Real-time PCR was performed using a StepOnePlus real-time PCR system (Applied Biosystems) with Fast SYBR Green Master Mix (Thermo Fisher) and specific primer sets (Zifp37-RT_Fw; ATACGAGACTTAGAAGCTGCAG; Zifp37-RT_Rv; TCAGACTACTTTGGCGCTTGCCT). Relative gene expression was quantified with the ΔΔCT method and normalized to Hprt expression.

ChIP-seq and RNA-seq data analysis. Data analysis for ChIP-seq was performed in the BioWadrobe Experiment Management System (https://biowadrobe.com/landing/). Briefly, reads were aligned to mouse genome mm10 with Bowtie (version 1.2.0), assigned to NCBI RefSeq genes or isoforms, and coverage was displayed on a local mirror of the UCSC genome browser. ChIP-seq peak calling was performed using MACS2 (version 2.1.20160309) with the default parameter setting for narrow peak detection in BioWadrobe. For the MACS2 analysis of H3K27ac, we permitted only peaks ±1 kb outside TSSs, that is, ‘distal peaks’, with a normalized enrichment value of ≥2. In this study, we refer to peaks ±1 kb outside TSSs as ‘distal peaks’ and peaks within ±2 kb of TSSs as ‘proximal peaks’. Pearson correlations for the genome-wide enrichment of H3K27ac peaks among ChIP-seq library replicates were analyzed using SeqMonk (Babraham Institute).

MAnorm, software designed for quantitative comparisons of ChIP-seq datasets, was used to compare the genome-wide ChIP-seq peaks among stages in spermatogenesis. Unique peaks were defined using the following criteria: (1) defined as ‘unique’ by the MAnorm algorithm; (2) P value <0.01; (3) raw counts of unique reads >10. Peaks common to two stages were defined using the following criteria: (1) defined as ‘common’ by the MAnorm algorithm and (2) raw read counts of both stages >10.

SEs were identified based on elevated H3K27ac enrichment in spermatogenesis using exactly the same criteria and algorithms as previously described149-150. The reads were normalized for each dataset before differential peak calling. We used normalized H3K27ac ChIP-seq data and MACS2 peak calls to rank all enhancers in spermatogenic cell type categories by increasing the total background (input)–subtracted ChIP-seq occupancy of H3K27ac; this was done by calling regions using the ROSE_main.py program157 with default parameters. We plotted the total background (input)–subtracted ChIP-seq occupancy of H3K27ac enhancers within 12.5 kb of each other were consolidated into a single entity. The visualized plot allowed us to define a clear inflection point to separate SEs in each cell type from all other enhancers. Enhancer entities enriched with an H3K27ac signal above this inflection point were defined as SEs. Average tag density profiles were calculated around TSSs for gene sets of somatic/progenitor genes, late spermatogenesis genes, constitutive active genes and constitutive inactive genes, as described previously4. Resulting graphs were smoothed in 200-bp windows. Enrichment levels for ChIP-seq experiments were calculated as 4 kb for low, promoter regions of genes (±2 kb surrounding TSSs) and enhancer regions. To normalize the tag value, read counts were multiplied by 1,000,000 and then divided by the total number of reads in each nucleotide position. The total amounts of tag values in promoter or enhancer regions were calculated as enrichment. The program ngs.plot was used to draw heatmaps for ChIP-seq read enrichment.

The k-means clustering of differential enhancer peaks was analyzed using Cluster 3.0 software. The results were further analyzed using JavaTreeview software7 to visualize as heatmaps.

MEME-ChIP was used for motif discovery as described in the text. For all motif analyses, we used only peak regions (±250 bp from the peak summit) outside of ±2 kb from (TSS/TS中) and a maximum of the lowest P values (P <0.01) via MAnorm analysis, and we extracted those sequences using the Table Browser.

HOMER motif analyses were performed using an expanded TF-binding motif library taken from the Cis-BP database. The HOMER software package24 was used for motif enrichment analyses using a customized version of HOMER that employs a log, scoring system and motifs contained in the Cis-BP motif database.

REL analysis was performed as described in ref. 8. In brief, genomic regions of interest (identified by HOMER motif analysis for wild-type PSs and RSs) were initially introduced in another study that analyzed the intersection of each dataset was calculated using REL.

Analyses of RNA-seq data were performed in the BioWadrobe Experiment Management System. The read counts were aligned by STAR alignment software (http://younglab.wi.mit.edu/software/STAR, 2.5.3a+) with default arguments except –outFilterMultimapNmax 1 and –outFilterMismatchNmax 2. The –outFilterMultimapNmax parameter was used to allow unique alignments only, and the –outFilterMismatchNmax parameter was used to allow a maximum of two errors. NCBI RefSeq annotation from the mm10 UCSC genome browser was used, and canonical TSSs (one TSS per gene) were analyzed. All reads from the resulting bam files were split into related isoforms with respect to RefSeq annotation. The expectation-maximization (EM) algorithm was then used to estimate the number of reads for each isoform. To detect differentially expressed genes between two biological samples, a read count output file was input to the DESeq2 package (version 1.16.1), then the program functions DESeqDataSetFromMatrix and DESeq were used to compare each gene's expression level between two biological samples. Differentially expressed genes were identified through binominal tests, thresholding Benjamini-Hochberg-adjusted P values to <0.01. To perform GO analyses, the functional annotation clustering tool in DAVID (version 6.8) was used and a background of all mouse genes was applied. Biological process term groups with a significance of P <0.05 (modified Fisher's exact test) were considered significant.

Statistics. Statistical methods and P values for each plot are listed in the figure legend and/or in the corresponding Methods section. In brief, all grouped data are represented as mean ± s.e.m. All box- and whisker plots are represented as: center lines, median; box limits, interquartile range (25th and 75th percentiles); whiskers, >90% of the data points (unless stated otherwise). Statistical significances for pairwise comparisons were determined using two-sided Mann–Whitney U-tests and unpaired t-tests. All quantitative analyses are represented as the mean ± s.e.m. of three biological replicates. Fisher exact test and hypergeometric test were used for detection of significantly enriched GO terms, genes and loci compared with backgrounds. Differentially expressed genes were determined in DESeq2 package. NGS data (RNA-seq and ChIP-seq) are based on two independent replicates. For all experiments, no statistical methods were used to predetermine sample size. Experiments were not randomized and investigator were not blinded to allocation during experiments and outcome assessments.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

Crosslinking H3K27ac ChIP-seq data reported in this study have been deposited at the Gene Expression Omnibus (GEO) under accession code GSE130652. H3K27ac native ChIP-seq data in WT and A-myb mutant PSs reported in this study are described in the accompanying study by Sakashita et al.46 and are deposited under accession code GSE142173. All other next-generation sequencing datasets used in this study are publicly available. RNA-seq data from THY1+ spermatogonia, PSs and RSs were downloaded from the GEO (accession no. GSE55060). ATAC-seq data from KIT+ spermatogonia and PSs were downloaded from the GEO (accession GSE102954). ChIP-seq data for A-MYB and RNA-seq data for A-myb mutant and control testes were downloaded from the GEO (accession no. GSE44690). ChIP-seq data for H3K4me3 and H3K4me2 and RNA-seq data from KIT+ spermatogonia were downloaded from the GEO (accession no. GSE89902). Although generated for and analyzed in this study, our H3K27ac ChIP-seq data for wild-type PSs and RSs were initially introduced in another study that analyzed active enhancers on the sex chromosomes2; ChIP-seq data for H3K27ac in wild-type PSs and RSs were downloaded from the GEO (GSE107398). ChIP-seq data for H3K27ac from embryonic stem cells were downloaded from the GEO (GSE29184). ChIP-seq data for H3K27ac from sperm were downloaded from the GEO (accession no. GSE79230). Source data are provided with this paper.

Code availability

Source code for all software and tools used in this study, with documentation, examples and additional information, is available at the following URLs: https://github.com/alexdobin/STAR (STAR RNA-seq aligner), http://younglab.wi.mit.edu/ super_enhancer_code.html (ROSE), https://crispor.tefor.net (CRISPOR), https://pypi.org/project/MACS2 (MACS2), https://bedtools.readthedocs.io/en/ latest/content/installation.html (BEDTools), https://bioconductor.org/packages/release/bioc/html/DESeq2.html (DESeq2), https://github.com/dfci/seqMonk (SeqMonk), https://github.com/shenlab-sinai/ngsplot (ngsplot), https://github.com/tydverse/ggplot2 (ggplot2), http://homer.ucsd.edu/homer (HOMER), http://great.stanford.edu/public/handle/GREAT), https://image.net/F[it][Downloads]
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Author contributions

S.M., A.S. and S.H.N. designed the study. S.M. performed crosslinking ChIP-seq experiments, and A.S. performed native ChIP-seq experiments. A.S. analyzed A-myb mutant mice with the help of K.T. A.S. and K.T. performed CRISPRa experiments. L.N. performed experiments with S.M. S.M., A.S., M.Y., X.C., K.G.A., M.T.W., A.B. and S.H.N. designed and interpreted the computational analyses. S.M., K.G.A. and S.H.N. wrote the manuscript with critical feedback from all other authors. S.M. and A.S. contributed equally to this work. S.H.N. supervised the project.

Competing interests

A.B. is a cofounder of Datirium, LLC.

Additional information

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Editor recognition statement Beth Moorefield was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.
Extended Data Fig. 1 | Biological replicates for H3K27ac ChIP-seq data. 

**a** Scatter plots show the reproducibility of H3K27ac ChIP-seq enrichment at individual peaks between biological replicates. Each peak was identified using MACS (P < 1x10^-5). H3K27ac ChIP-seq enrichment levels are shown in log2 RPKM values. The color scale indicates H3K27ac ChIP-seq peak density. Pearson correlation values (R) are shown. While generated for and analyzed in this study, our H3K27ac ChIP-seq data for wild-type PS and RS were initially introduced in another study that analyzed active enhancers on the male sex chromosomes (these data are adapted from Adams et al., PLOS Genet 2018). 

**b** Average tag density and heatmaps for H3K27ac ChIP-seq at proximal peak regions in KIT^+ and PS. Proximal peaks identified in Fig. 1d were used for this analysis.
Extended Data Fig. 2 | Identification of candidate TFs whose binding sites are enriched in meiotic SEs. Identification of candidate TFs whose binding sites are enriched in meiotic SEs. HOMER analyses identify 72 TFs that have binding motifs in meiotic SEs. Among the 72 TFs, 9 TFs are highly expressed in PS and RS (≥4-fold in comparison to their expression levels in spermatogonia).
Extended Data Fig. 3 | Spermatogenic phenotypes of A-myb mutant mice. a, Testis and epididymis sections from wild-type (WT) littermate control (left panels) and A-myb mutants (right panels) at 8 weeks of age. The sections were stained with hematoxylin and eosin. Scale bars: 200 μm. b, Testis sections from WT and A-myb mutant mice immunostained with antibodies raised against H1T. Scale bars: 200 μm. Numbers of H1T+ cells per seminiferous tubule as mean and whiskers, which indicate 25% (bottom) and 75% (top) of the data points from two independent littermate pairs (right panel). ***P < 0.001, unpaired t test. c, Chromosome spreads of wild-type and A-myb mutant PS immunostained with antibodies raised against SYCP3 and H3K27ac. Scale bars: 10 μm. Late pachytene spermatocytes were not detected (N.D.) in A-myb mutant samples. d, Scatter plot depicts relationship between A-MYB enrichments at promoters (TSS±1 kb) and dysregulation of meiotic SE-adjacent genes that were also differentially expressed in A-myb mutant testis (211 genes identified in Fig. 4d). Red line represents a regression line. R: Pearson correlation coefficient.
Extended Data Fig. 4 | Comparison of H3K27ac ChIP-seq enrichment between wild-type and Scml2-KO cells. a, MANorm analysis for H3K27ac peaks in THY1+ and KIT+ spermatogonia between wild-type and Scml2-KO. b, Track views of H3K27ac ChIP-seq enrichment on representative mitotic SEs in spermatogenesis. c, Average tag densities and heatmaps for H3K27ac ChIP-seq enrichment at genomic bivalent domains in PS and in RS.
Extended Data Fig. 5 | SCML2 binds to and regulates the resolution of mitotic SEs. a, Track views of SCML2 enrichment in GS cells and H3K27ac and H3K27me3 ChIP-seq enrichment at a representative mitotic SE in spermatogenesis. Light blue bars represent SCML2-binding sites with the peaks of H3K27ac in spermatogonia. After the mitosis-to-meiosis transition, SCML2 establishes H3K27me3 at these sites. b, MA norm analysis for SCML2 peaks in GS and THY1+ H3K27ac peaks. c, Pie charts represent the genomic-entity distributions of SCML2-H3K27ac-common distal peaks detected by MA norm. Meiotic SEs are shown apart from all other enhancers. d, Average tag densities and heatmaps for H3K27ac and H3K27me3 ChIP-seq signal at mitotic SEs that intersect with SCML2 peaks (n = 84). H3K27ac enrichment values: RPM.
Extended Data Fig. 6 | ChIP-seq enrichment at various genomic loci. 

**a** Enrichment of distal H3K27ac peaks around late spermatogenesis genes: 1,504 peaks

**b** Enrichment around TSSs
- Meiotic SE-adjacent late spermatogenesis genes: 652 genes
- Other late spermatogenesis genes: 1,971 genes

**c** Enrichment at distal H3K27ac peaks around RS-specific autosomal genes: 386 peaks

**d** Enrichment at distal H3K27ac peaks around RS-specific X-linked genes: 62 peaks

Box-and-whisker plots show the distributions of enrichment for ChIP-seq enrichment for the indicated genomic loci. Central bars represent medians, the boxes encompass 50% of the data points, and the whiskers indicate 90% of the data points.
Extended Data Fig. 7 | Enrichment of H3K4me2, H3K4me3, and H3K27me3 at the promoters of genes adjacent to SEs. Box-and-whisker plots show the distributions of ChIP-seq enrichment at TSSs ±2 kb for genes adjacent to SEs in spermatogenesis. Central bars represent medians, the boxes encompass 50% of the data points, and the whiskers indicate 90% of the data points.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- A description of all covariates tested
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Date were collected with GNU Wget and SRA Toolkit 'prefetch' from NCBI Gene Expression Omnibus (GEO).

Data analysis

Source code for all software and tools used in this study with documentation, examples and additional information, is available at following URLs:

- https://github.com/alexdoig/star/STAR RNA-seq aligner
- http://yougalab.wi.mit.edu/super_enhancer_code.html (ROSE)
- http://crrspor.tefor.net (CRISPOR)
- https://pypi.org/project/MAC52 (MAC52)
- https://bedtools.readthedocs.io/en/latest/content/installation.html (BEDTools)
- https://bioconductor.org/packages/release/bioc/html/DESeq2.html (DESeq2)
- https://david.ncifcrf.gov/summary.jsp (DAVID)
- https://www.bioinformatics.babraham.ac.uk/projects/seqmonk (SeqMonk)
- https://github.com/shenlab-sina/nextplot (nextplot)
- https://github.com/tidyverse/ggplot2 (ggplot2)
- http://home.ucsd.edu/home (HOMER)
- http://great.stanford.edu/public/html (GREAT)
- https://imagej.net/Fiji/Downloads (Fiji — Image)
- https://github.com/Weirauchlab/RELI (RELI)

Information for the BioWardrobe Experiment Management Platform, which is commercial software, is available at https://biowardrobe.com and https://github.com/Banski-lab/biowardrobe_hypergeometric p-value calculator (https://systems.crump.ucla.edu/hypergeometric/) was also used for hypergeometric probability tests.

For manuscripts utilizing custom algorithms or software that are not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All raw sequence data used in this study, including processed files for publication datasets, have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) as described in the method section.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences
☐ Behavioural & social sciences
☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical methods were used to predetermine sample sizes.

Data exclusions
No data were excluded from analyses.

Replication
We confirmed consistent results between three independent biological replicates for qPCR analysis. For Nextgen sequencing analysis, we obtain all biological or technical replicate samples from web databases (above mentioned), and confirmed consistent results between respective replicates. We also confirmed consistent results between two independent biological replicates for H3K27ac ChIP-seq experiments based on Pearson's correlation coefficient of each peak by SeqMonk.

Randomization
The experiments were not randomized.

Blinding
The experiments were not blinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a Involved in the study
☐ Antibodies
☒ Eukaryotic cell lines
☒ Palaeontology
☒ Animals and other organisms
☒ Human research participants
☒ Clinical data

Methods

n/a Involved in the study
☒ ChIP-seq
☐ Flow cytometry
☒ MRI-based neuroimaging

Antibodies

Antibodies used
Antibody against H3K27ac was obtained from Active Motif; 39133 and Abcam: ab4729.
Antibody against SYCP3 was obtained from Abcam; ab97672.
Antibody against H1T was provided by Dr. Mary Ann Handel (Jackson lab. Bar Harbor, ME, USA)

Validation
The antibodies used for ChIP-seq experiments was validated by the manufactures.
**Eukaryotic cell lines**

Policy information about cell lines

| Cell line source(s)                                                                 | Mouse J1 ES cells were obtained from Dr. Yuya Ogawa (Cincinnati Children’s Hospital Medical Center, Cincinnati, USA). CRISPRa ES cell lines have been generated in the Namekawa laboratory. |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Authentication                                                                      | Since these cells were easily distinguished based on colony morphologies, cell lines have been authenticated by microscopic inspection. CRISPRa ES cells were authenticated by the presence of functional transgenes (Details were described in Methods section). |
| Mycoplasma contamination                                                              | None of the cell lines used have been tested.                                                                                                                                                   |
| Commonly misidentified lines (See [ICLAC register](#))                                | Name any commonly misidentified cell lines used in the study and provide a rationale for their use.                                                                                        |

**Animals and other organisms**

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals                                                                 | Wild-type and Scml2-KO male mice on the C57BL/6J background (at least 12 independent mice at 90-120 days of age or at least 30 independent mice at 7 days of age) were used for isolation of germ cells for ChIP-seq experiments. Wild-type and A-myb mutant male mice on the C57BL/6J background (three independent mice, at 90-120 days of age) were used for histological analysis. |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Wild animals                                                                        | Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals. |
| Field-collected samples                                                             | For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field. |
| Ethics oversight                                                                    | Mice were maintained and used according to the guidelines of the Institutional Animal Care and Use Committee (protocol no. IACUC2018-0040) at Cincinnati Children’s Hospital Medical Center. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

**ChIP-seq**

**Data deposition**

- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

**Data access links**

| May remain private before publication. | H3K27ac ChIP-seq data reported in this study are deposited to the Gene Expression Omnibus (GEO) under the accession number GSE130652. Other RNA- and ChIP-seq data datasets used in this study were obtained from public databases. Accession codes for all raw sequencing files from databases were described in the method section. |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Files in database submission**

| Provide a list of all files available in the database submission. | Provide a link to an anonymized genome browser session for “Initial submission” and “Revised version” documents only, to enable peer review. Write “no longer applicable” for “Final submission” documents. |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Genome browser session (e.g. UCSC)**

| Provide a link to an anonymized genome browser session for “Initial submission” and “Revised version” documents only, to enable peer review. Write “no longer applicable” for “Final submission” documents. |

**Methodology**

| Replicates | Describe the experimental replicates, specifying number, type and replicate agreement. |
|------------|--------------------------------------------------------------------------------------|
| Sequencing depth | Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end. |
| Antibodies | Antibody against H3K27ac for ChIP-seq experiments was obtained from Active Motif; 39133. |
| Peak calling parameters | Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used. |
| Data quality | Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment. |
| Software | The Methods section of manuscript contains information on all procedures and softwares we used. |