

# Taf7l cooperates with Trf2 to regulate spermiogenesis

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TATA-binding protein (TBP)-associated factor 7l (*Taf7l*; a paralogue of *Taf7*) and TBP-related factor 2 (*Trf2*) are components of the core promoter complex required for gene/tissue-specific transcription of protein-coding genes by RNA polymerase II. Previous studies reported that *Taf7l* knockout (KO) mice exhibit structurally abnormal sperm, reduced sperm count, weakened motility, and compromised fertility. Here we find that continued backcrossing of *Taf7l*<sup>−/−</sup> mice from N5 to N9 produced KO males that are essentially sterile. Genome-wide expression profiling by mRNA-sequencing analysis of wild-type (WT) and *Taf7l*<sup>−/−</sup> (KO) testes revealed that *Taf7l* ablation impairs the expression of many postmeiotic spermatogenic-specific as well as metabolic genes. Importantly, histological analysis of testes revealed that *Taf7l*<sup>−/−</sup> mice develop postmeiotic arrest at the first stage of spermiogenesis, phenotypically similar to *Trf2*<sup>−/−</sup> mice, but distinct from *Taf4b*<sup>−/−</sup> mice. Indeed, we find that *Taf7l* and *Trf2* coregulate postmeiotic genes, but none of *Taf4b*-regulated germ stem cell genes in testes. Genome-wide ChIP-sequencing studies indicate that TAF7L binds to promoters of activated postmeiotic genes in testis. Moreover, biochemical studies show that TAF7L associates with TRF2 both in vitro and in testis, suggesting that TAF7L likely cooperates directly with TRF2 at promoters of a subset of postmeiotic genes to regulate spermiogenesis. Our findings thus provide a previously undescribed mechanism for cell-type-specific transcriptional control involving an interaction between a “nonprototypic” core promoter recognition factor (*Trf2*) and an orphan TAF subunit (*Taf7l*) in mammalian testis-specific gene transcription.

gene regulation | reproduction | RNA-seq | contraceptive medicine

Spermatogenesis is a cyclic process in which diploid spermatogonia differentiate into mature haploid spermatozoa. This process is mainly driven by two—pre- and postmeiotic—transcription waves that are tightly controlled by testis-specific transcription factors. During the premeiotic transcription phase, individual spermatogonia are committed to differentiating into primary spermatocytes that later undergo two meiotic divisions to generate haploid round spermatids connected by intercellular cytoplasmic bridges (1–3). During the postmeiotic transcription phase of spermiogenesis, haploid round spermatids are sculptured into the elongated shape of mature spermatozoa. These latter stages are accompanied by dramatic biochemical and morphological changes, including major remodeling of chromatin with protamines substituting for somatic histones to tightly pack DNA into the sperm nucleus.

Understanding the intricate mechanisms that control spermatogenesis has important implications for human health and reproduction. A key step in the regulation of spermatogenesis occurs at the level of transcription, starting with the use of distinct promoter elements (4) within uniquely reorganized chromatin (5) and driven by the action of several testis-specific transcription factors including cAMP-responsive element modulator (CREM) (6, 7) and the core promoter recognition factors required for global or gene-specific transcription such as testis-specific transcription factor IIA (TFIIAtau)/ALF (a paralogue of TFIIA) (8), TATA-binding protein (TBP)-associated factor 4b

(*Taf4b*; a homolog of *Taf4*) (9), TBP-related factor 2 (*Trf2*) (10, 11), and *Taf7l* (12, 13). For example, mice bearing mutant or deficient CREM showed decreased postmeiotic gene expression and defective spermiogenesis (14). Mice deficient in *Taf4b*, a testis-specific homolog of *Taf4*, are initially normal but undergo progressive germ-cell loss and become infertile by 3 mo of age with seminiferous tubules devoid of germ cells. *Taf4b* depletion blunted the expression of spermatogonial stem cell genes, indicating a critical role in maintenance of spermatogonial stem cells (9). The core promoter recognition factor *Trf2* is highly expressed in a finely regulated pattern in the mouse testis during spermatogenesis, and mice lacking *Trf2* are viable but sterile due to a complete arrest of late spermiogenesis with largely normal spermatogonia and spermatocytes (15, 16). *Taf7l*, originally identified in spermatogonia, is a testis- and adipose-specific X-chromosome gene (17). This orphan *Taf* is expressed throughout male germ-cell differentiation, while its intracellular localization is dynamically regulated from cytoplasm in spermatogonia to nucleus in late spermatogenesis (13). Early studies of *Taf7l*-deficient mice showed reduced fertility, abnormal sperm structure, low sperm count, and weakened motility (12). Although the spermatogenic defects of various mouse lines mentioned above have been extensively studied, the molecular mechanisms controlling testis-specific gene expression programs remain poorly understood. It has also remained unclear what, if any, functional relationship may exist between these testis-specific transcription factors, and potential cross-talk between these various regulators has remained elusive (15).

Here we report that backcrossed *Taf7l*<sup>−/−</sup> males from N5 to N9 leads to infertility and *Taf7l*<sup>−/−</sup> testes show obvious deficiencies

## Significance

TATA-binding protein (TBP)-associated factor 7l (*Taf7l*), *Taf4b*, and TBP-related factor 2 (*Trf2*) were found to be three important atypical testis-specific core promoter recognition factors. However, whether there was any functional cross-talk between them and at which stages of spermatogenesis remained uncharacterized. Here we report that *Taf7l*<sup>−/−</sup> becomes sterile rather than merely subfertile after backcrossing. Importantly, we found that *Taf7l* cooperates with *Trf2*, but not *Taf4b*, at late stages to direct the transcription of key spermatogenic genes. Our findings thus provide unique insights into the function of cell-type-specific *Tafs* and how two core promoter recognition factors mediate transcriptional control of spermiogenesis. The testis-specific role of *Taf7l* identifies this regulator as a potential target for male contraceptive intervention.

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The authors declare no conflict of interest.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo) (accession no. GSE50807).

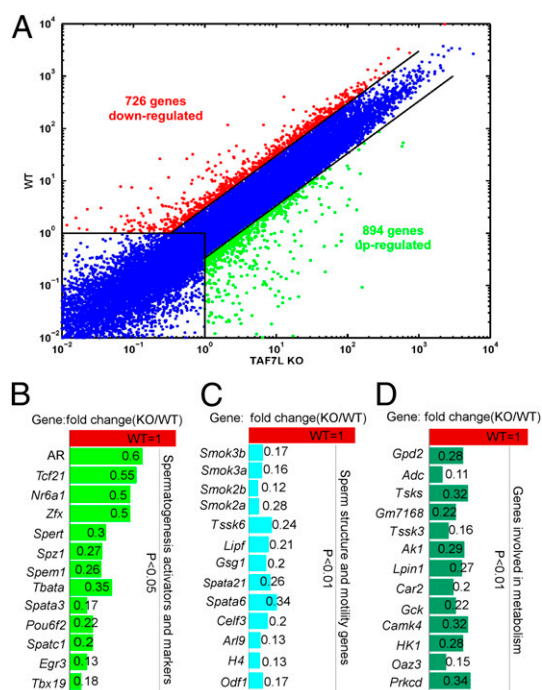
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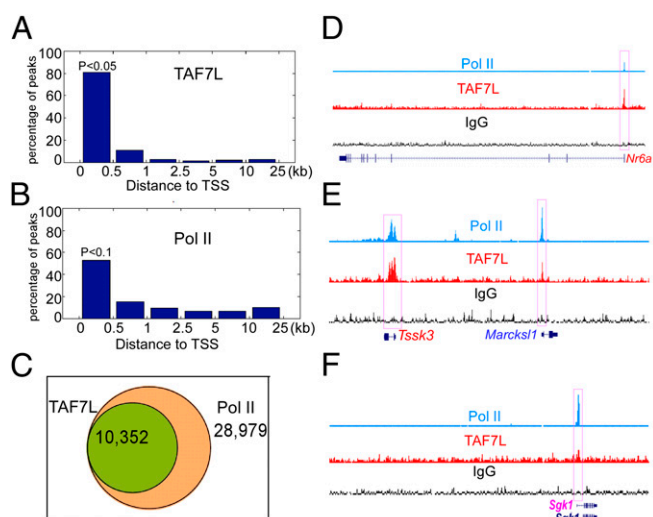




**Fig. 2.** *Taf7l* depletion dramatically deregulates testis gene expression by RNA-seq analysis. (A) Global gene expression of testis in *Taf7l*<sup>-/-</sup> (TAFL KO) (horizontal axis) and WT (vertical axis) mice. Red dots represent genes down-regulated above threefold (726 genes); green dots represent genes up-regulated above threefold (894 genes); blue dots represent genes with low expression and genes with unaltered expression. (B) Relative expression levels of representative spermatogenic activators and markers. (C) Relative expression levels of representative genes important for sperm structure and motility. (D) Relative expression levels of genes involved in metabolism. (B–D) Expression level of genes in WT testis were arbitrarily assigned a value of 1; their corresponding expression levels in KO testis are expressed relative to this value.

subgroups (i.e., the *Tssk3* gene that is involved in metabolism and sperm motility). One prominent group of genes dependent on *Taf7l* represents well-documented spermatogenic activators and markers such as *AR*, *Zfx*, *Spz1*, and *Spem1* (22–26). A second group of *Taf7l*-regulated genes [*Odf1*, testis-specific serine kinase 6 (*Tssk6*), sperm motility kinase 2a/b (*Smok2a/b*), and *Smok3a/b*] have been implicated in sperm structure and motility during late stages of spermatogenesis (Fig. 2 B and C) (27–30). Additionally, a group of genes that have generally been classified as ones involved in metabolism are significantly down-regulated in the testis upon loss of *Taf7l* and likely reflect both metabolic and spermatogenesis functions. These genes include the *Tssk3* or *Tsk3* testis-specific kinases (31, 32), as well as *Camk4*, a protein kinase involved in phosphorylating protamines (33); *Hk1* and *GCK* hexokinases involved in sperm glycolysis linked to motility (34); and *Oaz3* (ornithine decarboxylase antizyme) that controls polyamines required for proper sperm formation (35, 36) (Fig. 2D). To confirm the RNA-seq results, we carried out quantitative RT-PCR (RT-qPCR) on a handful of genes selected from these three representative classes (Fig. S3), providing additional evidence that *Taf7l* regulates a subset of spermatogenic and metabolic genes in testis. These findings also provide some insight into the relationship between metabolism and reproduction that has been reported in the study of various animal models (37–40) linking dysregulated metabolism with sterility. Our genome-wide expression data thus point to the likely involvement of *Taf7l* in regulating both metabolic and spermatogenesis genes in testis as being at least partly responsible for the observed infertility.

**TAF7L Binds to Promoters of Target Genes in Testis.** Given that hundreds of genes are down- or up-regulated by threefold or more in *Taf7l*<sup>-/-</sup> testis, we next set out to determine whether TAF7L directly binds to the promoters and/or enhancers of those spermatogenic and metabolic genes in testis. To this end, we carried out ChIP-seq (ChIP-seq) mapping of TAF7L-binding sites in WT testes, using RNA polymerase II (Pol II) binding sites as positive controls to mark the actively transcribed genes and IgG as negative controls. First, mapped ChIP tags from deep sequencing using Bowtie were analyzed by intersecting MACS and Grizzly Peak algorithms to identify binding regions for TAF7L and Pol II (41, 42). This analysis identified 28,979 significant peaks for Pol II, 10,352 significant peaks for TAF7L (Fig. 3C), and no significant peaks for IgG control. Next, we analyzed the distances of the TAF7L binding peaks to transcription start sites (TSS) and found that 95% of TAF7L peaks are within 1 kb of a TSS. Colocalization analysis between TAF7L and Pol II peaks revealed that almost all of the TAF7L peaks overlapped with Pol II peaks (Fig. 3 A and B), suggesting that TAF7L largely associates with the promoters of actively transcribed genes in the testis. In contrast to the *Taf7l*-dependent genes in testis, our direct binding data revealed that nearly all of the genes up-regulated by the loss of *Taf7l* in the testis (Fig. S1) likely resulted from indirect secondary effects of *Taf7l* depletion. We found that many down-regulated spermatogenic and metabolic genes identified by RNA-seq analysis bear direct TAF7L binding sites at or near their promoters. A few representative gene loci such as nuclear receptor subfamily 6, group A, member 1 (*Nr6a1*), *Tssk3*, and serine/threonine-protein kinase 1 (*Sgk1*) clearly show that TAF7L colocalizes with Pol II at promoter sites (Fig. 3 D–F). ChIP-qPCR analysis using WT and *Taf7l*-null testes confirmed that Pol II binding becomes dramatically diminished in *Taf7l*-null testes at target promoters (*Nr6a1*, testis-specific serine kinase 3 (*Tssk3*), *Sgk1*, protamine 1/2 (*Pm1/2*), and *Fscn3*), but not at a control actin intron (Fig. S4), suggesting that the presence of TAF7L is indeed critical for the proper expression of target genes in testis. Together these data suggest that TAF7L directly binds to gene promoters and that this binding is required for the expression of spermatogenic and metabolic genes in testis.



**Fig. 3.** ChIP-seq analysis identifies TAF7L and Pol II binding sites in testis. (A and B) Percentage of TAF7L (A) and Pol II (B) binding peaks vs. the distance of the peaks to transcription start sites (TSS) on genome-wide scale in testis. (C) Overlapping between TAF7L and Pol II binding peaks in testis. (D–F) Read accumulation of Pol II and TAF7L were shown on the *Nr6a1* (D), *Tssk3* (E), and *Sgk1* (F) gene loci. Vertical axis is 0–500 reads for each factor; colocalized peaks are marked with red solid boxes. IgG served as negative control for ChIP-seq analysis.



**Taf7l<sup>-/-</sup> Resembles Trf2<sup>-/-</sup> Mice and Targets Similar Postmeiotic Genes.** Having found that TAF7L binds to a specific subset of promoter regions of genes involved in spermatogenesis, we next set out to assess the possibility that *Taf7l* associates with other testis-specific core promoter recognition factors such as *Taf4b* and/or *Trf2* to work combinatorially to target genes directing spermatogenesis. First, we examined the spermatogenic defects of *Taf7l<sup>-/-</sup>* mice relative to *Taf4b<sup>-/-</sup>* and *Trf2<sup>-/-</sup>* mice. H&E staining of testes from 3-mo-old WT and *Taf7l<sup>-/-</sup>* (KO) mice revealed dramatically decreased elongated spermatids (Fig. 4A). The phenotype was highly reminiscent of *Trf2<sup>-/-</sup>* animals, but quite distinct from *Taf4b<sup>-/-</sup>* mice that showed defects in the maintenance of germ stem cells (9, 16). Next, we compared the target genes regulated by *Taf7l*, *Taf4b*, and *Trf2* in testis identified by RNA-seq (9, 16). As shown in Fig. S5, *Taf7l* and *Taf4b* appear to regulate largely nonoverlapping sets of genes expressed in the testis. For example, *GDNF*, stimulated by retinoic acid 8 (*Stra8*), *Stag3*, and disrupted meiotic cDNA 1 (*Dmc1*) are robustly down-regulated by the loss of *Taf4b* but are slightly increased in *Taf7l<sup>-/-</sup>* testes, suggesting that *Taf7l* is not required for maintenance of male germ stem cells. Instead, it appears that loss of *Taf7l* blocks spermatogenesis at a postmeiotic stage and that *Taf7l<sup>-/-</sup>* testes may actually accumulate more stem and meiotic cells (Fig. 4A and B).

We also examined the influence of *Taf7l* depletion on the expression of TFIID subunits by RNA-seq and RT-qPCR analysis and found that, except for *Taf7l* itself, all of the other TAFs were largely unaltered or slightly up-regulated (Fig. S6). These findings suggest that *Taf7l* is a “nonprototypical” testis-specific TAF subunit whose absence does not influence overall levels of TFIID. Strikingly, however, loss of *Taf7l*—like the loss of *Trf2*—down-regulates the same subset of genes directing spermatogenesis. For example, *Taf7l* does not influence the transcription of premeiotic genes such as *Proacrosin*, *H1.2*, and *Hsp70-2* but dramatically down-regulates *Trf2*-regulated postmeiotic genes

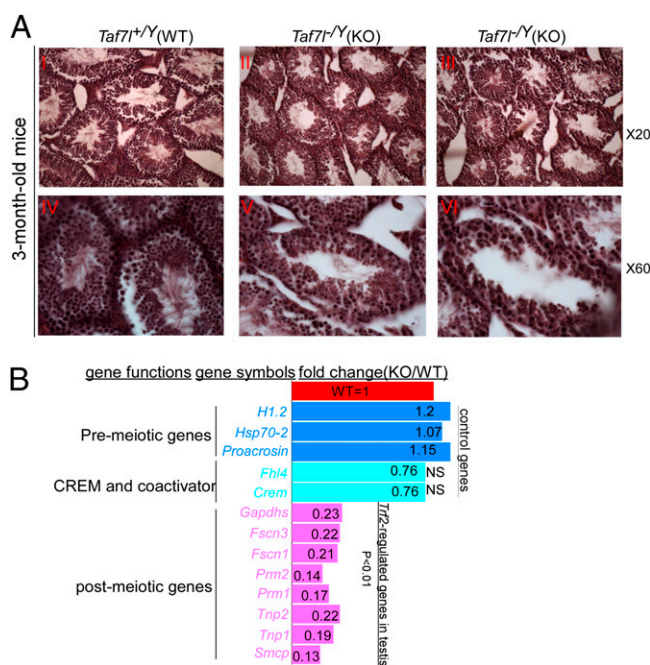
such as *Gapdh*s, transition proteins 1/2 (*Tnp1/2*), *Prm1/2*, *Fscn1/2*, and *Smcp* (Fig. 4B). This requirement for both *Taf7l* and *Trf2* for proper expression of postmeiotic genes is also consistent with the spermiogenesis defects observed in both *Taf7l<sup>-/-</sup>* and *Trf2<sup>-/-</sup>* testes (Fig. 4A). These findings suggest that *Taf7l* likely operates together with *Trf2*, but not *Taf4b*, to control postmeiotic genes, as deduced from our RNA-seq (Fig. 4B and Fig. S5), RT-qPCR (Fig. S7), and ChIP-seq analysis (Fig. 3 and Fig. S4). We found, for instance, that *Taf7l* binds efficiently to the TSS regions of *Trf2*-regulated gene *Gapdh*, but not at the promoter regions of *Taf4b*-regulated genes such as *Stra8* and *Dmc1* (Fig. S8).

We also assessed the expression levels and nuclear localization patterns of *Taf4b*, *Trf2*, and *Taf7l* during spermatogenesis to probe potential direct crosstalk between these factors based on previous studies. *Taf4b* is highly expressed in gonocytes, nuclei of spermatogonia, and spermatids, but not at other stages of spermatogenesis or in somatic cells (9). By contrast, *Taf7l* is expressed in germ cells, not testis somatic cells, during most stages of spermatogenesis, but it remains cytoplasmically localized in spermatogonia, and its expression becomes silenced at meiotic stages due to sex-chromosome inactivation (MSCI). *Taf7l* transcription becomes reactivated and translocated into nucleus of spermatids (13, 17) at a stage when *Trf2* is also specifically highly expressed (11, 20). Together, losses of *Taf7l* and *Trf2* exhibit a similar spermiogenesis deficiency phenotype; they appear in the nuclei of postmeiotic spermatids at similar times, bind to core promoter elements in an overlapping subset of genes, and regulate their expression in the testis. These findings raise the possibility that *Taf7l* and *Trf2* may actually work together in regulating spermatogenesis.

**Taf7l and Trf2 Coregulate a Subset of Postmeiotic Genes.** To further explore the possibility of interaction between TAF7L and TRF2 in regulating postmeiotic gene expression during spermatogenesis, we used FLAG-tagged TAF7L or TAF7 to co-IP with HA-tagged TRF2 coexpressed in 293T cells. These co-IP experiments showed that TAF7L could efficiently pull down TRF2 and vice versa (Fig. 5A); by contrast, TAF7 (a paralogue of TAF7L) was unable to coimmunoprecipitate TRF2 (Fig. 5B). To confirm that the association between TAF7L and TRF2 is directed by protein: protein interactions and not mediated via indirect DNA/chromatin interactions, we included benzonase treatment in our co-IP assays. Eliminating DNA in these co-IP experiments did not alter the binding interactions we observed between TAF7L and TRF2. Next, we used affinity-purified TAF7L antibody to coimmunoprecipitate endogenous TRF2 in WT and *Taf7l<sup>-/-</sup>* testes. The results showed that TAF7L could pull down endogenous TRF2 from WT testes, but not from *Taf7l<sup>-/-</sup>* testes (Fig. 5C), suggesting that TAF7L is associated with TRF2 in testes. The association between TAF7L and TRF2 in vitro and in vivo prompted us to explore further the relationship between TAF7L and TRF2 or TBP in testes. A previous study reported that TBP could be an interacting partner of TAF7L in testes (12). For these experiments, we used TAF7L and TBP antibodies to coimmunoprecipitate each other in WT and *Taf7l<sup>-/-</sup>* testes and found that only a small portion of TAF7L weakly associated with TBP in mouse testes (Fig. 5D). A direct comparison of TRF2 and TBP signals coimmunoprecipitated by TAF7L (Fig. 5C and D) confirmed that TAF7L associates more efficiently with TRF2 than with TBP in mouse testes when all three proteins are present. These studies suggest that two atypical testis-specific core promoter recognition factors, TAF7L and TRF2, likely work in concert to regulate a subset of postmeiotic genes required for spermiogenesis. Ablation of either factor results in a similar blockade of spermiogenesis, leading to male infertility.

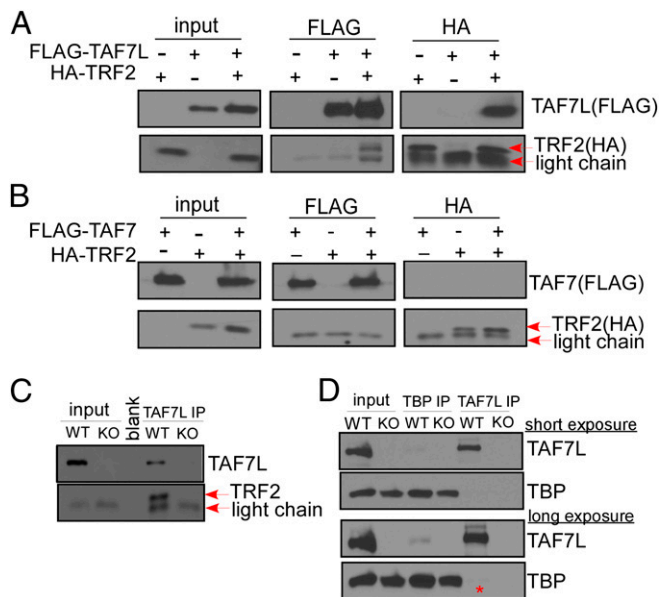
## Discussion

Cell-type-specific transcription is a key driver of tissue and organ formation during embryonic development. These complex



**Fig. 4.** *Taf7l* depletion blocks spermiogenesis and impairs postmeiotic gene expression in a similar way to *Trf2<sup>-/-</sup>* in testis. (A) H&E staining on 3-mo-old WT and KO testes at magnification  $\times 200$  (I–III) and magnification  $\times 600$  (IV–VI). (B) Expression of *Trf2*-regulated postmeiotic and control genes in *Taf7l<sup>-/-</sup>* testis was compared with WT testis from mRNA-seq analysis. Expression levels of genes in WT testis were arbitrarily assigned a value of 1, and their corresponding expression levels in KO testis are expressed relative to this value. NS, not significant.





**Fig. 5.** TAF7L associates with TRF2 both in vitro and in vivo. (A) FLAG-TAF7L and HA-TRF2 were overexpressed in 293T cells, and IPs were performed on both FLAG and HA antibodies, followed by Western blotting analysis with FLAG and HA antibodies. (B) The same procedures were performed on FLAG-TAF7 and HA-TRF2. (C) IPs were performed on WT and *Taf7l*<sup>-/-</sup> testis lysates with TAF7L antibody and followed by Western blotting with TAF7L and TRF2 antibodies. (D) IPs were performed on WT and *Taf7l*<sup>-/-</sup> testis lysates with either TAF7L or TBP antibody, followed by Western blotting with TAF7L and TBP antibodies. Both images are from the same Western blots with short (*Upper*) and long (*Lower*) exposure time. Red star marks the TBP signal from TAF7L IP.

expression networks are controlled by tissue-specific enhancer/promoter binding factors as well as core promoter recognition factors including *Tafs*, mediators, and TRFs. In several previous studies, *Taf7l* and *Trf2* have separately been found to function as testis-specific transcription factors. In this study, we provide unique evidence suggesting that these two atypical core promoter recognition factors work together to drive testis-specific gene expression.

Our model proposes that during the postmeiotic wave of transcription that occurs at the spermatogenesis–spermiogenesis transition, *Taf7l* is reactivated from MSC1 and translocated into the nuclei of pachytene/round spermatids where *Trf2* is highly expressed. We postulate that a testis-specific transcription pre-initiation complex containing both TAF7L and TRF2 is formed and targeted to a subset of “TATA-less” promoters to regulate postmeiotic *Trf2*-dependent gene expression (43–45) (Fig. S9). At the same time, prototypic TBP-containing core promoter recognition complexes can operate to direct TBP-dependent housekeeping genes and possibly some testes-specific genes. TAF7L, TRF2, and TBP all seem to be required for spermatogenesis, and each may provide some nonredundant testis-selective transcription function. Previous studies found that TRF2 can associate with multiple proteins to form a complex of >500 kDa (10). It will be interesting to see whether there is a TRF2–TAF complex of similar size in testes and eventually identify the other associated factors to more fully dissect the molecular mechanisms of *Taf7l*- and *Trf2*-driven testis-specific transcription. It will also be interesting to test whether, beyond its roles in testis-specific transcription control, *Taf7l* is required for maintaining chromatin architecture in round spermatids.

Given that *Taf7l* is highly expressed in germ cells, but not somatic cells in testis, we analyzed the global influence of *Taf7l* depletion in the whole testis. As a result, both up- and down-regulated genes found in this study by RNA-seq or RT-qPCR

were probably the direct result of *Taf7l* KO. It is also likely that diminished elongated spermatids observed are due to blockade of spermiogenesis upon loss of *Taf7l*. Although our ChIP-seq data supported a role for TAF7L in regulating testis-specific genes by direct binding to their promoters, future studies with stage-specific fractionated germ cells will be helpful to elucidate a more detailed molecular mechanism.

We recently reported that TAF7L associates with the adipocyte-specific transcription factor PPAR $\gamma$  during adipogenesis (21). Here in the context of testis-specific transcription, TAF7L teams up with TRF2, another testis-specific core promoter recognition factor, to direct male sperm formation. The association of an orphan TAF and a TRF is reminiscent of the TRF3/TAF3 scenario reported for myogenesis (46). These studies, taken in aggregate, suggest that various cell lineages take advantage of diversified core promoter factors in a combinatorial fashion to regulate tissue-specific programs of transcription.

Because of adverse side effects of female contraceptive medicines and the lack of male ones, an effective and safe therapeutic target has become a focus in the male germ-cell research field. Thus far, hundreds of genes have been found to influence spermatogenesis when mutated or depleted, including core promoter recognition factors *Trf2* and *Taf4b*, leptin *ob* and leptin receptor *db* genes, and other genes such as *Daz* and *Ddx4* (38, 47–49). However, most of these genes have relatively high expression levels and function in tissue or organs other than testes. Therefore, their depletion or inhibition often results in side effects and unacceptable complications that can range from minor to severe. For example, depletion of the *ob/db* gene results in obesity and diabetes (38). By contrast, *Taf7l* is more highly expressed in testis than all other tissues that were sampled, including adult white adipose tissue, and, not surprisingly, depletion of *Taf7l* causes >98% male infertility in mice. Most importantly, ablation of *Taf7l* leaves most of the male germ stem cells intact, suggesting that the effect of contraceptives targeting *Taf7l* will likely be reversible upon removal of the treatment. It is also interesting to note that *Taf7l* is an X-chromosome-linked gene; males carry only a single copy, and therefore the chances of introducing random mutations in *Taf7l* is twice as high as autosomal regulatory genes such as *Taf4b* and *Trf2*. These characteristics together may explain why multiple *Taf7l* mutations have been found in human infertile patients with oligozoospermia (low concentration of sperm). Our mouse studies thus may also help identify a potential candidate target gene affecting human male infertility.

## Materials and Methods

**Vectors and Plasmids.** *Taf7l*, *Taf7*, and *Trf2* full-length cDNAs were cloned into the pCS2+ vector with either HA or FLAG tag at their N terminus.

**RNA Isolation and Real-Time PCR Analysis.** A complete description of RNA isolation and real-time PCR analysis is provided in [SI Materials and Methods](#).

**Western Blot Analysis and IP.** A complete description of Western blot analysis and IP is provided in [SI Materials and Methods](#).

**Animal Care and Permission.** *Taf7l*-KO mice were generated previously (12). All animal experiments were performed in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health (50). All of the animals were handled according to approved animal use protocols (no. R007) by the Animal Care and Use Committee of the University of California, Berkeley.

**Animals Reproduction Capability Analysis.** Fifteen mating cages of three mating conditions were used as follows: WT males (*Taf7l*<sup>+/Y</sup>) with heterozygous *Taf7l*<sup>+/-</sup> females and *Taf7l*-null (*Taf7l*<sup>-/-</sup>) males with either *Taf7l*<sup>+/-</sup> or WT females were set up for over 7 mo. Males were started at 4 wk of age, and females were started at 6 wk of age in all of the mating cages, and mating cage numbers, total progeny produced, and total litter production times were recorded for 5 mo.



**ChIP, ChIP Library Preparation, and Deep Sequencing (ChIP-seq).** WT and *Taf7l* KO testis tissue from 10 mice at 3 mo of age was homogenized, followed by fixation with 1% (vol/vol) formaldehyde for 15 min at room temperature, 0.125 M glycine to stop the cross-linking for an additional 5 min, and collection of tissue by spinning down. The following steps are described in *SI Materials and Methods*.

**Mapping Sequencing Reads and Peak Calling Methods.** A complete description of sequencing reads mapping and peak calling methods is provided in *SI Materials and Methods*.

**mRNA-seq Library Preparation, Deep Sequencing, and Data Analysis.** A complete description of mRNA-seq library preparation, deep sequencing, and data analysis is provided in *SI Materials and Methods*.

**Data Availability.** Raw sequencing reads are available from the National Center for Biotechnology Information's GEO database ([www.ncbi.nlm.nih.gov/geo/](http://www.ncbi.nlm.nih.gov/geo/)) under accession no. [GSE50807](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE50807).

Additional materials and methods are described in *SI Materials and Methods*.

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