Genes Genet. Syst. (2022) **97**, p. 27–39

MEIOSIN directs initiation of meiosis and subsequent meiotic prophase program during spermatogenesis

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(Received 12 July 2021, accepted 14 September 2021; J-STAGE Advance published date: 25 December 2021)

Meiosis is a crucial process for spermatogenesis and oogenesis. Initiation of meiosis coincides with spermatocyte differentiation and is followed by meiotic prophase, a prolonged G2 phase that ensures the completion of numerous meiosis-specific chromosome events. During meiotic prophase, chromosomes are organized into axis-loop structures, which underlie meiosis-specific events such as meiotic recombination and homolog synapsis. In spermatocytes, meiotic prophase is accompanied by robust alterations of gene expression programs and chromatin status for subsequent sperm production. The mechanisms regulating meiotic initiation and subsequent meiotic prophase programs are enigmatic. Recently, we discovered MEIOSIN (Meiosis initiator), a DNA-binding protein that directs the switch from mitosis to meiosis. This review mainly focuses on how MEIOSIN is involved in meiotic initiation and the meiotic prophase program during spermatogenesis. Further, we discuss the downstream genes activated by MEIOSIN, which are crucial for meiotic prophase-specific events, from the viewpoint of chromosome dynamics and the gene expression program.

Key words: spermatogenesis, meiosis, chromosome, cohesin, gene expression

INTRODUCTION

During spermatogenesis, initiation of meiosis is one of the most noteworthy events that coincides with spermatocyte differentiation. Meiosis is a specialized cell cycle that produces haploid gametes from diploid cells. Meiotic entry occurs concomitantly with pre-meiotic S phase and is followed by meiotic prophase. Meiotic prophase is equivalent to G2 phase, but is prolonged to ensure the completion of numerous meiosis-specific chromosome events. During meiotic prophase, chromosomes are reorganized into axis-loop structures, which provide the structural framework for meiosis-specific events. Homologous chromosomes (homologs) then undergo pairing, synapsis and meiotic recombination, yielding crossovers called chiasmata that are physical linkages between the homologs (Baudat et al., 2013; Keeney et al., 2014; Zickler and Kleckner, 2015; Cahoon and Hawley, 2016). During these processes, chromosomes undergo dynamic movement to facilitate homolog pairing and synapsis, driven by telomeres attached to the nuclear envelope (Shibuya and Watanabe, 2014). In this way, the chromosome architecture and dynamics during meiotic prophase are markedly different from those in mitosis.

Completion of meiotic prophase is regulated by sexually dimorphic mechanisms, so the transcription and chromatin status are altered in the subsequent post-meiotic developmental program for sperm production and oocyte arrest/maturation. In spermatocytes, the completion of meiotic prophase is monitored under several layers of regulation such as the pachytene checkpoint and meiotic sex chromosome inactivation (Burgoyne et al., 2009; Ichijima et al., 2012; Turner, 2015). Male meiotic prophase is accompanied by robust alterations of gene expression programs (Schultz et al., 2003; Shima et al., 2004; Namekawa et al., 2006; Green et al., 2018; Grive et al., 2019) and epigenetic status (Kota and Feil, 2010; Sin et al., 2015; Maezawa et al., 2020) for post-meiotic spermiogenesis. However, the precise mechanisms by which this gene expression and epigenetic status are controlled under the meiotic prophase program is unknown.

Edited by Kenji Ichiyanagi
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DOI: https://doi.org/10.1266/ggs.21-00054

It has been a longstanding enigma what triggers the initiation of meiosis upon spermatocyte differentiation, and activates the subsequent meiotic prophase program. Recently, we discovered MEIOSIN, which directs the switching from mitosis to meiosis (Ishiguro et al., 2020). This review mainly focuses on the mechanism of meiotic initiation and the downstream meiotic prophase-specific events controlled by MEIOSIN.

INITIATION OF MEIOSIS DURING SPERMATOCYTE DIFFERENTIATION

Meiotic initiation coincides with spermatocyte differentiation. In mouse, retinoic acid (RA) and BMP signaling, at least in oocytes, synergistically induce meiotic transcription (Bowles et al., 2006; Koubova et al., 2006; Miyauchi et al., 2017; Nagaoka et al., 2020). Upon stimulation by RA, STRA8 (stimulated by retinoic acid gene 8) is transiently expressed in postnatal testis prior to the entry into meiosis (Oulad-Abdelghani et al., 1996) (Fig. 1). Since *Stra8* knockout (KO) germ cells fail to undergo normal meiosis in male and female (Baltus et al., 2006; Anderson et al., 2008; Mark et al., 2008; Dokshin et al., 2013), it has been assumed that STRA8 plays an essential role in the progression of meiosis. However, whereas *Stra8* KO germ cells fail to undergo meiosis in the C57BL/6 background (Baltus et al., 2006; Anderson et al., 2008), those in a mixed genetic background initiate but fail to complete meiotic prophase I (Mark et al., 2008). Thus, whether STRA8 is involved in the initiation of meiosis depends on genetic background. Furthermore, STRA8 is also transiently expressed in differentiating spermatogonia long before their differentiation into spermatocytes (Mark et al., 2008; Zhou et al., 2008a, 2008b; Endo et al., 2015) (Fig. 1). This indicates that *Stra8* expression alone is not sufficient for the induction of meiosis in spermatogonia, which raises the question of why meiosis is induced only in pre-leptotene spermatocytes, the phase of the second expression of STRA8, but not in spermatogonia despite the expression of STRA8. Thus, whether STRA8 is indeed required for meiotic initiation has been controversial.

By a proteomic approach, we screened STRA8-interacting factors from mouse testes and identified MEIOSIN (Meiosis initiator), which was encoded by a hypothetical gene, *Gm4969* (Ishiguro et al., 2020). As the gene information and the exon–intron prediction for *Gm4969* in the database were yet to be correctly annotated, even extensive studies in the field with transcriptome approaches may have failed to recognize *Gm4969*. MEIOSIN protein possesses basic helix–loop–helix (HLH) and high mobility group (HMG) box domains, implying a role as a DNA-binding protein (Fig. 2A). *Meiosin* mRNA showed a specific expression pattern in adult testis and embryonic ovary. Indeed, MEIOSIN and STRA8 were transiently co-expressed in pre-leptotene stage spermatocytes (Fig. 2B, 2C), which coincides with the S phase prior to meiotic prophase.

Whereas STRA8 is expressed both in differentiating spermatogonia and in pre-leptotene spermatocytes (Fig. 2C), expression of MEIOSIN is restricted to pre-leptotene spermatocytes, despite the existence of a RA response element in the 5′ region upstream of the *Meiosin* locus. It has been proposed that DMRT1 prevents premature

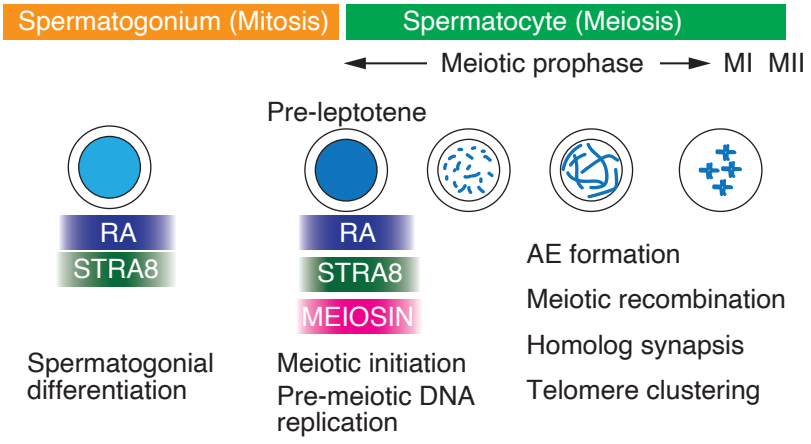


Fig. 1. MEIOSIN and STRA8 expression during spermatogenesis. Schematic illustration of spermatogenesis. The periods of STRA8 and MEIOSIN protein expression and retinoic acid (RA) execution are shown along the developmental stages. STRA8 protein is expressed in differentiating spermatogonia and in pre-leptotene spermatocytes. MEIOSIN protein is expressed at the transition toward meiotic initiation in pre-leptotene spermatocytes. The pre-leptotene stage is defined as the time point that coincides with the S phase starting shortly before meiotic prophase. RA is secreted from Sertoli cells in the stage VII–VIII seminiferous tubules, which contain differentiating spermatogonia and pre-leptotene spermatocytes. AE: axial element.

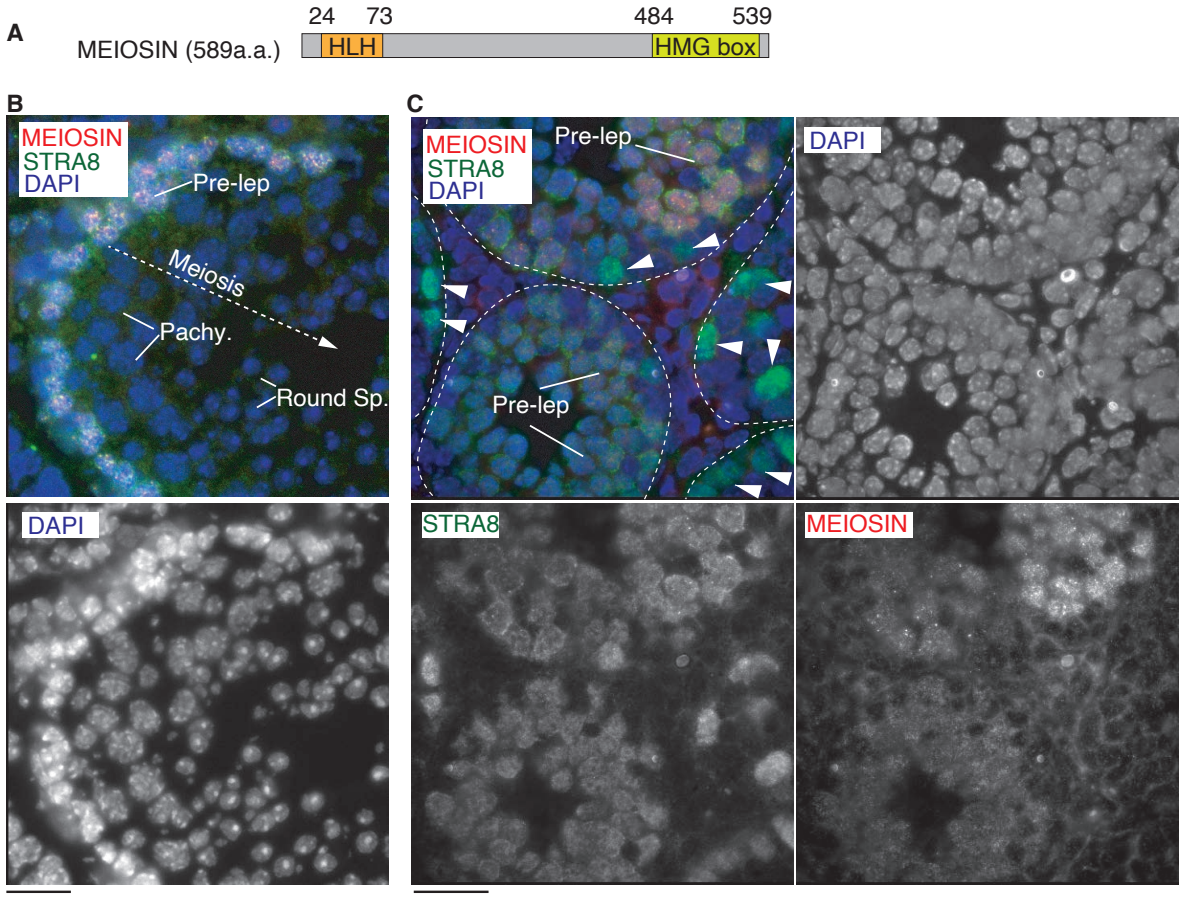


Fig. 2. MEIOSIN is a STRA8-binding factor. (A) Schematic illustration of the domains in mouse MEIOSIN protein. (B) Seminiferous tubule sections from adult WT mouse testis (eight-week-old) were stained as indicated. STRA8 and MEIOSIN proteins are co-expressed in the pre-leptotene nuclei. Pre-lep: pre-leptotene spermatocyte. Pachy: pachytene spermatocyte. Round Sp.: round spermatid. (C) WT neonatal male mice were subjected to consecutive injection of the RA synthesis inhibitor WIN 18,446 from postnatal day 5 (P5) to P11, followed by RA injection at P12 to enrich pre-leptotene spermatocytes. Seminiferous tubule sections at P13 were stained as indicated. Pre-lep: pre-leptotene spermatocyte. Arrowheads: STRA8-positive/MEIOSIN-negative differentiating spermatogonia. Boundaries of the seminiferous tubules are indicated by dashed lines. Scale bars: 25 μm. Samples for the photo images (B, C) were prepared and the images acquired as described previously (Ishiguro et al., 2020).

meiotic entry in spermatogonia by negatively regulating RA-dependent transcription (Matson et al., 2010). Since chromatin immunoprecipitation followed by sequencing (ChIP-seq) data showed that DMRT1 binds to the 5′ region upstream of the *Meiosin* locus (Murphy et al., 2015), DMRT1 may repress *Meiosin* expression in spermatogonia, which would explain why RA does not induce MEIOSIN expression and subsequent meiosis in differentiating spermatogonia. Although the precise mechanism of *Meiosin* expression is yet to be fully investigated, the temporal expression of MEIOSIN ensures the proper timing of meiotic initiation in testis.

MEIOSIN DIRECTS THE SWITCH FROM MITOSIS TO MEIOSIS

In *Meiosin*-deficient testes, spermatocytes later than pre-leptotene stage do not appear, and consequently postmeiotic spermatids or sperm are absent, resulting in severe infertility (Fig. 3A, 3B). In *Meiosin* KO testes, STRA8 is still expressed and *vice versa*, implying that *Meiosin* and *Stra8* expression are regulated independently of each other. Notably, despite the expression of STRA8, *Meiosin* KO spermatocytes fail to enter meiotic prophase (Fig. 3C). It is also worth noting that in *Meiosin* KO, STRA8 largely remains in the cytosol rather than in the nucleus, suggesting that MEIOSIN is required for nuclear localization of STRA8 in pre-leptotene spermatocytes. In differentiating spermatogonia, where MEIOSIN is not expressed, STRA8 localizes to the nucleus (Fig. 2C). This suggests that the nuclear localization of STRA8 is independent of MEIOSIN in differentiating spermatogonia, which further emphasizes that the expression of both MEIOSIN and STRA8 is required for the initiation of meiosis.

Consistent with this evidence, transcriptome analysis

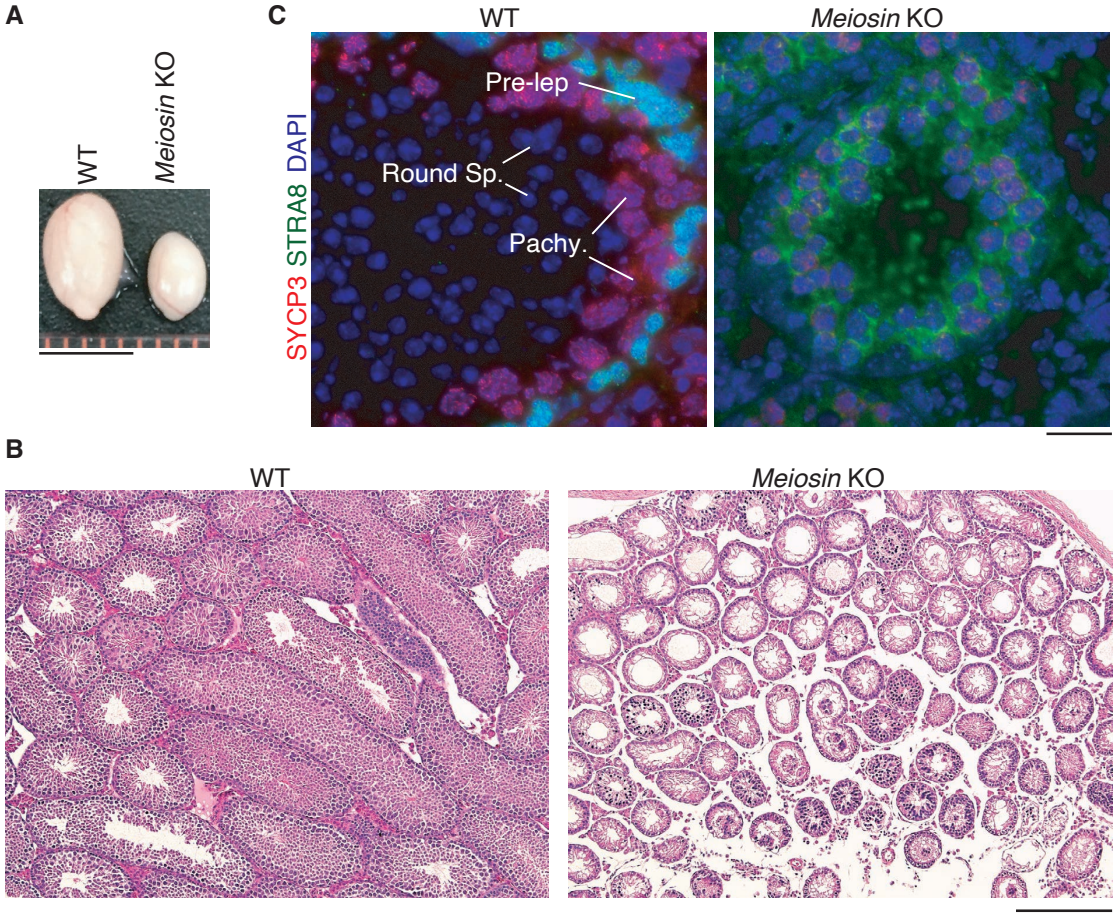


Fig. 3. MEIOSIN plays an essential role in meiotic initiation in testes. (A) Testes from eight-week-old WT and *Meiosin* KO. Scale bar: 2 mm. (B) Hematoxylin and eosin staining of sections from four-week-old WT and *Meiosin* KO testes. Spermatocytes in meiotic prophase and postmeiotic spermatids are absent in *Meiosin* KO testis. Scale bar: 250 μm. (C) Seminiferous tubule sections from eight-week-old WT and *Meiosin* KO testes were stained for SYCP3, STRA8 and DAPI. Spermatocytes that enter meiotic prophase are absent in *Meiosin* KO testes. Pre-lep: pre-leptotene spermatocyte; Pachy: pachytene spermatocyte. Round Sp.: round spermatid. Note that STRA8 is localized in the nuclei in WT testes, and mostly remains in the cytoplasm in *Meiosin* KO testes. Scale bar: 25 μm. Samples for the photo images (B, C) were prepared and the images acquired as described previously (Ishiguro et al., 2020).

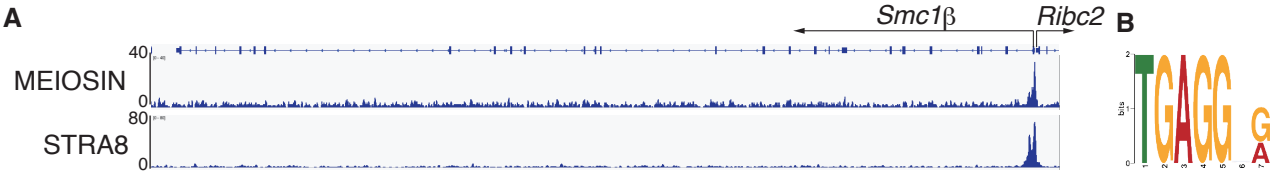
indicates that meiosis-associated genes, including those involved in the processes of meiotic chromosome organization, the meiotic cell cycle and spermatogenesis, are downregulated in *Meiosin* KO. This accounts for the failure of progression into meiotic prophase and subsequent maintenance of the meiotic cell cycle observed in *Meiosin* KO spermatocytes. Altogether, MEIOSIN plays a crucial role in the initiation of meiosis and the subsequent meiotic program. In *Stra8* KO, some spermatocytes still have the ability to enter meiosis but fail to complete meiotic prophase, indicating a phenotypic difference between *Meiosin* KO and *Stra8* KO testes. A subset of meiotic genes is more strongly downregulated in *Meiosin* KO than in *Stra8* KO, which may account for the phenotypic difference of a more severe pre-leptotene block in *Meiosin* KO than in *Stra8* KO.

Since their spermatocytes do not enter meiosis, what happens to *Meiosin* KO testes? In *Meiosin* KO testes, mitotic prometaphase-like cells marked by histone H3Ser10 phosphorylation accumulate to a high level. Moreover, the mitotic cyclin A2 is ectopically expressed in *Meiosin* KO spermatocytes, as if *Meiosin* KO spermatocyte-like cells are in the mitotic cell cycle, which are consequently eliminated by apoptosis. These observations suggest that *Meiosin* KO spermatocyte-like cells acquire precocious mitotic status soon after reaching the pre-leptotene-like stage. In summary, MEIOSIN is required for cell cycle switching from mitosis to meiosis.

MEIOSIN IN COLLABORATION WITH STRA8 ACTIVATES THE MEIOTIC PROPHASE PROGRAM

It has been suggested that the gene expression program for meiotic prophase is executed under RA signaling through either STRA8-dependent or -independent path-

ways (Koubova et al., 2014; Soh et al., 2015). However, it remained elusive how STRA8 might be involved in the expression program for meiotic prophase. MEIOSIN possesses the HMG box DNA-binding domain (Fig. 2A), and interacts with STRA8. Indeed, ChIP-seq analysis indicates that MEIOSIN and STRA8 share binding sites at promotor regions on the mouse genome (exemplified in Fig. 4A) with common DNA-binding motifs (Fig. 4B). MEIOSIN- and STRA8-binding sites overlap well at the transcription start site (TSS) regions, as revealed by CAGE-seq (cap analysis gene expression with sequencing) in the testis. Since meiotic gene promoters in spermatogonia are often poised by the enrichment of H3K4me2 and the loading of RNA polymerase II (Sin et al., 2015), one possibility is that at the target TSSs, MEIOSIN and STRA8 promote the release of paused RNA polymerase II, or stimulate the activity of the polymerase II-associated basal transcription machinery, for a rapid and synchronous burst of transcription. Indeed, it should be mentioned that some, if not all, of these MEIOSIN/STRA8-bound genes start to show weak expression just prior to meiotic entry, which is con-



C

Meiotic function	MEIOSIN/STRA8 target genes
Meiotic recombination	*Spo11, Spo16, Dmc1,Top6bl/Gm960, Prdm9, Meiob, Ankrd31, Msh5, Hsf2bp/Meilb2, Brca2, Mcmdc2, Rnf212b, Brme1/4930432K21Rik*
Synaptonemal complex	*Syce1, Syce2, Syce3, Tex12*
Chromosome axis	*Sycp3, Sycp2, Hormad1, Hormad2*
Meiotic cohesin	*Rad21l, Stag3, Smc1β*
Meiotic telomere/NE	*Kash5/Ccdc155, Sun1, Terb1, Majin*
RNA regulation	*Meioc, Ythdc2*
other	*Zfp541, M1ap*

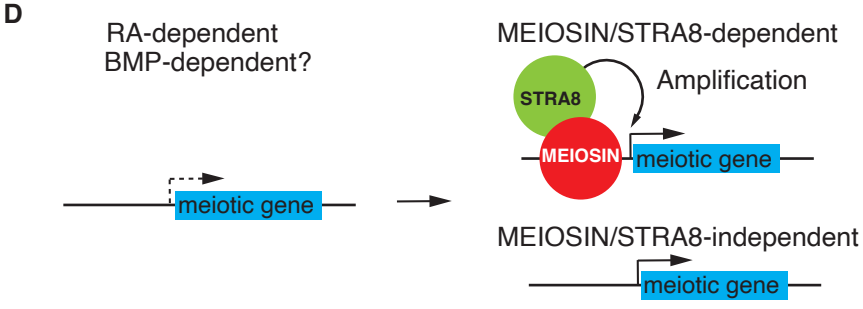


Fig. 4. MEIOSIN and STRA8 bind and activate meiotic genes. (A) Genomic view of MEIOSIN and STRA8 ChIP-seq data over a representative commonly bound meiotic gene (*Smc1*β) locus. Note that expression of the neighboring gene *Ribc2* is not regulated by MEIOSIN or STRA8. (B) Sequence motif enriched in MEIOSIN ChIP-seq and STRA8 ChIP-seq. (C) Meiotic genes that are bound by both MEIOSIN and STRA8. Note that some well-known meiotic genes are not listed. This is in part due to the statistical threshold used in our RNA-seq data set of WT vs. *Meiosin* KO to define the downregulated genes in *Meiosin* KO. (D) Schematic illustration of sequential activation of meiotic prophase genes by RA-dependent followed by MEIOSIN/STRA8-dependent processes. Meiotic genes are primed prior to meiotic entry in response to RA. STRA8 is upregulated in response to RA. MEIOSIN is possibly directly or indirectly upregulated in response to RA. ZGLP1 under BMP signaling also contributes to the induction of meiotic transcription, at least in part in female meiosis. How BMP signaling contributes to male meiotic initiation remains elusive. MEIOSIN and STRA8 amplify the transcription of meiotic genes and themselves. There are also MEIOSIN/STRA8-independent processes for meiotic gene transcription.

sistent with the observed upregulation of a broad range of genes after RA signaling (Koubova et al., 2014; Kojima et al., 2019). BMP signaling may also synergistically induce meiotic transcription through ZGLP1 (Nagaoka et al., 2020). Although the precise mechanism is yet to be investigated, MEIOSIN and STRA8 may amplify the transcription of these primed genes for meiotic entry.

Importantly, genes bound by both MEIOSIN and STRA8 are downregulated in *Meiosin* KO testes. Many of these genes have functions associated with meiotic prophase processes such as meiotic chromosome dynamics and recombination (Fig. 4C), which is consistent with the STRA8 ChIP data (Kojima et al., 2019). Notably, MEIOSIN and STRA8 bind to their own promoter regions, which may realize rapid expression during the short period of meiotic initiation by an autoactivation feedback loop. Interestingly, among the MEIOSIN- and STRA8-bound genes are the *Meioc* and *Ythdc2* genes, whose products are suggested to destabilize mitotic cell cycle-associated transcripts (Abby et al., 2016; Soh et al., 2017). In *Meioc* KO and *Ythdc2* KO mice, germ cells initiate but fail to maintain the meiotic prophase, showing misexpression of mitotic cyclin A2 and metaphase-like chromosome condensation (Abby et al., 2016; Soh et al., 2017; Jain et al., 2018). Since a similar mitotic status is also observed in *Meiosin* KO and *Stra8* KO mice (Mark et al., 2008; Ishiguro et al., 2020), the cell cycle switching program from mitosis to meiosis may be executed through the activation of *Meioc* and *Ythdc2*. This implies that a MEIOSIN–STRA8 complex directly activates the transcription of a subset of critical meiotic genes (Fig. 4D). Given that meiosis starts from the S phase of the cell cycle (Pratto et al., 2021), the meiotic prophase program may be installed on S phase by robust activation of MEIOSIN/STRA8 target genes.

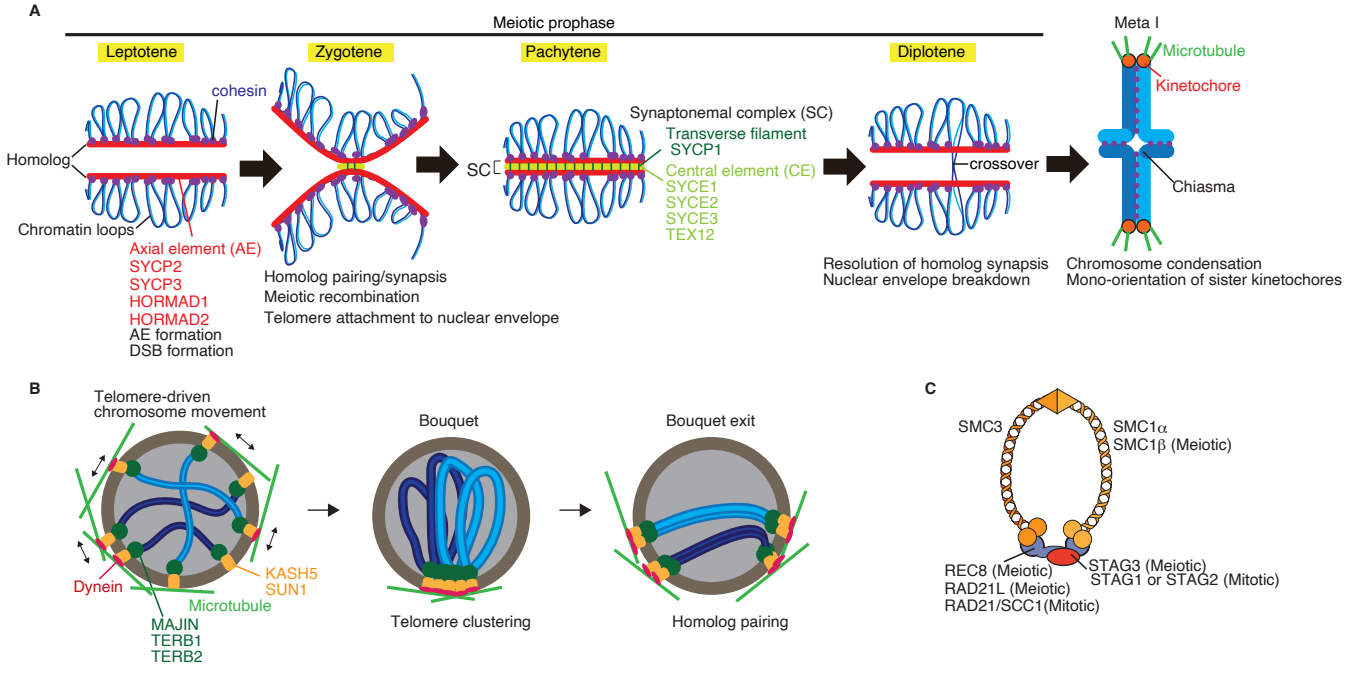


Fig. 5. Chromosome structure and dynamics during meiotic prophase. (A) Meiotic prophase I is a prolonged G2 phase divided into four substages according to the chromosome morphology. During meiotic prophase I, sister chromatids form an axial element (AE). Cohesins are loaded along sister chromatids. DSBs are generated at leptotene. Homologous chromosomes undergo pairing and synapsis through leptotene to zygotene. The synaptonemal complex (SC) is assembled between the homologous chromosomes during this process. When the SC is assembled between homologous chromosomes, the AE is called the lateral element (LE). Transverse filaments link two LEs. Cohesin locates at the inner side of the LE. Meiotic recombination machineries are loaded on the AE to repair DSBs and generate crossovers between homologous chromosomes, yielding physical linkages called chiasmata. At diplotene, the SC is disassembled. At metaphase I, sister kinetochores are mono-oriented, and chromosomes are condensed. (B) Telomeres are anchored to the nuclear envelope by meiosis-specific telomere-binding proteins (MAJIN, TERB1, TERB2, KASH5 and SUN1). By the movement of telomeres along the nuclear envelope, which is mediated by dynein on microtubules, chromosomes undergo dynamic movement to facilitate homolog pairing and synapsis. Chromosomes transiently exhibit extensive telomere clustering, called the bouquet. (C) Sister chromatids are held together by cohesin complexes in mitosis and meiosis. The cohesin complex consists of four core subunits that differ between meiosis and mitosis. In mitosis, it consists of SMC1α, SMC3, the kleisin family protein RAD21/SCC1, and STAG1 or STAG2. In meiosis of mammalian germ cells, it consists of SMC1β, SMC3, meiosis-specific kleisin subunits REC8 or RAD21L, and STAG3. Meiotic cohesin plays crucial roles in meiosis-specific chromosomal events during meiotic prophase I.

MEIOSIN/STRA8 TARGET GENES UNDERLIE MEIOTIC CHROMOSOME ARCHITECTURE

The chromosome structure during meiosis is markedly different to that in mitosis. The MEIOSIN–STRA8 complex directly activates the transcription of a subset of critical meiotic genes that are required for meiotic chromosome architecture (Fig. 4C). During meiotic prophase, chromosomes are organized into proteinaceous structures termed the axial element (AE) or chromosome axis, whose main components are encoded by MEIOSIN/STRA8 target genes, *Sycp2* (Yang et al., 2006), *Sycp3* (Yuan et al., 2000), *Hormad1* (Shin et al., 2010) and *Hormad2* (Wojtasz et al., 2009) (Fig. 5A). The AE provides a scaffold to recruit meiotic recombination machineries that promote double-strand break (DSB) introduction and DSB repair (Baudat et al., 2013). Many of the factors in these processes are encoded by MEIOSIN/STRA8 target genes. For example, SPO11 (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000), TOP6BL/Gm960 (Robert et al., 2016; Vrielynck et al., 2016), DMC1 (Pittman et al., 1998; Yoshida et al., 1998), PRDM9 (Baudat et al., 2010), MEIOB (Luo et al., 2013; Souquet et al., 2013; Xu et al., 2017), ANKRD31 (Boekhout et al., 2019; Papanikos et al., 2019), HSF2BP/MEILB2 (Zhang et al., 2019) and MSH5 (de Vries et al., 1999) are involved in meiotic recombination.

The AE also underlies the structural basis for the assembly of the synaptonemal complex (SC), which mediates tight association of homologous chromosomes and promotes recombination (Fig. 5A). SC components are also encoded by MEIOSIN/STRA8 target genes, such as SYCE1 (Costa et al., 2005), SYCE2 (Bolcun-Filas et al., 2007), SYCE3 (Schramm et al., 2011) and TEX12 (Hamer et al., 2006).

During these processes, telomeres are anchored to the nuclear envelope (NE) by meiosis-specific telomere and NE proteins (TERB1 (Shibuya et al., 2014), TERB2, MAJIN (Shibuya et al., 2015), KASH5/CCDC155 (Morimoto et al., 2012) and SUN1 (Ding et al., 2007)) that are also encoded by MEIOSIN/STRA8 target genes (Fig. 5B). By telomere-driven force, chromosomes undergo dynamic movement to facilitate homolog pairing and synapsis (Shibuya and Watanabe, 2014). It should be mentioned that some well-known meiotic genes such as *Hei10*, *Msh4*, *Six6OS* and *Rec8* are not bound by the MEIOSIN–STRA8

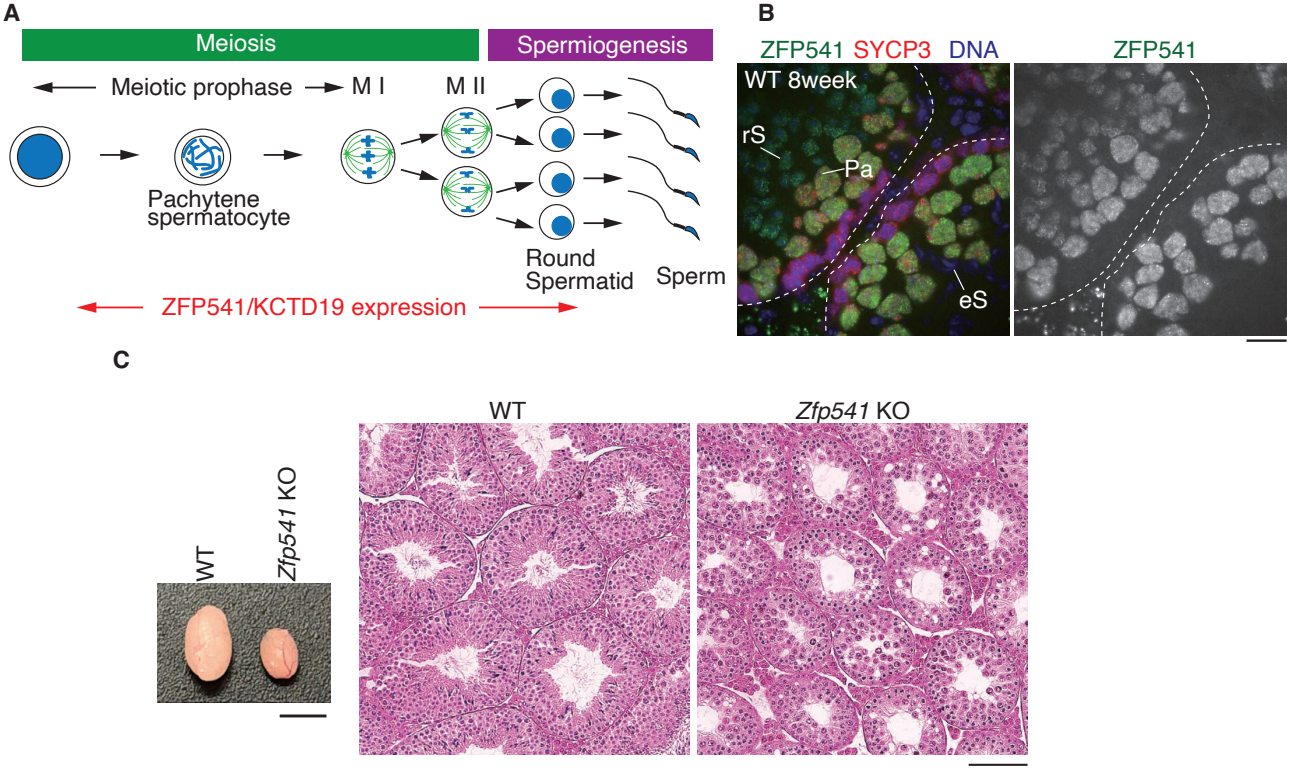
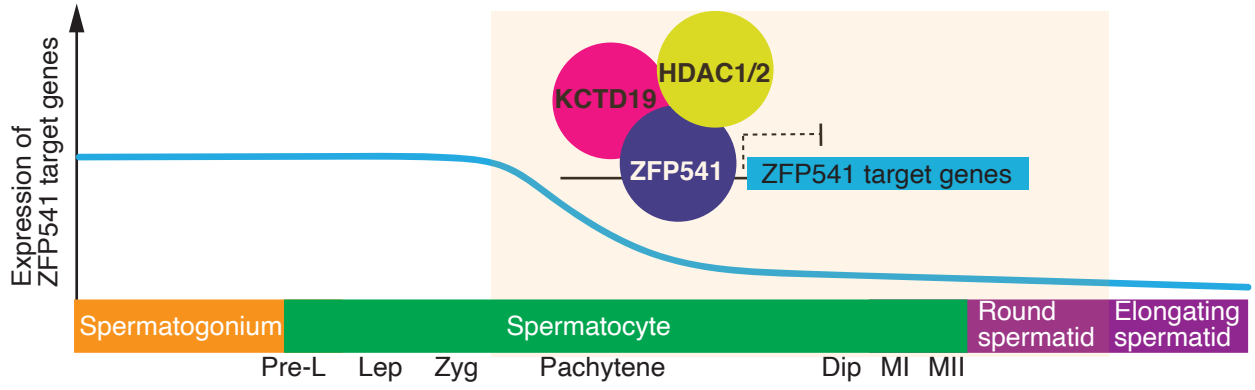


Fig. 6. ZFP541 plays an essential role in the completion of meiotic prophase. (A) The timing of ZFP541 and KCTD19 expression is shown along the developmental stages. (B) Seminiferous tubule sections (8 weeks old) were stained for SYCP3, ZFP541 and DAPI. Pa: pachytene spermatocyte, rS: round spermatid, eS: elongated spermatid. Boundaries of the seminiferous tubules are indicated by dashed lines. Scale bar: 15 μm. (C) Testes from 8-week-old WT and *Zfp541* KO mice (left; scale bar: 5 mm) and hematoxylin and eosin staining of the sections (right; scale bar: 50 μm). The samples for the photo images (B, C) were prepared and the images acquired as described previously (Horisawa-Takada et al., 2021).

complex, suggesting that a subset of the meiotic genes is regulated independently of MEIOSIN and STRA8, as in the case of *Rec8* (Koubova et al., 2014).

In meiosis, the cohesin complex plays crucial roles, not only in sister chromatid cohesion but also in numerous aspects of meiosis-specific chromosomal dynamics such as AE formation, homolog pairing/synapsis and meiotic recombination. Notably, the cohesin complex in meiosis differs from that in mitosis (Ishiguro, 2019) (Fig. 5C). In mammalian germ cells, there are two types of meiosis-specific cohesin complexes, one that contains REC8 (Bannister et al., 2004; Xu et al., 2005) and another that contains RAD21L (Herrán et al., 2011; Ishiguro et al., 2011, 2014; Lee and Hirano, 2011). In these cohesin complexes, mitotic cohesin subunits SMC1α and STAG1/STAG2 are replaced by meiosis-specific subunits, SMC1β (Revenkova et al., 2001; Biswas et al., 2018) and STAG3 (Fukuda et al., 2014; Hopkins et al., 2014; Llano et al., 2014; Winters et al., 2014; Ward et al., 2016), respectively. Expression of the genes encoding meiosis-specific cohesin subunits is activated by MEIOSIN/STRA8, except for *Rec8* whose expression seems to be regulated by RA (Koubova et al., 2014). It has been shown that a "cohesin axial core" is pre-formed between sister chromatids, which subsequently acts as an underlying framework for the formation of the AE (Pelttari et al., 2001; Fujiwara et al., 2020). Since AE formation is abolished in the absence of either of the meiosis-specific cohesin subunits (Llano et al., 2012; Ishiguro et al., 2014), the meiotic cohesin axial core assembled by meiosis-specific cohesin

A



B

ZFP541 target genes that are upregulated in *Zfp541* KO

Gene Ontlogy :0006355 regulation of transcription, DNA-templated	*ZFP326, RB1, CCNT2, ICE1, ZFP329, UBP1, AHR, RBPJ, IKZF2, RCBTB1, YY1, GPBP1, CPNE1, BBX, EP300, SOX4, SMARCC1, KDM2A, CSNK2A1, RALGAPA1, RFX3, SOX11, PIAS1, ERN1, HIC2, ZFP292, RFX7, KAT6A, RFX5, TIMELESS, AJUBA, CLOCK, CGGBP1, NOTCH2, NOTCH3, KHDRBS1, DNMT1, INO80D, PAXIP1, ANP32A, KMT2C, MYCBP2, PRDM15, ZFP629, HIF1A, FOXO1, MBTD1, ATXN3, NPAT, MIER1, HIVEP1, SCAI, ZBTB18, SMAD2, CREBBP, POU2F1, ZFHX2, ZBTB14, ZFP638, ZFP516, CBX3, PHF12, EAF1, PBX3, NFATC3, FOXN3, REX2, SMAD5, MED13L, CDK8, CNOT6, LIN28B, CNOT7, ZFP68, ZZZ3, TADA2A, YEATS2, FUBP1, SP4, AGO2, SP3, HNRNPD, BRWD1, EZH1, NFE2L2, EZH2, CREBZF, KDM5B, RNF10, PHTF2, GMEB1, CHD9, PHF20, CHD8, CHD7, CHD6, ZC3H8, TCF20, CHD3, WASL, CHD2, BMI1, AFF4, CHD1, SIN3A, CIC, JARID2, NEO1, GABPA, TRP53INP1, NCOA1, MKL2, NCOA2, ZFP532, CNOT6L, PDPK1, NCOA3, DNMT3A, TCF12, ASH1L, AXIN2, ARID1A, CBFA2T2, BAZ1B, SIRT1, ZFP518A, TGFBR1, NCOR2, PHF20L1, AEBP2, THRAP3, NFX1, LCOR, NCOA7, GM10778, ZFP652, ZMYND11, KDM7A, HDAC4, GTF2A1, NFAT5, ZFP148, PLAG1, VPRBP, AKAP8, MLLT1, ZBTB44, NR2C2, RNF2, ZBTB41, LRP6, RAD54B, PURB, PURA, BCLAF1, NSD1, TGS1, TRP53BP1, ZFP266, BRD4, BEND6, SUZ12, MLXIP, TCF7L2, MGA, FOXJ3, ATAD2, PCGF3, LRPPRC, CTCFL, REST, PER3, CARM1, CTNNB1, PKN2, NAA15, PAXBP1, LPIN2, SUPT16*
Gene Ontlogy :0016569 covalent chromatin modification	*HDAC4, RB1, KDM5B, DNMT1, CHD9, PHF20, KMT2C, CHD8, CHD7, VPRBP, CHD6, EHMT1, CHD2, BMI1, CHD1, RCBTB1, MBTD1, NSD1, UBN1, SMARCAD1, TLK1, JAK2, JARID2, BRD4, SUZ12, KDM6B, SMARCC1, USP49, KDM2A, CBX3, DNMT3A, ASH1L, DEK, ARID1A, CTCFL, KANSL1, AEBP2, CARM1, KAT6A, TET3, ANP32E, ZMYND11, KDM7A, EZH1, EZH2*

Fig. 7. ZFP541 represses chromatin-related genes during meiotic prophase. (A) Schematic model of the ZFP541–KCTD19–HDAC1/2-containing complex that suppresses a subset of genes for the completion of meiotic prophase. Overall expression level of ZFP541 target genes that are upregulated in *Zfp541* KO spermatocytes is shown along developmental progression. Pre-L: pre-leptotene; Lep: leptotene; Zyg: zygotene; Dip: diplotene; MI: meiotic division I; MII: meiotic division II. The pale shaded rectangle indicates the time frame when nuclear localization of ZFP541 and KCTD19 proteins is observed. (B) Representative ZFP541 target genes that are upregulated in *Zfp541* KO spermatocytes. The top two gene ontology categories of the ZFP541 target genes are indicated on the left.

complexes plays an essential role in AE formation. It is known that mitotic cohesin establishes chromatin loops by cooperating with CTCF, and forms topologically associated domains (TADs) in mitotic interphase nuclei (Zuin et al., 2014; Ghirlando and Felsenfeld, 2016; Gassler et al., 2017; Haarhuis et al., 2017; Wutz et al., 2017). Whether the meiosis-specific cohesins play a role in the formation of TADs during meiotic prophase is currently unknown.

GENE EXPRESSION OF CHROMATIN COMPONENTS IS ALTERED BY ZFP541 REPRESSOR COMPLEX PRIOR TO THE COMPLETION OF MEIOTIC PROPHASE

In spermatocytes, meiotic prophase is accompanied by robust alterations of gene expression programs (Schultz et al., 2003; Shima et al., 2004; Namekawa et al., 2006; Green et al., 2018; Grive et al., 2019) and chromatin status (Kota and Feil, 2010; Sin et al., 2015; Maezawa et al., 2020) as well as by reorganization of the chromatin structure (Alavattam et al., 2019; Patel et al., 2019; Wang et al., 2019), for sperm production. At the pachytene stage of spermatocytes, the transcriptional program for post-meiotic development starts to take place (da Cruz et al., 2016; Ernst et al., 2019). The germ cell-specific Polycomb protein SCML2 is, at least in part, responsible for the suppression of somatic genes and the activation of late-spermatogenesis-specific genes in spermatocytes and in round spermatids (Hasegawa et al., 2015; Maezawa et al., 2018a, 2018b). However, gene activation mechanisms during pachytene and late spermatogenesis largely remain unknown. Thus, for spermatocytes, pachytene exit is a critical developmental event for the subsequent spermatid differentiation, but how the completion of meiotic prophase is ensured prior to post-meiotic differentiation remained elusive.

The *Zfp541* gene, which encodes a zinc finger protein, has been identified as one of the MEIOSIN/STRA8 target genes (Horisawa-Takada et al., 2021). ZFP541 is also an interactor of KCTD19, which was discovered in KO screening of spermatogenic genes (Oura et al., 2021). ZFP541 is expressed in spermatocytes and in round spermatids (Fig. 6A, 6B), and interacts with HDAC1/2 and germ cell-specific KCTD19. Disruption of *Zfp541* leads to defects in the completion of meiotic prophase, with a severe impact on male fertility (Fig. 6C). Thus, ZFP541 plays a critical role in promoting developmental progression of meiotic prophase toward completion in spermatocytes. Chromatin binding analysis of ZFP541 combined with transcriptome analysis demonstrates that ZFP541 binds to and represses a broad range of genes whose biological functions are associated with the processes of transcriptional regulation and covalent chromatin modification (Fig. 7A). It is worth noting that most of these genes are generally expressed in broad cell types rather than being germ cell-specific. For example, these include *Dnmt1*, *Dnmt3A* (DNA methyltransferase), *Ino80D*, *Chd1*, *Chd2*, *Chd3*, *Chd6* (chromatin remodeling), *Kat6A*, *Kmt2C*, *Kmt2B*, *Kdm2A*, *Kdm5B*, *Ash1L*, *Bmi1*, *Jarid2*, *Ezh1*, *Ezh2*, *Ehmt1*, *Rnf2*, *Suz12* (histone modification) and *Ctcfl* (chromatin binding) (Fig. 7B). Notably, expression of these ZFP541-bound genes declines overall in prophase spermatocytes and post-meiotic round spermatids, compared to spermatogonia. This, at least in part, leads to reconstruction of chromatin status for spermiogenesis. Thus, an HDAC1/2-containing ZFP541–KCTD19 complex represses the transcription of a subset of critical genes that are involved in transcriptional regulation and chromatin modification prior to the completion of meiotic prophase. ZFP541 may trigger the reconstruction of the transcription network to promote the completion of prophase, finalize meiotic divisions, and proceed into spermatid production. Since ZFP541 and KCTD19 are conserved only in mammals, it will be interesting to investigate whether a similar mechanism exists for the reconstruction of the transcription network that promotes the completion of prophase in other species.

CONCLUSION

MEIOSIN in collaboration with STRA8 directs meiotic entry upon spermatocyte differentiation. The initiation of meiosis occurs after the second induction of STRA8 when both MEIOSIN and STRA8 are co-expressed. The temporal expression of MEIOSIN ensures proper timing of meiotic initiation in testis. The MEIOSIN–STRA8 complex directly binds and activates the transcription of a subset of meiotic genes that are required for meiotic chromosome dynamics. Furthermore, the MEIOSIN–STRA8 complex activates *Zfp541*, which plays a critical role in the regulation of the transcription network during meiotic prophase. Therefore, MEIOSIN together with STRA8 acts as a master transcription factor for establishing and maintaining the meiotic prophase program. Given that MEIOSIN and STRA8 bind to TSSs rather than to upstream enhancers, it will be interesting to investigate the molecular mechanism that triggers meiotic gene transcription. Since MEIOSIN and STRA8 are conserved in vertebrates, it is possible that the MEIOSIN/STRA8-mediated system acts for meiotic gene activation in other vertebrate species.

It should be mentioned that some targets of the MEIOSIN–STRA8 complex are hypothetical genes. These uncharacterized MEIOSIN/STRA8 target genes may play crucial roles in meiosis. Indeed, we have newly identified BRME1/4930432K21Rik among the MEIOSIN/STRA8 target genes, which acts for the recruitment of BRCA2-RAD51 during meiotic recombination (Takemoto et al., 2020). Further investigation of MEIOSIN/STRA8 target genes will shed light on new players in meiosis.

This work was supported in part by KAKENHI grants (#19H05743, #20H03265, #20K21504, #JP 16H06276) from MEXT, Japan; a grant from AMED PRIME (21gm6310021h0001); and grants from the Sumitomo Foundation, the Naito Foundation, the Astellas Foundation for Research on Metabolic Disorders, the Daiichi Sankyo Foundation of Life Science, the Uehara Memorial Foundation, the NOVARTIS Foundation (Japan) for the Promotion of Science, and the Takeda Science Foundation.

REFERENCES

Abby, E., Tourpin, S., Ribeiro, J., Daniel, K., Messiaen, S., Moison, D., Guerquin, J., Gaillard, J.-C., Armengaud, J., Langa, F., et al. (2016) Implementation of meiosis prophase I programme requires a conserved retinoid-independent stabilizer of meiotic transcripts. Nat. Commun. **7**, 10324.

Alavattam, K. G., Maezawa, S., Sakashita, A., Khoury, H., Barski, A., Kaplan, N., and Namekawa, S. H. (2019) Attenuated chromatin compartmentalization in meiosis and its maturation in sperm development. Nat. Struct. Mol. Biol. **26**, 175–184.

Anderson, E. L., Baltus, A. E., Roepers-Gajadien, H. L., Hassold, T. J., de Rooij, D. G., van Pelt, A. M. M., and Page, D. C. (2008) Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. Proc. Natl. Acad. Sci. USA **105**, 14976–14980.

Baltus, A. E., Menke, D. B., Hu, Y. C., Goodheart, M. L., Carpenter, A. E., de Rooij, D. G., and Page, D. C. (2006) In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat. Genet. **38**, 1430–1434.

Bannister, L. A., Reinholdt, L. G., Munroe, R. J., and Schimenti, J. C. (2004) Positional cloning and characterization of mouse mei8, a disrupted allelle of the meiotic cohesin Rec8. Genesis **40**, 184–194.

Baudat, F., Buard, J., Grey, C., Fledel-Alon, A., Ober, C., Przeworski, M., Coop, G., and de Massy, B. (2010) PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science **327**, 836–840.

Baudat, F., Imai, Y., and de Massy, B. (2013) Meiotic recombination in mammals: localization and regulation. Nat. Rev. Genet. **14**, 794–806.

Baudat, F., Manova, K., Yuen, J. P., Jasin, M., and Keeney, S. (2000) Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. Mol. Cell **6**, 989–998.

Biswas, U., Stevense, M., and Jessberger, R. (2018) SMC1α substitutes for many meiotic functions of SMC1β but cannot protect telomeres from damage. Curr. Biol. **28**, 249–261.e4.

Boekhout, M., Karasu, M. E., Wang, J., Acquaviva, L., Pratto, F., Brick, K., Eng, D. Y., Xu, J., Camerini-Otero, R. D., Patel, D. J., et al. (2019) REC114 partner ANKRD31 controls number, timing, and location of meiotic DNA breaks. Mol. Cell **74**, 1053–1068.e8.

Bolcun-Filas, E., Costa, Y., Speed, R., Taggart, M., Benavente, R., De Rooij, D. G., and Cooke, H. J. (2007) SYCE2 is required for synaptonemal complex assembly, double strand break repair, and homologous recombination. J. Cell Biol. **176**, 741–747.

Bowles, J., Knight, D., Smith, C., Wilhelm, D., Richman, J., Mamiya, S., Yashiro, K., Chawengsaksophak, K., Wilson, M. J., Rossant, J., et al. (2006) Retinoid signaling determines germ cell fate in mice. Science **312**, 596–600.

Burgoyne, P. S., Mahadevaiah, S. K., and Turner, J. M. A. (2009) The consequences of asynapsis for mammalian meiosis. Nat. Rev. Genet. **10**, 207–216.

Cahoon, C. K., and Hawley, R. S. (2016) Regulating the construction and demolition of the synaptonemal complex. Nat. Struct. Mol. Biol. **23**, 369–377.

Costa, Y., Speed, R., Öllinger, R., Alsheimer, M., Semple, C. A., Gautier, P., Maratou, K., Novak, I., Höög, C., Benavente, R., et al. (2005) Two novel proteins recruited by synaptonemal complex protein 1 (SYCP1) are at the centre of meiosis. J. Cell Sci. **118**, 2755–2762.

da Cruz, I., Rodríguez-Casuriaga, R., Santiñaque, F. F., Farías, J., Curti, G., Capoano, C. A., Folle, G. A., Benavente, R., Sotelo-Silveira, J. R., and Geisinger, A. (2016) Transcriptome analysis of highly purified mouse spermatogenic cell populations: gene expression signatures switch from meiotic-to postmeiotic-related processes at pachytene stage. BMC Genomics **17**, 294.

de Vries, S. S., Baart, E. B., Dekker, M., Siezen, A., de Rooij, D. G., de Boer, P., and te Riele, H. (1999) Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. Genes Dev. **13**, 523–531.

Ding, X., Xu, R., Yu, J., Xu, T., Zhuang, Y., and Han, M. (2007) SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. Dev. Cell **12**, 863–872.

Dokshin, G. A., Baltus, A. E., Eppig, J. J., and Page, D. C. (2013) Oocyte differentiation is genetically dissociable from meiosis in mice. Nat. Genet. **45**, 877–883.

Endo, T., Romer, K. A., Anderson, E. L., Baltus, A. E., de Rooij, D. G., and Page, D. C. (2015) Periodic retinoic acid-STRA8 signaling intersects with periodic germ-cell competencies to regulate spermatogenesis. Proc. Natl. Acad. Sci. USA **112**, E2347–E2356.

Ernst, C., Eling, N., Martinez-Jimenez, C. P., Marioni, J. C., and Odom, D. T. (2019) Staged developmental mapping and X chromosome transcriptional dynamics during mouse spermatogenesis. Nat. Commun. **10**, 1251.

Fujiwara, Y., Horisawa-Takada, Y., Inoue, E., Tani, N., Shibuya, H., Fujimura, S., Kariyazono, R., Sakata, T., Ohta, K., Araki, K., et al. (2020) Meiotic cohesins mediate initial loading of HORMAD1 to the chromosomes and coordinate SC formation during meiotic prophase. PLoS Genet. **16**, e1009048.

Fukuda, T., Fukuda, N., Agostinho, A., Hernández-Hernández, A., Kouznetsova, A., and Höög, C. (2014) STAG3-mediated stabilization of REC8 cohesin complexes promotes chromosome synapsis during meiosis. EMBO J. **33**, 1243–1255.

Gassler, J., Brandão, H. B., Imakaev, M., Flyamer, I. M., Ladstätter, S., Bickmore, W. A., Peters, J.-M., Mirny, L. A., and Tachibana, K. (2017) A mechanism of cohesin-dependent loop extrusion organizes zygotic genome architecture. EMBO J. **36**, 3600–3618.

Ghirlando, R., and Felsenfeld, G. (2016) CTCF: making the right connections. Genes Dev. **30**, 881–891.

Green, C. D., Ma, Q., Manske, G. L., Shami, A. N., Zheng, X., Marini, S., Moritz, L., Sultan, C., Gurczynski, S. J., Moore, B. B., et al. (2018) A comprehensive roadmap of murine spermatogenesis defined by single-cell RNA-Seq. Dev. Cell **46**, 651–667.e10.

Grive, K. J., Hu, Y., Shu, E., Grimson, A., Elemento, O., Grenier, J. K., and Cohen, P. E. (2019) Dynamic transcriptome profiles within spermatogonial and spermatocyte populations during postnatal testis maturation revealed by single-cell sequencing. PLoS Genet. **15**, e1007810.

Haarhuis, J. H. I., van der Weide, R. H., Blomen, V. A., Yáñez-Cuna, J. O., Amendola, M., van Ruiten, M. S., Krijger, P. H. L., Teunissen, H., Medema, R. H., van Steensel, B., et al.

(2017) The cohesin release factor WAPL restricts chromatin loop extension. Cell **169**, 693–707.e14.

Hamer, G., Gell, K., Kouznetsova, A., Novak, I., Benavente, R., and Höög, C. (2006) Characterization of a novel meiosis-specific protein within the central element of the synaptonemal complex. J. Cell Sci. **119**, 4025–4032.

Hasegawa, K., Sin, H.-S., Maezawa, S., Broering, T. J., Kartashov, A. V., Alavattam, K. G., Ichijima, Y., Zhang, F., Bacon, W. C., Greis, K. D., et al. (2015) SCML2 establishes the male germline epigenome through regulation of histone H2A ubiquitination. Dev. Cell **32**, 574–588.

Herrán, Y., Gutiérrez-Caballero, C., Sánchez-Martín, M., Hernández, T., Viera, A., Barbero, J. L., de Álava, E., de Rooij, D. G., Suja, J. Á., Llano, E., et al. (2011) The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. EMBO J. **30**, 3091–3105.

Hopkins, J., Hwang, G., Jacob, J., Sapp, N., Bedigian, R., Oka, K., Overbeek, P., Murray, S., and Jordan, P. W. (2014) Meiosis-specific cohesin component, *Stag3* is essential for maintaining centromere chromatid cohesion, and required for DNA repair and synapsis between homologous chromosomes. PLoS Genet. **10**, e1004413.

Horisawa-Takada, Y., Kodera, C., Takemoto, K., Sakashita, A., Horisawa, K., Maeda, R., Shimada, R., Usuki, S., Fujimura, S., Tani, N., et al. (2021) Meiosis-specific ZFP541 repressor complex promotes developmental progression of meiotic prophase towards completion during mouse spermatogenesis. Nat. Commun. **12**, 3184.

Ichijima, Y., Sin, H.-S., and Namekawa, S. H. (2012) Sex chromosome inactivation in germ cells: emerging roles of DNA damage response pathways. Cell. Mol. Life Sci. **69**, 2559–2572.

Ishiguro, K.-i. (2019) The cohesin complex in mammalian meiosis. Genes Cells **24**, 6–30.

Ishiguro, K.-i., Kim, J., Fujiyama-Nakamura, S., Kato, S., and Watanabe, Y. (2011) A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. EMBO Rep. **12**, 267–275.

Ishiguro, K.-i., Kim, J., Shibuya, H., Hernández-Hernández, A., Suzuki, A., Fukagawa, T., Shioi, G., Kiyonari, H., Li, X. C., Schimenti, J., et al. (2014) Meiosis-specific cohesin mediates homolog recognition in mouse spermatocytes. Genes Dev. **28**, 594–607.

Ishiguro, K.-i., Matsuura, K., Tani, N., Takeda, N., Usuki, S., Yamane, M., Sugimoto, M., Fujimura, S., Hosokawa, M., Chuma, S., et al. (2020) MEIOSIN directs the switch from mitosis to meiosis in mammalian germ cells. Dev. Cell **52**, 429–445.e10.

Jain, D., Puno, M. R., Meydan, C., Lailler, N., Mason, C. E., Lima, C. D., Anderson, K. V., and Keeney, S. (2018) *ketu* mutant mice uncover an essential meiotic function for the ancient RNA helicase YTHDC2. eLife **7**, e30919.

Keeney, S., Lange, J., and Mohibullah, N. (2014) Self-organization of meiotic recombination initiation: general principles and molecular pathways. Annu. Rev. Genet. **48**, 187–214.

Kojima, M. L., de Rooij, D. G., and Page, D. C. (2019) Amplification of a broad transcriptional program by a common factor triggers the meiotic cell cycle in mice. eLife **8**, e43738.

Kota, S. K., and Feil, R. (2010) Epigenetic transitions in germ cell development and meiosis. Dev. Cell **19**, 675–686.

Koubova, J., Hu, Y.-C., Bhattacharyya, T., Soh, Y. Q., Gill, M. E., Goodheart, M. L., Hogarth, C. A., Griswold, M. D., and Page, D. C. (2014) Retinoic acid activates two pathways required for meiosis in mice. PLoS Genet. **10**, e1004541.

Koubova, J., Menke, D. B., Zhou, Q., Capel, B., Griswold, M. D., and Page, D. C. (2006) Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc. Natl. Acad. Sci. USA **103**, 2474–2479.

Lee, J., and Hirano, T. (2011) RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. J. Cell Biol. **192**, 263–276.

Llano, E., Gomez-H, L., García-Tuñón, I., Sánchez-Martín, M., Caburet, S., Barbero, J. L., Schimenti, J. C., Veitia, R. A., and Pendas, A. M. (2014) *STAG3* is a strong candidate gene for male infertility. Hum. Mol. Genet. **23**, 3421–3431.

Llano, E., Herrán, Y., García-Tuñón, I., Gutiérrez-Caballero, C., de Álava, E., Barbero, J. L., Schimenti, J., de Rooij, D. G., Sánchez-Martín, M., and Pendás, A. M. (2012) Meiotic cohesin complexes are essential for the formation of the axial element in mice. J. Cell Biol. **197**, 877–885.

Luo, M., Yang, F., Leu, N. A., Landaiche, J., Handel, M. A., Benavente, R., La Salle, S., and Wang, P. J. (2013) MEIOB exhibits single-stranded DNA-binding and exonuclease activities and is essential for meiotic recombination. Nat. Commun. **4**, 2788.

Maezawa, S., Hasegawa, K., Alavattam, K. G., Funakoshi, M., Sato, T., Barski, A., and Namekawa, S. H. (2018a) SCML2 promotes heterochromatin organization in late spermatogenesis. J. Cell Sci. **131**, jcs217125.

Maezawa, S., Hasegawa, K., Yukawa, M., Kubo, N., Sakashita, A., Alavattam, K. G., Sin, H.-S., Kartashov, A. V., Sasaki, H., Barski, A., et al. (2018b) Polycomb protein SCML2 facilitates H3K27me3 to establish bivalent domains in the male germline. Proc. Natl. Acad. Sci. USA **115**, 4957–4962.

Maezawa, S., Sakashita, A., Yukawa, M., Chen, X., Takahashi, K., Alavattam, K. G., Nakata, I., Weirauch, M. T., Barski, A., and Namekawa, S. H. (2020) Super-enhancer switching drives a burst in gene expression at the mitosis-to-meiosis transition. Nat. Struct. Mol. Biol. **27**, 978–988.

Mark, M., Jacobs, H., Oulad-Abdelghani, M., Dennefeld, C., Féret, B., Vernet, N., Codreanu, C.-A., Chambon, P., and Ghyselinck, N. B. (2008) STRA8-deficient spermatocytes initiate, but fail to complete, meiosis and undergo premature chromosome condensation. J. Cell Sci. **121**, 3233–3242.

Matson, C. K., Murphy, M. W., Griswold, M. D., Yoshida, S., Bardwell, V. J., and Zarkower, D. (2010) The mammalian doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. Dev. Cell **19**, 612–624.

Miyauchi, H., Ohta, H., Nagaoka, S., Nakaki, F., Sasaki, K., Hayashi, K., Yabuta, Y., Nakamura, T., Yamamoto, T., and Saitou, M. (2017) Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. EMBO J. **36**, 3100–3119.

Morimoto, A., Shibuya, H., Zhu, X., Kim, J., Ishiguro, K.-i., Han, M., and Watanabe, Y. (2012) A conserved KASH domain protein associates with telomeres, SUN1, and dynactin during mammalian meiosis. J. Cell Biol. **198**, 165–172.

Murphy, M. W., Lee, J. K., Rojo, S., Gearhart, M. D., Kurahashi, K., Banerjee, S., Loeuille, G.-A., Bashamboo, A., McElreavey, K., Zarkower, D., et al. (2015) An ancient protein-DNA interaction underlying metazoan sex determination. Nat. Struct. Mol. Biol. **22**, 442–451.

Nagaoka, S. I., Nakaki, F., Miyauchi, H., Nosaka, Y., Ohta, H., Yabuta, Y., Kurimoto, K., Hayashi, K., Nakamura, T., Yamamoto, T., et al. (2020) ZGLP1 is a determinant for the oogenic fate in mice. Science **367**, eaaw4115.

Namekawa, S. H., Park, P. J., Zhang, L.-F., Shima, J. E., McCarrey, J. R., Griswold, M. D., and Lee, J. T. (2006) Postmeiotic sex chromatin in the male germline of mice. Curr.

Biol. **16**, 660–667.
Oulad-Abdelghani, M., Bouillet, P., Décimo, D., Gansmuller, A., Heyberger, S., Dollé, P., Bronner, S., Lutz, Y., and Chambon, P. (1996) Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J. Cell Biol. **135**, 469–477.
Oura, S., Koyano, T., Kodera, C., Horisawa-Takada, Y., Matsuyama, M., Ishiguro, K.-i., and Ikawa, M. (2021) KCTD19 and its associated protein ZFP541 are independently essential for meiosis in male mice. PLoS Genet. **17**, e1009412.
Papanikos, F., Clément, J. A. J., Testa, E., Ravindranathan, R., Grey, C., Dereli, I., Bondarieva, A., Valerio-Cabrera, S., Stanzione, M., Schleiffer, A., et al. (2019) Mouse ANKRD31 regulates spatiotemporal patterning of meiotic recombination initiation and ensures recombination between X and Y sex chromosomes. Mol. Cell **74**, 1069–1085.e1011.
Patel, L., Kang, R., Rosenberg, S. C., Qiu, Y., Raviram, R., Chee, S., Hu, R., Ren, B., Cole, F., and Corbett, K. D. (2019) Dynamic reorganization of the genome shapes the recombination landscape in meiotic prophase. Nat. Struct. Mol. Biol. **26**, 164–174.
Pelttari, J., Hoja, M. R., Yuan, L., Liu, J. G., Brundell, E., Moens, P., Santucci-Darmanin, S., Jessberger, R., Barbero, J. L., Heyting, C., et al. (2001) A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. Mol. Cell. Biol. **21**, 5667–5677.
Pittman, D. L., Cobb, J., Schimenti, K. J., Wilson, L. A., Cooper, D. M., Brignull, E., Handel, M. A., and Schimenti, J. C. (1998) Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for *Dmc1*, a germline-specific RecA homolog. Mol. Cell **1**, 697–705.
Pratto, F., Brick, K., Cheng, G., Lam, K.-W. G., Cloutier, J. M., Dahiya, D., Wellard, S. R., Jordan, P. W., and Camerini-Otero, R. D. (2021) Meiotic recombination mirrors patterns of germline replication in mice and humans. Cell **184**, 4251–4267.e20.
Revenkova, E., Eijpe, M., Heyting, C., Gross, B., and Jessberger, R. (2001) Novel meiosis-specific isoform of mammalian SMC1. Mol. Cell. Biol. **21**, 6984–6998.
Robert, T., Nore, A., Brun, C., Maffre, C., Crimi, B., Bourbon, H.-M., and de Massy, B. (2016) The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. Science **351**, 943–949.
Romanienko, P. J., and Camerini-Otero, R. D. (2000) The mouse *Spo11* gene is required for meiotic chromosome synapsis. Mol. Cell **6**, 975–987.
Schramm, S., Fraune, J., Naumann, R., Hernandez-Hernandez, A., Höög, C., Cooke, H. J., Alsheimer, M., and Benavente, R. (2011) A novel mouse synaptonemal complex protein is essential for loading of central element proteins, recombination, and fertility. PLoS Genet. **7**, e1002088.
Schultz, N., Hamra, F. K., and Garbers, D. L. (2003) A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. Proc. Natl. Acad. Sci. USA **100**, 12201–12206.
Shibuya, H., Hernández-Hernández, A., Morimoto, A., Negishi, L., Höög, C., and Watanabe, Y. (2015) MAJIN links telomeric DNA to the nuclear membrane by exchanging telomere cap. Cell **163**, 1252–1266.
Shibuya, H., Ishiguro, K.-i., and Watanabe, Y. (2014) The TRF1-binding protein TERB1 promotes chromosome movement and telomere rigidity in meiosis. Nat. Cell Biol. **16**, 145–156.
Shibuya, H., and Watanabe, Y. (2014) The meiosis-specific modification of mammalian telomeres. Cell Cycle **13**, 2024–2028.
Shima, J. E., McLean, D. J., McCarrey, J. R., and Griswold, M. D. (2004) The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. Biol. Reprod. **71**, 319–330.
Shin, Y.-H., Choi, Y., Erdin, S. U., Yatsenko, S. A., Kloc, M., Yang, F., Wang, P. J., Meistrich, M. L., and Rajkovic, A. (2010) *Hormad1* mutation disrupts synaptonemal complex formation, recombination, and chromosome segregation in mammalian meiosis. PLoS Genet. **6**, e1001190.
Sin, H.-S., Kartashov, A. V., Hasegawa, K., Barski, A., and Namekawa, S. H. (2015) Poised chromatin and bivalent domains facilitate the mitosis-to-meiosis transition in the male germline. BMC Biol. **13**, 53.
Soh, Y. Q. S., Junker, J. P., Gill, M. E., Mueller, J. L., van Oudenaarden, A., and Page, D. C. (2015) A gene regulatory program for meiotic prophase in the fetal ovary. PLoS Genet. **11**, e1005531.
Soh, Y. Q. S., Mikedis, M. M., Kojima, M., Godfrey, A. K., de Rooij, D. G., and Page, D. C. (2017) *Meioc* maintains an extended meiotic prophase I in mice. PLoS Genet. **13**, e1006704.
Souquet, B., Abby, E., Hervé, R., Finsterbusch, F., Tourpin, S., Le Bouffant, R., Duquenne, C., Messiaen, S., Martini, E., Bernardino-Sgherri, J., et al. (2013) MEIOB targets single-strand DNA and is necessary for meiotic recombination. PLoS Genet. **9**, e1003784.
Takemoto, K., Tani, N., Takada-Horisawa, Y., Fujimura, S., Tanno, N., Yamane, M., Okamura, K., Sugimoto, M., Araki, K., and Ishiguro, K.-i. (2020) Meiosis-specific C19orf57/4930432K21Rik/BRME1 modulates localization of RAD51 and DMC1 to DSBs in mouse meiotic recombination. Cell Rep. **31**, 107686.
Turner, J. M. (2015) Meiotic silencing in mammals. Annu. Rev. Genet. **49**, 395–412.
Vrielynck, N., Chambon, A., Vezon, D., Pereira, L., Chelysheva, L., De Muyt, A., Mézard, C., Mayer, C., and Grelon, M. (2016) A DNA topoisomerase VI-like complex initiates meiotic recombination. Science **351**, 939–943.
Wang, Y., Wang, H., Zhang, Y., Du, Z., Si, W., Fan, S., Qin, D., Wang, M., Duan, Y., Li, L., et al. (2019) Reprogramming of meiotic chromatin architecture during spermatogenesis. Mol. Cell **73**, 547-561.e6.
Ward, A., Hopkins, J., McKay, M., Murray, S., and Jordan, P. W. (2016) Genetic interactions between the meiosis-specific cohesin components, STAG3, REC8, and RAD21L. G3 (Bethesda) **6**, 1713–1724.
Winters, T., McNicoll, F., and Jessberger, R. (2014) Meiotic cohesin STAG3 is required for chromosome axis formation and sister chromatid cohesion. EMBO J. **33**, 1256–1270.
Wojtasz, L., Daniel, K., Roig, I., Bolcun-Filas, E., Xu, H., Boonsanay, V., Eckmann, C. R., Cooke, H. J., Jasin, M., Keeney, S., et al. (2009) Mouse HORMAD1 and HORMAD2, two conserved meiotic chromosomal proteins, are depleted from synapsed chromosome axes with the help of TRIP13 AAA-ATPase. PLoS Genet. **5**, e1000702.
Wutz, G., Várnai, C., Nagasaka, K., Cisneros, D. A., Stocsits, R. R., Tang, W., Schoenfelder, S., Jessberger, G., Muhar, M., Hossain, M. J., et al. (2017) Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. EMBO J. **36**, 3573–3599.
Xu, H., Beasley, M. D., Warren, W. D., van der Horst, G. T. J., and McKay, M. J. (2005) Absence of mouse REC8 cohesin

promotes synapsis of sister chromatids in meiosis. Dev. Cell **8**, 949–961.

Xu, Y., Greenberg, R. A., Schonbrunn, E., and Wang, P. J. (2017) Meiosis-specific proteins MEIOB and SPATA22 cooperatively associate with the single-stranded DNA-binding replication protein A complex and DNA double-strand breaks. Biol. Reprod. **96**, 1096–1104.

Yang, F., De La Fuente, R., Leu, N. A., Baumann, C., McLaughlin, K. J., and Wang, P. J. (2006) Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. J. Cell Biol. **173**, 497–507.

Yoshida, K., Kondoh, G., Matsuda, Y., Habu, T., Nishimune, Y., and Morita, T. (1998) The mouse *RecA*-like gene *Dmc1* is required for homologous chromosome synapsis during meiosis. Mol. Cell **1**, 707–718.

Yuan, L., Liu, J.-G., Zhao, J., Brundell, E., Daneholt, B., and Höög, C. (2000) The murine *SCP3* gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol. Cell **5**, 73–83.

Zhang, J., Fujiwara, Y., Yamamoto, S., and Shibuya, H. (2019) A meiosis-specific BRCA2 binding protein recruits recombinases to DNA double-strand breaks to ensure homologous recombination. Nat. Commun. **10**, 722.

Zhou, Q., Li, Y., Nie, R., Friel, P., Mitchell, D., Evanoff, R. M., Pouchnik, D., Banasik, B., McCarrey, J. R., Small, C., and Griswold, M. D. (2008a) Expression of stimulated by retinoic acid gene 8 (*Stra8*) and maturation of murine gonocytes and spermatogonia induced by retinoic acid in vitro. Biol. Reprod. **78**, 537–545.

Zhou, Q., Nie, R., Li, Y., Friel, P., Mitchell, D., Hess, R. A., Small, C., and Griswold, M. D. (2008b) Expression of stimulated by retinoic acid gene 8 (*Stra8*) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes. Biol. Reprod. **79**, 35–42.

Zickler, D., and Kleckner, N. (2015) Recombination, pairing, and synapsis of homologs during meiosis. Cold Spring Harb. Perspect. Biol. **7**, a016626.

Zuin, J., Dixon, J. R., van der Reijden, M. I. J. A., Ye, Z., Kolovos, P., Brouwer, R. W. W., van de Corput, M. P. C., van de Werken, H. J. G., Knoch, T. A., van, IJcken, W. F. J,, et al. (2014) Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc. Natl. Acad. Sci. USA **111**, 996–1001.