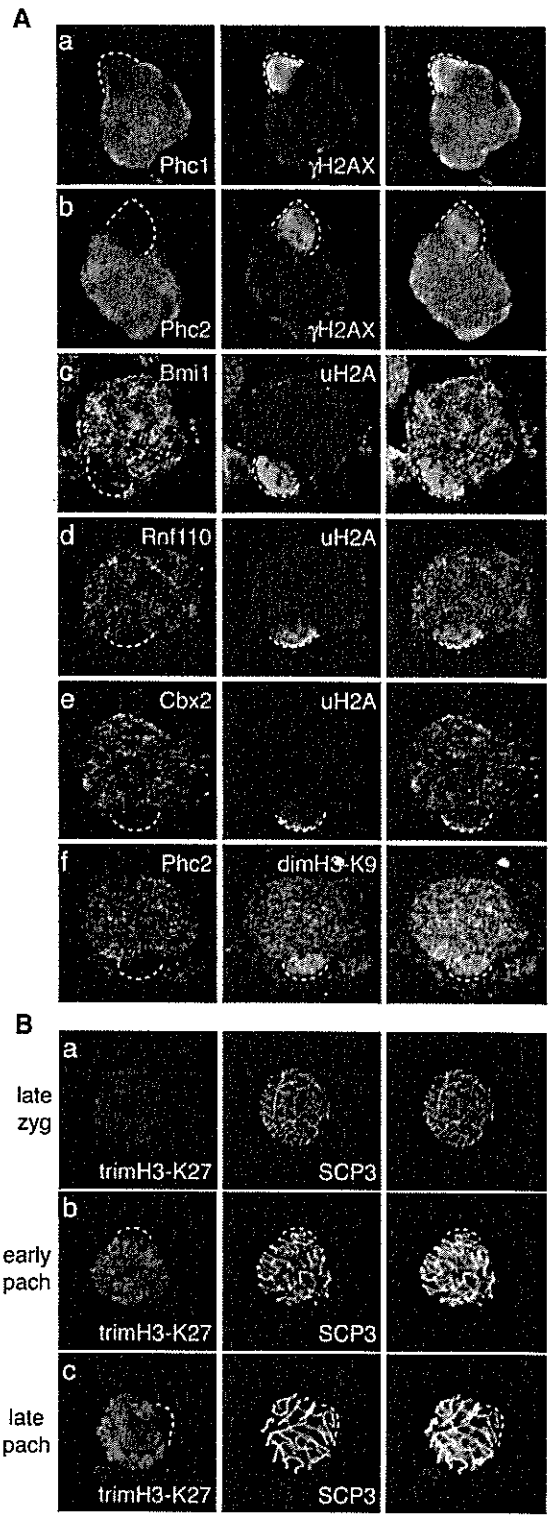

Fig. 2. Expression pattern of other PcG proteins and trimethylated H3-K27 at pachytene stage spermatocytes. (**A**) Immunocytochemical detection of Prc1 components in pachytene spermatocytes. (**Aa,Ab**) Phc1 (a) and Phc2 (b) were excluded from the XY body demarcated by extensive accumulation of γH2A.X. (**Ac-Ae**) Bmi1 (c), Rnf110 (d) and Cbx2 (e) were excluded from the XY body demarcated by extensive accumulation of uH2A. The XY body is indicated by dotted lines. (**Af**) Reciprocal subnuclear localization of Phc2 and dimethylated H3-K9 indicated exclusion of Scmh1 from the XY body. (**B**) Immunocytochemical detection of trimethylated H3-K27 from late zygotene to pachytene stage spermatocytes. Spermatocytes were immunostained by using anti-trimethylated H3-K27 (red) and anti-SCP3 (green). The X and Y chromosome territory is indicated by dotted lines.

We then examined *Scmh1*$^{-/-}$ testicular histology at various stages of first-wave spermatogenesis. Neither day 7 pp nor day 11 pp mutant mice exhibited any significant differences compared to wild type (Fig. 3B). The morphological changes in *Scmh1*$^{-/-}$ testes were observed in the seminiferous tubules as early as day 15 pp. At day 15 pp, most of the seminiferous tubules contained spermatogonia, Sertoli cells and several degenerating pachytene spermatocytes, whereas pre-leptotene to zygotene spermatocytes were seen rarely (see Fig. S3 in the supplementary material). Vacuoles were frequently seen in the luminal region. Based on these morphological parameters, days 15, 19, 25 and 30 pp testes were also examined. In conclusion, *Scmh1*$^{-/-}$ testes were progressively affected during first-wave spermatogenesis (Fig. 3B).

We went on to investigate the frequency of apoptosis during the progression of spermatogenesis by TUNEL labeling. In wild-type day 15 and 19 pp testes, a few TUNEL-labeled cells were clearly present but were seen only rarely in day 7 pp (Fig. 3Ca-c). In *Scmh1*$^{-/-}$ testes, a significant number of TUNEL-labeled cells were observed in the inner layers of seminiferous tubules at day 15 and 19 pp, but not at day 7 pp (Fig. 3Cd-h). These histological observations confirmed that postmitotic spermatocytes in meiotic prophase were predominantly affected in *Scmh1*$^{-/-}$ testes.

Finally, the expression of stage-specific molecular markers were examined by means of semi-quantitative RT-PCR analysis in wild-type and *Scmh1*$^{-/-}$ testes at day 35 pp, in order to address which stage of spermatogenesis was predominantly deleted in affected homozygous mutants (Fig. 3D). *CyclinA1*, *calmegin*, *Bmp8a* and *CREMτ* genes were used as markers for pachytene stage spermatocytes (Sweeney et al., 1996; Watanabe et al., 1994; Zhao and Hogan, 1996; Foulkes et al., 1992). These were reduced more than threefold in *Scmh1*$^{-/-}$ when compared with testes from wild type. In *Scmh1*$^{-/-}$ testes no change was observed in the expression of *A-myb*, *Dmc1*, *Mvh1* and *Scp3*, which are expressed before the pachytene stage (Mettus et al., 1994; Habu et al., 1996; Fujiwara et al., 1994; Tanaka et al., 2000; Klink et al., 1997). Taken together, in *Scmh1*$^{-/-}$ testes, postmitotic spermatocytes are predominantly depleted by apoptotic outbursts.

Apoptotic elimination of late pachytene spermatocytes occurs after synapsis of homologous chromosomes in *Scmh1*$^{-/-}$ testes

In order to further identify the meiotic substage at which *Scmh1*$^{-/-}$ spermatocytes are predominantly affected, immunolocalization studies were carried out in spread spermatogenic cells, prepared from day 18 pp males, by using antibodies against uH2A, γH2A.X and Scp3. Accumulation of uH2A on the XY body was seen in pachytene spermatocytes, whereas γH2A.X demarcates the XY body from late zygotene to diplotene stage (Baarends et al., 1999; Baarends et al., 2005; Mahadevaiah et al., 2001; Fernandez-

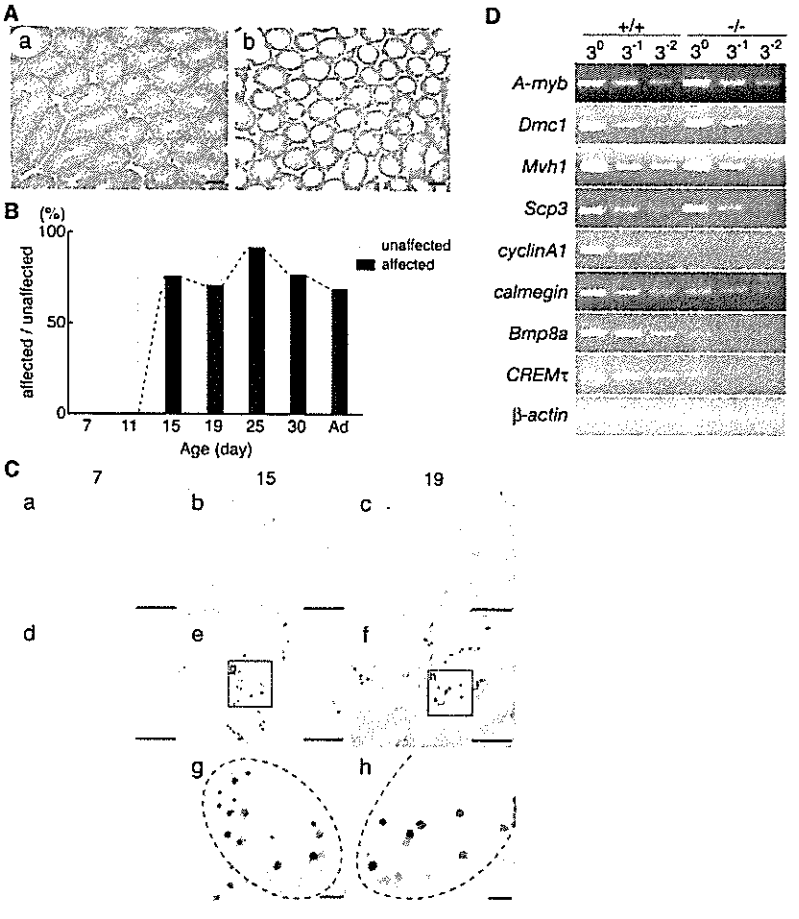

Fig. 3. Testicular abnormalities in *Scmh1*$^{-/-}$ mice. (**A**) Cross-sections of testes from day 35 pp wild-type (**Aa**) and *Scmh1*$^{-/-}$ (**Ab**) mice. Sections were stained with Hematoxylin and Eosin (HE). (**B**) The frequency of *Scmh1*$^{-/-}$ mice in which seminiferous tubules were morphologically affected during first-wave spermatogenesis. Days after birth are shown. At each age, more than ten mutants were examined. Mutants over 8 weeks of age were collected and indicated as adults (Ad). (**C**) Increased apoptotic spermatocytes in *Scmh1*$^{-/-}$ testes. (**Ca-Cc**) Incidence of apoptosis in wild-type testes at day 7, 15 and 19 pp. (**Cd-Cf**) Incidence of apoptosis in *Scmh1*$^{-/-}$ testes at day 7, 15 and 19 pp. (**Cg,Ch**) Higher magnification views of individual seminiferous tubules shown in e and f. Outline of seminiferous tubules are indicated by dotted lines. (**D**) The expression of stage-specific markers during spermatogenesis in wild-type and unaffected and affected *Scmh1*$^{-/-}$ testes at day 35 pp, as revealed by semi-quantitative RT-PCR. *β-actin* was used as a standard to verify the equal amounts of cDNA. Primers used in each reaction are shown in Table 1. Scale bars: 100 μm in A,B,Ca-Cf; 10 μm in Cg,Ch.

Capetillo et al., 2003; Xu et al., 2003). In particular, the degree of uH2A association to the XY body was intriguing, as the Rnf2 component of class 2 PcG has been shown to be an E3 component of ubiquitin ligase for histone H2A (Wang et al., 2004; de Napoles et al., 2004). We did not see any significant difference between *Scmh1*$^{-/-}$ and wild-type testes in the frequency of the spermatocytes, in which uH2A and γH2A.X localized on the XY bodies. This implies entry into pachytene stage was not affected in *Scmh1*$^{-/-}$ (Y.T., unpublished). Using Scp3 staining and morphology, we substaged further the spermatocytes, in which uH2A was accumulated on the XY body, into early and late pachytene stages (Fig. 4A). The frequency of early pachytene spermatocytes was 37% in wild type and 66% in *Scmh1*$^{-/-}$ (Fig. 4A). This suggests that *Scmh1*$^{-/-}$ spermatocytes were incompletely depleted by late pachytene.

During meiosis, synapsis is essential for proper chromosome segregation, and is monitored by various meiotic checkpoints (Cohen and Pollard, 2001). As proper chromosome alignment and segregation in the first meiotic division are ensured by recombination between homologous chromosomes, we examined the localization of Mlh1, a mismatch-repair protein, that forms foci at sites of meiotic crossover in mid- to late-pachytene spermatocytes (Celeste et al., 2002). Mlh1 foci were distributed on the synaptonemal complexes in late pachytene spermatocytes of both wild type and *Scmh1*$^{-/-}$ (Fig. 4B). Consistent with this observation, late pachytene spermatocytes remained in *Scmh1*$^{-/-}$ testes exhibited normal Scp3 distribution, including PAR of sex chromosomes (see Fig. S4 in the supplementary material). Taken together, Scmh1 is dispensable for pairing and synapsis of homologous chromosomes.

The role of Scmh1 at the XY body in pachytene spermatocytes

Apart from homologous autosomes, the X and Y chromosomes pair along PAR and undergo extensive and sequential remodeling into heterochromatin, thus forming the XY body, which is associated with transcriptional inactivation. Failure to form the XY body has been shown to coincide with male sterility and arrest of spermatogenesis, although it is not yet definitely proven whether the XY body is required for survival and fertility of male germ cells (Fernandez-Capetillo et al., 2003). Scmh1 and other PcG components were excluded at the transition from early to late pachytene stage. *Scmh1*$^{-/-}$ spermatocytes were affected at a stage that was temporally similar to that concerning the exclusion of PcG proteins from the XY chromatin domain. These observations prompted us to focus on whether spermatogenic arrest in *Scmh1*$^{-/-}$ testes is accompanied by changes in chromatin remodeling at the XY body. We first examined the degree of H3-K9 methylation, acetylation and phosphorylated RNA pol II association to the XY bodies, which have been shown to change during the pachytene stage (Richler et al., 2000; Khalil et al., 2004). In wild type, 76 and 62% of the XY body marked by uH2A were hyperdi- and hypermonomethylated at H3-K9, respectively, compared with 36 and 18%, respectively, in *Scmh1*$^{-/-}$ testes (Fig. 5Aa,b).

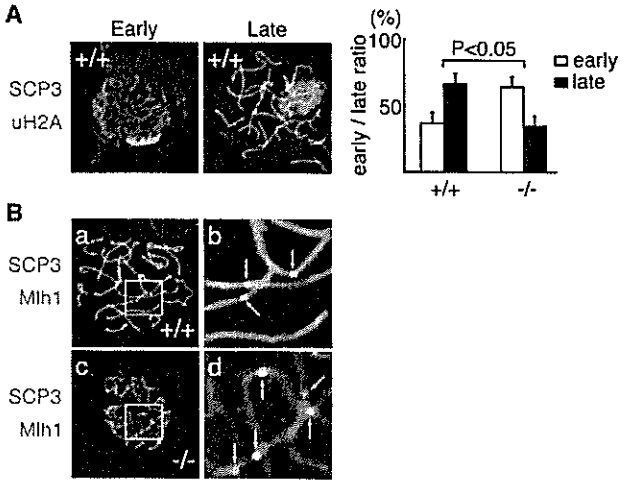

Fig. 4. Significant reduction of late pachytene spermatocytes in *Scmh1*$^{-/-}$ testes. (**A**) Reduction of late pachytene spermatocytes in spermatocyte spread prepared from day 18 pp *Scmh1*$^{-/-}$ testes in comparison with wild type. Spermatocytes were immunostained using anti-Scp3 (red) and uH2A (green). (Left) Wild-type spermatocytes, in which uH2A is enriched at the XY body, were examined at early or late pachytene stage, based on Scp3 immunostaining and morphology. (Right) Frequency of early and late pachytene spermatocytes was compared between the wild-type and *Scmh1*$^{-/-}$ testes. (**B**) Localization of Mlh1 in spermatocytes. (**Ba**) Spermatocytes of wild type were immunostained by using anti-Scp3 (red) and Mlh1 (green). (**Bb**) Higher magnification view of chromosomes shown in a. (**Bc**) Localization of Mlh1 in *Scmh1*$^{-/-}$ mutant testes. (**Bd**) Higher magnification view of chromosomes shown in c. Arrows in b and d indicate Mlh1 foci.

Phosphorylated RNA pol II was excluded from the XY body in 51% of the *Scmh1*$^{-/-}$ spermatocytes compared with 90% of wild type (Fig. 5Ac) (Richler et al., 2000; Khalil et al., 2004). These results suggest that elimination of late pachytene spermatocytes in *Scmh1*$^{-/-}$ testes is coincidental with the stage at which the XY body undergoes chromatin remodeling. It is also noteworthy that underacetylation of H3-K9 at the XY body was observed to a similar extent between the wild-type and *Scmh1*$^{-/-}$ spermatocytes (Fig. 5Ad). Changes in such specific chromatin modifications at the XY body of *Scmh1*$^{-/-}$ spermatocytes imply that they may not solely represent their developmental arrest at late pachytene stage. We therefore postulated a regulatory role for Scmh1 in sequential chromatin modifications of the XY body.

To address this possibility, we extended the analyses to other epigenetic modifications, which could potentially be influenced by *Scmh1* mutation. We first examined the localization of PRC1 components and trimethylated H3-K27, which was bound by PRC1, in *Scmh1*$^{-/-}$ testes. The frequency of meiotic spermatocytes exhibiting reciprocal localization of Phc1 or Phc2 and γH2A.X in *Scmh1*$^{-/-}$ spermatocytes was examined. In 79% of wild-type spermatocytes, Phc1 and Phc2 were excluded from the XY body demarcated by γH2A.X (Fig. 5Ba,b). In *Scmh1*$^{-/-}$ spermatocytes, the frequency of spermatocytes in which Phc1 and Phc2 were excluded from the XY body was reduced to 40 and 27%, respectively (Fig. 5Ba,b). Similarly, trimethylated H3-K27 was excluded from the XY body demarcated by uH2A in 88% of wild-type spermatocytes compared with 39% in *Scmh1*$^{-/-}$ spermatocytes (Fig. 5Bc). Therefore meiotic spermatocytes, in which the sequential exclusion of trimethylated H3-K27 and Prc1 components from the XY body had failed, may be predominantly depleted in *Scmh1*$^{-/-}$ testes. As the exclusion of trimethylated H3-K27 from the XY body is shown to precede the exclusion of PRC1 components in wild type, this result could be interpreted as recurrence of H3-K27 trimethylation in the late pachytene stage. We therefore substaged the spermatocytes in which trimethylated H3-K27 was excluded from the XY body into early and late pachytene stages by using Scp3 staining and morphology. In early pachytene stage, trimethylated H3-K27 was excluded from the XY body to a similar extent between the wild type and *Scmh1*$^{-/-}$ (Fig. 5Bd). In contrast, the frequency of late pachytene spermatocytes, in which trimethylated H3-K27 was excluded, was significantly reduced in *Scmh1*$^{-/-}$ testes compared to wild type (Fig. 5Bd). This suggests that Scmh1 is required to maintain the exclusion of trimethylated H3-K27 from the XY body in late pachytene spermatocytes but not in early pachytene.

We went on to examine the localization of monomethylated histone H3 at K4 (H3-K4) and dimethylated histone H4 at K20 (H4-K20) because the mbt repeats, which are also found in the Scmh1 N-terminal, have been shown to exhibit specific binding to mono- and dimethylated H3-K9, monomethylated H3-K4 and mono- and dimethylated H4-K20 (Kim et al., 2006; Klymenko et al., 2006). Neither hypermonomethylation of H3-K4 nor underdimethylation of H4-K20 at the XY body were significantly different between wild-type and *Scmh1*$^{-/-}$ spermatocytes (Fig. 5Ca,b). It is particularly noteworthy that H4-K20 underdimethylation at the XY body, which was exclusively seen in the late pachytene spermatocytes in wild type, was not affected in the mutants (Y.T., unpublished). Taken together, these results show that apoptotic elimination of late pachytene spermatocytes in *Scmh1*$^{-/-}$ testes is preceded by failure in hypermethylation of H3-K9, exclusion of phosphorylated RNA pol II and Prc1 components and undermethylation of H3-K27 at the XY body, whereas it is not accompanied by changes in H3-K9 acetylation or methylation of H3-K4 or H4-K20. These results support the idea that changes in chromatin modifications at the XY body of *Scmh1*$^{-/-}$ spermatocytes are not simply a consequence of apoptotic elimination of late pachytene spermatocytes. Instead, Scmh1 was suggested to play the regulatory role for the sequential changes in chromatin modifications of the XY body.

Phc2 mutation alleviates spermatogenic defects in *Scmh1*$^{-/-}$ spermatocytes

We postulated that Scmh1 functions via its direct interaction with Prc1 in pachytene spermatocytes, as Scmh1 has been identified as a constituent of Prc1 components because, in general, mutant interactions of *PcG* alleles have been shown to modify the respective phenotypes in mammals as well (Bel et al., 1998; Akasaka et al., 2001; Isono et al., 2005). We have generated *Scmh1;Phc2* double mutants (dko) as Phc2 protein binds to Scmh1 via its SPM domain and the homozygous mutants were viable and fertile (Isono et al., 2005) (Y.T., unpublished). dko mice were viable and born according to the principles of Mendelian inheritance, although some of them exhibited growth retardation (Y.T., unpublished). The fertility of ten normal-sized dko and *Scmh1*$^{-/-}$ males was tested by natural mating to approximately 10-week-old C57BL/6 females. Strikingly, all the dko males were fertile, whereas half the *Scmh1*$^{-/-}$ males were sterile (Fig. 6B). Histological inspections revealed that all the dko testes were morphologically indistinguishable from wild type at day 35 pp and the frequency of apoptotic outbursts in dko was significantly reduced in comparison with littermate *Scmh1*$^{-/-}$ testes (Fig. 6A,C). Significant restorations of late pachytene spermatocytes were also revealed by substaging spermatocytes using antibodies against di-

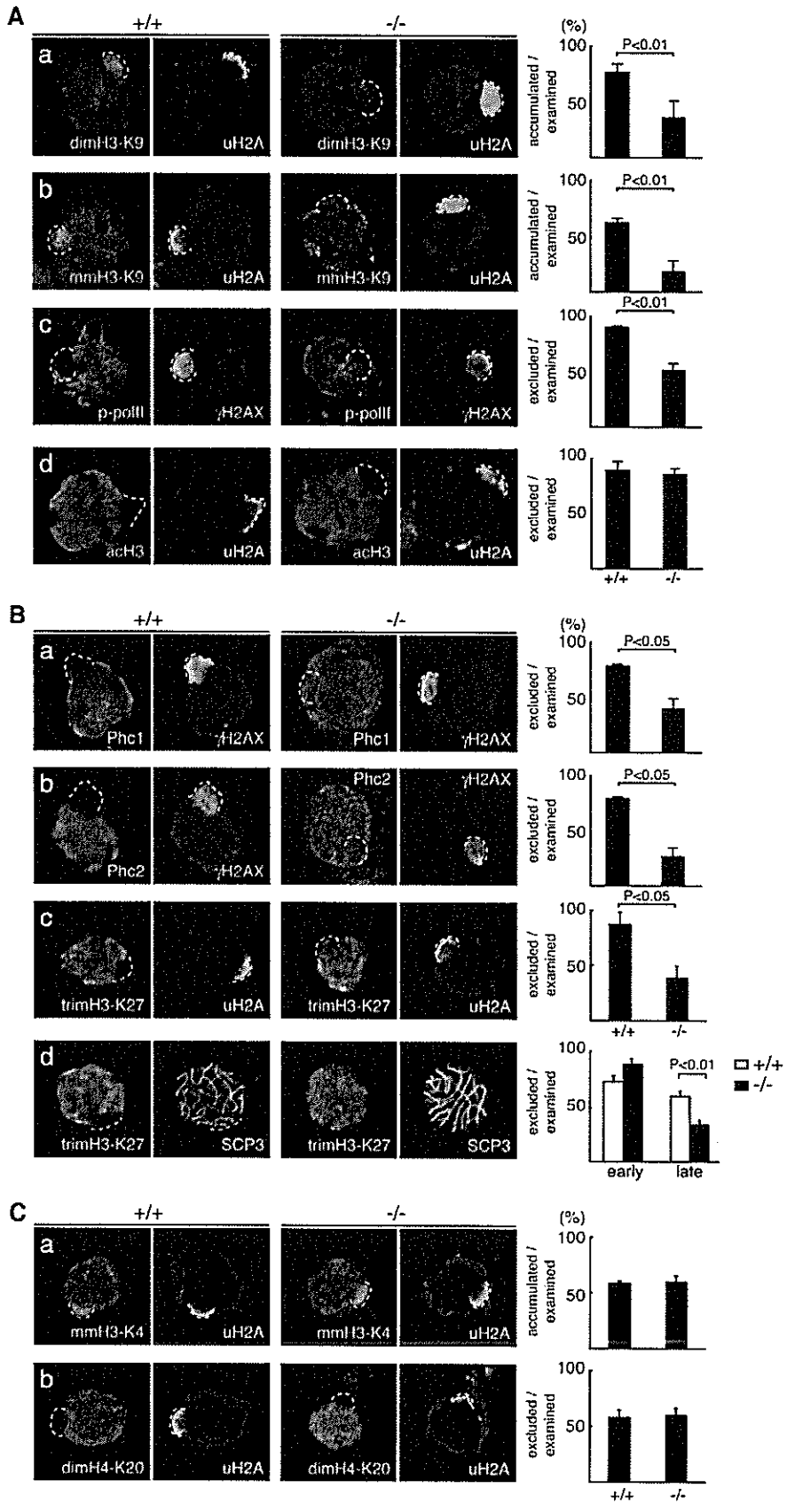

Fig. 5. Altered chromatin modifications at the XY body in *Scmh1*$^{-/-}$ spermatocytes.
(**A**) Immunostaining for dimethylated H3-K9, monomethylated H3-K9, phosphorylated RNA pol II, γH2A.X and uH2A in the spermatocyte spread. (**Aa**,**Ab**) Frequency of spermatocytes, in which dimethylated (a) or monomethylated (b) H3-K9 was enriched at the XY body demarcated by uH2A accumulation, was compared (left) and results were summarized (right). (**Ac**,**Ad**) Frequency of spermatocytes, in which phosphorylated RNA pol II (c) or acetylated H3-K9 (d) were excluded from the XY body, was compared (left) and results were summarized (right). (**Ba**,**Bb**) Frequency of spermatocytes, in which Phc1 (a) or Phc2 (b) were excluded from the XY body, was compared (left) and results were summarized (right). (**Bc**,**Bd**) Frequency of spermatocytes, in which trimethylated H3-K27 were excluded from the XY body was compared. Scp3 was used to substage the spermatocytes. (**C**) Frequency of spermatocytes, in which monomethylated H3-K4 (**Ca**) and dimethylated H4-K20 (**Cb**) were accumulated on the XY body, was compared (left) and results were summarized (right).

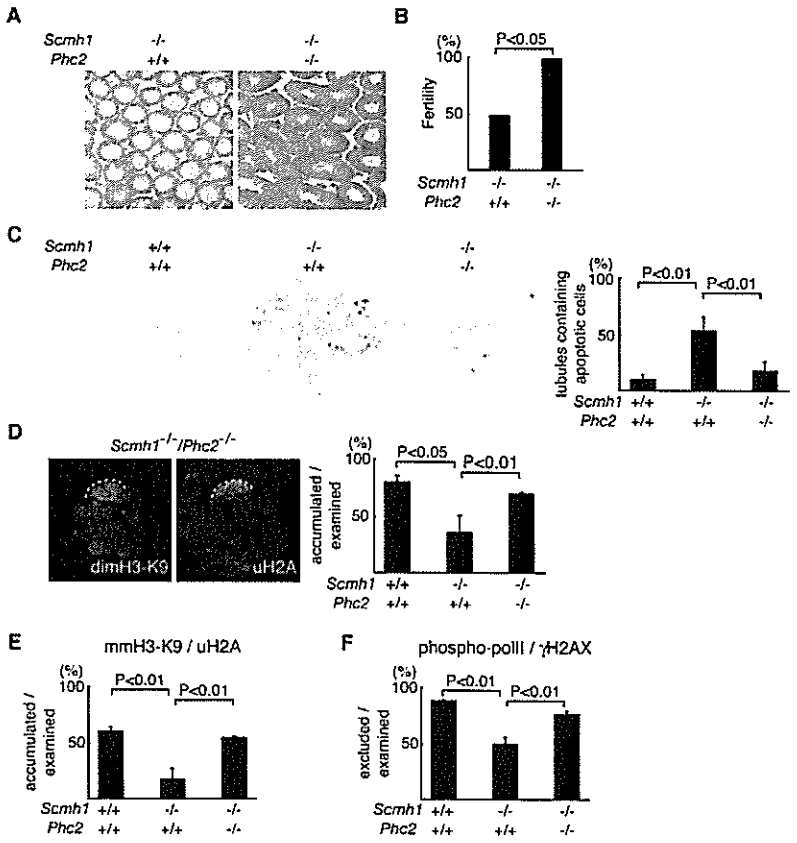

Fig. 6. Restoration of spermatogenic defects in *Scmh1*$^{-/-}$ testes by *Phc2* mutation.
(**A**) Restoration of morphological defects in *Scmh1*$^{-/-}$ testes by *Phc2* mutation. (**B**) Restoration of fertility in *Scmh1*$^{-/-}$;*Phc2*$^{-/-}$ mice. Results from ten mice with respective genotypes were summarized. (**C**) Significant reduction of apoptotic outbursts in *Scmh1*$^{-/-}$;*Phc2*$^{-/-}$ testes compared with *Scmh1*$^{-/-}$ single mutants. (Left) Incidence of apoptosis was examined in wild-type, *Scmh1*$^{-/-}$ and *Scmh1*$^{-/-}$;*Phc2*$^{-/-}$ testes at day 19 pp by TUNEL staining. (Right) Three hundred seminiferous tubules derived from five mice with respective genotypes were analyzed for the presence of TUNEL-positive cells and the results were summarized. (**D**) Restoration of spermatocytes, in which dimethylated H3-K9 was enriched at the XY body in *Scmh1*$^{-/-}$;*Phc2*$^{-/-}$ testes (left). Frequency of spermatocytes, in which dimethylated H3-K9 was accumulated on the XY body, was summarized (right). (**E**,**F**) Frequency of spermatocytes, in which monomethylated H3-K9 was enriched at (E) and phosphorylated pol II was excluded from (F) the XY body in *Scmh1*$^{-/-}$;*Phc2*$^{-/-}$ testes was compared.

and monomethylated H3-K9, the phosphorylated form of RNA pol II and Phc1 (Fig. 6D-F and Y.T., unpublished). Defects in spermatogenesis were also significantly alleviated in *Scmh1*$^{-/-}$*Phc2*$^{+/-}$ albeit to a lesser extent than dkos (Y.T., unpublished). Therefore *Phc2* mutation coincidentally restored aberrant chromatin modifications seen in the XY body of *Scmh1*$^{-/-}$ spermatocytes and their developmental arrest at the late pachytene stage. Taken together, this evidence suggests that Scmh1 is a regulatory component of Prc1 that mediates exclusion of Prc1 from the XY body at the pachytene stage of meiosis. It is likely that the lack of Phc2 components may accelerate this exclusion irrespective of Scmh1.

MSCI is not affected in *Scmh1*$^{-/-}$ spermatocytes

Transcriptional inactivation of sex chromosomes during spermatogenesis is accompanied by sequential changes in their histone modifications, which are notably affected in *Scmh1*$^{-/-}$ spermatocytes. We thus examined the degree of MSCI in *Scmh1*$^{-/-}$ testes, by performing genome-wide microarray-based analysis from three independent preparations of wild-type and *Scmh1*$^{-/-}$ spermatocytes at days 15, 18 and 20 pp and Cot1 RNA fluorescence in situ hybridization (FISH) (Fernandez-Capetillo et al., 2003; Turner et al., 2004; Turner et al., 2005). Average expression levels of genes located on the autosomes and sex chromosomes were compared between the wild type and *Scmh1*$^{-/-}$ after conventional normalization. No significant changes were found in the expression of autosomal and sex chromosomal genes in *Scmh1*$^{-/-}$ spermatocytes (see Fig. S5A in the supplementary material). Concordant with this result, the XY chromatin domain enriched in γH2A.X was negative for Cot-1 RNA in *Scmh1*$^{-/-}$ spermatocytes as well as the wild type (see Fig. S5B in the supplementary material). In conclusion, sequential chromatin modifications mediated by Scmh1 are not required to maintain MSCI.

DISCUSSION

In this study, we generated *Scmh1* mutant mice and identified Scmh1 as an indispensable component of Prc1, based on the axial homeosis and premature senescence of MEFs in the homozygous mutants. We have further identified the role of Scmh1 in mediating the survival of late pachytene spermatocytes. Apoptotic elimination of *Scmh1*$^{-/-}$ spermatocytes is accompanied by the preceding failure of several specific chromatin modifications at the XY body, whereas synapsis of homologous autosomes is not affected. Therefore, it is suggested that Scmh1 is involved in regulating the sequential changes in chromatin modifications at the XY chromatin domain of the pachytene spermatocytes but is not required to maintain MSCI. Restoration of defects in *Scmh1*$^{-/-}$ spermatocytes by *Phc2* mutation indicates that Scmh1 exerts its molecular functions via its interaction with Prc1. Therefore, for the first time, we have been able to indicate a functional involvement of Prc1 during the meiotic prophase of male germ cells and a regulatory role of Scmh1 for Prc1, which presumably involves sex chromosomes.

Based on the present observations, we postulate that Scmh1 could primarily promote the exclusion of Prc1 components from the XY body in the pachytene spermatocytes because Scmh1 itself is a functional component of Prc1. By contrast, failure to maintain exclusion of trimethylated H3-K27 and to undergo H3-K9 methylation at the XY body in *Scmh1*$^{-/-}$ spermatocytes may occur

secondarily to the failure to exclude Prc1 from the XY body. At many loci, epistatic engagement of Prc1 by Prc2 has been shown to be essential for the mediation of transcriptional repression (Lee et al., 2006; Boyer et al., 2006; Fujimura et al., 2006). Preceding exclusion of trimethylated H3-K27, which represents Prc2 actions, for Prc1 exclusion from the XY body, is consistent with epistatic roles of Prc2 for Prc1 at the XY body. Therefore, Scmh1 may affect H3-K27 trimethylation at the XY body through the Prc1-Prc2 engagement. It is noteworthy that H3-K27 trimethylation has been shown to be regulated by Prc1 at the XY body. This may imply that Prc1-Prc2 engagement is a reciprocal rather than epistatic process at the XY body. This possibility should be addressed by using conditional mutants for Prc2 components. We also hypothesize a functional correlation between Prc1 exclusion and H3-K9 methylations at the XY body because the indispensable H3-K9 methyltransferase complex, composed of G9a and GLP, is constitutively associated with E2F6 complexes, which share at least Rnf2 and Ring1 components with Prc1. Moreover, several components of respective complexes are structurally related to each other (Ogawa et al., 2002; Trimarchi et al., 2001). Intriguingly, although Prc1 components, apart from Rnf2, have been shown to be excluded from the XY body at late pachytene stage, components of E2F6 complexes including Rnf2, RYBP, HP1γ and G9a are retained (Y.T., K.I. and H.K., unpublished). The most attractive scenario would be that exclusion of Prc1 is a prerequisite for the functional manifestation of E2F6 complexes to mediate the hypermethylation of H3-K9 at the XY body. We thus propose that Scmh1-mediated exclusion of Prc1 from the XY body might be a prerequisite for maintaining appropriate chromatin structure to undergo subsequent sequential chromatin remodeling of the XY chromatin in pachytene spermatocytes.

We also suggest that sequential changes in chromatin modifications of the sex chromosomes in the pachytene spermatocytes might be monitored by some meiotic checkpoint mechanisms. This is supported by the temporal concurrence of Prc1 exclusion from the XY body and apoptotic depletion of meiotic spermatocytes, their coincidental restorations by *Phc2* mutation, and normal oogenesis and fertility in *Scmh1*$^{-/-}$ females (Y.T. and H.K., unpublished). In addition, defects in the XY body formations have been shown to correlate with apoptotic depletion of meiotic spermatocytes by studies using *H2A.X* and *Brca1* mutants, although developmental arrests occurred by early pachytene stage (Fernandez-Capetillo et al., 2003; Xu et al., 2003). However, this link has not been substantially demonstrated.

Although Scmh1 has been shown to act together with Prc1, the role of Scmh1 for Prc1 might be modified in a tissue- or locus-specific manner because spermatogenic defects by *Scmh1* mutation are restored by *Phc2* mutation, whereas premature senescence of MEFs is enhanced mutually by both mutations (Y.T. and H.K., unpublished). This is supported by an immunofluorescence study revealing the co-localization of Scmh1 with other class 2 PcG proteins in subnuclear speckles in U2OS cells, whereas in female trophoblastic stem (TS) cells it is excluded from the inactive X chromosome domain, which is intensely decorated by Rnf2, Phc2 and Rnf110 (see Fig. S6A,B in the supplementary material) (Plath et al., 2004; de Napoles et al., 2004). It may be possible to postulate some additional factors that modify the molecular functions or subnuclear localization of Scmh1. Indeed, most of the soluble pool of SCM in *Drosophila* embryos is not stably associated with Prc1, although SCM is capable of assembling with the Polyhomeotic protein by their respective SPM domains in the Polycomb core complex (Peterson et al., 2004). As the SPM domain is shared, not only by *polyhomeotic* homologs, but also by multiple paralogs of the *Drosophila Scm* gene, namely *Scml1*, *Scml2*, *Sfmbt*, *l(3)mbt3* and others in mammals, these structurally related gene products may potentially interact with Scmh1 and modulate its functions. Conservations of crucial amino acid residues required for the mutual interaction of SPM domains and multiple mbt repeats in these proteins may further suggest functional overlap with Scmh1. It is notable that phenotypic expressions of *Scmh1* mutation are quite variable during spermatogenesis and axial development even after more than five times backcrossing to a C57Bl/6 background. This incomplete penetrance might involve multiple paralogs of the canonical Scm proteins, which may act in compensatory manner for *Scmh1* mutation, as revealed between *Rnf110* and *Bmi1* or *Phc1* and *Phc2* (Akasaka et al., 2001; Isono et al., 2005).

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/3/579/DC1

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