Distinct requirements for the COMPASS core subunits Set1, Swd1, and Swd3 during meiosis in the budding yeast Saccharomyces cerevisiae

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Abstract

Meiosis-specific chromatin structures, guided by histone modifications, are critical mediators of a meiotic transient transcription program and progression through prophase I. Histone H3K4 can be methylated up to three times by the Set1-containing COMPASS complex and each methylation mark corresponds to a different chromatin conformation. The level of H3K4 modification is directed by the activity of additional COMPASS components. In this study, we characterized the role of the COMPASS subunits during meiosis in Saccharomyces cerevisiae. In vegetative cells, previous studies revealed a role for subunits Swd2, Sdc1, and Bre2 for H3K4me2 while Spp1 supported trimethylation. However, we found that Bre2 and Sdc1 are required for H3K4me3 as yeast prepare to enter meiosis while Spp1 is not. Interestingly, we identified distinct meiotic functions for the core COMPASS complex members that required for all H3K4me, Set1, Swd1, and Swd3. While Set1 and Swd1 are required for progression through early meiosis, Swd3 is critical for late meiosis and spore morphogenesis. Furthermore, the meiotic requirement for Set1 is independent of H3K4 methylation, suggesting the presence of nonhistone substrates. Finally, checkpoint suppression analyses indicate that Set1 and Swd1 are required for both homologous recombination and chromosome segregation. These data suggest that COMPASS has important new roles for meiosis that are independent of its well-characterized functions during mitotic divisions.

Keywords: histone H3K4 methylation; meiosis; COMPASS complex

Introduction

Histone proteins responsible for packaging DNA in the nucleus are subject to an array of post-translational chemical modifications, including acetylation, phosphorylation, and methylation, that are critical regulators of diverse cellular processes. Histone H3Lys4 methylation (H3K4me) is one of the best-studied of the histone modifications. In the budding yeast Saccharomyces cerevisiae, all H3K4me is catalyzed by the Set1-containing COMPASS complex (Briggs et al. 2001; Roguev et al. 2001). COMPASS is an evolutionarily conserved protein complex that is comprised of Set1 and at least six other subunits including Swd1, Swd2, Swd3, Bre2, Sdc1, and Spp1 (Miller et al. 2001; Roguev et al. 2001; Nagy et al. 2002; Santos-Rosa et al. 2004). H3K4 can be mono- (me1), di- (me2), or trimethylated (me3) and each methylation level requires specific COMPASS complex members (Schneider et al. 2005; Dehe et al. 2006). Set1, Swd1, and Swd3 form an enzymatic core that mediates COMPASS complex stability and all H3K4me (Schneider et al. 2005; Dehe et al. 2006; Mersman et al. 2012). In addition, Sdc1, Swd2, and Bre2 are required for H3K4me2 and me3, while Spp1 is important for H3K4me3 (Schneider et al. 2005; Dehe et al. 2006; Mersman et al. 2012). Initial studies of COMPASS function in yeast largely focused on mutations of Set1 that eliminate all H3K4me and, in many cases, uncovered somewhat conflicting results. For example, despite H3K4me3 enrichment at the 5' ends of actively transcribed genes, Set1 is also required for transcriptional silencing at telomeres and the rDNA locus (Nislow et al. 1997; Krogan et al. 2002). More recent studies focusing on separate roles for COMPASS subunits during stress response suggested that COMPASS is remodeled to accommodate transcriptional regulation, but the mechanisms underlying this process remain elusive (Margaritis et al. 2012; D’Urso et al. 2016). Together, these studies indicate that COMPASS-mediated H3K4me is sensitive to growth conditions and regulates cellular processes in a locus-specific manner.

Meiosis is a specialized cell division that produces haploid gametes through one round of DNA replication followed by two rounds of chromosomal division. Meiosis is induced in diploid yeast that is starved for nitrogen and fermentable carbon (Mitchell 1994). Underlying this process is a temporally restricted meiotic transcriptional program that is generally divided into
three stages termed early, middle, and late (Chu et al. 1998; Primig et al. 2000). Post-translational histone modifications regulate the precise timing of gene repression and induction. For example, many early meiotic genes are repressed during vegetative growth by the Ume6 DNA-binding protein, which recruits the Sin3-Rpd3 histone deacetylase to maintain closed chromatin (Strich et al. 1994; Kadosh and Struhl 1997). Early gene activation requires Ume6 degradation, which is catalyzed in a two-step mechanism by the histone acetyltransferase Gcn5 (Mallory et al. 2007; Mallory et al. 2012; Law et al. 2014). Similarly, middle meiotic gene repression implicates both histone deacetylation by the Sum1-Rfn1-Hst1 complex and histone H3K4me2 by the COMPASS complex (Xie et al. 1999; McCord et al. 2003; Jaiswal et al. 2017). This repression is relieved upon Sum1 dissociation from chromatin, allowing Ndt80-mediated middle meiotic gene activation and cellular commitment to the meiotic divisions (rev. in Winter 2012). Interestingly, despite being dispensable for vegetative growth and having opposing functions, sin3A, rpd3A, gcn5A, and set1A mutants arrest early in meiosis (Vidal and Gaber 1991; Vidal et al. 1991; Burgess et al. 1999; Sollier et al. 2004), emphasizing the importance of regulating histone modifications during both meiotic entry and commitment.

While the majority of COMPASS investigations have focused on its roles as a transcriptional regulator, multiple studies have determined that Set1 and Spp1 play transcription-independent roles that are critical for progression through early meiosis (Sommermeyer et al. 2013; Jaiswal et al. 2017; Adam et al. 2018). Following DNA replication, chromosomes are subject to programmed double-stranded DNA breaks (DSBs), homologous chromosome alignment, and genetic recombination. Histone H3K4me3 marks recombination sites by recruiting the Spo11 in Winter 2012). Interestingly, despite being dispensable for vegetative growth and having opposing functions, Sin3, Rdh3A, and Gcn5A, and Set1A mutants arrest early in meiosis (Vidal and Gaber 1991; Vidal et al. 1991; Burgess et al. 1999; Sollier et al. 2004), emphasizing the importance of regulating histone modifications during both meiotic entry and commitment.

Here, we investigated the role of individual COMPASS subunits in the meiotic program in yeast. These studies revealed that Bre2 and Sdc1 are required for efficient progression through the meiotic program, but are not essential for sporulation. Interestingly, we identified distinct functions for the core COMPASS subunits Set1, Swd1, and Swd3 in executing early and late meiosis, respectively. While Set1 and Swd1 are required for timely progression through prophase I and proper chromosome segregation, Swd3 is important for spore formation. Furthermore, we found that the meiotic requirement of Set1 is independent of H3K4me, suggesting that COMPASS regulates meiotic progression through either a structural role or by targeting nonhistone substrates.

**Materials and methods**

**Yeast strains and growth conditions**

Genotypes for yeast strains used in this study are listed in Table 1. All strains are in the high-sporulating SK1 genetic background. Homozygous diploid deletion mutants were generated with one-step replacement using the kanamycin (kanMX6)- or the hygromycin (hphMX4)-resistance marker (Rothstein 1991; Goldstein and McCusker 1999). Transformations were performed using the lithium acetate procedure (Gietz and Woods 2002). Construction of diploid homozygous deletion yeast mutants utilized transformation of haploid deletion mutants with Ycp50-HO. Vegetative yeast was cultured in YEPD (1% Yeast Extract, 2% Peptone, and 1% Dextrose) to a density of ~5 x 10^7 cells per ml. Pre-meiotic yeast was cultured in YEPA (1% Yeast Extract, 2% Peptone, and 2% Potassium Acetate) to a density of ~9 x 10^8 cells per ml. Meiotic time courses were performed as previously described (Cooper et al. 1997). Briefly, yeast was cultured in YEPA to a density of ~1.2 x 10^7 cells per ml, harvested, washed twice in ddH2O, and resuspended in 1/5th volume of SPII (2% Potassium Acetate, pH = 7.0). Time points were then harvested for subsequent analyses as indicated. All cell densities were determined by light sonication of culture samples followed by microscopic quantification using a hemocytometer.

**Meiotic phenotype analyses**

**Terminal meiotic phenotypes**

Meiotic completion was determined by quantifying the spore percentage of triplicate cultures 24 h after meiotic induction using bright-field microscopy. For each experiment, at least 200 cells were counted.

**Spore viability analyses**

Spore viability was scored by macroscopic colony formation on rich medium following tetrad dissection as previously described (Amberg et al. 2005). Ascospore wall digestion was accomplished using zymolyase (5 U per ml, Zymo Research) treatment for 5–10 min at room temperature. Viability of at least 80 spores per strain was quantified. Aneuploidy was confirmed using PCR analyses of the mating-type locus HO as previously described (White and Haber 1990).

**Meiotic progression**

Meiotic progression was monitored by fixing cells in 70% ethanol然后 staining with 4’,6-diamidino-2-phenylindole (DAPI) as described (Pringle et al. 1989). At least 200 cells per time point were counted to monitor progression through MI (bi-nucleated cells) and MII (3–4 nucleated cells). Quantification of DAPI-staining cells for each single deletion mutant was performed for the first 12 h of the time course and a final sample was analyzed after 24 h in SPM. Double deletion mutants were assayed for meiotic progression after 12- and 24-h in SPM.

**Microscopy**

Bright field and fluorescence microscopy were performed using a Leica DM4000B microscope equipped with a Leica DFC450 C digital CCD camera. Calcofluor White and Eosin Y staining was performed on cells that were sporulated in liquid SPM for 24 h as previously described (Lin et al. 2013). Cells were first harvested and washed in 1 ml McIlvaine’s buffer (0.2 M Na2HPO4/0.1 M citric acid, pH 7.0).
acid, pH = 6.0). Staining was then performed using 30 µl Eosin Y disodium salt (5 mg/ml Sigma) in 500 µl McIlvaine’s buffer for 10 min at room temperature in the dark. Cells were washed twice in McIlvaine’s buffer and resuspended in 200 µl McIlvaine’s buffer containing 1 µl of 1 mg/ml Calcoflour White solution (Sigma). Fluorescence of Calcofluor White and Eosin Y was examined using the DAPI and FITC filters, respectively. At least 200 cells were counted for the presence of internalized Eosin Y staining.

**Ethanol resistance assay**

Ethanol resistance was assayed essentially as previously described with the following modifications (Lin et al. 2013). Cells were sporulated for 24 h in liquid SPM, harvested, and resuspended in sterile ddH2O. Cell densities were then equilibrated and either resuspended in sterile ddH2O or ether for 2 min of exposure. 10-fold serial dilutions of ether-treated and -untreated samples were resuspended in sterile ddH2O or ether for 2 min of exposure. 10 µl of each serial dilution was spotted onto YEPD agar, incubated at 30°C for 24 h, then incubated at room temperature in the dark. Images were collected. 

**Western blot analysis**

Protein extracts were prepared from vegetative, pre-meiotic, or meiotic cultures as described previously (Cooper et al. 1997). Either 100 or 25 µg of whole-cell protein extract was used for α-myc-Set1 or histone H3 modifications, respectively. Following transfer onto PVDF, membranes were incubated with α-H3 C-terminal domain (1:5000, Abcam ab1791), α-H3K4me1 (1:2500, Abcam ab8895), α-H3K4me2 (1:2500, Abcam ab11946), or α-H3K4me3 (1:2500, Abcam ab8580). Myc-epitope tagged Set1 protein levels were monitored using α-myc (1:3000, Abcam ab322) with α-alpha Tubulin (1:5000, Abcam ab184970) or α-PGk (1:5000, Abcam ab113687) as a loading control. Secondary antibodies were conjugated with alkaline phosphatase (1:5000; α-mouse Abcam ab6790; α-rabbit Abcam ab97097) and signal was detected using CDp star detection reagent.

**RT-qPCR**

Total nucleic acids were prepared from 2 ml of sporulation culture using Trizol (Thermo Fisher) and mechanical lysis with glass beads according to the manufacturer’s instructions. Approximately 1 µg of total nucleic acid preparations were treated with DNase I (New England Biolabs) and then reverse transcribed using Transcripto II reverse transcriptase (New England Biolabs) in oligo-dT primed reactions according to the manufacturer’s instructions. Subsequent qPCR reactions were prepared using the Power SYBR Master mix (Applied Biosystems) containing primers listed in Table 2. All Ct values were normalized first to ACT1, then to wild-type values at t = 0 (ΔΔCt). Values

| Strain | Genotype | Source |
|--------|----------|--------|
| MLY1   | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | (Strich et al. 1994) |
| MLY20  | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA SET1-9myc-TRP1/SET1-9myc-TRP1 | This study |
| MLY86  | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: KanMX set1:: KanMX | (Law and Ciccaglione 2015) |
| MLY234 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY370 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA pp1::HphMX/pp1::HphMX | This study |
| MLY372 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA HHT1::KanMX/HHT1::KanMX HHT2-K4A/HHT2-K4A | This study |
| MLY373 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA adc1:: KanMX/MX/sdc1::KanMX | This study |
| MLY374 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA bre2:: KanMX/bre2::KanMX | This study |
| MLY385 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY386 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY544 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: KanMX set1:: KanMX swd3::HphMX/swd3::HphMX | This study |
| MLY585 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: HphMX set1:: HphMX mad2::KanMX/mad2::KanMX | This study |
| MLY614 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY621 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY622 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY634 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: HphMX set1:: HphMX mad2::KanMX/mad2::KanMX | This study |
| MLY635 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: HphMX set1:: HphMX pch2::KanMX/pch2::KanMX | This study |
| MLY641 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA set1:: HphMX set1:: HphMX spo11::KanMX/spo11::KanMX | This study |
| MLY642 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA | This study |
| MLY646 | MATa/MATα lys2/lys2 trp1:hisG/trp1:hisG ura3/ura3 LYS2::hoA/LYS2::hoA HHT1::KanMX/HHT1::KanMX HHT2-K4A/HHT2-K4A set1:: HphMX set1:: HphMX | This study |

Table 1 Strains used in this study
reported are the average of three or more independent biological replicates; error bars represent the standard error of the mean.

Results

The COMPASS complex and H3K4me are sensitive to pre-meiotic growth conditions

Commitment to meiosis is a step-wise process that begins as cells are cultured in pre-meiotic growth conditions. Multiple lines of evidence support this, including (1) transcription of the Inducer of Meiosis (IME1) is elevated, (2) mitotic cyclins, whose function is replaced by the meiosis-specific factor IME2, are downregulated, (3) Ume6 protein, a major repressor of early meiotic genes whose degradation is required for meiotic entry, is decreased by 50%, and (4) H3K4me3 patterns associated with meiotic recombination sites are established (Mai and Breeden 2000; Mallory et al. 2007; Borde et al. 2009; Strudwick et al. 2010). To determine the contribution of specific COMPASS subunits in maintaining H3K4me in pre-meiotic growth conditions, we performed Western blot analyses measuring H3K4me2 or me3 in wild-type or yeast mutants lacking individual COMPASS complex members. We chose not to evaluate the impact of deleting SWD2 in this investigation, due to its essential contributions as part of the cleavage and polyadenylation factor complex (Roguev et al. 2001; Cheng et al. 2004; Dichtl et al. 2004). These experiments were performed on yeast cultured in either vegetative or pre-meiotic conditions (see Materials and Methods). Wild-type cells display similar levels of H3K4me2 and me3 regardless of growth condition, suggesting that global COMPASS complex activity was intact (Figure 1A). Consistent with previous reports, SWD1 and SWD3 are required for H3K4me2 and me3 in vegetative conditions (Dehe et al. 2006; Mersman et al. 2012; Kim et al. 2013) and this requirement is maintained in pre-meiotic growth conditions, indicating that both SWD1 and SWD3 are essential for COMPASS-mediated H3K4me (Figure 1A). We found that H3K4me2 levels are greatly diminished for bre2Δ and sdc1Δ mutants cultured in vegetative conditions, which agrees well with other reports (Figure 1A; Dehe et al. 2006; Mersman et al. 2012; Kim et al. 2013). In contrast, we observed more subtle defects in H3K4me2 for these mutants during pre-meiotic growth, suggesting a growth condition-dependent alteration in COMPASS behavior (Figure 1A). Finally, while SPP1 is required for maximal H3K4me3 levels in vegetative growth conditions, it is dispensable for this modification during pre-meiotic growth (Figure 1A). This finding indicates that COMPASS can maintain H3K4me3 in pre-meiotic growth conditions independently of Spp1. Together, these data suggest that the requirement for Bre2, Sdc1, and Spp1 for H3K4me2 and me3 is sensitive to pre-meiotic growth conditions.

Previous studies have determined that Set1 protein levels are undetectable in the absence of SWD1 and SWD3 and are greatly diminished in sdc1Δ mutants (Dehe et al. 2006; Mersman et al. 2012). To determine the requirement of COMPASS subunits for maintaining Set1 protein levels in pre-meiotic growth conditions, we performed Western blot analyses measuring myc-epitope tagged Set1 in wild-type or COMPASS deletion yeast mutants. As described above, these experiments were performed on yeast cultured to mid-logarithmic phase in either vegetative or pre-meiotic cultures. We observed moderately increased Set1 protein levels in wild-type yeast cultured in pre-meiotic growth conditions, indicating that both SWD1 and SWD3 are required for H3K4me3 in pre-meiotic growth conditions as compared to rich, vegetative cultures (Figure 1B). This is in contrast to similar levels of H3K4me2 and me3 observed across both growth conditions (Figure 1A). Consistent with other reports, we found that Set1 protein levels are drastically decreased in vegetative cultures for sdc1Δ, sdc3Δ, and spp1Δ mutants (Figure 1B, left panel; Dehe et al. 2006; Mersman et al. 2012). Interestingly, we observed a modest increase in Set1 protein levels for bre2Δ, sdc1Δ, and spp1Δ mutant yeast in pre-meiotic cultures relative to vegetative growth conditions (Figure 1B). This observation is similar to the increased Set1 protein levels for wild-type, pre-meiotic yeast cultures, suggesting that Set1 expression is sensitive to culture conditions. Despite these increases in Set1 protein, yeast lacking Brev2 or SDC1 are unable to catalyze H3K4me3 (Figure 1A). This is in contrast to SPP1, which is dispensable for H3K4me3 in pre-meiotic cultures (Figure 1A). Together, these data suggest that BRE2 and SDC1 are required for H3K4me3 in pre-meiotic growth conditions, while SPP1 is not. This is consistent with previous work indicating that Spp1 plays COMPASS-independent functions during stress response and in early meiosis (D’Urso et al. 2016; Adam et al. 2018). Finally, we found that SWD1 and SWD3 are required for maintaining Set1 protein levels, regardless of the growth condition (Figure 1B). These results indicate that Set1 protein levels are increased as yeast are preparing to enter meiosis and support a model in which some of the well-characterized interactions between COMPASS complex subunits that occur during vegetative growth may be altered to accommodate meiosis.

COMPASS complex subunits play separate roles in meiosis

We found that COMPASS subunits have distinct roles during pre-meiotic growth. Therefore, we next tested whether this was also the case during meiosis. To address this question, we performed time-course experiments for wild-type and COMPASS deletion mutants measuring their progression through, and successful
completion of meiosis (see Materials and Methods for details). While spore percentages allow determination of a cell’s ability to progress through meiosis and form microscopically visible ascospores, they do not provide an indication of the kinetics of meiosis in individual genotypes. Similarly, cells that fail to form spores might have arrested at any point in the meiotic program prior to this final step. Therefore, we quantify DAPI staining bodies in individual cells to indicate progression through the MI and MII divisions, thus allowing us to discriminate between mutants that fail to form spores but have progressed through one or both meiotic divisions. Finally, cells might form ascospores containing aneuploid spores due to mistakes in chromosome segregation, which can be determined using measurements of spore viability. Consistent with previous reports, wild-type and spp1Δ mutants complete meiosis (Figure 2A) with similar kinetics (Figure 2B) and produce viable gametes (Table 3; Acquaviva et al. 2013; Sommermeyer et al. 2013). In contrast, we observed bre2Δ and sdc1Δ single mutants display an ~50% reduction in spore formation after 24 h in SPM (Figure 2A). Despite a delay in meiotic divisions, >80% of bre2Δ or sdc1Δ mutants have completed both MI and MII prior to 24 h in SPM, suggesting that BRE2 and SDC1 are important for efficient completion of meiosis, but are not essential for meiosis (Figure 2, B and C; Supplementary Figure S1). In support of this, we observed 97.5% viable spores in sdc1Δ mutants, while bre2Δ mutants showed a mild reduction to 67.5% viability (Table 3). Finally, we observed disparate roles for the core COMPASS complex members SET1, SWD1, and SWD3 during meiosis. First, SET1 is required for efficient sporulation and meiotic divisions as ~10% of the set1Δ mutants form spores and ~15% of these mutants have completed MI and MII after 24 h in SPM (Figure 2). Interestingly, the set1Δ mutants that are able to complete meiosis form viable spores, which agrees well with previous investigations (Sollier et al. 2004) and indicates compensatory mechanisms are in place to ensure meiosis occurs without functional Set1 (Table 3). Second, SWD1 is required for efficient sporulation by 12 h; however, swd1Δ mutants exhibit delayed, but not absent sporulation (Figure 2). In contrast to set1Δ mutants, swd1Δ mutants have 42.5% viable spores, suggesting that Swd1 is important for meiotic chromosome segregation (Table 3). Finally, swd3Δ mutants display delays in MI and MII and reduced spore numbers that are more similar to those observed in bre2Δ and sdc1Δ mutants than set1Δ or swd1Δ mutations (Figure 2). Importantly, spore viability in swd3Δ mutants is moderately impaired, indicating that Swd3 is less important for chromosome segregation than Swd1 (Table 3). These observations are in stark contrast to previous studies performed in multiple labs indicating that Set1, Swd1, and Swd3 have overlapping requirements for H3K4 methylation and chromosome behavior (Krogan et al. 2002; Schneider et al. 2005; Dehe et al. 2006; South et al. 2010; Mersman et al. 2012; Soares et al. 2014; Thornton et al. 2014). The remainder of this study focuses on further characterizing the roles of these core COMPASS complex members during yeast meiosis.

**SET1, but not histone H3K4 methylation, is required for meiosis.**

Along with Set1, Swd1 and Swd3 form an enzymatic core for the COMPASS complex that is essential for all H3K4me and Set1 protein stability (Krogan et al. 2002; Schneider et al. 2005; Dehe et al. 2006; South et al. 2010; Mersman et al. 2012; Soares et al. 2014; Thornton et al. 2014). To determine if the differences in Swd1- and Swd3-dependent meiosis are related to histone H3K4me catalysis, we performed Western blot analyses measuring H3K4me1 levels in wild type, set1Δ, swd1Δ, or swd3Δ yeast mutants during meiosis (Figure 3A). We decided to focus on H3K4me1 as this modification is a prerequisite for subsequent H3K4me2 and me3 marks (Schneider et al. 2005; Dehe et al. 2006; Kim et al. 2013). As cells enter meiosis, wild-type yeast exhibit low levels of H3K4me1 that increase 4 h into the meiotic program (Figure 3A). Importantly, Set1, Swd1, and Swd3 are all required for H3K4me1 in meiosis, indicating that the differences we

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**Table 3 Spore viability for compass deletion mutant strains**

| Strain genotype | % viable spores | Number of spores analyzed |
|-----------------|-----------------|---------------------------|
| Wild type       | 97.5            | 240                       |
| set1Δ           | 100             | 80                        |
| swd1Δ           | 42.5            | 240                       |
| swd3Δ           | 70.4            | 240                       |
| bre2Δ           | 67.5            | 120                       |
| sdc1Δ           | 97.5            | 120                       |
| spp1Δ           | 88.4            | 120                       |
| H3K4A           | 96.3            | 120                       |

Figure 2 Distinct roles for COMPASS subunits during meiosis. Terminal meiotic phenotypes and meiotic progression were monitored in wild-type or yeast harboring COMPASS deletion mutations. (A) Spore percentages were quantified 12 or 24 h after meiotic induction using bright field microscopy. Graphs depict the average for three independent biological replicates; error bars show the standard error of the mean. (B) Kinetics of meiotic divisions were monitored by quantifying the number of DAPI staining nuclei per cell. Time points were harvested as indicated and the number of cells containing 2 or 4 nuclei are shown. A representative time course experiment is shown. (C) Quantification of DAPI staining nuclei for yeast harboring the indicated deletion mutations was performed 24 h after meiotic induction.
The lack of measurable H3K4me1 in swd3Δ mutants combined with their ability to form viable gametes raised the possibility that H3K4me is dispensable for meiosis. To test this possibility, we measured both sporulation percentage and spore viability of a yeast mutant harboring the chromosomally integrated H3K4A mutation (Figure 3B and Table 3). Consistent with previous investigations (Govin et al. 2010), we found that H3K4a is dispensable for spore formation and viability, suggesting that the requirement of Set1 for meiosis is independent of H3K4me. ‘To test this possibility, we performed meiotic analyses in a H3K4a set1Δ mutant. Interestingly, we found that set1Δ mutants are not viable even when methylation of histone H3K4 is not possible (Figure 3B), suggesting that set1Δ methylates a nonhistone substrate or plays a structural role in coordinating progress through meiotic prophase.

**Figure 3** Meiotic phenotypes for the COMPASS core complex are independent of histone H3K4me. (A) The COMPASS core complex is required for H3K4me1 during meiosis. Western blot analyses measuring histone H3K4me1 for wild-type or yeast mutants harboring the indicated deletion mutations. Total protein extracts were prepared from yeast grown in pre-meiotic conditions or 4 h post-meiotic induction. Membranes were incubated with anti-H3K4me1 antibody with anti-Pgk1 serving as the loading control. (B) H3K4me is dispensable for meiosis. Yeast harboring chromosomally integrated histone H3K4A point mutations with wild-type or set1Δ deletion mutations were induced to enter meiosis. Graphs represent the average spore percentage for three independent biological replicates; error bars show the standard error of the mean.

**Figure 4** SET1 and SWD1 are required for meiotic transcriptional timing

Meiosis and spore morphogenesis require a transient transcriptional program generally divided into three stages termed early, middle, and late. Early meiotic genes, induced by the master regulator of meiosis IME1, are responsible for initiating meiotic DNA replication and homologous recombination (Kassir et al. 1988; Smith and Mitchell 1989; rev. in Winter 2012). Middle gene expression, coordinated by the meiosis-specific transcription factor Ndt80, results in commitment to the meiotic divisions and initiates enclosure of each haploid nucleus in the prospore membrane (Chu and Herskowitz 1998; Hepworth et al. 1998; Pak and Segall 2002; Sopko et al. 2002). Late genes stimulate spore maturation, chromatin compaction, and spore wall assembly, allowing the development of a mature ascus (Neiman 2011). Misregulating early meiotic gene transcription can result in meiotic prophase arrest, preventing Ndt80 activation, middle meiotic gene expression and chromosome division (Chu and Herskowitz 1998; Hepworth et al. 1998; Tung et al. 2000). In contrast, errors in middle or late meiosis permit chromosome segregation while causing specific defects in spore wall assembly (Neiman 1998; Suda et al. 2009; Neiman 2011; Lin et al. 2013).

To determine the requirement of the COMPASS core for executing this program we performed meiotic time course experiments and measured gene expression using RT-qPCR. Our analyses centered on the expression of IME1, IME2, and NDT80, three genes that play central roles in meiotic progression. Expression of the master regulator of meiosis IME1 peaks in wild-type yeast at 4.5 h and decreases through the remainder of the time course (Figure 4A). IME1 expression was delayed by ∼3 h in set1Δ mutants suggesting that, despite their failure to perform MI and MII, SET1 is not required for the initiation of meiosis (Figure 4A). Both swd1Δ and swd3Δ mutants have reduced expression of IME1; however, IME1 expression peaks in swd1Δ mutants at 12 h, while the pattern of IME1 expression in swd3Δ mutants is more similar to wild-type cells (Figure 4A). This indicates that while both Swd1 and Swd3 are required for maximal IME1 expression, Swd1 is more important for the timing of IME1 induction. Together, these data suggest that the meiotic defects observed in COMPASS core mutants are not due to a failure to induce the meiotic program through IME1 expression, but might be caused by reduced IME1 expression levels.

We next measured the transcription of IME2, an early meiotic gene that encodes a protein kinase required for progression through meiotic prophase (rev. in Winter 2012). Wild-type yeast induce IME2 1.5 h into meiosis and expression peaks at 7.5 h (Figure 4B). Yeast lacking SET1 have greatly reduced, but not...
absent, IME2 expression (Figure 4B). In contrast, SWD1 is required for IME2 expression, suggesting that Set1 and Swd1 may play different roles in early meiosis (Figure 4B). Finally, SWD3 is necessary for the proper kinetics of IME2 expression, consistent with its role in the timing of MI and MII.

NDT80 expression is stimulated by Ime2 and represents a key event in the cellular commitment to the meiotic divisions. Wild-type yeast induce low-level NDT80 expression at 4.5 h, consistent with their progression through MI at this time point (Figures 2B and 4C). Similar to our observations for IME2 expression, we observed delayed and reduced NDT80 in swd3Δ mutants, supporting its role in efficient meiotic timing and completion (Figure 4C). Finally, we found that Set1 and SWD1 are required for NDT80 expression, which agrees well with their requirement for progression through MI and MII (Figure 4C). Together, these data suggest that SET1 and SWD1 are required for distinct steps during early meiosis that lead to meiotic divisions, while SWD3 is more important for the appropriate timing of meiotic transcription and commitment to form gametes.

**SET1 and SWD1 are required for meiotic prophase and spindle assembly**

Our observations indicate that SET1 and SWD1 are required for NDT80 expression and that this requirement may occur via two different mechanisms. Yeast lacking SET1 allow low-level induction of IME2 whereas swd1Δ mutants fail to induce IME2. These data could be explained by locus-specific differences in H3K4me, leading to misregulation of gene expression. However, since H3K4 is dispensable for sporulation, this possibility is eliminated. Alternatively, the observed deficiencies in the meiotic transcription program could be due to a requirement for SET1 and SWD1 to progress past early meiotic checkpoints. To test this possibility, we performed meiotic analyses of yeast lacking SET1 or SWD1 in combination with deletion mutations of three checkpoint-related genes RAD9, PCH2, or MAD2. Rad9 will arrest meiotic cells that have no measurable defects in meiotic progression, homologous recombination frequency, spor formation, or spore viability (Weber and Byers 1992). Interestingly, yeast harboring set1Δrad9Δ mutations fail to sporulate, but swd1Δrad9Δ mutants sporulate with ~90% efficiency (Figure 5A). Quantification of DAPI staining nuclei in the set1Δrad9Δ mutants indicated an approximately twofold increase in progression through MI and MII relative to set1Δ mutants alone, suggesting a role for RAD9 in suppressing the set1Δ-associated meiotic phenotype (Figures 5B and 2C). Both the set1Δrad9Δ and swd1Δrad9Δ mutants have reduced spore viability, indicating improper chromosome segregation (Table 4). These results suggest that Swd1 either prevents DNA damage during replication or is important for post-replicative DNA repair, but Set1 does not.

The pachytene checkpoint ensures that chromosome segregation is inhibited in the presence of meiotic recombination or chromosome synopsis errors (rev in. Roeder and Baillie 2000). Pch2 is a meiosis-specific component of the pachytene checkpoint pathway that monitors synaptonemal complex (SC) formation and is dispensable for normal homologous recombination, chromosome segregation, and sporulation (San-Segundo and Roeder 1999). We examined the requirement for PCH2 in the meiotic arrest of set1Δ and swd1Δ yeast mutants by testing the ability of set1Δpch2Δ and swd1Δpch2Δ mutants to form viable spores. After 12 h in sporulation medium, we found that set1Δpch2Δ mutants produce 38% spores while swd1Δpch2Δ mutants produce 30% spores (Figure 5B). These spore percentages increase for both strains after 24 h of sporulation. These data indicate a partial requirement for PCH2 in mediating set1Δ and swd1Δ mutant meiotic arrest. In support of this conclusion, we found that ~20% of set1Δpch2Δ and swd1Δpch2Δ mutants remain mononucleated after 24 h in sporulation conditions (Figure 5B). Interestingly, despite increased sporulation efficiency, both set1Δpch2Δ and swd1Δpch2Δ mutants have reduced spore viability, indicating defects in chromosome segregation in these mutants (Table 4).

The suppression of set1Δ and swd1Δ mutant meiotic arrest by pch2Δ mutations suggests that Set1 and Swd1 mediate SC formation. To determine if this is an indirect result of impaired DSB formation, we performed meiotic analyses in yeast lacking the

**Table 4 Spore viability for checkpoint double mutants**

| Strain genotype | % viable spores | Number of spores analyzed |
|-----------------|-----------------|---------------------------|
| set1Δrad9Δ      | 32.5            | 80                        |
| swd1Δrad9Δ      | 40              | 120                       |
| set1Δpch2Δ      | 57.5            | 120                       |
| swd1Δpch2Δ      | 35              | 120                       |
| set1Δmad2Δ      | 47.5            | 120                       |
| swd1Δmad2Δ      | 17.5            | 120                       |
| set1Δspo11Δ     | 8.4             | 120                       |
| swd1Δspo11Δ     | 0               | 120                       |

Figure 5 SET1 and SWD1 are required for different steps of meiotic commitment. (A) Spore percentages and (B) DAPI staining bodies were quantified for yeast harboring deletion mutations in SET1 or SWD1 combined with the checkpoint genes RAD9, PCH2, or MAD2. (A) Spore percentages are reported 12 and 24 h post-meiotic induction and include deletion mutations for the SPO11 endonuclease. (B) DAPI staining nuclei were quantified following 24 h in SPM. Graphs represent the average spore percentage for independent biological triplicates; error bars depict the standard error of the mean.
meiosis-specific endonuclease Spo11. Previous studies indicated that SET1 is required for the normal distribution of Spo11-induced DSBs, but it is not essential for DSB formation (Sollier et al. 2004; Borde et al. 2009). Yeast lacking Spo11 fail to initiate DSBs and bypass the recombination and chromosome synopsis checkpoints (Klapholz et al. 1985; Keeney et al. 1997). To determine if Spo11 is required for the set1Δ and swd1Δ meiotic arrest phenotype, we performed meiotic analyses of set1Δspo11Δ and swd1Δspo11Δ mutants (Figure 5). These experiments revealed that ~25% of set1Δspo11Δ mutants form spores after 12 h in sporulation medium, and this number increases to ~40% of after 24 h (Figure 5). These data suggest that the set1Δ meiotic arrest is partially dependent on Spo11-mediated DSB formation. We observed a similar, but more robust, rescue of sporulation in swd1Δspo11Δ mutants with ~40% spores after 12 h of growth in sporulation medium that increases to >70% sporulation after 24 h (Figure 5). These data suggest that DSB formation plays a more significant role in the meiotic arrest observed in swd1Δ mutants. In both cases, microdissection of the resulting spores revealed that they are inviable, consistent with previous investigations indicating that Spo11 is required for meiotic chromosome segregation (Klapholz et al. 1985; Wagstaff et al. 1985; Cao et al. 1990). Together, these data are consistent with a role in homologous recombination for both Set1 and Swd1.

Finally, we investigated the role of the spindle assembly checkpoint (SAC) in mediating SET1- and SWD1-dependent meiotic arrest. Similar to mitosis, chromosome segregation during meiosis requires proper spindle assembly and attachment to kinetochores (Li and Nicklas 1995; Biggins and Murray 2001). The SAC ensures that these steps are faithfully completed to allow proper chromosome segregation and avoid aneuploidy. The SAC component Mad2 is important for proper meiotic chromosome segregation and regulates the timing of cellular progression through MI (Shonn et al. 2003; Tsuchiya et al. 2011). Importantly, previous investigations of mitotic chromosome division demonstrated that Set1-mediated H3K4me negatively regulates Mad2 until the spindle attaches to chromosomes (Schibler et al. 2016).

To determine if the absence of SET1 and SWD1 lead to hyperactivation of Mad2 and meiotic arrest, we performed meiotic analyses of set1Δmad2Δ and swd1Δmad2Δ mutants. Similar to our analyses of PCH2, we found that set1Δmad2Δ and swd1Δmad2Δ mutants produce ~20% spores after 12 h in sporulation medium and that the spore percentages increase to ~65% at the 24-h time point (Figure 5A). Consistent with a partial role for MAD2 in SET1- and SWD1-dependent meiotic progression, 25% of set1Δmad2Δ and swd1Δmad2Δ mutants remain mononucleated after 24 h in sporulation medium (Figure 5B). Microdissection of the resulting ascii revealed defects in spore viability, as 47.5% of set1Δ mad2Δ mutant spores were viable and only 17.5% of swd1Δmad2Δ mutant spores survived (Table 4). The reduced spore viability in the set1Δmad2Δ and swd1Δmad2Δ yeast mutants also reflects the high rates of nondisjunction observed when Mad2 is removed in isolation (Shonn et al. 2000, 2003). Our results suggest that both Set1 and Swd1 are important for spindle attachment to the kinetochore during the meiotic divisions. Together, our data support a model in which both Set1 and Swd1 are critical for the faithful execution of multiple steps during prophase I, including recombination and spindle assembly. Furthermore, Swd1 displays a Set1-independent role in regulating replicative chromosomal lesions, as supported by the Rad9-dependent arrest in swd1Δ mutants (Figure 5A).

SWD3 is involved in late spore morphogenesis

Following the completion of MI, yeast undergo morphogenetic pathway directing prospore membrane formation and spore wall assembly thus protecting the newly formed haploid gametes from environmental stressors (Briza et al. 1990). Spore wall assembly occurs sequentially in a process that first deposits mannan and beta-glucan, whose orientation is reversed relative to vegetative cells, and the spore-specific chitosan and dityrosine layers (Kreger-Van Rij 1978; Briza et al. 1988; Smits et al. 2001). Importantly, spore wall formation is dependent upon both the expression of Ndt80-dependent middle and late meiotic genes and the activity of Smk1 and Sp2 protein kinases (Chu et al. 1998; Chu and Herskowitz 1998; Primig et al. 2000; Omerza et al. 2018). Spore sensitivity to ether is a frequently used assay that allows measurement of spore wall assembly. While ether is toxic to vegetative cells, yeast gametes with properly formed spore walls are resistant to ether exposure (Dawes and Hardie 1974). To determine if COMPASS is required for spore wall formation, we performed ether sensitivity assays on the COMPASS deletion mutants (Figure 6A and Supplementary Figure S2). Consistent with previous reports, we found that wild-type yeast spores are resistant to ether exposure (Figure 6A). Both set1Δ and swd1Δ cause increased ether sensitivity, however, both of these strains also have reduced spore numbers. For example, while set1Δ mutant spores are ~100-fold more sensitive to ether than wild-type spores, the mutant strains have a 10-fold reduction in total spore numbers (Figures 2A and 6A). Similarly, swd1Δ mutants display ~10–100-fold increases in ether sensitivity yet sporulate with approximately threefold reduced rates relative to wild type (Figures 6A and 2A). In contrast, swd3Δ mutants form approximately two-fold fewer spores than wild-type, these deletion mutants are >1000-fold more sensitive to ether exposure (Figures 2A and 6A).

![Figure 6 SWD3 supports late spore morphogenesis and spore resistance to stress.](image-url)
These data suggest that SWD3 is important for proper spore wall formation.

To further characterize the role of Swd3 in spore wall assembly, we performed fluorescence microscopy of wild-type, swd1Δ and swd3Δ mutant spores stained with both Calcofluor white and Eosin Y. Calcofluor white is a chitin/chitosan stain that can only access spores if there is a defective dityrosine layer, while Eosin Y will specifically stain chitosan. We eliminated set1Δ mutants from these analyses due to the low spore numbers in this mutant (Figure 2A). In all strains analyzed, we observed minimal Calcofluor white staining indicating that the dityrosine layer is intact in these spores (Figure 6B; Tachikawa et al. 2001). Consistent with previous reports, Eosin Y staining of wild-type cells revealed a chitosan layer surrounding each individual spore (Figure 6B; Baker et al. 2007; Lin et al. 2013). Interestingly, while we observed robust Eosin Y staining in both the swd1Δ and swd3Δ mutants, the distribution of the stain showed a unique pattern (Figure 6B). In these mutant strains, an aberrant Eosin Y staining pattern was visible inside of individual spores. Quantification of this irregular staining distribution revealed that ~5% of swd1Δ mutant spores contain Eosin Y staining that is internalized, whereas ~73% of swd3Δ mutant spores have this staining pattern (Figure 6B). Furthermore, the internalized Eosin Y staining pattern appeared to be random, as the same ascus could contain anywhere from 1 to 4 spores displaying this unique spore morphology. Together, these data suggest that the increased ether sensitivity observed for swd3Δ mutants may be due to improper chitosan distribution in the spore wall, as opposed to failures in chitosan production.

Discussion

This study reveals new roles for the core COMPASS complex members Set1, Swd1, and Swd3 in executing meiosis. Our findings are consistent with a model in which both Set1 and Swd1 promote progression through meiotic prophase by acting at multiple execution points. Checkpoint suppression analyses indicated that Set1 is required for homologous recombination and meiotic spindle assembly. These results are consistent with previous reports suggesting that Set1 is required for efficient meiotic DSB distribution and mitotic spindle assembly (Sollier et al. 2004; Borde et al. 2009; Schibler et al. 2016). Interestingly, our results indicate that the role for Set1 during prophase is independent of H3K4me, suggesting the presence of nonhistone substrates or an important structural role for the COMPASS complex during meiosis. In contrast to Set1, Swd1 appears to act earlier in the meiotic program and is involved in the post-replicative repair pathway. Finally, we find that Swd3 is dispensable for meiotic prophase, but plays an important role during late meiosis. Specifically, yeast mutants lacking Swd3 are hypersensitive to ether exposure and display errors in ascospore wall assembly. Together, these results indicate that Set1, Swd1, and Swd3 have previously undescribed roles in efficient meiotic timing and gametogenesis that are independent of their well-characterized functions for catalyzing H3K4me catalysis.

Meiosis-specific functions for COMPASS subunits

In contrast to the well-studied functions for COMPASS during vegetative growth, investigations focused on meiosis have been more limited (Borde et al. 2000; Sollier et al. 2004; Borde et al. 2009; Sommermeyer et al. 2013; Adam et al. 2018). Similar to other histone-modifying complexes, Set1 is dispensable for mitotic divisions, but is required for meiosis (Sollier et al. 2004; Borde et al. 2009; Jaiswal et al. 2017). This highlights the sensitivity of the temporally regulated meiotic program to the balance in histone modification complex activity. In addition to targeting histone substrates, this investigation and others indicate that modifications of nonhistone proteins are also critical regulators of meiosis (Mallory et al. 2012; Law et al. 2014). Furthermore, our results suggest that meiosis-specific COMPASS functions are a key aspect of meiotic timing and commitment. In support of this, previous studies have found that Spp1 stimulates H3K4me3 by interacting with the COMPASS complex during vegetative growth. As cells enter meiotic prophase, Spp1 plays COMPASS-independent roles to coordinate DSBs by forming a molecular bridge spanning both H3K4me3 and the chromosomes axis protein Mer2 (Acquaviva et al. 2013; Sommermeyer et al. 2013; Adam et al. 2015). Interestingly, despite the importance of both H3K4me3 and Spp1 to efficient DSB formation, yeast mutants lacking either Set1 or Spp1 form re-distributed DSBs at reduced levels (Borde et al. 2009). These data indicate that multiple compensatory mechanisms are in place to ensure that genetic recombination occurs during meiosis, thus resulting in genetically diverse haploid gametocytes. Identifying other factors that direct Spo11 to initiate DSBs will be an important step to resolving this key step in gametogenesis.

Similar to Spp1, the results presented here are consistent with meiosis-specific functions for Swd1 and Swd3. While most studies have indicated that Swd1 and Swd3 are genetically and biochemically inseparable during vegetative growth, transcriptomic analyses indicate minor differences in their requirement for gene repression (Margaritis et al. 2012). In contrast to this subtle distinction, our results indicate dramatically different requirements for Swd1 and Swd3 in early and late meiosis respectively. Interestingly, our data suggest that Swd1 and Swd3 function during meiosis is independent of their roles in catalyzing H3K4me as part of the COMPASS complex.

These results are consistent with at least two models that describe the requirement of COMPASS for meiosis. In the first model, COMPASS catalyzes methylation of nonhistone substrates whose modification is required for meiosis. The only nonhistone substrate for COMPASS identified to date is Dam1, a component of a heterodecameric protein complex that comprises the kinetochore (Cheeseman et al. 2001; Janke et al. 2002; Li et al. 2002; Westermann et al. 2005; Zhang et al. 2005). Interactions between the kinetochore and microtubules stimulate sister chromatid separation during both mitosis and MI and therefore must be modified to accommodate homologous chromosome separation during MI (Marston and Amon 2004). During MI kinetochores of sister chromatids are co-oriented and attach to microtubules from the same spindle pole utilizing a protein complex termed monopolin (Toth et al. 2000). Interestingly, the timing of microtubule-kinetochore attachments appears to be a critical regulator of the chromosome divisions during meiosis. For example, inhibition of these attachments through ectopic expression of the meiosis-specific factor Mam1 during mitosis results in M-like reductive divisions (Miller et al. 2012). Conversely, inducing microtubule-kinetochore attachments prematurely during MI results in sister chromatid segregation as observed during mitosis (Miller et al. 2012). Therefore, one model to explain the H3K4me-independent functions of COMPASS during meiosis implicates Dam1 methylation as a key regulator of microtubule attachments during MI. While a precise role for Dam1 methylation has not yet been identified, previous studies indicate that methylation inhibits phosphorylation of neighboring serines by the Aurora kinase Ipl1 and is important for a “methyl-phospho”
switch (Zhang et al. 2005). Interestingly, kinetochore attachment to microtubules requires Dam1 phosphorylation, suggesting that failure to methylate Dam1 may allow premature phosphorylation and cause defects in chromosome segregation during MI. This is supported by our findings of aneuploidy in gametes formed from set1Δ and set1A yeast mutants harboring deletions in the Mad2 SAC protein. Interestingly, while previous work found that both Set1 and Swd1 are required for Dam1 methylation, the role of Swd3 in catalyzing this modification was not reported (Latham et al. 2011). This raises the possibility that Dam1 methylation occurs independently of Swd3, thus resulting in the disparate requirements for Set1, Swd1, and Swd3 during MI.

A second model that could explain our results involves noncatalytic, structural roles for COMPASS in orchestrating progression through meiotic prophase. Recent, high-resolution structural studies of the COMPASS complex indicate the presence of extensive interactions with the nucleosome (Worden et al. 2020). For example, Set1/Bre2 interact directly with nucleosomal DNA on one arm of the complex while Swd1/Spp1 bind to DNA on the opposite arm (Worden et al. 2020). This establishes a COMPASS/nucleosome interface that positions both Swd1 and Set1 in close proximity to the histone core. Point mutations of key basic residues in Swd1 that are responsible for contacting DNA cause a moderate reduction in H3K4me2 and me3, indicating that maintaining these interactions is a key component of catalytic activity (Worden et al. 2020). In contrast to Set1 and Swd1, Swd3 appears away from DNA on the backside of the COMPASS complex and does not contribute to direct interactions between COMPASS and the nucleosome (Worden et al. 2020). These data suggest that eliminating either Set1 or Swd1 would cause dissociation of the COMPASS complex from nucleosomes, perhaps influencing local chromatin structure independently of methyltransferase activity. It is intriguing that swd3Δ mutants cause destabilization of Set1, yet do not phenocopy set1Δ mutants during meiosis. This observation raises questions about locus-specific COMPASS assemblies and their ability to maintain local chromatin structures via both catalytic and structural mechanisms. Future experiments aimed at discriminating between a structural or catalytic role for COMPASS during meiosis will be a crucial next step in determining how this highly conserved protein complex regulates gametogenesis. In support of this, separation of function alleles for the COMPASS during meiosis will be a crucial next step in determining how this highly conserved protein complex regulates gameto-

**Genetic interactions between COMPASS and HORMA-domain proteins**

Our results and others indicate that COMPASS genetically interacts with proteins that harbor a common protein domain termed HORMA (Hop1, Rev7, Mad2; Aravind and Koonin 1998; Schibler et al. 2016). HORMA domain-containing proteins are required for coordinating meiotic prophase and are characterized by an N-terminal core domain and a C-terminal “safety belt” (Rosenberg and Corbett 2015). The safety belt region interacts with the HORMA core domain in two distinct conformations termed “open” and “closed,” which in turn mediates protein-protein interactions. HORMA proteins interact with binding partners in the “closed” state and these interactions are critical for their meiotic functions. How these conformational changes are regulated throughout meiotic prophase remains poorly understood.

Some insight into how COMPASS regulates HORMA proteins can be gleaned from the checkpoint suppression analyses reported in this study. First, we found that the requirement for SET1 and SWD1 during meiotic prophase is suppressed by mutation of PCH2. Pch2 is a conserved protein that mediates Hop1 removal following successful SC formation (San-Segundo and Roeder 1999). Hop1 interacts with Spo11 via the linker protein Red1 and these interactions are critical for normal DSB formation (Smith and Roeder 1997; de los Santos and Hollingsworth 1999; Panizza et al. 2011). Our data suggest that in the absence of SET1 or SWD1, Hop1 is unable to establish the SC, leading to Pch2-mediated meiotic arrest. This is consistent with the requirement of Set1 for DSB distribution (Sollier et al. 2004; Sommermeyer et al. 2013) and is further supported by our findings that spo11Δ suppresses set1Δ and swd1Δ meiotic defects.

Second, we found that deletion of the HORMA protein Mad2 suppresses set1Δ and swd1Δ mutant meiotic arrest. Mad2 is a component of the SAC that monitors microtubule-kinetochore attachments during chromosome divisions in both mitosis and meiosis (Li and Murray 1991; Pesin and Orr-Weaver 2008; Lau and Murray 2012; Jia et al. 2013). The SAC is deactivated when microtubules properly attach to the kinetochore and establish tension, permitting Anaphase Promoting Complex/Cyclosome (APC/C) activation (Jia et al. 2013). The APC/C is an E3 ubiquitin ligase that targets protein substrates, such as Pds1, for degradation by the 26S proteasome (Jia et al. 2013). Both APC/C activation and substrate specificity are mediated by auxiliary factors such as Cdc20 and Cdh1 and the meiosis-specific protein Ama1 (Dawson et al. 1995; Visintin et al. 1997; Cooper et al. 2000). Previous work indicates that H3K4me inactivates the SAC during mitosis by directly interacting with Mad2 and separating it from APC/C (Schibler et al. 2016). This interaction relieves APC/C/Cdc20 repression, allowing cells to progress through anaphase. In this model, COMPASS restricts Cdc20-mediated proteolysis until the proper conditions for chromosome separation have been met. Our results partially support a similar role for H3K4me in regulating Mad2 activity and progression through anaphase I of meiosis. For example, our data indicate that the subunits required for H3K4me2, BRE2 and SDC1, are important for both meiotic timing and completion (Figure 2). Furthermore, we found that both set1Δmad2Δ and swd1Δmad2Δ mutants progress past meiosis I, but have increased aneuploidy (Figure 5 and Table 4). These data suggest both Set1 and Swd1 are important for proper chromosome segregation during meiosis, consistent with a role in spindle attachment to the kinetochore. This points to important roles for COMPASS mediated methylation of both histone H3K4 and non-histone substrates in regulating meiotic chromosome segregation.

Finally, our data indicate that Swd1 is involved in Rad9-dependent post-replicative DNA damage repair during meiosis. One mechanism that cells can utilize to bypass DNA lesions involves error-prone translesion DNA synthesis by the DNA Polymerase Zeta complex, which contains the HORMA protein
Rev7 (Polç, Nelson et al. 1996; Andersen et al. 2008). Yeast mutants lacking Rad9 display a Rev7-dependent hypermutability phenotype, indicating that Rad9 antagonizes the error-prone repair pathway in favor of an error-free one (Murakami-Sekimata et al. 2010). In addition to its well-characterized role in translesion DNA synthesis, Rev7 is also implicated in meiotic DSB processing. Intriguingly, recent reports indicate Spo11-induced DSBs during meiosis are associated with Polç-mediated repair mechanisms (Rattray et al. 2015). The net result of this DNA repair is an increased mutation rate at DSB hotspots, which increases the genetic variation in the haploid gametes. These results suggest that Swd1 may be an important regulatory factor in directing Rev7 activity to repair DSBs during meiosis. Identifying how changes in both COMPASS composition and locus-specific activity contribute to genetic recombination during meiosis will be an important question in the future.

Data availability

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplementary material is available at G3 online.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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