Copyright © 1997 by the Genetics Society of America

Progression Into the First Meiotic Division Is Sensitive to Histone H2A-H2B Dimer Concentration in *Saccharomyces cerevisiae*

Kochung Tsui,* Lee Simon* and David Norris*,†

**The Waksman Institute and †The Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855-0759*

Manuscript received June 28, 1996
Accepted for publication November 22, 1996

ABSTRACT

The yeast *Saccharomyces cerevisiae* contains two genes for histone H2A and two for histone H2B located in two divergently transcribed gene pairs: *HTA1-HTB1* and *HTA2-HTB2.* Diploid strains lacking *HTA1-HTB1* (*hta1-htb1*Δ/*hta1-htb1*Δ, *HTA2-HTB2/HTA2-HTB2*) grow vegetatively, but will not sporulate. This sporulation phenotype results from a partial depletion of H2A-H2B dimers. Since the expression patterns of *HTA1-HTB1* and *HTA2-HTB2* are similar in mitosis and meiosis, the sporulation pathway is therefore more sensitive than the mitotic cycle to depletion of H2A-H2B dimers. After completing premeiotic DNA replication, commitment to meiotic recombination, and chiasma resolution, the *hta1-htb1*Δ/*hta1-htb1*Δ, *HTA2-HTB2/HTA2-HTB2* mutant arrests before the first meiotic division. The arrest is not due to any obvious disruptions in spindle pole bodies or microtubules. The meiotic block is not bypassed in backgrounds homozygous for *spo13*, *rad50*Δ, or *rad9*Δ mutations, but is bypassed in the presence of hydroxyurea, a drug known to inhibit DNA chain elongation. We hypothesize that the deposition of H2A-H2B dimers in the mutant is unable to keep pace with the replication fork, thereby leading to a disruption in chromosome structure that interferes with the meiotic divisions.

THE fundamental subunit of eukaryotic chromatin is the nucleosome core particle, consisting of two superhelical turns of DNA wrapped around an octamer of histones; the octamer, in turn, is constructed from three subunits, a single H3-H4 tetramer and two H2A-H2B dimers (Eickbush and Moudrianakis 1978; Pederson *et al.* 1986; Moudrianakis and Arents 1993; Wang *et al.* 1994). Genetic analyses have shown that these suboctameric particles function not only as structural components of chromatin, but also as important regulators of chromatin assembly. For instance, in the yeast *Saccharomyces cerevisiae,* the rate of chromosome loss increased dramatically in cells overexpressing either the dimer or the tetramer; the rate did not increase, however, in cells overexpressing the dimer and tetramer together or in cells overexpressing the core histones individually (Meeks-Wagner and Hartwell 1986). These results have been interpreted to mean that the eukaryotic chromosome exists in a state of dynamic equilibrium, one which is ultimately regulated by the intracellular concentrations of the dimer and tetramer (Meeks-Wagner and Hartwell 1986). If true, then the equilibrium is not disrupted by increasing the absolute levels of the dimer or tetramer *per se,* but rather by altering the balance or stoichiometry between them.

How do cells regulate intracellular levels of H2A-H2B dimers and H3-H4 tetramers, both in relation to each other and in relation to newly replicated DNA? What stages of the life cycle are most sensitive to changes in dimer and tetramer stoichiometry? We are taking a genetic approach to address these questions using yeast as our experimental organism. In particular, we are focusing on the regulation of H2A-H2B dimers.

Yeast contains two genes for histone H2A (*HTA1* and *HTA2*) and two genes for histone H2B (*HTB1* and *HTB2*) located in two divergently transcribed gene pairs: an *HTA1-HTB1* pair on chromosome *IV* and an *HTA2-HTB2* pair on chromosome *II* (Hereford *et al.* 1979). The *HTA1-HTB1* and *HTA2-HTB2* loci are not functionally identical. Haploid *hta1-htb1*Δ, *HTA2-HTB2* mutants grow slowly, exit from stationary phase inefficiently, and germinate poorly; morever, they manifest changes in chromatin structure, in promoter site selection by RNA polymerase, and in the heat shock response (Norris and Osley 1987; Clark-Adams *et al.* 1988; Norris *et al.* 1988). By comparison, *HTA1-HTB1, hta2-htb2*Δ mutants exhibit no observable phenotypes. This dramatic difference reflects the fact that the expression of *HTA1-HTB1,* but not *HTA2-HTB2,* modulates in response to the intracellular level of H2A-H2B dimers (Norris and Osley 1987; Moran *et al.* 1990). Thus, in the *HTA1-HTB1, hta2-htb2*Δ mutant, the expression of *HTA1-HTB1* increases to compensate for the *hta2-htb2*Δ mutation. As a result of this phenomenon, known as dosage compensation, H2AH2B dimers are produced at an appropriate level to maintain fully functional chromatin. However, in the *hta1-htb1*Δ, *HTA2-HTB2* mutant, the remaining *HTA2-HTB2* locus

Corresponding author: David Norris, The Waksman Institute, P.O. Box 759, Piscataway, NJ 08855-0759.
E-mail: norris@mbcl.rutgers.edu

does not compensate for the *hta1-htb1Δ* mutation. Therefore, in the latter mutant, insufficient H2A and H2B transcripts are produced, and the cell exhibits the described phenotypes.

Notwithstanding their highly pleiotropic phenotypes, *hta1-htb1Δ, HTA2-HTB2* mutants successfully complete the mitotic cell cycle. In contrast, *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* diploids fail to complete the nonvegetative process of sporulation (NORRIS and OSLEY 1987). In this report, we examined why sporulation was more sensitive to changes in *HTA1-HTB1* copy number than mitotic growth. We tested three models:

1. The first model hypothesizes that the histone subtypes encoded by *HTA1-HTB1* are specifically required for sporulation. The H2A subtypes encoded by *HTA1* and *HTA2* differ by two amino acids, while the H2B subtypes encoded by *HTB1* and *HTB2* differ by four amino acids (WALLIS *et al.* 1980). The differences in H2B subtypes are particularly intriguing in that three of the affected amino acids are located in the flexible N-terminal arm. Since these arms are subject to extensive posttranslational modification, and since similar arms in H3 and H4 have been shown to regulate higher order chromatin in yeast, it seemed possible that H2B1 and H2B2 might play different roles in the regulation of chromatin structure during sporulation (JOHNSON *et al.* 1992; MANN and GRUNSTEIN 1992; ROTH *et al.* 1992; THOMPSON *et al.* 1994a,b; FISHER-ADAMS and GRUNSTEIN 1995; HECHT *et al.* 1995).
2. The second model hypothesizes that *HTA2-HTB2* is transcribed at a lower basal rate during sporulation, *i.e.*, *HTA2* and *HTB2* messages comprise a smaller fraction of the total *HTA* and *HTB* transcripts. As a result, the removal of *HTA1-HTB1* would be predicted to have a more pronounced effect on the intracellular levels of histone transcripts during sporulation.
3. The third model hypothesizes that some aspect of sporulation itself is particularly sensitive to decreases in *HTA1-HTB1* copy number. Since sporulation and vegetative growth differ dramatically, many stages of sporulation are unique and, hence, could be potential *hta1-htb1Δ*-sensitive steps (ESPOSITO and KLAPHOLZ 1981).

We show here that diploid yeast cells, under appropriate genetic conditions, sporulate in the absence of *HTA1-HTB1* subtypes, ruling out the first model. We also show that the expression levels and dosage compensation patterns of *HTA1-HTB1* and *HTA2-HTB2* are nearly identical during sporulation and vegetative growth, ruling out the second model. Therefore, our results are most consistent with the third model. To determine why sporulation exhibited this enhanced sensitivity, we further analyzed the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant under sporulation conditions. We found that the cells completed meiotic recombination, although it was somewhat delayed, and then arrested before the first meiotic division. The arrest was not bypassed in the presence of homozygous *spo13, rad50Δ,* or *rad9Δ* mutations, but a significant increase in sporulation was observed after the addition of small amounts of hydroxyurea, a drug known to slow the replication fork.

MATERIALS AND METHODS

Strains: The isogenic strains used in this study are listed in Table 1. To isolate strains bearing the *hta1-htb1Δ* and *hta2-htb2Δ* mutations, *hta1-htb1Δ::URA3* and *hta2-htb2Δ::URA3* constructs were introduced into cells by one step gene transplacement as described (ROTHSTEIN 1983; NORRIS and OSLEY 1987). To isolate strains bearing a duplication of *HTA2-HTB2,* an *HTA2-HTB2::URA3::HTA2-HTB2* construct was introduced into cells by integration of YIp5-TRT2 into *HTA2-HTB2* as described (ROTHSTEIN 1983; NORRIS and OSLEY 1987). To isolate strains bearing the *rad50Δ* mutation, the 1.1-kb *SacI-BglII* fragment of *RAD50* was substituted with a *LEU2* biosynthetic marker, and the resulting construct was introduced into cells by one step gene transplacement (ROTHSTEIN 1983). To isolate strains bearing the *HTA1-htb1-50* locus, a three step procedure was followed. First, the *SacI-BamHI* fragment of *HTB1* was cloned into pBluescript, and alanine-2, lysine-3, and threonine-27 were changed to serine, alanine, and valine, respectively (KUNKEL 1985). Second, the mutagenized fragment was cloned into the yeast shuttle vector YIp5, and the resulting plasmid was integrated into the *HTA1-HTB1* locus (ROTHSTEIN 1983). Third, revertants arising by intrachromosomal recombination were isolated after plating on 5-fluoroorotic acid (BOEKE *et al.* 1984). Retention of the appropriate amino acids in the revertants was confirmed by PCR and DNA sequencing. All diploids and double mutants were isolated by crosses and tetrad dissection.

Chemicals and enzymes: Restriction enzymes were from New England BioLabs. S1 nuclease was from Life Technologies. Bacto yeast extract, Bactopeptone, Dextrose, and potassium acetate were from Fisher. Hydroxyurea was from Sigma.

Isolation of synchronously sporulating cells: Isolation of synchronously sporulating cells was performed by a slight modification of the method of PADMORE *et al.* (1991). Strains were grown to saturation in 5 ml YPD medium [1% (w/v) Bacto yeast extract, 2% (w/v) Bacto peptone, 2% (w/v) dextrose]. An aliquot was resuspended at an OD600 of 0.2 in 350 ml YPA medium [1% (w/v) Bacto yeast extract, 2% (w/v) Bacto peptone, 1% (w/v) potassium acetate] in a 2-liter flask. The flask was shaken vigorously until the culture just completed its final division (typically ~16 hr). Cells were collected by filtration, washed, and resuspended in 350 ml SPM medium [1% (w/v) potassium acetate]. For the hydroxyurea experiments, the same procedure was followed with the exception that the SPM medium contained the designated amount of hydroxyurea. For RNA extraction, aliquots (35 ml) were removed at various times after transfer to SPM, and RNA was prepared as described (NEIGEBORN and MITCHELL 1991).

S1 nuclease assay: Quantitative S1 nuclease assays were performed according to the method of OSLEY *et al.* (1986). The probe for *HTB1* was a *HindIII-SacI* fragment, extending from −379 to +239 with respect to the initiator ATG. The probe for *HTB2* was a *HindIII-AccI* fragment, extending from −461 to +239 with respect to the initiator ATG. The probes were end-labeled with T4 polynucleotide kinase, heat denatured,

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
DN1000	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG*	R. KOLODNER
DN1018	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG his4x/his4b hta1-htb1Δ::URA3/ hta-1-htb1Δ::URA3*	This study
DN1023	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 his4x/his4b*	N. KLECKNER
DN1034	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG his4x/his4b hta1-htb1Δ::URA3/ hta1-htb1Δ::URA3 HTA2-HTB2::URA3::HTA2-HTB2/HTA2-HTB2::URA3::HTA2-HTB2*	This study
DN1068	α *lys2 ura3 ho::LYS2 leu2::hisG HIS4::LEU2*	N. KLECKNER
DN1069	**a** *lys2 ura3 ho::LYS2 leu2::hisG his4x::LEU2-URA3*	N. KLECKNER
KT003	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG HTA1-htb1-50/HTA1-htb1-50*	This study
KT048	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG hta1-htb1Δ::URA3/hta1-htb1Δ::URA3 spo13::hisG/spo13::hisG*	This study
KT056	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG HIS4::LEU2/his4x::LEU2-URA3 hta1-htb1Δ::URA3/hta1-htb1Δ::URA3*	This study
KT060	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG hta2-htb2Δ::URA3/hta2-htb2Δ::URA3*	This study
KT063	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG HIS4::LEU2/his4x::LEU2-URA3*	This study
KT112	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG hta1-htb1Δ::URA3/ hta1-htb1Δ::URA3 rad50Δ::LEU2/rad50Δ::LEU2*	This study
KT195	**a**/α *lys2/lys2 ura3/ura3 ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG hta1-htb1Δ::URA3/ hta1-htb1Δ::URA3 rad9Δ::LEU2/rad9Δ::LEU2*	This study

and hybridized at 48° for 12 hr to total cellular RNA. The resulting RNA:DNA mixture was treated with S1 nuclease (12 units/μg RNA) for 60 min at 15° and then electrophoresed through a 4% polyacrylamide gel.

Physical analysis of reciprocal exchange: To examine reciprocal exchange, we used the physical assay described by CAO *et al.* (1990). These authors showed that a strong hot spot for meiotic reciprocal exchange is created by the insertion of a 2.8-kb segment containing the *LEU2* gene at a site adjacent to the *HIS4* gene. The diploid strain used in our studies contains this *HIS4::LEU2* construct on one copy of chromosome *III*. On the other copy, a *URA3* gene was integrated immediately adjacent to this locus, and the *Xho*I site in the *HIS4* portion was filled in to generate a *his4-X::LEU2-URA3* locus. As a result of these manipulations, *Xho*I digests of genomic DNA produce an 11.8-kb fragment containing the *HIS4::LEU2* locus and a 17.7-kb fragment containing the *his4-X::LEU2-URA3* locus. A reciprocal exchange that resolves at this locus generates two new fragments that are 13.2 and 16.3 kb long. In the Southern analysis shown in Figure 5, the 17.7-kb parental fragment and the 16.3-kb recombinant fragment resolve poorly.

Electron microscopy: The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant was subjected to the standard sporulation regime. After 16 hr in SPM medium, the cells were fixed and embedded as described by BYERS and GOETSCH (1991). The cells were then sectioned with a diamond knife, and the resulting thin sections were stained with aqueous 1% uranyl acetate followed by lead citrate as described by REYNOLDS (1963).

Other techniques: DAPI staining was performed by the method of KASSIR and SIMCHEN (1991). Fluorescence-activated cell sorting (FACS) to determine DNA content was performed by the method of HUTTER and EIPEL (1979). Commitment to genetic recombination was analyzed by an established "pull-back" protocol (ESPOSITO and ESPOSITO 1974; SHERMAN and ROMAN 1963).

RESULTS

Sporulation in yeast is not dependent upon the H2A1 or H2B1 subtypes: As described in the Introduction, the subtype model hypothesizes that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant fails to sporulate because it lacks the H2A1 and/or H2B1 subtypes. We used two genetic assays to test this model. The first examined whether increasing the copy number of *HTA2-HTB2* loci could suppress the phenotype. If the subtype model were correct, then increasing the *in vivo* concentration of H2A2 and H2B2 by increasing their gene dosages would not be expected to lead to suppression. On the other hand, if the phenotype resulted from the general underexpression of H2A-H2B dimers, then increasing the copy number *HTA2-HTB2* might result in at least a partial suppression.

To carry out these experiments, three control strains and one experimental strain were subjected to identical sporulation regimes, and the asci were quantitated 4 days later by differential interference microscopy (Table 2). The control strains consisted of a wild-type diploid, which sporulated at an efficiency of 87.8%, the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant, which sporulated at an efficiency of <1%, and the *HTA1-HTB1/HTA1-HTB1, hta2-htb2Δ/hta2-htb2Δ* mutant, which sporulated at an efficiency of 82.6%. These results are in agreement with our earlier findings and indicate that the phenomenon under study is not background-dependent (NORRIS and OSLEY 1987). The final diploid was the experimental strain, which had the following genotype: *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2*. This diploid sporulated at an efficiency of 88.9%. Thus, increasing the gene dosage of *HTA2-HTB2* suppresses the sporulation phenotype caused by homozygous *hta1-htb1Δ* mutations. This result disproves the subtype model and indicates that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/*

TABLE 2

Sporulation frequencies of histone mutants

Genotype	Sporulation (%)	Incomplete asci (%)
HTA1-HTB1/HTA1-HTB1 HTA2-HTB2/HTA2-HTB2	87.8	5
hta1-htb1Δ/hta1-htb1Δ HTA2-HTB2/HTA2-HTB2	<1	—
HTA1-HTB1/HTA1-HTB1 hta2-htb2Δ/hta2-htb2Δ	82.6	—
hta1-htb1Δ/hta1-htb1Δ HTA2-HTB2/HTA2-HTB2 HTA2-HTB2/HTA2-HTB2	88.9	33
HTA1-htb1-50/HTA1-htb1-50 HTA2-HTB2/HTA2-HTB2	85.6	5

HTA2-HTB2 mutant fails to sporulate because of an underexpression of H2A-H2B dimers.

The sporulation seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant, however, was somewhat aberrant. The asci in a wild-type population of cells were symmetrical and contained four sharply distinguishable spores, but many of the asci in the mutant were incomplete. Thus, while only 5% of the wild-type asci contained less than four spores (19 immature asci/374 total asci), 33% of the asci in the mutant contained less than four (125 immature asci/382 total asci). Moreover, the asci in the mutant were "disorganized," meaning that the spores within the asci had variable sizes and were packaged asymmetrically. We dissected five four-spored asci and 13 three-spored asci from the mutant and found that germination occurred at wild-type frequencies, indicating that the spores were phenotypically normal, at least at a superficial level.

We were intrigued by the observation that sporulation in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant was slightly aberrant. Formally, these results could indicate that the H2A1 or H2B1 subtypes, while not playing dramatic roles, have more subtle functions during sporulation. Alternatively, they might indicate that the expression patterns of histone genes in the latter mutant differ from those in wild-type cells. To distinguish between these possibilities, we used a second genetic assay that examined the sporulation of a diploid strain in which three of the four unique amino acids of H2B1, specifically those located in the flexible N-terminal arm, were changed to the analogous amino acids in H2B2. This strain had the following genotype: *HTA1-htb1-50/HTA1-htb1-50, HTA2-HTB2/HTA2-HTB2,* where the *htb1-50* mutation codes for a protein in which the three unique amino acids in the flexible arm of H2B1 have been changed into the analogous amino acids from H2B2.

We found that this latter mutant sporulated at an efficiency of 85.6% (Table 2). Importantly, the frequency of asci with less than four spores dropped to ~5%. These results argue that the aberrant sporulation seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant did not reflect functional differences between the N-terminal arms of H2B1 and H2B2. We cannot rule out the possibility that the remaining unchanged amino acids in H2B1 or H2A1 are specifically required for sporulation, but these latter residues are located within the core region of the histones and, hence, are unlikely to play a dramatic role in chromatin regulation (Wallis *et al.* 1980). Instead, the sporulation phenotype in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant is most likely due to a subtle defect in histone gene expression patterns. Further evidence for this conclusion is presented below.

Expression patterns of *HTA1-HTB1* and *HTA2-HTB2* are similar in the mitotic and meiotic cell cycles: The second model hypothesized that the two H2A-H2B gene pairs are expressed differently in vegetative and sporulating cells and that this difference is responsible for the block in sporulation exhibited by the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant. To address this model, we examined the expression of *HTA1-HTB1* and *HTA2-HTB2* in sporulating cells. Specifically, we examined whether the two loci exhibit periodic transcription, as is true during mitotic growth; whether their dosage compensation patterns are the same as in mitotic cells; and whether their relative expression levels are similar in mitotic and sporulating cells.

First, we established by Northern analysis the baseline expression patterns of *HTA1-HTB1* and *HTA2-HTB2* during a wild-type sporulation pathway. These experiments, and all others in this work, were performed in the SK-1 strain background (Kane and Roth 1974), which sporulated efficiently and synchronously when subjected to a simple regime developed by Padmore *et al.* (1991). As controls, we also examined the expression of *IME1* and *IME2*, two genes expressed early in meiosis that positively activate the sporulation pathway (Kassir *et al.* 1988; Smith and Mitchell 1989). Importantly for these experiments, *IME1* and *IME2* are known to be regulated temporally during sporulation and can therefore be used to monitor the meiotic synchrony of the sporulating cells. Finally, we examined the expression of the gene that hybridizes to the pC-4 probe (Law and Segall 1988). Although the function of this latter gene remains unknown, it is expressed constitutively throughout sporulation and vegetative growth and, hence, can be used to normalize the amount of loaded RNA.

As expected, *HTA1-HTB1* and *HTA2-HTB2* were ex-

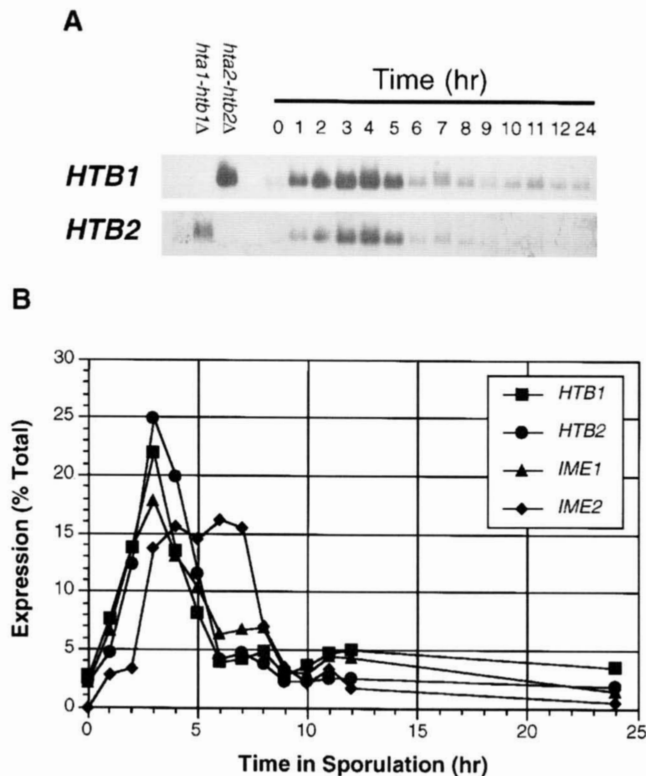

FIGURE 1.—Expression of the *HTB1* and *HTB2* genes exhibits cell-cycle regulation during sporulation. Yeast strain DN1000 was sporulated synchronously as described in MATERIALS AND METHODS. (A) RNA was isolated at the indicated times and subjected to Northern analysis using an *HTB1*-specific probe extending from −176 to +245 relative to the initiator ATG. After exposure of the autoradiogram, probe was stripped from the membrane by boiling, and the blot was rehybridized with an *HTB2*-specific probe extending from −206 to +245 relative to the initiator ATG. (B) The meiotic levels of *HTB1, HTB2, IME1,* and *IME2* transcripts were quantitated on a phosphorimager and normalized against the transcript recognized by the pC-4 probe (MATERIALS AND METHODS) (LAW and SEGALL 1988).

pressed in a cell cycle-dependent fashion in sporulating cells (Figure 1). The period of expression, however, lasted >3 hr during meiosis, as compared to ~30 min during mitosis. This increase in the period of expression most likely reflects the increased time required for premeiotic S phase (PADMORE *et al.* 1991). The *IME1* and *IME2* genes were also expressed in a meiotic cell cycle pattern, with the expression of *IME1* preceding that of *IME2* (Figure 1B). These latter results are in agreement with published studies (KASSIR *et al.* 1988; SMITH and MITCHELL 1989).

Second, we examined the dosage compensation properties of the two H2A-H2B gene pairs during sporulation. To do so, we measured by Northern analysis how the remaining *HTB* genes in various deletion mutants were expressed during sporulation. The results were in general agreement with the predictions. Thus, the steady-state level of *HTB1* transcripts increased approximately twofold in an *HTA1-HTB1/HTA1-HTB1, hta2-htb2Δ/hta2-htb2Δ* mutant (Figure 2B). Therefore, as predicted, the expression of *HTA1-HTB1* exhibits dosage compensation. The steady-state level of *HTB2* transcripts, however, remained low in a *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant (Figure 2A). Finally, the steady-state levels of *HTB2* transcripts increased in a *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2* mutant (Figure 2C). This last result further argues that the original *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant fails to sporulate because the steady-state levels of HTA and HTB transcripts are too low, strongly arguing that its sporulation deficiency results from the underexpression of H2A-H2B dimers.

Notwithstanding the general agreement between prediction and result, the experiments did hold some surprises. For instance, in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant, the initiation of *HTB2* expression was delayed by 2–3 hr when compared to wild-type cells. As we show below, initiation of premeiotic S phase is likewise delayed in the mutant (Figure 7). The reason for the delay remains unclear, but the results are in general agreement with earlier findings showing that strains carrying the *hta1-htb1Δ* mutation are unable to efficiently transit from one growth state to another (NORRIS and OSLEY 1987). In this case, the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant transited slowly from aerobic, vegetative growth into the sporulation pathway. In further support of this interpretation, the peak of *HTB2* expression in Figure 2A was lower and broader than expected, suggesting that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant entered the sporulation pathway more asynchronously than the other strains.

HTB2 expression also differed slightly from prediction in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant. Specifically, transcription of *HTB2* initiated earlier, and stayed on longer, than in wild-type cells. Since the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant sporulated with the same kinetics as wild-type cells (data not shown), we do not attribute this broadening of the peak to a lesser degree of meiotic synchrony. Rather, we favor a model in which the *HTA2-HTB2* duplication is regulated less stringently than a lone *HTA2-HTB2* locus. While the molecular nature of this difference remains unclear, it may explain the increase in aberrant tetrads seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2* mutant (Table 2).

As controls in the preceding Northern analyses, we examined *IME1* expression (Figure 2D). We were surprised to observe that the expression of *IME1* mimicked in several important ways the expression of the H2A-H2B gene pairs. Thus, in the *HTA1-HTB1/HTA1-HTB1, hta2-htb2Δ/hta2-htb2Δ* mutant, the steady-state level of the *IME1* transcript increased approximately twofold

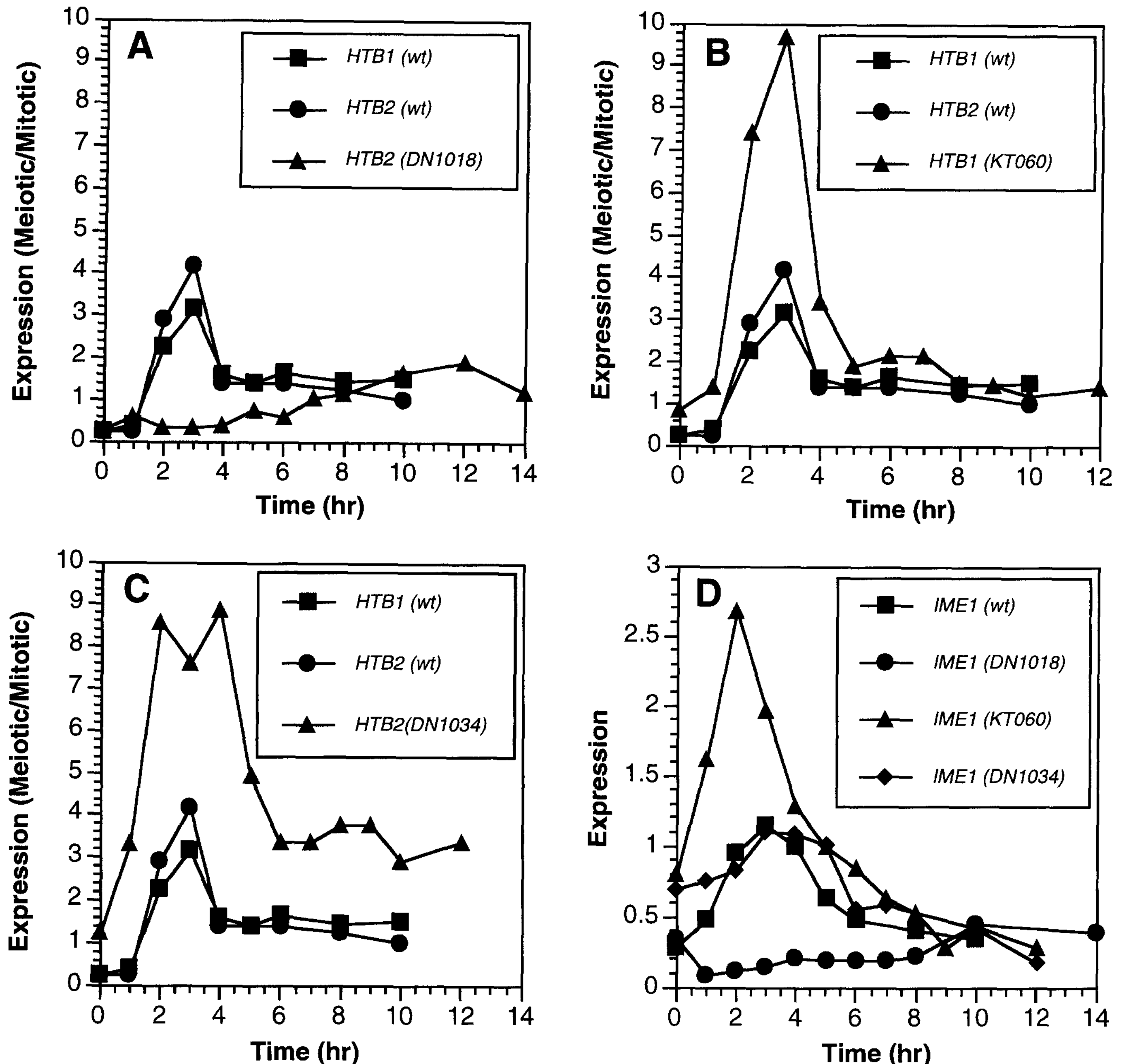

FIGURE 2.—Dosage compensation patterns of *HTA1-HTB1* and *HTA2-HTB2*. All strains were sporulated synchronously (MATERIALS AND METHODS), RNA was extracted at the indicated times and analyzed by Northern blots using the probes described in Figure 1. Quantitation was performed on a phosphorimager, normalizing against a single mitotic RNA control included on each gel. (A) Expression of *HTB1* and *HTB2* in wild-type cells compared to the expression *HTB2* in DN1018 (*hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2*). (B) Expression of *HTB1* and *HTB2* in wild-type cells compared to the expression *HTB1* in KT060 (*HTA1-HTB1/HTA1-HTB1, hta2-htb2Δ/hta2-htb2Δ*). (C) Expression of *HTB1* and *HTB2* in wild-type cells compared to the expression *HTB2* in DN1034 (*hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2*). (D) Expression of *IME1* in wild-type, DN1018, KT060, and DN1034 strains.

over its level in wild-type cells, similar to the dosage-compensated *HTB1* message. In the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant, the initiation of *IME1* transcription was delayed, similar to the delay in the initiation of *HTB2* transcription. Finally, the expression of *IME1* in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant started earlier and extended longer than in wild-type cells, similar to the expression pattern of *HTB2* seen in this mutant. The reasons for these changes in *IME1* expression are unknown, but the observation that *IME1* may be coregulated with the two H2A-H2B gene pairs suggests a potential functional linkage between their gene products.

Third, we determined the mitotic and meiotic levels of the *HTB1* and *HTB2* transcripts using a quantitative

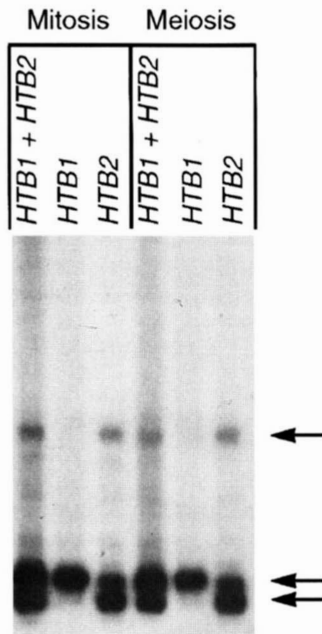

FIGURE 3.—The *HTB1* and *HTB2* genes are expressed equivalently in mitosis and meiosis. RNA was extracted from strain DN1000 during logarithmic growth (labeled Mitosis) or 4 hr after entrance into sporulation (labeled Meiosis). The extracted RNA was then subjected to quantitatiive S1 analysis according to the method of OSLEY *et al.* (1986). The lane designations (*HTB1, HTB2,* and *HTB1+HTB2*) indicate the DNA probes used in the S1 reactions (MATERIALS AND METHODS). The arrows indicate the positions of the 5′ ends of the *HTB2* transcripts.

S1 nuclease assay (Figure 3) (OSLEY *et al.* 1986). To do so, RNA was extracted from logarithmic cells and from meiotic cells 4 hr into sporulation. The RNA samples were then hybridized simultaneously to two probes, one homologous to the 5′ end of the *HTB1* transcript and the other homologous to the 5′ end of the *HTB2* transcript. The resulting RNA:DNA hybrids were treated with S1 nuclease and subsequently electrophoresed through a polyacrylamide gel, and the intensities of the S1-resistant bands were quantitated on a phosphorimager and normalized to the specific activities of the individual *HTB* probes. To identify the specific transcripts, we also carried out control reactions in which the purified RNA was hybridized only to the *HTB1* probe or to the *HTB2* probe.

The results of this analysis indicated that transcription of *HTB1* and *HTB2* initiated at the same sites in mitosis and meiosis (Figure 3). *HTB2* had three major initiation sites (marked by the arrows in Figure 3). *HTB1* had one site, which generated an S1-resistant fragment that comigrated with the middle *HTB2* transcript. Thus, in the experimental samples that were hybridized to both probes, the middle band arose from *HTB1* and *HTB2.* The final quantitation, taking account of the fact that the middle band represented both messages, indicated that *HTB1* comprised 42% of the total *HTB* transcript during mitosis and 45% of the total *HTB* transcript during meiosis. Therefore, the two H2A-H2B gene pairs are expressed at approximately the same ratios in meiosis and mitosis.

In summary, the expression patterns of the H2A-H2B gene pairs are similar in the mitotic and meiotic cell cycles. Therefore, we have no evidence to support the second model in the Introduction. Instead, the third model, which postulates that some step in the sporulation pathway is uniquely sensitive to the partial depletion of H2A-H2B dimers, most likely accounts for the enhanced sensitivity seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant.

The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrests before the first meiotic division: To gain insight into why sporulating cells are more sensitive than mitotic cells to decreases in dimer levels, we examined the sporulation deficiency of the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant in more detail. The first step in this analysis was to determine where in the sporulation pathway it arrested. We therefore subjected the mutant to sporulation conditions and determined the fate of particular landmark events. We started by staining aliquots of arrested cells with the DNA-intercalating dye DAPI to determine whether the mutants passed through the meiotic divisions (KASSIR and SIMCHEN 1991). After 24 hr in sporulation conditions, 282 cells (86.5%) arrested with one nucleus, 12 cells (3.7%) arrested with two nuclei, 12 cells (3.7%) arrested with three nuclei, and 20 cells (6.1%) arrested with four nuclei. Cells after 48 hr of arrest exhibited similar cell types (data not shown). The nuclei in all cells were unelongated, indicating that the block did not occur during the actual segregation of chromosomes. From these results, we conclude that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrested primarily before the first meiotic division, although some cells were able to proceed through the divisions.

Meiotic recombination immediately precedes the first meiotic division in wild-type cells (PADMORE *et al.* 1991). To directly test whether meiotic recombination is affected in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant, we subjected it to sporulation conditions and examined whether recombination occurred. Since the mutant was unable to sporulate, recombination between genetic markers could not be quantitated by standard tetrad analysis. Recombination could be analyzed, however, with a return-to-growth protocol (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974). This approach is based upon the observation that cells in the early stages of meiosis reverse back into the mitotic cycle when challenged with rich medium. Importantly, commitment to meiotic recombination occurs before the point in the meiotic cycle at which cells are no longer able to reverse. Thus, if the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrests after commitment to recombination, gene

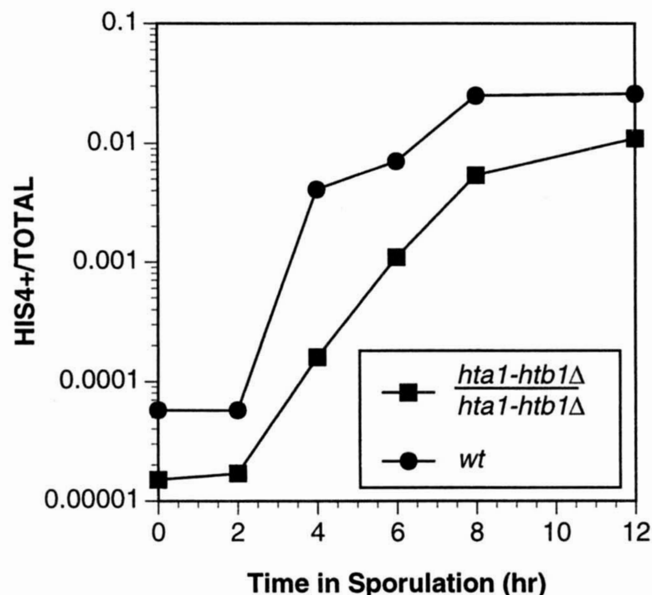

FIGURE 4.—The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrests after commitment to meiotic recombination. Strains DN1018 (wild type) and DN1023 (*hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2*) were sporulated, aliquots were removed at the indicated times, and gene conversion between *his4* heteroalleles was analyzed by a return to growth protocol (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974).

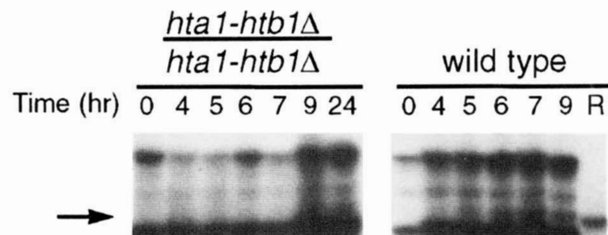

FIGURE 5.—The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrests after reciprocal exchange. Strains KT056 (*hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HIS4::LEU2/ his4x::LEU2-URA3*) and KT063 (*HTA1-HTB1/HTA1-HTB1, HTA2-HTB2/HTA2-HTB2, HIS4::LEU2/his4-x::LEU2-URA3*) were sporulated, aliquots were removed at the indicated times, and reciprocal exchange was assayed by a Southern blotting protocol (MATERIALS AND METHODS). The dark upper and lower bands represent the original parental alleles. The arrow indicates a reciprocal exchange product resolving at the locus. R represents a haploid tetrad segregant with the selected crossover. The additional band represents cross-hybridization with unknown DNA.

conversion between heteroalleles will be dramatically induced.

We therefore determined whether commitment to meiotic recombination occurs in cells homozygous for the *hta1-htb1Δ* deletion by examining nonreciprocal exchange between two *his4* heteroalleles (ALANI *et al.* 1990). A strain of genotype *his4x/his4b, hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* was placed in sporulation medium, and aliquots were removed at various times and plated on either a rich medium or a medium that selected for histidine prototrophs. The frequency of gene conversion (nonreciprocal exchange) was then calculated by dividing the number of histidine prototrophs by the number of viable cells (Figure 4). Gene conversion was induced to approximately the same level in the mutant as in wild-type control strains. Therefore, the commitment to genetic recombination occurs normally in the mutant under sporulation conditions, although it again exhibited an apparent delay.

In a typical meiotic cycle, heteroduplex DNA is processed into Holliday junctions that are then resolved into reciprocal exchanges or crossovers (PADMORE *et al.* 1991). PADMORE *et al.* (1991) showed that the formation of heteroduplex DNA occurs significantly before its subsequent processing into reciprocal exchanges. We next examined whether reciprocal exchanges occurred in the mutant. Since this again was impossible to examine by standard tetrad analysis, we used a restriction fragment length polymorphism assay developed by CAO *et al.* (1990). This assay monitors recombination between two homologous genetic loci that have different restriction maps. A crossover resolving at this locus generates new combinations of restriction fragments that can be monitored by Southern blotting (see MATERIALS AND METHODS).

We constructed a diploid strain that was homozygous for the *hta1-htb1Δ* mutation and heterozygous at the polymorphic locus. This strain was subjected to sporulation conditions, aliquots were removed at various times, and DNA from each sample was digested with *Xho*I and subjected to Southern analysis. To monitor the appearance of the appropriate recombination event, we also included on our gels genomic DNA from a tetrad segregant that had undergone an appropriate reciprocal exchange. After 8–9 hr in sporulation medium, the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant began to show reciprocal exchange events, which were still present after 24 hr (Figure 5). In wild-type cells, however, crossovers were first observable after 4 hr in sporulation medium, indicating that they were significantly delayed in the mutant, as was true for conversion and histone gene transcription (Figure 5).

We conclude from these studies that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant arrests primarily after the resolution of crossovers but before the first meiotic division. One way to account for this inability to progress into MI is to hypothesize a defect in microtubules or spindle pole bodies. To address this possibility, we examined thin sections of arrested cells by electron microscopy (Figure 6). Two classes of spindle pole bodies were observed: unduplicated spindle pole bodies (Figure 6, E and F) and duplicated but unseparated spindle pole bodies (Figure 6, A–D). Both classes were associated with what appeared to be structurally normal microtubules. Thus, the meiotic block observed in the mutant is not attributable to a gross change in spindle pole bodies, although more subtle

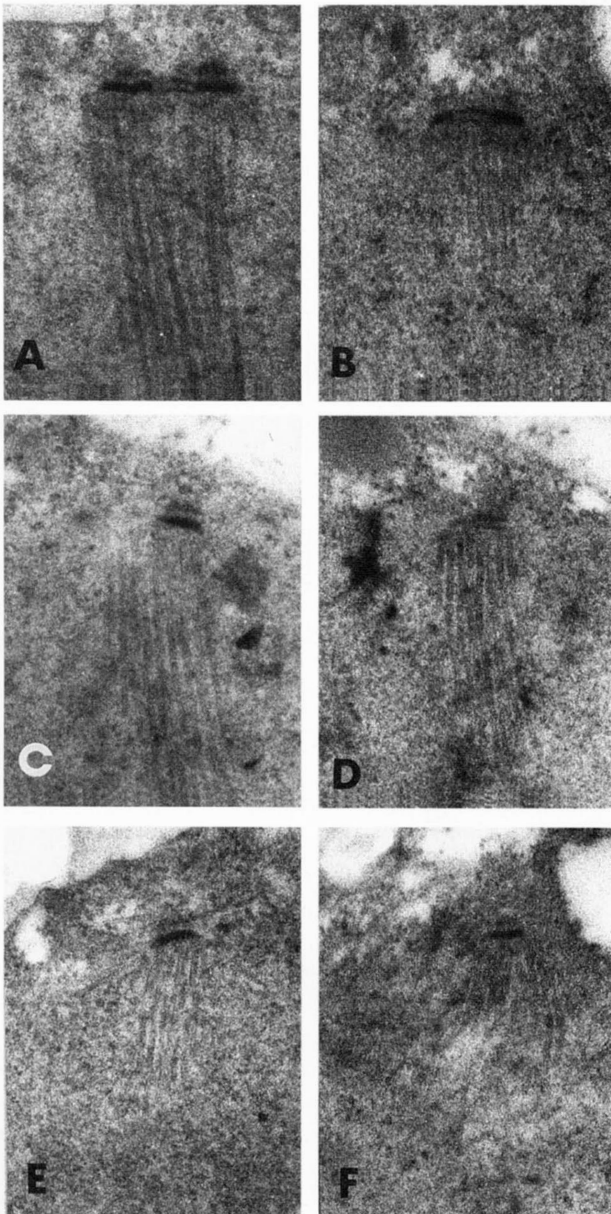

FIGURE 6.—Structure of spindle pole bodies in the arrested *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant. The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant was subjected to sporulation conditions as described in MATERIALS AND METHODS. After 16 hr, cells were fixed, embedded, thin-sectioned and stained as described (MATERIALS AND METHODS). (A–D) Duplicated but unseparated spindle pole bodies. In C and D, the membrane plaques are not within the individual sections, but their positions can be inferred from the flanking parallel microtubules. (E and F) Unduplicated spindle pole bodies. Magnification in all panels is ×40,000.

changes cannot be ruled out. Moreover, the spindle pole body morphology in arrested cells confirms that the block occurs before anaphase, as predicted by the earlier DAPI-staining experiments.

Bypass studies on the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant: The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant exhibits essentially wild-type levels of recombination (the observed delay in meiotic recombination most likely reflects a prior delay in the initiation of DNA replication). We were surprised by this finding since meiotic recombination correlates with, and is dependent upon, remarkable structural alterations in chromosomes (VON WETTSTEIN *et al.* 1984; DRESSER and GIROUX 1988; ROEDER 1995).

To further verify this feature of the mutant, we carried out two genetic bypass experiments. Specifically, we asked whether the mutant would sporulate in the presence of homozygous mutations in the *SPO13* or *RAD50* genes. *spo13/spo13* cells exhibit normal levels of meiotic recombination, but skip the first meiotic division to produce two viable diploid spores (KLAPHOLTZ and ESPOSITO 1980; MALONE and ESPOSITO 1981). Many mutations that block cells during meiotic recombination can be bypassed in *spo13/spo13* backgrounds (ROEDER 1995). *rad50Δ/rad50Δ* mutants do not initiate meiotic recombination, although they proceed through the meiotic divisions to form four inviable spores. Therefore, some mutants that arrest during the middle or late stages of meiotic recombination have been shown to sporulate in *rad50Δ/rad50Δ* backgrounds (CAO *et al.* 1990). We found that neither of these mutations bypassed the arrest seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant, providing further evidence that the arrest was not due to a defect in meiotic recombination.

The meiotic phenotype of the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant is reminiscent of the meiotic phenotypes of *top2/top2* and *cdc28/cdc28* mutants (see DISCUSSION). Analysis of these latter mutants suggested the existence of a major regulation point, or checkpoint, in the meiotic cycle that occurs immediately before the first meiotic division (SHUSTER and BYERS 1989; ROSE and HOLM 1993). Recently, BYERS and colleagues demonstrated at least two *RAD9*-dependent checkpoints in meiosis that occur before the induction of recombination (WEBER and BYERS 1992; THORNE and BYERS 1993). Although these checkpoints precede the arrest point described in this report, we nevertheless wondered whether the *RAD9* gene product might also mediate the recognition of an aberrant chromatin structure in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant and block further progression through sporulation. We found, however, that an *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, rad9Δ/rad9Δ* mutant sporulated at a frequency of <5%. Thus, the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant mutant did not block at a *RAD9*-dependent checkpoint. This does not rule out the possibility that the mutant arrests at a checkpoint regulated by other genes (see DISCUSSION).

The sporulation deficiency in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant occurs because of an underexpression of H2A-H2B dimers. Since dimers are deposited in chromatin during DNA replication, we asked whether slowing the replication fork dur-

TABLE 3

Hydroxyurea partially bypasses the sporulation phenotype of the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant

Strain	[HU] (mM)	Sporulation (%)
Wild type	0	66
	1.5	63
	2.5	61
	5	21.5
hta1-htb1Δ/hta1-htb1Δ	0	<1
	1.5	2
	2.5	18
	5	12.3

ing premeiotic S phase might suppress the meiotic phenotype. To examine this possibility, small amounts of hydroxyurea, a drug known to inhibit DNA chain elongation, were added to cultures of *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutants during their incubation in sporulation medium. As seen in Table 3, the addition of hydroxyurea to a final concentration of 2.5 mM had little effect on the sporulation of wild-type cells. However, the frequency of sporulation in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant increased from <1% to 18%.

To provide further support for the model, we analyzed the progression of premeiotic S phase in wild-type and mutant cells in the presence or absence of 2.5 mM hydroxyurea (Figure 7). In the absence of the drug, the bulk of replication in wild-type cells occurred between 0–3 hr after resuspension in SPM. Under the same conditions, the bulk of replication in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant occurred between 3–5 hr after resuspension in SPM, showing that the mutant delays entrance into the meiotic pathway. Moreover, replication in the mutant appears to be less efficient than in wild-type cells, as a larger proportion of cells failed to replicate at all.

The presence of hydroxyurea had two effects on premeiotic S phase in wild-type cells. First, the intiation of replication was delayed at least 2 hr. Second, the G1 peak moved more slowly to the G2 position, indicative of slower replication. The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant likewise showed a slower replication, although the delay in initiation appeared less pronounced. Thus, as predicted, the addition of hydroxyurea slows DNA replication during premeiotic S phase in wild-type and *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant cells.

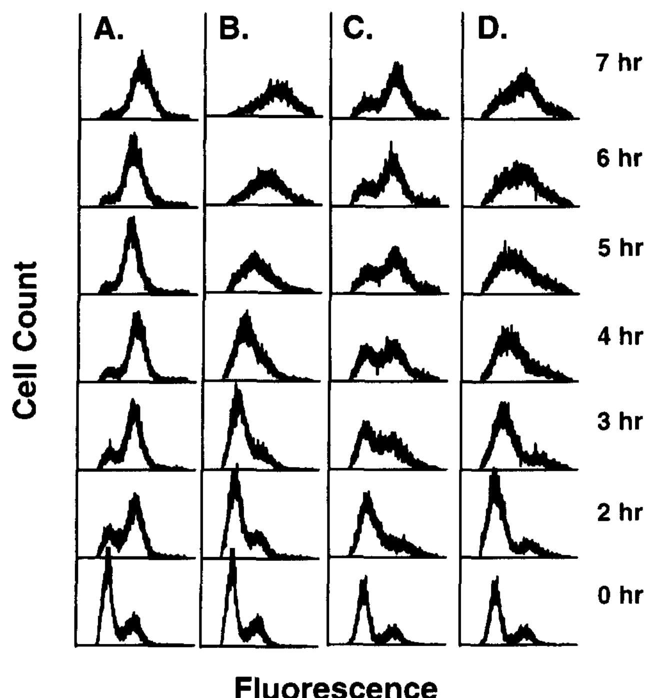

FIGURE 7.—Addition of hydroxyurea slows premeiotic S phase. Cells were subjected to standard sporulation conditions (MATERIALS AND METHODS). At various times, aliquots were removed, and DNA content was analyzed on a fluorescence-activated cell sorter (MATERIALS AND METHODS). (A) Wild-type cells. (B) Wild-type cells supplemented with 2.5 mM hydroxyurea. (C) The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant. (D) The *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant supplemented with 2.5 mM hydroxyurea. Time in sporulation conditions is shown on the right of the graph.

DISCUSSION

In this report, we showed that the partial depletion of H2A-H2B dimers in yeast blocks progression into the first meiotic division. Since the expression patterns of the two H2A-H2B gene pairs are nearly identical in mitotic and meiotic cells, these results argue that the MI division is more sensitive than the mitotic division to changes in histone stoichiometry. In addition, we showed that the sporulation phenotype of the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant could be partially suppressed in the presence of hydroxyurea. These latter results suggest that the deposition of H2A-H2B dimers in the mutant cannot keep pace with the advancing replication fork during premeiotic S phase, and that this uncoupling of replication and chromatin deposition leads to a disruption in chromosome structure that interferes with the first meiotic division. Alternatively, the delay in DNA replication caused by the drug may lead to an increase in *HTA2-HTB2* expression, thereby suppressing the sporulation phenotype. Further experimentation will be required to verify these models.

One of the distinguishing characteristics of meiosis in yeast and other eukaryotes is the dramatic induction of genetic recombination that occurs before the first meiotic division. The induction of recombination is contemporaneous with remarkable structural alterations in chromosomes, most notably their pairing into synaptonemal complexes (VON WETTSTEIN *et al.* 1984;

PADMORE *et al.* 1991; ROEDER 1995). Two results suggest that the depletion of dimers has at most a small effect on meiotic recombination. First, commitment to meiotic levels of nonreciprocal and reciprocal recombination occur at wild-type levels in the mutant before its arrest. Second, mutations that typically bypass recombination or synaptonemal complex defects do not bypass the arrest seen in the histone mutant. We therefore as yet have no evidence that meiotic recombination is grossly affected in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant.

Instead, the main problem in the mutant appears to be its inability to transit through the meiotic divisions. In retrospect, this phenotype should not have been unexpected, as it has been observed in mitosis as well. The following studies indicate that mitotic chromsome segregation is aberrant in histone mutants: (1) disrupting the stoichiometry between the dimer and tetramer decreases the fidelity of mitotic chromosome segregation (MEEKS-WAGNER and HARTWELL 1986), (2) complete depletion of H4 arrests mitotic progression at the G2/M border (KIM *et al.* 1988), (3) mutations in the N-terminal arms of H3 and H4 delay progression through G2 and M (MEGEE *et al.* 1990, 1995; MORGAN *et al.* 1991; SMITH *et al.* 1996), (4) complete depletion of dimers leads to a cell cycle arrest in mitosis (HAN *et al.* 1987), and (5) partial depletion of dimers delays progression through mitosis (NORRIS and OSLEY 1987). Our results extend these results to sporulating cells, indicating that the meiotic divisions may be even more sensitive to disruptions in chromatin structure than the mitotic division.

The observation that the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant displays different mitotic and meiotic phenotypes prompted us to initiate these studies. Two other studies have examined mutations that confer different phenotypes in mitosis and meiosis, and, intriguingly, both of these mutations block meiotic progression at the G2/MI border as well. The first of these mutations is in the *TOP2* gene, which encodes topoisomerase II. Haploid strains carrying a conditional mutation in *TOP2* lose viability extremely rapidly during vegetative growth when shifted to nonpermissive condtions (HOLM *et al.* 1985). This inviability results from the fact that, in the absence of the enzyme, chromosomes fail to deconcatenate during mitosis and, hence, are physically torn apart as the chromosomes segregate (HOLM *et al.* 1985). By comparison, *top2/top2* mutants retain high viability when shifted to nonpermissive conditions during sporulation because the cells arrest at the G2/MI border, before entrance into the meiotic divisions (ROSE and HOLM 1993). These results led HOLM and colleagues to postulate the existence of a major cell-cycle regulation point immediately before the first meiotic division that senses chromosomal structure. The meiotic arrest seen in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant is obviously very similar to that seen in the *top2/top2* mutant, and suggests that the histone mutant may likewise fail to transit through this regulation point. It is important to state, however, that the arrests in the two strains are not identical, as a *top2/top2, rad50Δ/rad50Δ* strain will sporulate. In other words, unlike the histone mutant, the meiotic arrest in *top2/top2* strains is bypassed under conditions that eliminate genetic recombination.

The second study examined diploid strains homozygous for temperature-sensitive mutations in the *CDC28, CDC36,* or *CDC39* genes (SHUSTER and BYERS 1989). In mitotic cells, these mutants arrest in nonpermissive conditions at START, *i.e.,* before entrance into S phase. Surprisingly, however, in sporulating cells, the same mutants arrest after premeiotic S phase but before MI. Like the histone mutant, they also exhibited full commitment to meiotic levels of intragenic recombination (resolution of reciprocal recombinants was not examined). Synaptonemal complexes were directly examined in the arrested cells, and they appeared structurally normal. SHUSTER and BYERS used these results to also argue for the existence of a major regulation point at the G2/MI border (SHUSTER and BYERS 1989).

Three other mutants, *cdc5-1/cdc5-1, cdc14-1/cdc14-1,* and *spo1-1/spo1-1,* arrest under nonpermissive conditions after premeiotic replication and recombination, but before nuclear division. The first two mutants block in meiosis after spindle pole body duplication and separation and, thus, after the arrest point in the histone mutant (SCHILD and BYERS 1980). The *spo1-1/spo1-1* mutant arrests earlier, with a single, unduplicated spindle pole body (MOENS *et al.* 1974). The relationship between these mutants and the histone mutant remains to be ascertained. It is particularly intriguing to note, however, that the promoter of *CDC14,* which encodes a putative tyrosine phosphatase, shares a region of high sequence homology with the promoter of *HTA1-HTB1,* suggesting that the two loci may be regulated by similar mechanisms (WAN *et al.* 1992).

How might chromatin depleted of H2A-H2B dimers lead to the observed meiotic arrest? One possibility is that a specific chromosomal structure required for the first meiotic division is disrupted in the mutant. A good candidate for such a structure is the centromere. It has been shown that the complete depletion of either H2B or H4 *in vivo* dramatically disrupts centromere structure (SAUNDERS *et al.* 1990), although the partial depletion of dimers has less profound effects (NORRIS *et al.* 1988). Moreover, a multi-copy plasmid carrying *CSE4,* an H3 variant related to the mammalian CENP-A kinetochore antigen, suppresses the mitotic chromosome segregation phenotype of an H4 mutant (SMITH *et al.* 1996). As described above, another possibility is that the disruption of chromatin structure in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant is sensed by a "chromosomal checkpoint" gene product, which prohibits further progression through sporulation (SMITH

et al. 1996). If true, then this checkpoint would have to be independent of the *RAD9* gene product. The final possibility is that the disruptions in chromatin structure affect the expression of a gene(s) that is required specifically for the transition into the first meiotic division. We are currently testing these potential models.

Finally, one of the enigmatic characteristics of the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant is the pronounced delay it exhibits before entering premeiotic S phase. One simple way to account for this is to assume that a "faulty" chromatin structure was set up during its prior growth in YPA presporulation medium. We have recently found, however, that the expression of the *HTA2-HTB2* locus, which fails to exhibit dosage compensation in rich YPD growth medium, does exhibit dosage compensation in YPA presporulation medium (K. Tsui and D. Norris, unpublished data). As a result, the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant grows at the same rate as a wild-type strain in YPA, indicating that it produces sufficient amounts of H2A-H2B dimers during growth in presporulation medium. It is also unlikely that the delay results from the loss of a particular histone subtype, as the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2, HTA2-HTB2/HTA2-HTB2* mutant transcribes its histone genes at the appropriate time (Figure 2).

Instead, we hypothesize that the delay reflects the transcriptional properties of the two H2A-H2B gene pairs. Previous work by Osley and Hereford showed that DNA replication and histone transcription are interdependent processes, with the transcription of *HTB1* preceding entrance into mitotic S phase (Hereford *et al.* 1981, 1982; Osley and Hereford 1981). We can rationalize the observed delay in premeiotic S phase by speculating that DNA replication and the transcription of *HTA2-HTB2* both require the prior expression of *HTA1-HTB1*. By this scenario, the *HTA1-HTB1* locus is expressed first to generate a pool of free H2A-H2B dimers, which then positively activates DNA replication and transcription of *HTA2-HTB2*. The delay that we observed in the *hta1-htb1Δ/hta1-htb1Δ, HTA2-HTB2/HTA2-HTB2* mutant would therefore reflect the increased time required by the cell to generate a sufficient intracellular pool of H2A-H2B dimers to initiate replication. We have recently carried out detailed time courses that suggest that the transcription of *HTA1-HTB1* indeed precedes that of *HTA2-HTB2* during meiosis (K. Tsui and D. Norris, unpublished data).

We are grateful to Andrew Vershon and Ruth Steward for careful review of this manuscript. This work was supported by National Institutes of Health grant GM-52192-01A1 and grant number 3978 from The Council for Tobacco Research, USA.

LITERATURE CITED

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Boeke, J. D., F. Lacroute and G. R. Fink, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–347.

Byers, B., and L. Goetsch, 1991 Preparation of yeast cells for thin sectioning. Methods Enzymol. **194:** 602–608.

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae.* Cell **61:** 1089–1101.

Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler, F. Winston, 1988 Changes in histone gene dosage alter transcription in yeast. Genes Dev. **2:** 150–159.

Dresser, M. E., and C. N. Giroux, 1988 Meiotic chromosome behavior in spread preparations of yeast. J. Cell Biol. **106:** 567–573.

Eickbush, T. H., and E. N. Moudrianakis, 1978 The histone core complex: an octamer assembled by two sets of protein-protein interactions. Biochemistry **17:** 4955–4964.

Esposito, M. S., and R. E. Esposito, 1974 Genetic recombination and commitment to meiosis in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **71:** 3172–3176.

Esposito, R. E., and S. Klapholz, 1981 Meiosis and ascospore development, pp. 211–288 in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fisher-Adams, G., and M. Grunstein, 1995 Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the *GAL1* promoter. EMBO J. **14:** 1468–1477.

Han, M., C. Chang, U.-J. Kim and M. Grunstein, 1987 Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication, and transcription. Cell **48:** 589–597.

Hecht, A., T. Laroche, S. Strahl-Bolsinger, S. M. Gasser and M. Grunstein, 1995 Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:** 583–592.

Hereford, L., K. Fahrner, T. Woolford, M. Rosbash and D. Kaback, 1979 Isolation of yeast histone genes H2A and H2B. Cell **18:** 1261–1271.

Hereford, L. M., M. A. Osley, J. R. Ludwig and C. McLaughlin, 1981 Cell cycle regulation of yeast histone mRNA. Cell **24:** 367–375.

Hereford, L., S. Bromley and M. A. Osley, 1982 Periodic transcription of yeast histone genes. Cell **30:** 305–310.

Holm, C., T. Goto, J. C. Wang and D. Botstein, 1985 DNA topoisomerase II is required at the time of mitosis in yeast. Cell **41:** 553–563.

Hutter, K. J., and H. E. Eipel, 1979 Microbial determinations by flow cytometry. J. Gen. Microbiol. **113:** 369–375.

Johnson, L. M., G. Fisher-Adams and M. Grunstein, 1992 Identification of a non-basic domain in the histone H4 N-terminus required for repression of the yeast silent mating loci. EMBO J. **11:** 2201–2209.

Kane, S., and R. Roth, 1974 Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118:** 8–14.

Kassir, Y., and G. Simchen, 1991 Monitoring meiosis and sporulation in *Saccharomyces cerevisiae.* Methods Enzymol. **194:** 94–110.

Kassir, Y., D. Granot and G. Simchen, 1988 *IME1*, a positive regulatory gene of meiosis in S. cerevisiae. Cell **52:** 853–862.

Kim, U. J., M. Han, P. Kayne and M. Grunstein, 1988 Effects of histone H4 depletion on the cell cycle and transcription of *Saccharomyces cerevisiae.* EMBO J. **7:** 2211–2219.

Klapholtz, S., and R. E. Esposito, 1980 Recombination and chromosome segregation during the single division meiosis in *spo12–1* and *spo13–1* diploids. Genetics **96:** 589–611.

Kunkel, T. A., 1985 Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl Acad. Sci. USA **82:** 488–492.

Law, D. T. S., and J. Segall, 1988 The *SPS100* gene of *Saccharomyces cerevisiae* is activated late in the sporulation process and contributes to spore wall maturation. Mol. Cell. Biol. **8:** 912–922.

Malone, R. E., and R. E. Esposito, 1981 Recombinationless meiosis in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **1:** 891–901.

MANN, R. K., and M. GRUNSTEIN, 1992 Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene *in vivo*. EMBO J. **11:** 3297–3306.

MEEKS-WAGNER, D., and L. H. HARTWELL, 1986 Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44:** 43–52.

MEGEE, P. C., B. A. MORGAN, B. A. MITTMAN and M. M. SMITH, 1990 Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science **247:** 841–845.

MEGEE, P. C., B. A. MORGAN and M. M. SMITH, 1995 Histone H4 and the maintenance of genome integrity. Genes Dev. **9:** 1716–1727.

MOENS, P. B., R. E. ESPOSITO and M. S. ESPOSITO, 1974 Aberrant nuclear behavior at meiosis and annucleate spore formation by sporulation-deficient (spo) mutants of *Saccharomyces cerevisiae*. Exp. Cell Res. **83:** 166–174.

MORAN, L., D. NORRIS and M. A. OSLEY, 1990 A yeast H2A-H2B promoter can be regulated by changes in histone gene dosage. Genes Dev. **4:** 752–763.

MORGAN, B. A., B. A. MITTMAN and M. M. SMITH, 1991 The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. Mol. Cell. Biol. **11:** 4111–4120.

MOUDRIANAKIS, E. N., and G. ARENTS, 1993 Structure of the histone octamer core of the nucleosome and its potential interactions with DNA. Cold Spring Harbor Symp. Quant. Biol. **58:** 273–279.

NEIGEBORN, L., and A. P. MITCHELL, 1991 The yeast *MCK1* gene encodes a protein kinase homolog that activates early meiotic gene expression. Genes Dev. **5:** 533–548.

NORRIS, D., and M. A. OSLEY, 1987 The two gene pairs encoding H2A and H2B play different roles in the *Saccharomyces cerevisiae* life cycle. Mol. Cell. Biol. **7:** 3473–3481.

NORRIS, D., B. DUNN and M. A. OSLEY, 1988 The effect of histone gene deletions on chromatin structure in *Saccharomyces cerevisiae*. Science **242:** 759–761.

OSLEY, M. A., and L. HEREFORD, 1981 Yeast histone genes show dosage compensation. Cell **24:** 377–384.

OSLEY, M. A., J. GOULD, S. KIM, M. KANE and L. HEREFORD, 1986 Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell **45:** 537–544.

PADMORE, R., L. CAO and N. KLECKNER, 1991 Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell **66:** 1239–1256.

PEDERSON, D. S., F. THOMA and R. T. SIMPSON, 1986 Core particle, fiber, and transcriptionally active chromatin structure. Annu. Rev. Cell Biol. **2:** 117–147.

REYNOLDS, E. S., 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17:** 208–214.

ROEDER, G. S., 1995 Sex and the single cell: meiosis in yeast. Proc. Natl. Acad. Sci. USA **92:** 10450–10456.

ROSE, D., and C. HOLM, 1993 Meiosis-specific arrest revealed in DNA topoisomerase II mutants. Mol. Cell. Biol. **13:** 3445–3455.

ROTH, S. Y., M. SHIMIZU, L. JOHNSON, M. GRUNSTEIN and R. T. SIMPSON, 1992 Stable nucleosome positioning and complete repression by the yeast $\alpha 2$ repressor are disrupted by amino-terminal mutations in histone H4. Genes Dev. **6:** 411–425.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101C:** 202–210.

SAUNDERS, M. J., E. YEH, M. GRUNSTEIN and K. BLOOM, 1990 Nucleosome depletion alters the chromatin structure of *Saccharomyces cerevisiae* centromeres. Mol. Cell. Biol. **10:** 5721–5727.

SCHILD, D., and B. BYERS, 1980 Diploid spore formation and other meiotic effects of two cell-division-cycle mutations of *Saccharomyces cerevisiae*. Genetics **96:** 859–876.

SHERMAN, F., and H. ROMAN, 1963 Evidence for two types of allelic recombination in yeast. Genetics **48:** 255–261.

SHUSTER, E. O., and B. BYERS, 1989 Pachytene arrest and other meiotic effects of the start mutations in *Saccharomyces cerevisiae*. Genetics **123:** 29–43.

SMITH, H. E., and A. P. MITCHELL, 1989 A transcriptional cascade governs entry into meiosis in yeast. Mol. Cell. Biol. **9:** 2142–2152.

SMITH, M. M., P. YANG, M. SOLEDAD SANTISTEBAN, P. W. BOONE, A. T. GOLDSTEIN *et al.*, 1996 A novel histone H4 mutant defective in nuclear division and mitotic chromosome transmission. Mol. Cell. Biol. **16:** 1017–1026.

THOMPSON, J. S., L. M. JOHNSON and M. GRUNSTEIN, 1994a Specific repression of the yeast silent mating locus HMR by an adjacent telomere. Mol. Cell. Biol. **14:** 446–455.

THOMPSON, J. S., X. LING and M. GRUNSTEIN, 1994b Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. Nature **369:** 245–247.

THORNE, L. W., and B. BYERS, 1993 Stage-specific effects of X-irradiation on yeast meiosis. Genetics **134:** 29–42.

VON WETTSTEIN, D., S. W. RASMUSSEN and P. B. HOLM, 1984 The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18:** 331–413.

WALLIS, J. W., L. HEREFORD and M. GRUNSTEIN, 1980 Histone H2B genes of yeast encode two different proteins. Cell **22:** 799–805.

WAN, J., H. XU and M. GRUNSTEIN, 1992 *CDC14* of *Saccharomyces cerevisiae*. Cloning, sequence analysis, and transcription during the cell cycle. J. Biol. Chem. **267:** 11274–11280.

WANG, B. C., J. ROSE, G. ARENTS and E. N. MOUDRIANAKIS, 1994 The octameric histone core of the nucleosome: structural issues resolved. J. Mol. Biol. **236:** 179–188.

WEBER, L., and B. BYERS, 1992 A *RAD9*-dependent checkpoint blocks meiosis of *cdc13* yeast cells. Genetics **131:** 55–63.

Communicating editor: F. WINSTON