

***GCN5*-dependent histone H3 acetylation and *RPD3*-dependent histone H4 deacetylation have distinct, opposing effects on *IME2* transcription, during meiosis and during vegetative growth, in budding yeast**

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ABSTRACT Diploid yeast undergo meiosis under certain conditions of nutrient limitation, which trigger a transcriptional cascade involving two key regulatory genes. *IME1* is a positive activator of *IME2*, which activates downstream genes. We report that Gcn5, a histone H3 acetylase, plays a central role in initiation of meiosis via effects on *IME2* expression. An allele, *gcn5-21*, was isolated as a mutant defective in spore formation. *gcn5-21* fails to carry out meiotic DNA replication, recombination, or meiotic divisions. This mutant also fails to induce *IME2* transcription; *IME1* transcription, however, is essentially normal. Further investigation shows that during wild-type meiosis the *IME2* promoter undergoes an increase in the level of bound acetylated histone H3. This increase is contemporaneous with meiotic induction of *IME2* transcription and is absent in *gcn5-21*. In contrast, the *RPD3* gene, which encodes a histone H4 deacetylase and is known to be required for repression of basal *IME2* transcription in growing yeast cells, is not involved in induction of *IME2* transcription or *IME2* histone acetylation during meiosis. These and other results suggest that Gcn5 and Rpd3 play distinct roles, modulating transcription initiation in opposite directions under two different cellular conditions. These roles are implemented via opposing effects of the two gene products on acetylation of two different histones. Finally, we find that *gcn5* and *rdp3* single mutants are not defective in meiosis if acetate is absent and respiration is promoted by a metabolically unrelated carbon source. Perhaps intracellular acetate levels regulate meiosis by controlling histone acetylation patterns.

A constellation of environmental conditions triggers meiosis and spore formation in the budding yeast, *Saccharomyces cerevisiae* (1). Limited levels of nitrogen and a fermentable carbon source, conditions associated with starvation, and the presence of a nonfermentable carbon source (2) lead to the transcriptional activation of *IME1* and *IME2*, two key regulators of meiosis-specific gene expression (3, 4). *IME1* acts as a positive activator of *IME2* transcription (4, 5). Expression of *IME2* is required for meiotic DNA replication, meiotic recombination, two nuclear divisions, and spore formation (1).

Transcription of *IME2* is repressed during vegetative growth (4, 5). *RPD3* is required for this *IME2* repression and encodes a histone deacetylase (6–8). In an *rdp3Δ* mutant, acetylated histone H4 levels increase specifically at the *IME2* promoter, suggesting that repression is coupled to histone deacetylation at this promoter (8, 9).

In contrast, hyper-acetylation of promoter-bound histones has been implicated in transcription activation (10). *GCN5* encodes a histone acetyltransferase (11) and is required for the

activation of a number of genes involved in amino acid biosynthesis, respiration, and other pathways controlled by the *SWI/SNF* gene products (12–15). Thus far, *GCN5*-dependent transcription activation has been specifically correlated, *in vivo*, with hyperacetylation of bound histone H3 in one case, the promoter for the *HIS3* gene (16).

Here we describe the identification and analysis of an allele, *gcn5-21*, isolated as a sporulation-defective mutant in *S. cerevisiae*. We find that *IME2* expression requires *GCN5* function. We also explore the functional interplay between a histone acetylase and a histone deacetylase acting on a single promoter under conditions of repressed and activated transcription.

MATERIALS AND METHODS

Media. YPD is 1% yeast extract, 2% peptone, 2% glucose supplemented with 0.04% Tryptophan. YPA and YPG are the same as YPD except that 2% potassium acetate and 3% glycerol are substituted for glucose, respectively. Sporulation media (SPM) is 0.3% KOAc, 0.02% raffinose unless otherwise noted. Plates were made by adding 2% Bacto agar (Difco).

Strains. All yeast strains were stored at –80°C and recovered by incubation at 30°C for 12–16 hr on YPG plates. Strains are derivatives of SK1 containing the markers *lys2*, *ura3*, *leu2::hisG*, *trp1::hisG*, *arg4-nsp*, *arg4-bgl*, *his4X::LEU2*, and *his4B::LEU2* (17), or *ime2-lacZ-URA3* (18). The *rdp3Δ::KanMx* allele was constructed by using the following oligos for PCR synthesis to create a *KanMx4* marker (19) flanked by *RPD3* sequences: 5'-ACAATTGCGCCATACAAAACATT-CGTGGCTACAACCTCGATATCCGTGCAGGTCGAGGG-ATCC-3' and 5'-CTTTTGTTCACATTATTTATATTCG-TATATACTTCCAACCTCTTTTTCGCGCATAGGCCAC-TAGT-3'. The PCR product was transformed into NKY3222. Disruption of the *RPD3* locus was confirmed by PCR.

The following strains contain the markers *ho::LYS2*, *lys2*, *leu2::hisG*, *ura3*, and *trp1::hisG* unless otherwise noted. NKY3198 *MATa/MATα HO//TRP1//ste7-1//gcn5-21//*; NKY3200 *MATa his4B::LEU2 arg4-Bgl gcn5-21*; NKY3201 *MATα his4X::LEU2 arg4-Nsp gcn5-21*; NKY3202 *MATa/MATα his4X::LEU2/his4B::LEU2 arg4-Bgl/arg4-Nsp gcn5-21//*; NKY3203 *MATa/MATα his4X::LEU2/his4B::LEU2 arg4-Bgl/arg4-Nsp gcn5-21/GCN5*; NKY3210 *MATa/MATα his4X::LEU2(Bam)-URA3/his4B::LEU2 arg4-Bgl/arg4-Nsp*; NKY3212 *MATa/MATα his4X::LEU2(Bam)-URA3/his4B::LEU2arg4-Bgl/arg4-Nsp gcn5-21//*; NKY3221 *MATa/*

Abbreviations: YPD, yeast extract/peptone/glucose; YPA, yeast extract/peptone/potassium acetate; SPM, sporulation media; IP, immunoprecipitation; WT, wild type; DSB, double-strand break.

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MAT α ho::hisG/' *gcn5-21*/'; NKY3222 *MAT α /MAT α ho::hisG*/'; NKY3227 *MAT α /MAT α ho::hisG*/' *rdp3 Δ* /'; NKY3228 *MAT α /MAT α ho::hisG*/' *gcn5-21*/' *rdp3 Δ* /'; NKY3219 *MAT α /MAT α TRP1*/' *his4X::LEU2/his4 B::LEU2 spo11::hisG-URA3-hisG*/' *gcn5-21*/'; NKY3220 *MAT α /MAT α TRP1 his4X::LEU2/his4B::LEU2 spo11::hisG-URA3-hisG*/'.

Cloning of *NDT21/GCN5*. Genetic methods have been described (20). The *ndt21-1* mutation was isolated in the same screen described in refs. 18 and 21. Four DNA clones able to complement the *ndt21-1* sporulation-defective phenotype were recovered from a Ycp50-based yeast genomic library and share a 4.5-kb region in common (18). Confirmation that the cloned region covers the mutated segment of DNA and not a suppressor was made in two steps: First, the region in common to all four complementing clones was deleted in one wild-type (WT) clone and filled in by gap repair in a *ndt21-1* mutant cell (NKY3198; ref. 22). Second, the mutant allele on the gap-repaired plasmid was used to replace the WT allele in two different unmutagenized haploid cell backgrounds by two-step allele replacement (22). Restoration of a sporulation-defective phenotype in diploids generated from these cells indicated that the *NDT21* locus had been cloned. All *ndt21-1* mutant strains are derived from NKY3200 and NKY3201, two haploid strains generated in this way. The Spo⁻ phenotype conferred by *ndt21-1* was complemented by a 1.2-kb *PstI-XhoI* fragment containing *GCN5* that was carried on pRS314 (23).

Cytology. Sporulation on solid media was carried out by patching single colonies from a YPD plate to SPM and incubating at 30°C for 24–36 hr. Spore formation, scored as asci with one or more spores, was assessed by light microscopic analysis of cells resuspended in water. Cells from liquid cultures were scored similarly. Meiotic divisions were monitored by staining cells fixed in 40% ethanol with 1 μ g/ml of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and by using fluorescence and/or phase-contrast microscopy. At least 100 cells were analyzed for each cytological analysis.

Meiotic Time Courses. The method of cell synchronization for meiotic time courses has been described (24, 25).

Nucleic Acid Techniques. Meiotic double-strand breaks near the *THR4* locus were assayed as described (26). RNA isolation and Northern blot analysis were carried out as described (27, 28). Hybond-N (Amersham Pharmacia) was used to immobilize total RNA. ³²P-labeled DNA probes were prepared by using a random priming kit (Stratagene). The 240-bp *HindIII-HindIII* fragment containing the *IME1* coding region from pPL139 (29), the *BamHI-EcoRI* fragment containing the *IME2* coding region from pMH6 (4), and the 9-kb fragment containing the rRNA gene cluster (pOL73) were used to probe the same membrane sequentially. Probes were stripped from the membrane before each hybridization.

Analysis. DNA replication was monitored by using a Beckton Dickinson FACScan Analyzer. Cells were prepared for flow cytometry as described (30).

Chromatin Immunoprecipitation (IP). Chromatin IP was carried out as described (31). Anti-H3.AcLys(9/14) and anti-H4.AcLys(5/8/12/16) were purchased from Upstate Biotechnology. Cells grown in YPD were harvested at OD₆₀₀ 1.0. Meiotic cells were harvested 2 hr after transfer into SPM. Immunoprecipitated DNA and total preimmunoprecipitated DNA (10% of that subjected to IP) were applied to Hybond N⁺ (Amersham) by using a slot-blot apparatus and hybridized sequentially (32) to randomly labeled probes for the *IME2* promoter region (nucleotides -751 to -124; obtained by PCR using the oligos: 5'-ACTGGTAACCCAGATACC-3' and 5'-ATTCTCTAGACAGCCACC-3'), total genomic DNA isolated from SK1 (25) and for the *IME1* promoter region (nucleotides -620 to -33, *HindIII-XbaI* fragment from YEpK26-7) (3). Probes were stripped from the membrane

between hybridizations. A Fuji PhosphorImager was used for quantitation.

RESULTS

Isolation of *gcn5-21*. A mutation, *ndt21-1*, was isolated in a screen for mutants defective in spore formation (21). The *ndt21-1* mutation was found to segregate as a single Mendelian trait. Further analysis revealed *ndt21-1* to be an allele of *GCN5*; it was thus renamed *gcn5-21* (*Materials and Methods*).

A *gcn5-21* Mutant Does Not Carry Out Meiotic Nuclear Divisions and Does Not Initiate Meiotic DNA Replication or Meiotic Recombination. Events of meiosis were assayed in parallel in WT and *gcn5-21* mutant cells. For each assay, a population of cells was synchronized (24). Cells cultured in rich media plus glucose (YPD) were used to inoculate rich media plus potassium acetate (YPA). Cell cultures were grown \approx 13.5 hr at which point >90% of cells arrest in G₁ immediately before the entry into meiosis. Cells then were transferred into SPM containing acetate after which the major events of meiosis occur. Cells prepared in this way proceed sequentially and relatively synchronously through meiotic DNA replication, recombination, divisions, and finally spore formation (24).

In WT meiosis, DNA replication is complete in most cells by 4 hr and in essentially all cells by 6 hr, as assayed by flow cytometry (FACS); both meiotic divisions are complete in most and all cells by \approx 7 and \approx 10 hr, respectively, as assayed by light microscopic examination of 4',6-diamidino-2-phenylindole dihydrochloride-stained cells (24). In the *gcn5-21* mutant, no replication was observed after 6 hr (Fig. 1A), or even after incubation in SPM for up to 24 hr (data not shown), and no nuclear divisions were observed at any time point measured through 24 hr (Fig. 1B).

In general, mutants defective in carrying out S phase also are defective in carrying out meiotic recombination (1), which holds for *gcn5-21*. Meiotic recombination was assayed in two ways. First, the frequency of His⁺ and Arg⁺ prototrophs arising from recombination between *his4* and *arg4* heteroalleles was measured by a "return-to-growth" assay (33). In a *gcn5-21* mutant, His⁺ and Arg⁺ prototroph levels were reduced 100- and 300-fold, respectively, compared with WT, indicating no commitment to heteroallelic recombination (Fig. 1C and data not shown). Second, the occurrence of DNA double-strand breaks (DSBs), thought to be the initiating event of meiotic recombination (34), was measured. DSBs are transiently detectable in WT cells several hours after transfer into SPM, as illustrated here (Fig. 1D) for breaks at the *THR4* locus (25, 35). In contrast, the *gcn5-21* mutant failed to form detectable levels of DSBs at any time point after transfer into SPM (Fig. 1D).

Some mutants are proficient for initiation of recombination but exhibit meiotic arrest, apparently in response to a subsequent block in the recombination process. Accordingly, this type of arrest is alleviated by mutations that block DSB formation (e.g., *spo11 Δ* ; see ref. 21). Because *gcn5-21* is blocked before the initiation of meiotic recombination, it should not be subject to such alleviation. This expectation is fulfilled: WT and *spo11 Δ* cells sporulated on solid media at 85% and 79% efficiency, respectively, whereas *gcn5-21* and *gcn5-21 spo11 Δ* cells gave spores at less than 1% efficiency (NKY3210, NKY3219, NKY3212, and NKY3220, respectively).

***GCN5* Is Required for Meiotic Induction of *IME2* Transcription.** We tested whether *GCN5* might be required for the activation of transcription of important meiotic regulatory gene(s) *IME1* and/or *IME2* (*Introduction*). First, *IME2* transcript levels relative to rRNA were measured in WT and mutant cells at various times after transfer to SPM (Fig. 2, *Left*). In the *gcn5-21* mutant, *IME2* transcript levels were reduced 10- to 15-fold from WT levels at all time points after the transfer of cells to SPM. The same effect is observed with

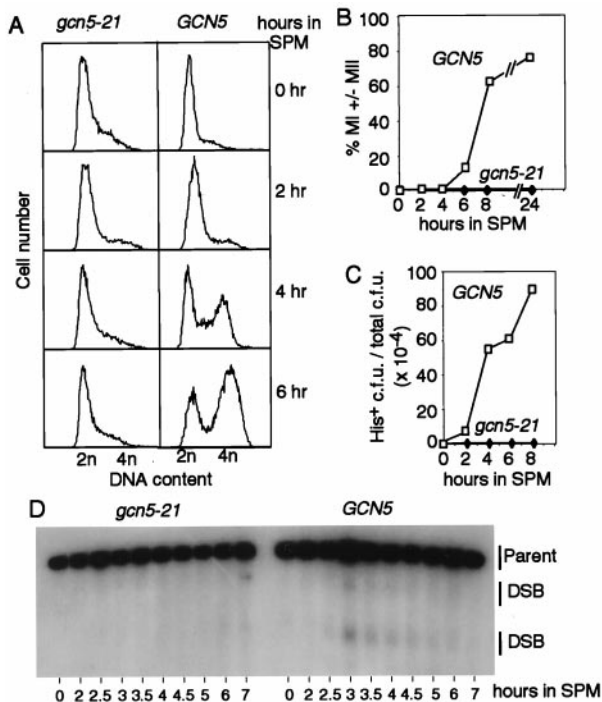


Fig. 1. Events of meiosis in WT and *gcn5-21* mutant cells. (A) Meiotic DNA replication in WT (NKY3221) and *gcn5-21* mutant cells (NKY3222) at the indicated time points after transfer to SPM. (B) Meiotic divisions (MI ± MII) in the same time course as A. (C) HIS⁺ prototroph formation in *GCN5/gcn5-21* cells (NKY3203) and *gcn5-21/gcn5-21* mutant cells (NKY3202) returned to vegetative growth at the indicated times after transfer into SPM. Cells were plated onto synthetic complete media (SC) and onto media lacking histidine (SC-HIS). (D) Meiotic DNA double-strand breaks near the *THR4* locus in *gcn5-21* (NKY3198) and WT (NKY895) cells at the indicated time points after transfer to SPM assayed by Southern blot analysis.

an *IME2-lacZ* transcriptional fusion integrated at the *IME2* locus. In the *gcn5-21* mutant, the level of β -galactosidase activity was reduced 40-fold from the WT level when measured 6 hr after transfer into SPM (data not shown).

In contrast, *IME1* transcripts were readily detectable in both WT and *gcn5-21* mutant cells after overnight growth in YPA; transcript levels did not increase on further incubation in SPM (Fig. 2, Right). Only a slight decrease in *IME1* transcript levels was observed for all time points in the *gcn5-21* mutant. Other studies have reported very low levels of *IME1* transcript in cells grown in YPA with a >30-fold induction on transfer to SPM (e.g., ref. 4). Differences in strain background and/or premeiotic growth conditions may account for the early expression of *IME1* transcript in this study.

GCN5- and RPD3-Mediated Modulation of Histone Acetylation in Vegetative and Meiotic Cells. We wanted to determine whether *GCN5* mediates its effects on *IME2* expression directly via its acetylase activity and to further explore the role of *RPD3* and its functional relationship to *GCN5*. We therefore used chromatin IP to determine the levels of acetylated histone H3 and acetylated histone H4 bound to different DNA populations in vegetative and meiotic cells, in WT and *gcn5* and *rpd3* single mutants.

IP was carried out with antibodies specific to the acetylated forms of the two histones on sheared chromatin present in whole-cell extracts from formaldehyde-treated cultures; an aliquot of chromatin removed before IP served as a control. Formaldehyde crosslinks then were removed in both the "IP" and "preIP" samples, and DNA was isolated and applied to a nylon membrane. The membrane then was probed sequentially with three labeled DNAs corresponding, respectively, to total

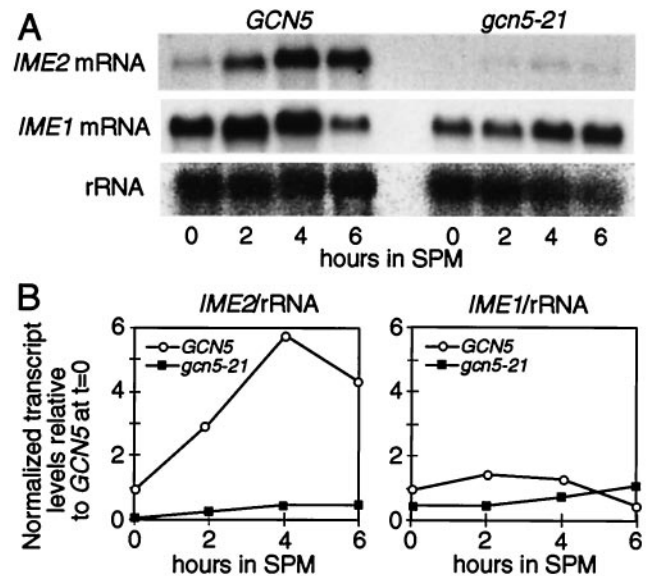


Fig. 2. (A) Northern blot analysis of *IME2* and *IME1* transcripts levels in WT cells (NKY3210) and *gcn5-21* mutants (NKY3212). (B) Relative transcript levels of *IME2* (Left) and *IME1* (Right) from WT and *gcn5-21* mutant cells normalized to rRNA levels. Sporulation of WT cells occurred at 87% efficiency, and no sporulation of *gcn5-21* cells occurred (<1%) after 24 hr in SPM.

genomic sequences, the *IME1* promoter region, and the *IME2* promoter region. For each hybridization sample, the level of "bound" acetylated histone was determined by comparing the absolute hybridization signal to the signal obtained from one-tenth volume of a comparable aliquot of total DNA (IP/preIP; Fig. 3B). To simplify consideration of the signals obtained in diverse cell conditions, the efficiency of each relative IP signal was further normalized to that observed for the same probe in WT cells under vegetative growth conditions (Fig. 3C).

At the IME2 promoter, specifically, the level of bound acetylated histone H3 increases during early meiosis. In WT cells, the level of immunoprecipitated *IME2* promoter region associated with acetylated histone H3 was about 2-fold higher in early meiotic cells than in vegetative cells (compare sample 3 with 6, Fig. 3C, Upper Left). In contrast, the levels of immunoprecipitated genomic DNA and *IME1* promoter were decreased about 2-fold compared with vegetative cells (compare samples 1 and 2 with 4 and 5, Fig. 3C, Upper Left). This pattern of effects suggests that the *IME2* promoter region undergoes meiosis-specific acetylation of histone H3 and that this effect is specific to the *IME2* promoter region.

GCN5 mediates the IME2-specific increase in bound acetylated histone H3 and also mediates global histone H3 acetylation in both vegetative and meiotic cells. The *gcn5-21* mutant exhibited a dramatic decrease (3- to 17-fold) in the levels of acetylated H3-bound DNA for all three probed DNAs in both vegetative cells and during meiosis (Fig. 3C, Upper Center). In addition, the *gcn5-21* mutant exhibits no *IME2*-specific increase in acetylated H3 levels during meiosis. The second effect implies that Gcn5 mediates the meiosis-specific increase in acetylated H3 levels that occurs uniquely at the *IME2* promoter as seen in WT cells. The first effect implies that Gcn5 also has more global role(s) on the level of DNA-bound acetylated H3, which are strong regardless of which cellular program is occurring and which can be seen for at least one locus that is not subject to meiosis-specific modulation.

RPD3 mediates histone H4 deacetylation specifically at the IME2 promoter during vegetative growth but not during meiosis. The levels of immunoprecipitated DNA bound to acetylated histone H4 varied little among all of the samples analyzed, with

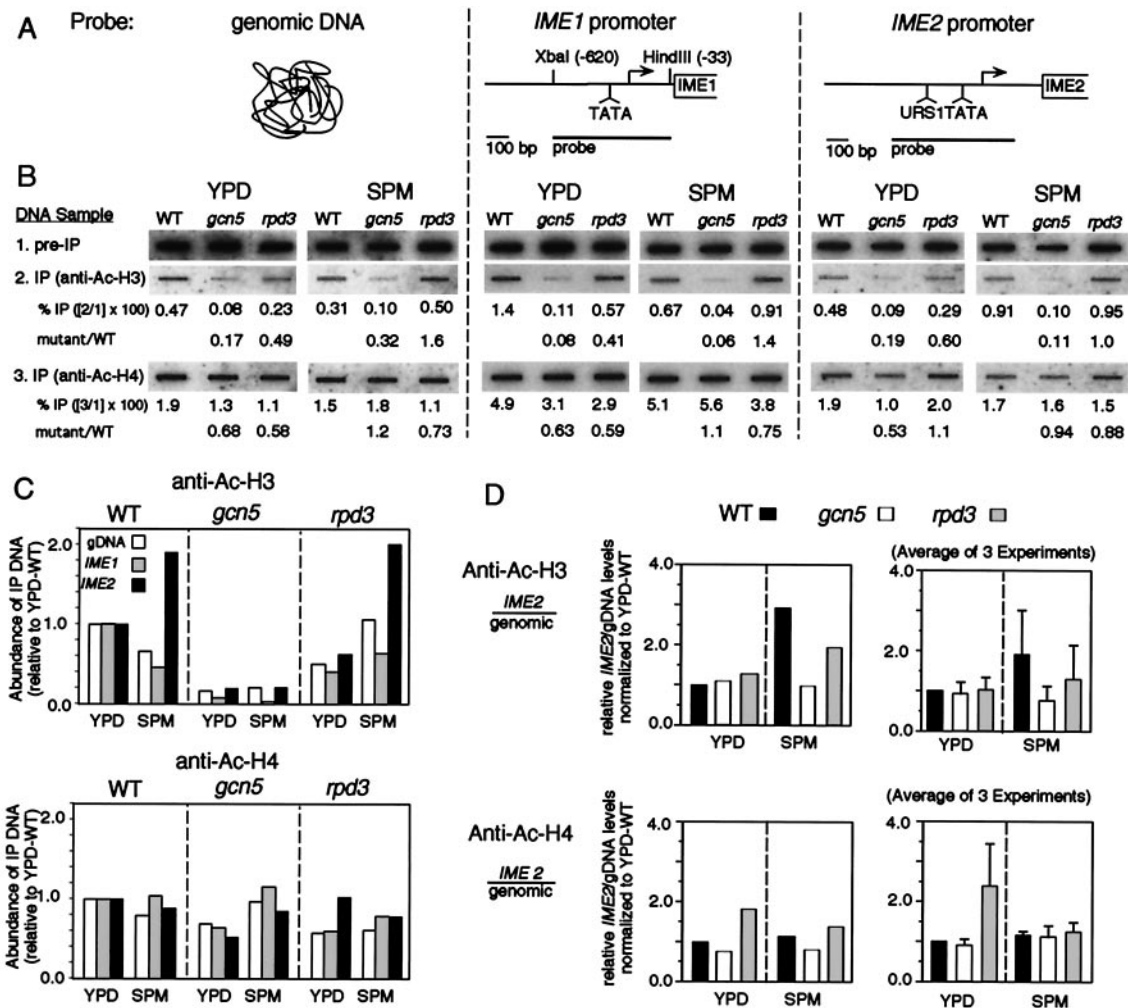


FIG. 3. Acetylation of histone H3 and histone H4 in total DNA and at the *IME1* and *IME2* promoters under vegetative and meiotic conditions. (A) Schematic of probes used. (B) Results of chromatin IP using antibodies to acetylated histone H3 or H4. WT cells (NKY3222) sporulated at 88% efficiency and *gcn5-21* (NKY3221) and *rdp3Δ* (NKY3227) mutants sporulated at <1% efficiency after 24 hr in SPM. (C) Relative IP levels are shown after normalization to YPD-WT levels. (D) The ratio of precipitated *IME2* fragment to precipitated genomic DNA from the experiment shown in B (Left) and from three independent experiments (\pm SD; Right).

one exception: the *IME2* promoter region during vegetative growth. In the *rdp3Δ* mutant, the acetylation level at the *IME2* promoter region was specifically increased 2- to 3-fold relative to the other two probed DNAs (Fig. 3C, Lower Right, compare sample 3 with samples 1 and 2). This result confirms that *RPD3*-dependent histone H4 deacetylation at the *IME2* promoter during vegetative growth, previously reported for haploid cells, also occurs in diploid cells. No such effect is observed during meiosis.

There also is no evidence that *RPD3* directly mediates any strong global effect on histone acetylation levels analogous to that seen for *GCN5*. If anything, an *rdp3Δ* mutation causes a slight decrease in the levels of H4 acetylation for total DNA and the *IME1* promoter in vegetative cells and for all three probed DNAs in meiosis. Interestingly, the *rdp3Δ* mutant also exhibits a slight increase in the level of bound acetylated H3 in total DNA and at the *IME1* promoter.

In summary, *GCN5* promotes histone H3 acetylation at many loci, but affects the *IME2* promoter uniquely, only as cells enter meiosis. *RPD3* function is also specific for the *IME2* promoter, but promotes a decrease in histone H4 acetylation and only during vegetative growth. These effects on *IME2* are clearly seen when the ratios of immunoprecipitated *IME2* promoter and total genomic DNA are plotted for each mutant for each condition in the experiment described above (Fig. 3D,

Right). These effects also are seen when averaged over three independent experiments, including one using a different method to prepare whole-cell extract (ref. 36; Fig. 3D, Right).

***GCN5* and *RPD3* Affect, Respectively, Activated/Meiotic *IME2* Transcription and Repressed/Vegetative *IME2* Transcription.** The patterns of histone acetylation at *IME2* described above raise the possibility that *RPD3* and *GCN5* might play distinct roles in *IME2* transcription during vegetative growth and early meiosis, respectively. To address this possibility, *IME2* transcript levels were examined in WT and single and double mutant diploid cells in both situations.

In vegetative cells, a *rdp3Δ* single mutant exhibits an elevated level of *IME2* transcription relative to WT, as observed previously in haploid cells (8). No such increase is observed in a *gcn5-21* mutant, which exhibits a WT transcript level; furthermore, a *gcn5-21 rdp3Δ* double mutant exhibits the same elevated transcript level as the *rdp3Δ* single mutant (Fig. 4A).

In WT meiotic cells, *IME2* transcription begins just after transfer to SPM and increases more or less continuously thereafter. In a *gcn5-21* mutant, *IME2* transcription is drastically reduced at all time points, confirming requirements of *GCN5* for meiotic induction as presented above.

In an *rdp3Δ* mutant, in contrast, *IME2* transcript levels are higher than in WT cells at $t = 0$, are similar to WT at early times ($t = 2$ hr) and appear to be somewhat reduced as

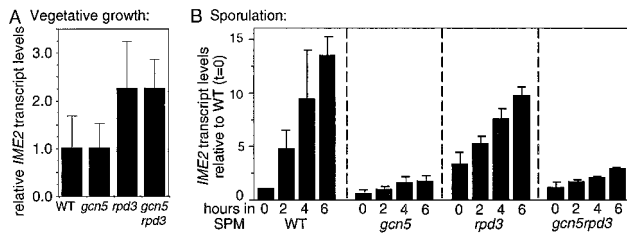


FIG. 4. *IME2* transcript levels in vegetative and meiotic WT cells (NKY3222) and *gcn5-21* (NKY3221), *rdp3Δ* (NKY3227), and *gcn5-21 rdp3Δ* (NKY3228) mutant cells. (A) Average *IME2* transcript levels from Northern blot analysis (not shown) of three independent cultures (\pm SD) normalized to rRNA levels. (B) Average *IME2* transcript levels from Northern blot analysis (not shown) of two independent meiotic time course experiments normalized to rRNA levels.

compared with WT at intermediate/late times ($t = 4$ and 6 hr; Fig. 4B). And in a *gcn5-21 rdp3Δ* double mutant, transcript levels are essentially indistinguishable from the *gcn5-21* single mutant. Meiotic DNA replication patterns correlate with the presence or absence of the *gcn5-21* mutation only; replication occurs in an *rdp3Δ* mutant but is absent in the *gcn5-21* and *gcn5-21 rdp3Δ* double mutants (Fig. 5).

Complex Roles of *GCN5* and *RPD3* for Sporulation under Nonstandard Conditions. In the course of these studies we found that *gcn5-21* and *rdp3Δ* single mutants both are proficient for meiosis, yielding nearly WT levels of viable spores when they undergo meiosis on agar plates supplemented with glycerol as a nonfermentable carbon source rather than acetate (or the related carbon source, ethanol; Table 1). Furthermore, the double mutant is strongly sporulation defective (Table 1), suggesting that, under these particular nutritional conditions *GCN5* and *RPD3* are functionally redundant. Moreover, addition of acetate to agar plates containing glycerol restores the phenotype observed under "standard" sporulation conditions: *gcn5-21* and *rdp3Δ* single mutants are both defective in formation of spores (Table 1; ref. 37). Thus, the presence of acetate creates more stringent requirements for *GCN5* and *RPD3* regardless of whether another carbon source is present or not. Notably, when no additional carbon source is present, sporulation is the same as that observed on glycerol alone (Table 1). Presumably, another carbon source in the medium is sufficient under this condition.

DISCUSSION

***GCN5* Is Required for Entry into Meiosis and for Induction of *IME2* Expression.** The *gcn5-21* mutation confers a block on meiosis at a very early step, before any DNA replication detectable by flow cytometry. This block explains the absence of meiotic recombination initiation, meiotic divisions, and spores, which are known not to occur if S phase is blocked.

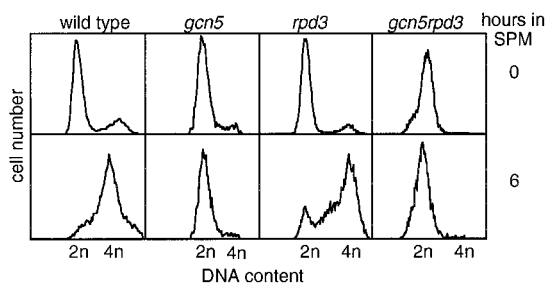


FIG. 5. Flow cytometry analysis of cells arrested before meiosis in YPA and 6 hr after the transfer of cells into SPM. Strains used are the same as in Fig. 4. After 24 hr in SPM, 61% of WT cells sporulated, and in each single and double mutant strain <1% of cells sporulated.

The *gcn5-21* mutation also confers a strong block to induction of *IME2* transcription. This block is likely because of a direct role of *GCN5* in establishing an active *IME2* promoter. *GCN5* is a histone H3 acetylase; the *IME2* promoter, uniquely, exhibits an early meiosis-specific increase in H3 acetylation; and no such increase occurs in the mutant.

The *gcn5-21* defect in *IME2* transcription should be sufficient to explain the failure of mutant cells to enter meiosis. It cannot be excluded, however, that other defects (e.g., altered bulk chromatin status) also contribute and/or also would by themselves be sufficient. In support of this idea, the *gcn5-21* mutation is not an exact phenocopy of *ime2Δ*; whereas *gcn5-21* cells do not carry out meiotic DNA replication, *ime2Δ* cells eventually do (ref. 38; S.M.B. and N.K., unpublished work). In addition, effects on histone acetylation by *Gcn5* extend to chromosomal loci that are not also affected for transcription (e.g., the *IME1* locus).

***GCN5* and *RPD3*, Independently, Mediate Programmed, Locus-Specific, Histone Subunit-Specific Modulation of Histone Acetylation at the *IME2* Promoter with Resultant Effects on Transcription Initiation.** The results presented above suggest that induction of *IME2* transcription during meiosis requires the presence of *GCN5*-acetylated histone H3 and is independent of *RPD3*-mediated deacetylation of histone H4. Conversely, repression of basal *IME2* transcription during vegetative growth requires *RPD3*-dependent deacetylation of histone H4, as shown previously, and is independent of *GCN5*.

These results provide an additional example of a positive correlation between histone acetylation and transcriptional activity. More particularly, regulation of the *IME2* promoter involves several highly specialized features. Two different specific constellations of bound histones are achieved at the same promoter in two different cell types (vegetative and meiotic). In both cases, the acetylation level of one particular histone subunit was critical, but with two different subunits being relevant in the two different cell types. Also, locus-specific modulation of bound histone status was achieved over and above the background of general histone acetylation, implying that locus-specific factors must participate in setting up the observed arrays. Presumably, different sets of such factors would be important in different cell types, e.g., *IME1* in the case of meiotic activation of *IME2*.

Do *GCN5* and *RPD3* Play Multiple Roles during Meiosis? Under standard conditions, *GCN5* is required for entry into the meiotic program (above); *RPD3*, in contrast, is required at later step(s) (39), and it is unknown whether *GCN5* does or does not participate in those events. On SPM lacking acetate (or a metabolically related compound), however, a different set of *gcn5* and *rdp3* mutant phenotypes is observed. These findings imply that the nature of the nonfermentable carbon source present during meiosis affects the roles of these gene products.

The ability of acetate to uncover an early role for *GCN5* in meiosis, even in the presence of glycerol, is also interesting because the substrate molecule for *GCN5*-mediated histone acetylation, acetyl Co-A, is a direct metabolite of acetate (40). This result raises the possibility of a direct link between the presence of acetate in the medium and the activation of *IME2* expression. This idea is further supported by the finding that ethanol has the same effect as acetate, because metabolism of ethanol also leads directly to acetyl Co-A (whereas metabolism of glycerol does not; ref. 40). In this regard, it may be relevant that the *SNF1*-kinase-dependent pathway(s), which enable cells to use acetate, appear to act at the same two steps of meiosis affected by the *gcn5* and *rdp3* mutations (41).

During meiosis in the absence of acetate, *GCN5* and *RPD3* are functionally redundant: *gcn5* and *rdp3* single mutants are proficient whereas only the double mutant is defective. This situation is quite paradoxical given that the two gene products are thought to have opposing effects on histone acetylation

Table 1. Sporulation efficiency on nonfermentable carbon sources

Strain	Relevant genotype	Acetate	Ethanol	Glycerol	Acetate + glycerol	No carbon	Spore viability*
NKY3222	<i>GCN5 RPD3</i>	88%	49%	70%	55%	71%	96%
NKY3221	<i>gcn5 RPD3</i>	<1%	<1%	26%	3%	25%	94%
NKY3226	<i>GCN5 rpd3</i>	<1%	2%	39%	4%	24%	90%
NKY3227	<i>gcn5 rpd3</i>	<1%	<1%	<1%	<1%	<1%	No sporulation

Cells were grown on solid YPD media for 2 days before patching on sporulation plates. All media contain 0.2 M Mes, pH 5.5, 0.1× amino acid drop out powder, 2% agar plus 0.3% of each carbon source. Sporulation was assayed after 3 days.

*Twelve four-spore asci were dissected from glycerol-containing media onto YPD plates to measure spore viability.

status. We find that *GCN5* plays a role in bulk chromatin acetylation both during vegetative growth and during early meiosis in the presence of acetate. Thus it is likely that acetylation of bulk chromatin also is affected in the *gcn5* mutant in no-acetate meiosis. We are intrigued by the possibility that, in the presence of acetate, the target of *Gcn5* is the status of the *IME2* promoter, whereas no-acetate meiosis reveals a role for acetylation of bulk chromatin structure. In particular, the presence of hypoacetylated bulk chromatin in meiosis in the *gcn5* mutant might uncover or create an additional role for *RPD3*.

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