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Nutritional Regulation of Late Meiotic Events in *Saccharomyces cerevisiae* through a Pathway Distinct from Initiation†

RITA H. LEE AND SAUL M. HONIGBERG*

Department of Biology, Syracuse University, Syracuse, New York 13244-1210

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The *IME1* gene is essential for initiation of meiosis in the yeast *Saccharomyces cerevisiae*, although it is not required for growth. Here we report that in stationary-phase cultures containing low concentrations of glucose, cells overexpressing *IME1* undergo the early meiotic events, including DNA replication, commitment to recombination, and synaptonemal complex formation and dissolution. In contrast, later meiotic events, such as chromosome segregation, commitment to meiosis, and spore formation, do not occur. Thus, nutrients can repress the late stages of meiosis independently of their block to initiation. Cells arrested at this midpoint in meiosis are relatively stable and can resume meiotic differentiation if transferred to sporulation conditions. Resumption of meiosis does not require repression of *IME1* expression, since *IME1* RNA levels stay high after transfer of the arrested cells to sporulation medium. These results suggest that meiosis in *S. cerevisiae* is a paradigm of a differentiation pathway regulated by signal transduction at both early and late stages.

The yeast *Saccharomyces cerevisiae* can undergo several types of differentiation in response to specific environmental signals, including mating, pseudohypha production, and meiosis-sporulation. As in other organisms, differentiation in *S. cerevisiae* involves the coordination of a series of different cellular events. This is particularly apparent during meiosis and sporulation. Once meiosis has initiated, cells undergo DNA replication, chromosome pairing, DNA recombination, and two cycles of chromosome segregation and are finally packaged into haploid spores. The extracellular signals that trigger this change in cell type are relatively simple: to sporulate efficiently, cells need to be starved for an essential growth nutrient (such as nitrogen). In addition, glucose must be absent from the medium, whereas nonfermentable carbon sources (such as acetate) actually promote meiosis.

Our understanding of the molecular mechanism of meiotic initiation has expanded greatly in the last few years (reviewed in reference 30). Activation of the *IME1* gene is a critical event in this process (20). Haploids are prevented from initiating meiosis, even under starvation conditions, at least in part because the *RME1* protein, which is expressed only in haploid cells, binds to and directly represses the *IME1* gene (7, 32, 41). In diploids, nutrients repress the *IME1* gene through a signal transduction pathway involving *RAS2* and cyclic AMP-dependent protein kinase (27, 48). When a diploid cell is starved for nutrients, the *IME1* gene is derepressed and its product, a transcription factor (25, 47), causes the expression of other early meiosis-specific genes (31). Whereas *IME1* is required for meiosis and sporulation, it is not sufficient; overexpression of this gene does not overcome the nutritional block to sporulation in most strains and does not allow expression of late meiosis-specific genes (21, 43, 48). Remarkably, overexpression of *IME1* in growth medium does induce high levels of recombination in growth medium (48), as well as promote the transcription of several genes expressed early in meiosis (49, 54). Furthermore, this recombination is "meiotic" in nature, since it depends on the presence of a gene, *SPO11*, which acts specifically in meiotic recombination (48).

Once meiotic differentiation has initiated, a progression of cellular events ensues in which each event is precisely timed relative to other events. A fascinating aspect of this downstream control is the flexibility of the differentiation pathway in response to changes in extracellular signals. During the early stages of meiotic differentiation, cells will revert directly to the mitotic cycle if nutrients are resupplied (14, 23). Even after cells have initiated meiotic recombination, they are able to reenter the growth cycle, resulting in cells that have undergone high levels of recombination but remain diploid (10, 44, 46). In contrast, after meiosis I chromosome segregation, cells complete meiosis and sporulation even when nutrients are restored. Thus, cells in the later stages of meiotic differentiation are said to be "committed to meiosis."

In higher organisms, differentiating cells also remain capable of assuming alternative fates during the initial stages of differentiation, only becoming committed to assume a single fate at a later stage in the program (reviewed in reference 16). Recent evidence suggests that the transition from uncommitted to committed cells during meiosis in *S. cerevisiae* is a regulatory rather than a structural change in the cell, since committed cells that are blocked from completing meiosis eventually regain the ability to reenter mitotic growth (17). A similar situation may exist in *Caenorhabditis elegans*, where the *gld-1* (null) mutation blocks oogenesis at prophase of meiosis I, allowing reentry of these oocytes into the cell cycle and causing a tumorous germ line phenotype (12).

Meiosis and sporulation in diploid yeast cells can be considered a single, continuous differentiation pathway, since the complete process is triggered by a single external signal—nutrient deprivation. Here we report that overexpression of the *IME1* gene in medium containing low levels of glucose allows early but not late meiotic stages. This implies that meiosis in

* Corresponding author. Dept. of Biology, Syracuse University, Lyman Hall/108 College Pl., Syracuse, NY 13244-1210. Phone: (315) 443-1299. Fax: (315) 443-1405. Electronic mail address: shonigbe@mailbox.syr.edu.

† Dedicated to the memory of our friend and colleague Kevin Van Doren.

yeast cells is regulated by nutrients through different signaling pathways at early and late stages and it suggests a mechanism for the switch between committed and uncommitted cells. Thus, although meiosis and sporulation in yeast cells is a continuous process, it nevertheless involves two distinct signaling pathways acting at different stages.

MATERIALS AND METHODS

Strains and plasmids. The high-copy-number plasmid bearing the *IME1* gene used in this study, YEpK26-7 (20), is called pIME1 in this report. In addition to being present at a high copy number, the *IME1* gene in this plasmid lacks some of the upstream regulatory region required for nutrient repression (15). All of the experiments in this study were done with yeast strain SH777 containing either pIME1 or the vector YEp24.

SH777 is isogenic to W303 and has the genotype *MAT***a**/*MAT*α *ade2-1*/*ade2-1 can1:ADE2:CAN1*/*can1:ADE2:CAN1 his3-11,15*/*his3-11,15 leu2-3,112*/*leu2-3,112 lys2*(*3′del*):*HIS3*:*lys2*(*5′del*)/*LYS2 trp1-1*/*trp1-3′del ura3-1*/*ura3-1*. It was constructed from the parents of W303 (*MAT***a**/*MAT*α *ade2-1*/*ade2-1 can^{r}1-100*/*can^{r}1-100 his3-11,15*/*his3-11,15 leu2-3,112*/*leu2-3,112 trp1-1*/*trp1-1 ura3-1*/*ura3-1*) in three steps. First, pADECAN, an integrating vector containing both the *ADE2* and *CAN^{s}1* genes (constructed by C. Fritze, University of Chicago) was linearized at the *Nae*I site and integrated into the *can^{r}1-100* locus to create *can^{r}1*:*ADE2*:*CAN^{s}1* alleles in both parents. Second, plasmid pRB1210, containing an internal fragment of *LYS2* and the entire *HIS3* gene (6), was linearized at the *Stu*I site and integrated into the *LYS2* gene in the *MAT***a** parent to create the *lys2*(*3′del*):*HIS3*:*lys2*(*5′del*) allele. Finally, pRS19, containing *URA3* and an allele of *trp1* with a 400-bp 3′ deletion (51), was linearized with *Bgl*II and used in a two-step gene replacement (39) to create the *trp1-3′del* allele in the *MAT*α parent.

MIN medium is standard yeast minimal medium and contains 0.17% yeast nitrogen base (without amino acids or ammonium sulfate), 2% glucose, and 0.5% $(NH_4)_2SO_4$. SPII (sporulation) medium is 2% potassium acetate (pH 7.0). MIN(0.5%) and MIN(0%) are identical to MIN medium except that they contain 0.5 and 0% glucose, respectively, rather than 2% glucose. IM(0.1%) and IM(0%) are identical to MIN medium except that they contain 0.1 and 0% glucose, respectively, and lack $(NH_4)_2SO_4$. SC, YPA, and various dropout media have been described previously (38). For this study, SPII, MIN, MIN(0.5%), MIN(0%), IM(0.1%), and IM(0%) media contained histidine, leucine, lysine, and tryptophan at standard concentrations (38) to complement the auxotrophies of SH777. SC medium lacking histidine, lysine, and tryptophan (SC-HLT) was used to assay for diploid recombinants, and SC medium lacking arginine and containing canavanine (60 μg/ml) was used to assay for committed meiotic cells. In addition, to maintain selection for the plasmids, all liquid and solid media lacked uracil.

Conditions for growth and meiosis. Unless otherwise noted, cells were inoculated at approximately 10^5/ml into SC-Ura medium and grown at 30°C for 24 h with vigorous shaking and then harvested, sonicated, resuspended in the same (spent) medium, and grown under the same conditions for an additional 12 h. After this treatment, approximately 90% of the cells were unbudded after a further round of sonication. Cells from this stage were washed once and transferred to IM(0.1%), sporulation, or another medium, and incubation was continued at 30°C with vigorous shaking. At various times after this transfer, 1-ml aliquots were removed from these cultures, washed once in distilled H_2O, and placed on ice for up to 24 h before being assayed for meiotic events as described below.

Assays for replication, recombination, ploidy, and commitment. DNA replication was monitored by flow cytometry after cells had been fixed in ethanol, treated with RNase A and proteinase K, and stained with propidium iodide (40) with a Becton-Dickinson FACScan 4 apparatus and LYSYS II software.

In the strains used in this study, intragenic recombination between *trp1* heteroalleles can generate Trp^+ prototrophs. Thus, recombination frequency was monitored by plating cells on medium lacking tryptophan (SC-Trp). Whereas cells complete meiosis as haploids, diploid recombinants can arise as transient intermediates in meiosis, reflecting cells that are committed to meiotic recombination but not yet committed to haploidization (Fig. 1A). To allow us to detect these intermediates, our strains had the *LYS2* gene disrupted by the *HIS3* gene in one copy of chromosome II. Since only diploids can be both His^+ and Lys^+, diploid recombinants were selected by plating cells on synthetic complete medium lacking histidine and lysine, as well as tryptophan (SC-HLT). Although the *lys2*:*HIS3* disruption is unstable, recombination at this locus is rare and does not significantly affect either the signal or the background. Verification of this assay is described below.

In the course of this study, we also developed a highly selective genetic assay for meiotic commitment as follows. SH777 has a tandem duplication containing a *CAN^{s}1* allele and a *can^{r}1* allele on each copy of chromosome V. The *CAN^{s}1* gene confers sensitivity to the drug canavanine and is dominant to the recessive *can^{r}1* allele. Can^r cells can only be generated efficiently in meiosis, that is, by a recombination event at the *CAN1* locus causing loss of the *CAN^{s}1* allele from one chromatid, followed by haploidization (Fig. 1B). Thus, the frequency of cells that form colonies on plates containing canavanine is directly proportional to the frequency of cells that are committed to complete (or have already completed) meiosis. Although Can^r diploids can be generated during mitotic growth, this requires two independent recombination events and the frequency with which this occurs is very low. Therefore, Can^r cells occur in vegetative growth at a frequency approximately 10,000-fold less than in meiosis. Hence, the assay is much more selective than earlier assays for committed cells that were based entirely on the reduction of heterozygosity (44). For example, when three isolates of SH777 were grown to stationary phase in SC medium and then plated on medium containing canavanine, the mean frequency of Can^r cells was $(2.5 \pm 1.6) \times 10^{-6}$. After transfer to sporulation medium for 48 h, these same three cultures yielded Can^r colonies at a mean frequency of $(2.2 \pm 0.4) \times 10^{-2}$. Since Can^r colonies identify committed cells, these colonies should be haploid, and this was routinely verified by mating type tests. In one typical experiment, in which SH777 was placed in sporulation medium for 24 h and then plated on canavanine-containing medium, 200 of the 200 Can^r colonies were maters.

We verified that colonies growing on SC-HLT were diploid by using two different criteria: mating type tests (38) and an assay for chromosome ploidy developed for this study. The ploidy assay was necessary because *MAT*-homozygous diploids are indistinguishable from haploids by mating type assays. Our ploidy assay is based on the *can^{r}1:ADE2:CAN^{s}1* allele described in the previous paragraph; since diploids require two independent recombination events to generate Can^r cells during vegetative growth, while haploids require only one recombination event, the frequency of Can^r haploid cells is much higher. Thus, haploids are distinguishable from diploids by the high frequency of papillations generated when a patch of haploid cells is replica plated to medium containing canavanine. In one experiment, an 8-h sporulation culture of SH777 was plated on SC-HLT and 200 colonies were patched onto a master plate and then tested for mating type and ploidy by comparison with control patches on the same plate. Of the 200 colonies growing on this medium, 190 were nonmaters and all 200 were found to be diploid by the ploidy assay. The 10 diploid maters were likely derived from reciprocal exchange between the centromere and the *MAT* locus followed by segregation to yield *MAT*-homozygous diploids. In tests of intergenic recombination in strain SH777(pIME1) (described in Results), mating type and ploidy tests were carried out after each isolate was cured of its *URA3* plasmid by replica plating to 5-fluoroorotic acid-containing medium (38).

For both recombination and commitment assays, cells were also plated on SC and SC-Ura media to measure viability and plasmid stability, respectively. All colonies were counted after 3 days at 30°C. Recombination and commitment were calculated as the frequency of either event per viable cell retaining the plasmid (CFU). All data for recombination and commitment in this study are averages of three independent cultures. The error bars shown in Fig. 2 and 3 represent the standard errors of the means (SEM), and the data in Table 1 and the text are expressed as means ± SEM.

Cytology. Cell concentration and percent spore formation were determined with a hemacytometer. Synaptonemal complexes were visualized in accordance with published procedures (8, 24). Briefly, cells were treated with Zymolyase 100T (ICN) until spheroplasts were formed, burst with Lipsol detergent (LIP, Ltd.), fixed with formaldehyde, and allowed to spread while drying. Synaptonemal complexes were stained with $AgNO_3$ and visualized by light microscopy. The staining methods of Loidl et al. (24) and Dresser and Giroux (8) gave similar quantitative results; in this study, the former technique yielded better resolution but varied in effectiveness with different batches of nylon mesh. Quantitation of the fraction of cells that have undergone the meiotic divisions by staining of cells with 4′,6′-diamidino-2-phenylindole (DAPI) and fluorescent visualization of nuclei has been described previously (17). The fraction of cells at a given time that had completed synaptonemal complex formation, the meiotic divisions, or spore formation was determined by assaying 200 to 300 cells. The data in Fig. 5 and 6 and the text are averages of three independent cultures, the data in the text are given as means ± SEM, and the error bars in the figures represent the SEM.

RNA analysis. RNA was isolated by vortexing 2×10^8 yeast cells with glass beads and phenol as previously described (9). RNase protection analysis with nuclease S1 was performed as previously described (2), 20 μg of RNA was used in each reaction mixture, and both probes were present in excess. Probes were prepared by SP6 in vitro transcription; the probe prepared from pPL139 protected the 0.23-kb *Pst*I-*Sac*I region of the *IME1* gene, and the probe prepared from pDED1 protected the 0.25-kb *Bam*HI-*Afl*II region of the *DED1* gene. The sizes of the protected fragments were verified by comparison with end-labeled, *Hin*fI-digested YRp7 DNA.

RESULTS

Effects of *IME1* expression on growth and recombination. Although *IME1* is required for meiotic initiation, overexpression of this gene does not inhibit cell growth in standard growth media containing glucose, nor does it promote spore formation in these media. Interestingly, however, overexpression of *IME1* does promote high levels of recombination in

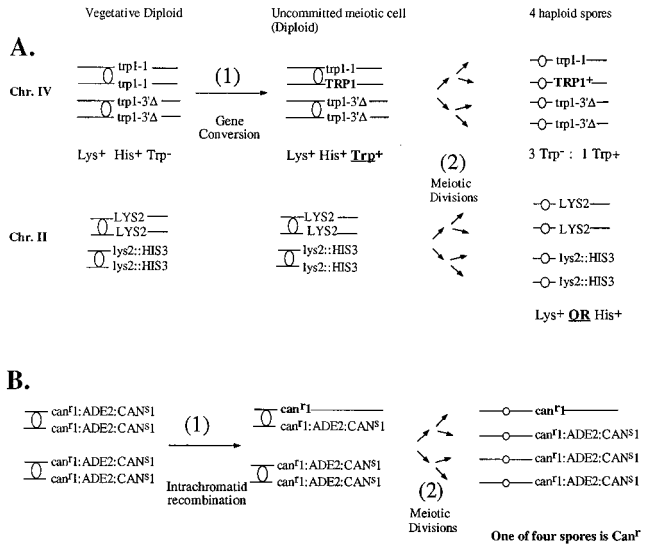

FIG. 1. Genetic assays for uncommitted and committed meiotic SH777 cells. (A) Assay for diploid recombinants. The parent strain (SH777) is a diploid in which the *LYS2* gene on one of the two copies of chromosome (Chr.) II is disrupted with the *HIS3* gene. In addition, the strain is heteroallelic at the *trp1* locus (chromosome IV). Diploid recombinants—cells that have undergone recombination at the *trp1* locus but not haploidization—are selected on His^- Lys^- Trp^- medium. These diploid recombinants can arise as meiotic intermediates (uncommitted meiotic cells) which have undergone gene conversion to yield Trp^+ prototrophs (step 1) but are not yet committed to segregating the *LYS2* and *HIS3* copies of chromosome II (step 2). (B) Assay for commitment to meiosis. A committed cell undergoes high levels of recombination at the duplicated *can*^{r}*1*:*ADE2*:*CAN*^{s}*1* gene (step 1), followed by haploidization (step 2), to generate a spore that lacks any copies of the *CAN*^{s}*1* allele. As a result, committed cells can be detected by plating cells on medium containing canavanine.

growth medium, particularly when the growth medium contains diminished amounts of glucose. Since these cells do not sporulate, the recombinants remain diploid (48). How does *IME1* expression induce recombination without blocking growth or inducing spore formation? Our investigation began by determining whether this induced recombination occurs during vegetative growth or only after cells reach stationary phase. To distinguish recombinants which remain diploid from the haploid recombinants generated during meiosis, we developed an assay which specifically selects for the former class. In brief, diploid recombinants were monitored by simultaneously selecting for recombination at the *trp1* locus and for the continued presence of both copies of chromosome II (diagrammed in Fig. 1A and described in Materials and Methods).

Wild-type cells containing the *IME1* gene on a high-copy plasmid, SH777(pIME1), were grown to stationary phase in MIN growth medium (standard growth medium containing only the required amino acids). Cells from this stationary-phase culture were then used to inoculate MIN(0.5%) medium at a low cell density. This latter medium allows cell growth but contains a lower glucose concentration than is typically used (see Materials and Methods). Growth and recombination were measured at various times after transfer to MIN(0.5%) medium and compared with those of an isogenic control strain carrying only the vector plasmid, SH777(YEp24) (Fig. 2). Our results confirmed that an increased *IME1* gene dosage does not affect the growth rate or the viability of the cultures (20) but does induce recombination to a level only slightly lower than the frequency seen in meiosis (48).

Two observations confirmed that these recombinants remained diploid. First, the frequency of diploid Trp^+ recombinants in this culture (i.e., His^+ Lys^+ Trp^+ prototrophs) did not decrease during the 36 h shown in Fig. 2. Second, mating type and ploidy tests of 100 Trp^+ colonies derived from a 36-h MIN(0.5%) culture revealed that all 100 were diploid. In agreement with this, only minimal levels of asci were seen in stationary-phase cultures (0.1% ± 0.1% asci after 36 h).

Most importantly, comparison of the kinetics of growth and recombination showed that recombination only initiated once cells reached stationary phase. This explains why induction of recombination did not affect the growth rate: recombination was only induced after the cells had stopped growing. Although recombinants began to appear almost immediately after the culture had stopped growing, it is difficult to assess whether there is a time interval between the two events since cells in an asynchronous culture stop dividing at different times. The timing of induction is addressed more fully in the following sections.

Nutritional parameters of induction of recombination by *IME1*. Cells typically arrest in stationary phase when one or more essential nutrients are limiting. Since the induction of recombination was first noticed in MIN(0.5%) medium, which contains lowered concentrations of glucose, the effect of this nutrient on induction was examined. Recombination occurred much less efficiently in stationary-phase SH777(pIME1) cells in standard MIN medium, i.e., 2% glucose, than in cells in MIN(0.5%) medium, even when the cells were in the former medium for a total of 72 h (Table 1, compare rows 5 and 6). Nevertheless, the level of recombination seen in 2% glucose was significantly higher than in the control strain, suggesting that the repression by a high concentration of glucose was incomplete (Table 1, compare rows 2 and 6). Although 2% glucose can prevent efficient induction of recombination even when a high dosage of the *IME1* gene is present, this block is not due to transcriptional repression of *IME1* by the higher glucose concentrations, since levels of *IME1* RNA accumulation were similar at high and low glucose concentrations (see below). The repression of meiotic initiation in SH777(pIME1) at high glucose concentrations could result from the proposed posttranslational control of *IME1* expression (4, 43).

To simplify analysis of the *IME1*-mediated induction of re-

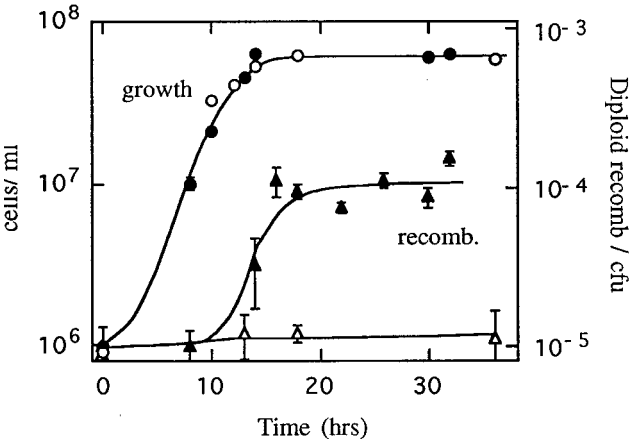

FIG. 2. Growth and recombination (recomb.) in SH777(pIME1) and SH777(YEp24) cells. Recombinants which remain diploid were measured as Trp^+ His^+ Lys^+ prototrophs (Fig. 1). SH777(pIME1) and SH777(YEp24) cells were placed in MIN(0.5%) medium, and growth and recombination were monitored over time. Cell viability remained high throughout this time course. Symbols: ●, SH777(pIME1) cell concentration; ○, SH777(YEp24) cell concentration; ▲, SH777 (pIME1) diploid recombinants; △, SH777(YEp24) diploid recombinants.

TABLE 1. Recombination and sporulation at increased *IME1* dosage

Strain and medium[a]	Mean no. of Trp^+ recombinants/10^6 CFU ± SEM		Mean % sporulation[b] ± SEM
	Total recombinants[c]	Diploid recombinants[d]	
YEp24			
MIN(0.5%)	7.5 ± 5.9	11 ± 5.6	<0.1
MIN	6 ± 1	6 ± 1	<0.1
IM(0.1%)	8 ± 1	10 ± 2	<0.1
SP[e]	440 ± 50	13 ± 2	25 ± 2
pIME1			
MIN(0.5%)	220 ± 20	150 ± 15	0.1 ± 0.1
MIN	64 ± 8	58 ± 12	<0.1
IM(0.1%)	390 ± 80	210 ± 50	3.5 ± 1
SP	250 ± 60	2 ± 1	51 ± 2

[a] The strain used was SH777 containing either plasmid YEp24 or pIME1. Cells were grown in MIN medium for 36 h as described in Materials and Methods and then transferred to MIN(0.5%), IM(0.1%), or sporulation medium or left in MIN medium. After an additional 36 h, cells were assayed for recombination and sporulation.

[b] Percentage of cells that formed asci.

[c] Intragenic recombination at the *trp1* locus measured as the frequency of Trp^+ prototrophs.

[d] Frequency of cells that were Trp^+ prototrophs and remained diploid.

[e] SP, sporulation medium.

combination, a condition was identified that allows this induction without prior growth. SH777(pIME1) was grown to stationary phase in MIN medium, a condition under which recombination is not induced efficiently, and then transferred to a medium containing low concentrations of glucose but lacking a nitrogen source, termed IM(0.1%) medium. As expected, since the medium lacked nitrogen, further growth was prevented yet cell viability remained high after 36 h (data not shown). Strikingly, after 36 h in this medium, recombination was induced to levels comparable to that seen in MIN(0.5%) or sporulation medium and only low levels of asci were formed (Table 1, row 7). Note that in the strain containing pIME1, the frequency of diploid recombinants remained high after 36 h in IM(0.1%) whereas in sporulation medium most recombinants were haploid by this time (Table 1, compare rows 7 and 8). In the control strain, SH777(YEp24), neither recombination nor spore formation was observed in IM(0.1%) medium, although both occurred relatively efficiently in sporulation medium (Table 1, compare rows 3 and 4). The presporulation conditions used in this study were chosen to maintain selection on the plasmid; they yield only 40 to 50% of the asci that optimal presporulation conditions do. Since IM(0.1%) medium allows induction of recombination in SH777(pIME1) without prior growth, it was used throughout the remainder of this study.

To further examine the effects of different nutrients on both the initiation and the completion of meiosis, we did a series of experiments with media lacking various nutrients. When SH777(pIME1) was transferred from MIN medium to medium identical to IM(0.1%) except completely lacking glucose [IM(0%) medium], not only were full levels of recombination induced ($[3.9 \pm 0.7] \times 10^{-4}$ Trp^+ recombinants per CFU) by 24 h, but significant levels of spore formation (12% ± 2% asci) were also seen. This result is consistent with the idea that the small amount of glucose present in IM(0.1%) medium is able to repress completion of meiosis even when *IME1* is overexpressed. However, it is worth pointing out that spore formation, unlike recombination, occurs significantly less efficiently in IM(0%) medium (or in the same medium lacking yeast nitrogen base [data not shown]) than it does in sporulation medium (Table 1, row 8), which contains 2% potassium acetate. Thus, completion of meiosis and sporulation in SH777(pIME1) may be promoted by acetate, a nonfermentable carbon source, as well as repressed by glucose.

Since IM(0.1%) medium lacks nitrogen, yet SH777(pIME1) cells in this medium fail to complete meiosis, nitrogen is not essential for blocking the late stages of meiosis. More directly, MIN[0%) medium, which is identical to IM(0%) medium except that it contains nitrogen, allows 14% ± 1% of SH777(pIME1) cells to form asci, approximately the same fraction of cells as form asci in IM(0%) medium. This result suggests that unlike glucose, nitrogen is unable to prevent the completion of meiosis when a high dosage of *IME1* is present. In agreement with these results and as previously reported (20), cells bearing plasmid pIME1 sporulate efficiently after 24 h in rich acetate medium (YPA), which contains nitrogen but no glucose [43% ± 2% asci in SH777(pIME1) versus <0.1% asci in SH777(YEp24)].

Timing of recombination in SH777(pIME1). Since nearly meiotic levels of recombination are induced by *IME1* in growth medium and this recombination is dependent on meiotic recombination genes (48), we determined whether the timing of recombination induced by pIME1 in IM(0.1%) medium is similar to the timing of recombination induced in sporulation medium. For this purpose, SH777(pIME1) was grown to stationary phase in MIN medium and then transferred to IM(0.1%) medium or sporulation medium as described in the previous section. Total recombinants appeared at approximately the same rate and to the same extent in both media (Fig. 3A), suggesting that meiosis initiated in the low-glucose medium, as is discussed in more detail below. Diploid recombinants are a transient intermediate in sporulation medium, reflecting the fact that cells undergo recombination in meiosis several hours before they undergo haploidization. As expected, the frequency of diploid recombinants reached a peak in sporulation medium and then declined for either SH777(pIME1) (Fig. 3B) or the control strain (data not shown). In contrast, diploid recombinants were formed stably in IM(0.1%) medium; they appeared with the same kinetics as in sporulation medium, but their levels did not decrease (Fig. 3B). In summary, an increased dosage of the *IME1* gene induces recombination in medium with lowered concentrations of nutrients, particularly glucose. Although this recombination occurs with timing and levels similar to the induction of recombination during meiosis, neither spore formation nor haploidization occurs.

Intergenic recombination is also induced by expression of the *IME1* gene. Intragenic recombination, such as was measured at the *trp1* locus, derives primarily from gene conversion, whereas intergenic recombination derives mostly from reciprocal recombination (11). To determine whether *IME1* expression also induces intergenic recombination in IM(0.1%) medium, recombination was measured in the *CENIII-MAT* interval. This was achieved by determining the frequency of diploid cells in the culture that were able to mate. Such cells derive from *MAT***a**/*MAT*α diploids by recombination in the *CENIII-MAT* interval followed by segregation of the recombinant chromatids after the cells resume growth, yielding diploids homozygous at the *MAT* locus. SH777(pIME1) was incubated in IM(0.1%) medium for 12 h and then plated on medium selecting diploid Trp^+ intragenic recombinants. Mating type and ploidy tests of 134 of these colonies (see Materials and Methods) revealed that all were diploid and 19 were maters. Since segregation of recombinant or nonrecombinant chromosomes in mitosis is random, 50% of the recombination events are hidden because of cosegregation of recombinant

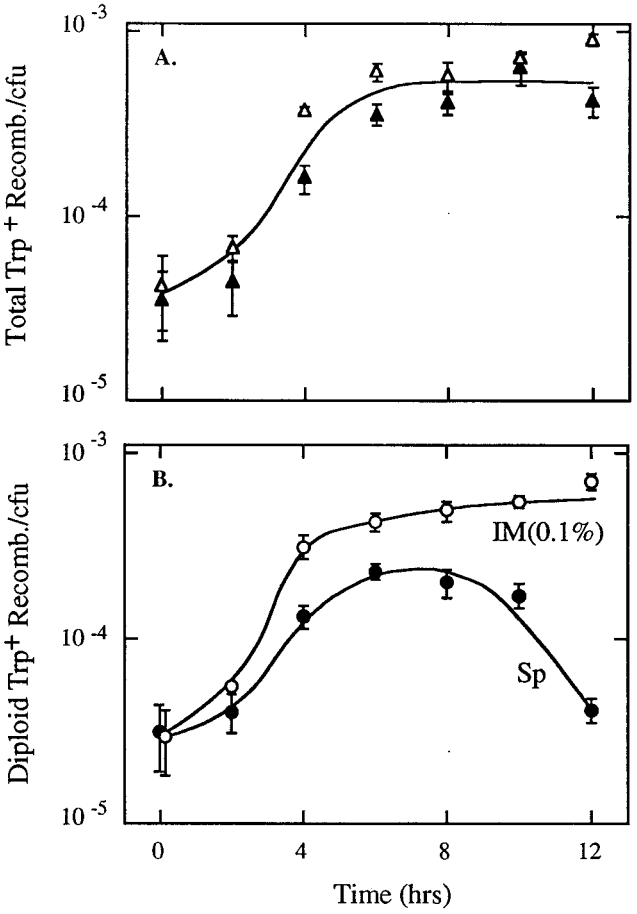

FIG. 3. Accumulation of total Trp^+ recombinants and diploid Trp^+ recombinants in IM(0.1%) or sporulation medium. Strain SH777(pIME1) was grown to stationary phase as described in Materials and Methods and then transferred to either IM(0.1%) or sporulation medium. (A) The total frequency of *trp1* allele recombinants (either haploid or diploid) was monitored as the frequency of Trp^+ prototrophs in the culture at various times after transfer. Symbols: △, total recombinants in IM(0.1%) medium; ▲, total recombinants in sporulation medium. (B) Frequency of diploid recombinants monitored as the frequency of Trp^+ His^+ Lys^+ prototrophs at various times after transfer. Symbols: ○, diploid recombinants in IM(0.1%) medium; ●, diploid recombinants in sporulation medium.

chromosomes. Thus, the frequency of recombination between *CENIII* and *MAT* at 12 h is (19 × 2)/134 = 28%. This frequency is comparable to the frequency of meiotic recombination expected in this region from the map distance of 29 centimorgans (34).

To confirm that high levels of intergenic recombination are induced by *IME1* expression, recombination in a second interval, *CENII-LYS2/HIS3*, was examined. Cells from a 12-h culture of SH777(pIME1) in IM(0.1%) medium were plated on Trp^- medium. Ploidy tests and tests for His^- or Lys^- auxotrophs revealed that 98% of the colonies were diploids (233 of 238) and that 15% (34 of 233) of the diploids had become homozygous at the *LYS2/HIS3* locus. As described above, the recombination frequency can be calculated as (34 × 2)/233 = 29%, comparable to the 50% meiotic recombination frequency expected between *CENII* and *LYS2/HIS3*, which are 68 centimorgans apart on chromosome II (34).

Intergenic recombination in the *CENII-LYS2/HIS3* region accounts, in part, for the twofold excess of total Trp^+ recombinants over diploid recombinants mentioned previously (Table 1, row 7). Since diploids that have become homozygous at the *LYS2/HIS3* locus do not grow on Trp^- Lys^- His^- medium, the assay for diploid recombinants underestimates the real number of diploid recombinants by approximately 15 to 25%. A further reason for the excess of total over diploid recombinants after long incubation in IM(0.1%) is discussed in a subsequent section of Results ["Commitment to meiosis in SH777(pIME1)"]. The major conclusion from these experiments is that expression of the *IME1* gene allows high levels of intergenic, as well as intragenic, recombination even when 0.1% glucose is present in the medium.

Expression of the *IME1* gene induces both DNA replication and synaptonemal complex formation. The identical timing and extent of recombination in 0.1% glucose medium and sporulation medium suggest that overexpression of *IME1* in the presence of glucose causes initiation of meiotic differentiation. To test this hypothesis, we examined other early meiotic events—specifically, DNA replication and synaptonemal complex formation—to determine whether they also are induced by *IME1* expression.

DNA replication is among the earliest meiotic landmarks, preceding the initiation of meiotic recombination by several hours. To test whether this event was also initiated when the *IME1* gene was present at a high copy number, SH777(pIME1) and SH777(YEp24) were grown in MIN medium to stationary phase and then transferred to IM(0.1%) medium under our standard conditions. Samples were removed after 10 h, and the DNA content was measured by flow cytometry. The control culture, SH777(YEp24), arrested primarily in G_1 of the cell cycle (2C DNA content), as expected for cells in stationary phase (Fig. 4A). In contrast, slightly more than half of the cells containing the *IME1*-containing plasmid arrested with 4C DNA content, indicating that they had undergone replication (Fig. 4B). In control experiments, both cultures underwent replication in sporulation medium (data not shown). These results are consistent with the idea that overexpression of *IME1* causes initiation of meiosis when low levels of nutrients are present.

In *S. cerevisiae*, and also in most other organisms, darkly staining protein structures termed synaptonemal complexes form along the length of the paired chromosomes during the pachytene stage of meiosis. These structures disappear before the onset of meiosis I chromosome segregation and may act to

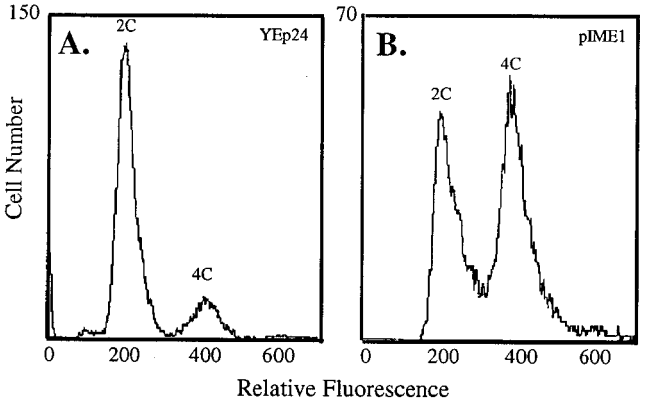

FIG. 4. Effect of overexpression of *IME1* on DNA content in stationary-phase cultures. Cells were grown in MIN medium as described in Materials and Methods and then transferred to IM(0.1%) medium for an additional 10 h. DNA content was analyzed by flow cytometry. Peaks reflecting unreplicated diploid (2C) and replicated diploid (4C) cells are indicated. A, control strain SH777(YEp24); B, SH777(pIME1).

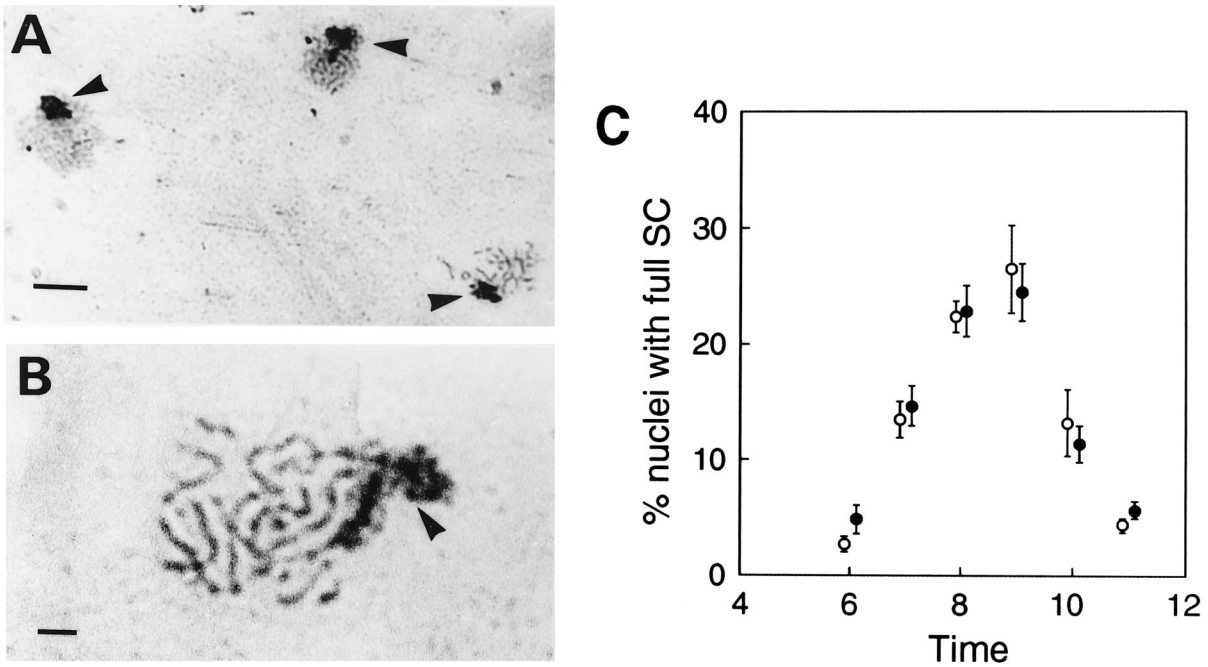

FIG. 5. Synaptonemal complex formation in SH777(pIME1) cells. Cells were grown in accordance with our standard protocol (see Materials and Methods) and then transferred to either sporulation medium or IM(0.1%) medium. Aliquots were removed, and synaptonemal complexes were visualized by silver staining and light microscopy. To determine the fraction of cells containing synaptonemal complexes, each spread nucleus associated with a visible nucleolus (arrowheads in A and B) was counted in one of three classes: diffusely staining nuclei, those with a partial synaptonemal complex structure, or those with a full synaptonemal complex structure. (A) SH777(YEp24) after 7 h in sporulation medium. On the left is a diffusely staining nucleus, in the center is a nucleus with a partial synaptonemal complex structure, and on the right is a nucleus with a full synaptonemal complex. Bar, 5 μm. (B) A representative synaptonemal complex seen in strain SH777(pIME1) after 7 h in IM(0.1%) medium. Bar, 1 μm. (C) Fraction of SH777(pIME1) cells containing full synaptonemal complexes in either IM(0.1%) or sporulation medium. Symbols: ○, IM(0.1%) medium; ●, sporulation medium.

distribute crossovers evenly among the chromosomes (22, 53). Typically, synaptonemal complexes form after cells have already become committed to recombination (35) and at about the time or slightly before they become committed to completing meiosis and spore formation (18). To determine whether expression of the *IME1* gene allows synaptonemal complex formation in IM(0.1%) medium, a stationary-phase culture of strain SH777(pIME1) was transferred to either IM(0.1%) or sporulation medium. Samples were removed at various times after this transfer, and the fraction of cells containing synaptonemal complexes was determined by silver staining and light microscopy. These experiments revealed that synaptonemal complexes formed and dissociated in the IM(0.1%) medium with the same kinetics as in sporulation medium (Fig. 5C). Furthermore, the synaptonemal complexes formed in IM(0.1%) medium had the same appearance as those formed in sporulation medium (Fig. 5A and B).

In summary, when cells containing the *IME1* gene at a high copy number are placed in media containing lowered concentrations of glucose, they initiate meiosis and undergo the characteristic early meiotic events: DNA replication, commitment to recombination, formation of synaptonemal complexes, and dissolution of synaptonemal complexes.

The nutritional block to late meiotic events is reversible. synaptonemal complexes dissociate in meiosis shortly before chromosome segregation initiates in the meiosis I division. Since the recombinants formed in IM(0.1%) medium remained diploid, we expected that these cells had not undergone the meiotic divisions. To test this directly, SH777(pIME1) was grown in MIN medium and then transferred to IM(0.1%) medium as described in Materials and Methods. At various times after this transfer, the fraction of cells which have completed the first meiotic division was monitored by fluorescent microscopy. As a positive control, the same strain was transferred from MIN medium to sporulation medium. As expected, cells underwent meiosis I segregation in sporulation medium but not in IM(0.1%) medium; for example, 61% ± 6% of cells had undergone the meiosis I division by 12 h in sporulation medium, whereas only 3% ± 1% of cells had undergone the first division after 18 h in IM(0.1%) medium.

Since overexpression of *IME1* in IM(0.1%) medium allows early but not late meiotic events, we determined whether such cells are reversibly arrested at a midpoint in meiosis. If they are, then they should be able to undergo chromosome segregation immediately after transfer from IM(0.1%) to sporulation medium. SH777(pIME1) was grown in MIN medium, placed in IM(0.1%) medium for 12 h, and finally transferred to sporulation medium. Not only were these cells able to resume meiosis when transferred to sporulation medium; they underwent the first division almost immediately after transfer (Fig. 6, open triangles). When cells were instead transferred directly from MIN to sporulation medium (so that initiation does not occur efficiently prior to exposure to sporulation medium), the divisions occurred 4 to 6 h later than when they were transferred from IM(0.1%) (compare open triangles with open circles). The second meiotic division and spore wall formation were also advanced in the IM(0.1%) culture relative to cells transferred directly from MIN medium (data not shown). Since we used separate assays to measure replication, recombination, and synaptonemal complex formation in SH777(pIME1) cells in the previous experiments, it is possible that these different events occurred in different populations of cells. However, since the arrested cells remained viable and were able to resume meiosis synchronously and efficiently after transfer to

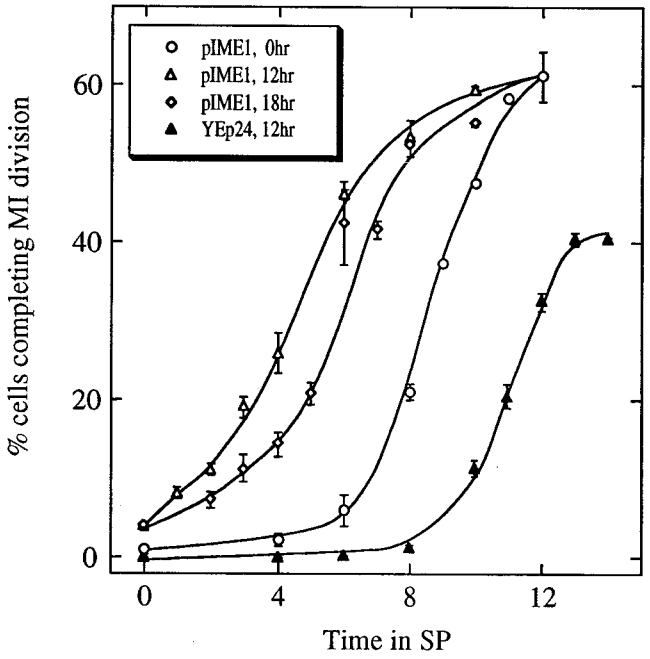

FIG. 6. Timing of the first meiotic division after transfer into sporulation medium. SH777(pIME1) or SH777(YEp24) cells were grown to stationary phase under standard conditions and then transferred to IM(0.1%) medium. After various times in IM(0.1%) medium, cells were transferred to sporulation medium (SP). Completion of meiosis I chromosome segregation was monitored by fluorescence microscopy at various times after the transfer to sporulation medium. Symbols: △, SH777(pIME1) after 12 h of preincubation in IM(0.1%); ◇, SH777(pIME1) after 18 h of preincubation in IM; ○, SH777(pIME1) without preincubation in IM(0.1%); ▲, SH777(YEp24) after 12 h of preincubation in IM(0.1%).

sporulation medium, it is likely that the entire series of early meiotic events progresses normally in IM(0.1%) medium. Thus, cells overexpressing *IME1* in 0.1% glucose are arrested just prior to meiosis I segregation and this arrest is reversible when cells are transferred to sporulation medium.

To determine whether the meiotic arrest in 0.1% glucose is stable, cells carrying pIME1 were placed in IM(0.1%) medium for 18, rather than 12, h before transfer to sporulation medium and meiosis I segregation was monitored as before. The longer preincubation in IM(0.1%) medium slightly delayed the timing of commitment but did not affect efficiency, indicating that cells reach a relatively stable arrest in 0.1% glucose, at least during the time frame tested here (Fig. 6, compare open diamonds with open triangles). It is not known what caused the slight delay in resumption of meiosis in cells preincubated for the longer time.

As a negative control for this series of experiments, a strain containing only the vector plasmid was preincubated in IM(0.1%) and then transferred to sporulation medium as described above. As expected, and in contrast to SH777(pIME1), 12 h of preincubation in IM(0.1%) did not lead to immediate chromosome segregation once cells were transferred to sporulation medium (closed triangles in Fig. 6). Interestingly, even when SH777 (pIME1) cells were grown in MIN medium, where meiotic initiation does not take place efficiently, and then transferred to sporulation medium, the onset of meiosis I segregation was 2 to 3 h earlier than in the SH777(YEp24) control (Fig. 6, compare filled triangles with circles). This difference probably reflects the time required for induction of *IME1* in wild-type cells (see below), and it suggests that induction of the *IME1* gene is rate limiting for the initiation of meiosis under these conditions.

Commitment to meiosis in SH777(pIME1). At approximately the same time as meiosis I chromosome segregation, cells become committed to meiosis; that is, they will complete meiosis after this time even when transferred to growth conditions (see introduction). Since the majority of cells overexpressing *IME1* reenter growth as diploids rather than haploids, they appear to be uncommitted to the completion of meiosis at the time of their arrest. To examine this point further, a simple genetic assay for committed cells was developed (see Materials and Methods). In brief, the frequency of cells that form colonies on canavanine-containing medium is proportional to the frequency of cells that are committed to completing meiosis (or have already completed meiosis), since haploidization, as well as recombination, is required to generate a canavanine-resistant genotype (Fig. 1B).

As a control, the timing of commitment of SH777(pIME1) cells in sporulation medium was measured. The majority of cells became committed by 12 h in sporulation medium (open circles in Fig. 7). In contrast, when the same cultures were transferred to IM(0.1%) medium rather than sporulation medium, only relatively low levels of commitment (2 to 3% of the maximum) were seen (triangles in Fig. 7). When cells were preincubated in IM(0.1%) medium for 12 h and then transferred to sporulation medium, the timing of commitment was clearly advanced relative to that of cells transferred directly from MIN medium (Fig. 7, compare open circles with triangles), indicating that cells initiated meiosis in the IM(0.1%) medium but were blocked from commitment until they were "released" into sporulation medium. Interestingly, when SH777(pIME1) cells were released into sporulation medium, a larger fraction of the resulting asci were dyads relative to the control strain under the same conditions [44% ± 7% dyads for

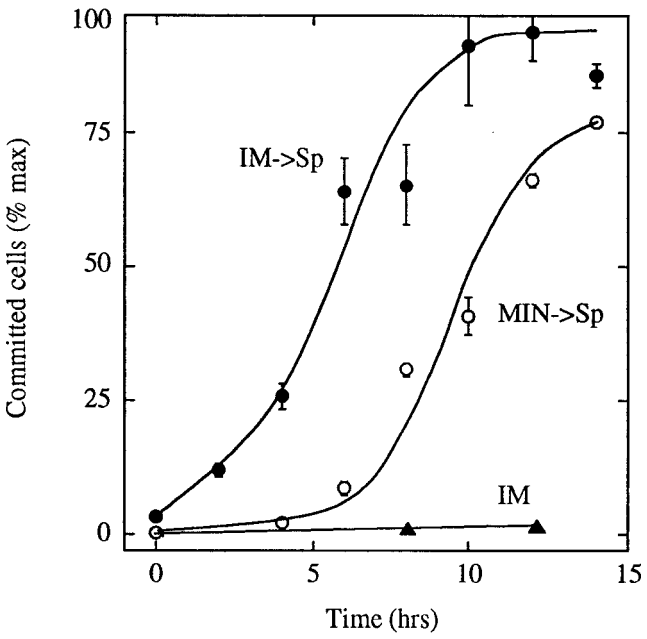

FIG. 7. Timing of meiotic commitment after transfer of meiotically arrested cells into sporulation medium. SH777(pIME1) cells were grown under standard conditions and then transferred to IM(0.1%) or sporulation (Sp) medium. Commitment was then monitored over time in IM(0.1%) medium (▲), in sporulation medium (○), or in sporulation medium after 12 h of preincubation in IM(0.1%) medium (●). Committed cells (% max) = [(frequency of Can^r cells)/(maximum frequency of Can^r cells in sporulation medium)] × 100%.

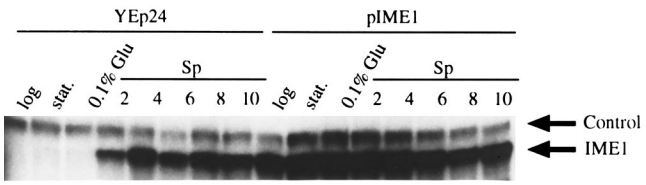

FIG. 8. Expression of the *IME1* gene under growth, initiation, or sporulation conditions. SH777(YEp24) (lanes 1 to 8) and SH777(pIME1) (lanes 9 to 16) cells were grown as described in Materials and Methods. Samples were harvested after 14 h (log phase, 10^6 cells per ml; lanes 1 and 9) and 36 h (stationary [stat.] phase, 5×10^7 cells per ml; lanes 2 and 10) in MIN medium and after transfer to IM(0.1%) medium for a further 10 h (time zero; lanes 3 and 11). Finally, the IM(0.1%) cultures were transferred into sporulation medium and samples were removed after 2, 4, 6, 8, and 10 h (lanes 4 to 8 and 12 to 16). RNA was isolated from each sample, and *IME1* expression was determined by an RNase protection assay (see Materials and Methods). The control RNA is the *DED1* transcript, which is present at a constant level through meiosis and in vegetative growth. A weak *IME1* protected band of approximately 210 bases is now shown; it was presumably caused by transcription stopping during preparation of the *IME1* probe.

SH777(pIME1) cells compared with 13% ± 2% dyads for the control strain]. This result suggests that *IME1* overexpression interferes with the meiotic divisions and/or spore formation. Mating type tests confirmed that the Can^r colonies derived from cells newly released into sporulation medium indeed reflect committed cells. For example, 2 h after cells were transferred from IM(0.1%) medium to sporulation medium, they were plated on medium containing canavanine. Of the 100 colonies that grew on this plate and were tested for mating type, 99% (99 of 100) were maters.

While commitment to meiosis was largely blocked by the IM(0.1%) medium (Fig. 7, triangles), a fraction of SH777 (pIME1) cells did become committed after much longer incubations in this medium (13% ± 2% of the cells were committed at 36 h), whereas the control strain remained uncommitted (0.1% ± 0.1% of the cells were committed at 36 h). The fraction of cells that became committed to meiosis after 36 h in IM(0.1%) medium contributed to the twofold excess of total recombinants over diploid recombinants noted previously (Table 1, row 7). As described in a previous section, intergenic recombination accounts for some of this difference; ploidy tests revealed that committed cells, which do not form diploid recombinants, account for most of the rest (data not shown).

Taken together, our results show that expression of the *IME1* gene in medium containing low levels of glucose allows cells to proceed through the early stages of meiosis, including DNA replication, meiotic recombination, and synaptonemal complex formation and dissolution, but not through the commitment transition or into the meiotic divisions or spore formation. Furthermore, this meiotic arrest is stable and reversible: once the cells are completely deprived of glucose, they immediately undergo commitment, complete the meiotic divisions, and form spores.

Expression of the *IME1* gene before and after transfer to sporulation medium. The finding that overexpression of *IME1* is sufficient for the early meiotic events in growth medium while transfer to sporulation medium is required for later meiotic events suggests that *IME1* gene expression is not directly involved in regulation of the later events. An alternative view is that *IME1* must be repressed for the cells to progress through late stages of meiosis, and this repression is achieved only after cells are transferred to sporulation medium. This latter idea is consistent with the known expression pattern of the single-copy *IME1* gene in meiosis: it is expressed early and turned off (via a negative-feedback loop) later in meiosis (42, 48). To distinguish between these two possibilities, *IME1* transcript accumulation was measured in both SH777(pIME1) and SH777(YEp24) cells by RNase protection. The *DED1* transcript, which is present at constant levels during both meiosis and vegetative growth, was monitored as an internal control.

First, RNA accumulation was compared in log- and stationary-phase cells (MIN medium) and after the stationary-phase cultures were transferred to IM(0.1%). As expected, the control strain, SH777(YEp24), showed little or no expression of the endogenous *IME1* gene in either medium (Fig. 8, lanes 1 to 3). In the cells bearing pIME1, high levels of expression were seen at all times, i.e., in log- and stationary-phase cells grown in MIN and after a further 10 h of incubation in IM(0.1%) (Fig. 8, lanes 9 to 11). Thus, the presence of plasmid pIME1 is sufficient for transcriptional expression of the *IME1* gene even under growth conditions.

Next, the accumulation of RNA was measured at various times after cells had been transferred from IM(0.1%) medium to sporulation medium, i.e., after release of SH777(pIME1) cells from meiotic arrest. In the control strain, as expected on the basis of previous studies (20, 48), *IME1* expression was seen 2 h after transfer to sporulation medium, reached a maximum after 6 h, and remained high for at least another 4 h (Fig. 8, lanes 4 to 8). In strain SH777(pIME1), *IME1* RNA, which was already present in the vegetative cultures, remained at high levels when the cells were transferred to sporulation conditions. In fact, *IME1* RNA accumulation did not change appreciably for at least 10 h after transfer to sporulation medium (Fig. 8, lanes 9 to 16), and this was verified in shorter exposures of the gel. Since both commitment and the meiotic divisions have occurred in the majority of cells by this time, significant transcriptional repression of *IME1* must not be required for this transition. This suggests that chromosome segregation and commitment to meiosis are independent of the continued presence of the *IME1* product. Alternatively, the commitment tran-

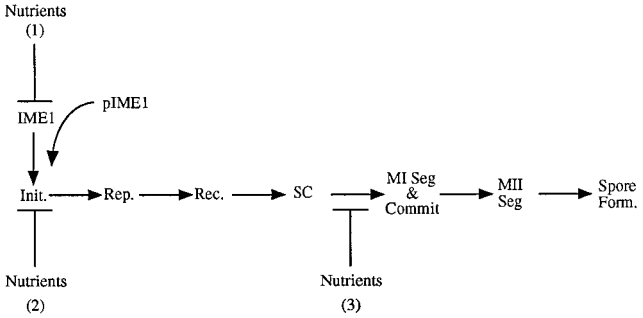

FIG. 9. Model for early and late nutritional control of meiosis. Abbreviations of cellular events in meiosis pathway: Init., initiation of meiosis; Rep., DNA replication; Rec., initiation of (commitment to) recombination; SC, synaptonemal complex formation and dissolution; MI Seg, chromosome segregation in the meiosis I division; Commit, commitment to completion of meiosis and sporulation; MII Seg, chromosome segregation in the meiosis II division; Spore Form., spore wall formation around each haploid genome. Nutritional control points during meiosis are as follows. (Point 1) The genomic copy of the *IME1* gene is transcriptionally repressed by either glucose or nitrogen. This control can be overcome by the presence of plasmid pIMEI (curved arrow), which causes high *IME1* transcript levels even when large amounts of these nutrients are present. (Point 2) Even when *IME1* is expressed, the presence of high levels of glucose prevents cells from efficiently initiating meiosis; this suggests a second control of meiotic initiation, perhaps posttranscriptional control of *IME1* expression (4, 43). In contrast, when only low concentrations of glucose are present, the presence of plasmid pIME1 is sufficient to allow initiation of meiosis. (Point 3) Under the latter conditions, the low levels of glucose are still sufficient to prevent later meiotic events from occurring; this suggests that nutrients act on late meiotic events through a separable pathway.

sition may involve posttranscriptional modification of *IME1* expression.

DISCUSSION

Starvation activates the transcription of the *IME1* gene in diploid yeast cells, and the product of this gene, itself a transcription factor, is critical for the induction of meiosis. The principal finding reported here is that forcing expression of the *IME1* gene in cells exposed to low levels of nutrients allows the early, but not the later, stages of meiotic differentiation. In particular, DNA replication, early recombination events, and the formation and dissolution of synaptonemal complexes occur almost as efficiently in medium containing low concentrations of glucose as they do in sporulation medium, whereas later meiotic events, such as commitment to meiosis, the meiotic divisions, and spore formation, occur at only minimal levels in this medium. Since low concentrations of glucose block the later meiotic events even when *IME1* is being expressed, there is likely to be a nutrient-signaling pathway acting late in meiosis which is distinguishable from the *IME1*-mediated initiation pathway (Fig. 9). Our results correlate well with the earlier finding that some genes specifically expressed late in meiotic differentiation (*SPS1*, *SPS2*, and *SGA1*) are still repressed by nutrients when *IME1* is overexpressed (21, 49). In fact, the resumption of meiosis when SH777(pIME1) cells are transferred to sporulation conditions occurs at about the same point in meiosis (the initiation of first-division segregation) at which some of these genes become derepressed in wild-type strains (13, 36).

Nutritional repression of the *IME1* gene is mediated, at least in part, through repression by a pathway involving Ras2 protein and cyclic AMP-dependent protein kinase (26, 48, 55). Other nutrient signal transduction pathways may also be involved in meiotic repression (5, 33). The finding that a relatively high concentration of glucose is required to block the initiation of meiosis in strain SH777(pIME1), whereas 0.1% glucose is sufficient to block meiosis at a later stage, suggests that the nutrient-sensing functions acting at the two stages are in some way different. Also consistent with this view is our finding that while nitrogen effectively blocks initiation of meiosis in wild-type strains, it cannot prevent completion of meiosis when *IME1* is being overexpressed. Moreover, while a mutant with a constitutively active cyclic AMP-dependent protein kinase (*bcy1*) cannot sporulate at all (5), addition of plasmid pIME1 to this mutant almost completely restores the early stages of meiosis but not the later stages (27). This suggests that the cyclic AMP-dependent pathway acts on the later meiotic events independently of its action on *IME1* expression. Thus, nutritional control at different stages in meiosis may involve either different signal transduction pathways or differential modulation of a single pathway.

Expression of the *IME1* gene is not sufficient for initiation of meiosis, since SH777(pIME1) cells exposed to standard growth medium express high *IME1* transcript levels but do not initiate meiosis efficiently. Thus, initiation of meiosis itself may be repressed at an additional level beyond repression of the *IME1* gene (Fig. 9), as suggested earlier (43). Interestingly, the presence of pIME1 promotes both the initiation and the completion of meiosis and sporulation in synthetic growth medium in a different strain background (15). The difference between this result and ours could be explained if repression of the late meiotic events was inactive in the strains used in the earlier study.

The meiotic arrest caused by overexpressing the *IME1* gene in low-nutrient medium is reversible; if arrested cells are transferred to sporulation medium, they resume meiosis and rapidly become committed. The arrest point is also relatively stable: cells placed in this medium for 18 h resume meiosis with approximately the same timing and efficiency as cells left in the medium for only 12 h. Although the *IME1* gene is usually repressed at later stages of meiosis, when the arrested SH777(pIME1) strain is transferred to sporulation medium, it can become committed while the *IME1* transcript is still present at high levels. Since an increased *IME1* transcript level leads to increased translation under most conditions (43), Ime1 protein is likely to be present in these cells at the time they become committed. Taken together, these results suggest that the regulation of late meiotic events is independent of the presence of Ime1 protein. Nevertheless, we cannot rule out the possibility that posttranslational regulation of *IME1* is important in the transition to the later stages of meiosis; in fact, the *IME1* gene product is thought to be activated posttranslationally by the action of a protein kinase, Rim11 (4). Furthermore, *IME1* expression may interfere with the late meiotic events in subtle ways. For example, we observed that increased *IME1* expression led to a larger fraction of asci that contained only two spores.

The stage of the meiotic arrest induced by *IME1* expression in low-glucose medium is particularly intriguing. Mutations in any of several different genes (*CDC28*, *CDC36*, *CDC39*, and *NDT80*) cause meiotic arrest at the pachytene stage, when synaptonemal complexes are fully formed (45, 56). In contrast, mutations in *ZIP1* lead to a prophase arrest in which the synaptonemal complex is only partially assembled, and mutations in *DMC1* cause delayed assembly of the synaptonemal complex (3, 37, 52). The prophase arrest described in this report is different from any of these other arrests, since synaptonemal complexes are able to form and dissociate normally. Since an arrest prior to chromosome segregation can occur with partial synaptonemal complexes (*zip1*), complete synaptonemal complexes (*cdc28* and *ndt80*), or synaptonemal complexes forming and disassembling normally (overexpressed *IME1*), proper synaptonemal complex assembly-disassembly is probably a necessary but not sufficient condition for meiosis I segregation.

Meiosis is reversible in its early stages in the sense that when cells are refed with nutrients, they leave the meiotic pathway directly and reenter the vegetative growth cycle. In contrast, meiosis and sporulation become irreversible at approximately the time of the meiosis I division, and cells complete differentiation even when nutrients are restored; the transition between these two stages is termed commitment to meiosis. While the functions involved in commitment are still unknown, the framework of two signaling pathways proposed here suggests a model to explain how meiosis is reversible only in its early stages, as follows: just as the *IME1* gene is induced by the absence of nutrients at the initiation of meiosis, the continued absence of nutrients at a later stage could lead to the activation of a gene product that specifically blocks reentry into the cell cycle. Once this function is activated, the cell no longer responds to the addition of nutrients; i.e., it is committed to meiosis. In this light, it is interesting to compare the regulation of meiosis in *S. cerevisiae* with that of the fission yeast *Schizosaccharomyces pombe*. Unlike *S. cerevisiae*, *S. pombe* yeast cells become committed to the completion of meiosis at nearly the same time that meiosis is initiated (1, 19). This suggests that fission yeast cells do not have a separate nutritional control acting on late meiotic events.

Differentiation during development in multicellular organisms can involve a series of separately regulated stages (re-

viewed in references 28, 29, and 50). In contrast, meiosis and sporulation in yeast cells is normally a single, continuous process: once diploid cells are placed in sporulation medium, they progress through each stage of meiosis until differentiation is complete. For this reason, it is at first remarkable that nutritional signals control the process at late stages, as well as at initiation. Potentially, having two different pathways mediating meiotic repression ensures that cells do not undergo haploidization or spore formation under inappropriate conditions. Alternatively, having early and late meiotic events under separate control might provide an advantage by allowing wild-type cells under some conditions to undergo the high levels of variability introduced by genetic recombination without the long growth delay caused by spore formation.

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