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Positive Control of Sporulation-Specific Genes by the *IME1* and *IME2* Products in *Saccharomyces cerevisiae*

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In the yeast *Saccharomyces cerevisiae*, meiosis and spore formation require the induction of sporulation-specific genes. Two genes are thought to activate the sporulation program: *IME1* and *IME2* (inducer of meiosis). Both genes are induced upon entry into meiosis, and *IME1* is required for *IME2* expression. We report here that *IME1* is essential for expression of four sporulation-specific genes. In contrast, *IME2* is not absolutely essential for expression of the sporulation-specific genes, but contributes to their rapid induction. Expression of *IME2* from a heterologous promoter permits the expression of these sporulation-specific genes, meiotic recombination, and spore formation in the absence of *IME1*. We propose that the *IME1* and *IME2* products can each activate sporulation-specific genes independently. In addition, the *IME1* product stimulates sporulation-specific gene expression indirectly through activation of *IME2* expression.

Sporulation in the yeast *Saccharomyces cerevisiae* is a program of cellular differentiation that includes genetic recombination, meiotic divisions, and spore formation (7). Sporulation is induced by starvation and is restricted to one type of cell, the **a**/α cell. The other two types of cells, **a** and α cells, express an inhibitor of meiosis that blocks sporulation (14). Several genes that are essential for sporulation are expressed only in sporulating cells: they are induced by starvation in **a**/α cells, not in **a** or α cells (1, 4, 13, 15, 16, 20, 23). Control of sporulation is thus achieved, at least in part, through control of sporulation-specific gene expression.

Two genes are thought to play a central role in the decision to enter meiosis and to sporulate: *IME1* and *IME2* (inducer of meiosis [11, 18]). Null mutations in either gene block sporulation, and an increased dosage of either gene can partially relieve both genetic and environmental controls over sporulation. (A third gene with these properties, *IME3*, has recently been characterized [L. Neigeborn and A. P. Mitchell, manuscript in preparation].) *IME1* and *IME2* transcript levels increase more than 30-fold upon entry into meiosis, and mutations that alter genetic or nutritional regulation of meiosis alter *IME1* and *IME2* expression in parallel. The *IME1* product is required for *IME2* expression, and the presence of *IME2* on a multicopy plasmid can partially relieve the requirement for *IME1* in sporulation (18). We thus proposed that genetic and environmental signals govern *IME1* expression and that the *IME1* product activates meiosis through activation of *IME2* expression. Because the *IME2* plasmid suppressed an *ime1* deletion weakly, we suggested that the *IME1* product may have a second role in meiosis, in addition to activation of *IME2*.

In the present study, we examined sporulation-specific gene expression in strains with deletions of *IME1* or *IME2* and in a strain that expresses *IME2* in the absence of *IME1*. Our results indicate that each *IME* product plays a unique role in activation of sporulation-specific genes and that the *IME1* product also stimulates sporulation-specific genes indirectly through activation of *IME2*.

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MATERIALS AND METHODS

Strains and genetic markers. Yeast strains were all derived from the SK1 genetic background and are shown in Table 1.

The *ime2-2* deletion was constructed as follows. Plasmid pHS101, carrying the 4-kilobase-pair (kbp) *IME2 Xho*I fragment, was digested with *Bgl*II to release a 2.5-kbp fragment (Fig. 1A). The 3-kbp *Bgl*II *LEU2* fragment was ligated between these *Bgl*II sites, creating plasmid pAM412 (Fig. 1B). For transformation into *S. cerevisiae*, pAM412 was digested with *Bam*HI and *Pst*I, and Leu$^+$ transformants were selected. The *ime2-2* deletion was initially transformed into a diploid; meiotic analysis revealed that the deletion caused no growth defect. In subsequent experiments, the deletion was transformed directly into haploids, whose genotype was then confirmed by Southern analysis and by their failure to complement *ime2-1* mutants. We point out that the 2.5-kbp segment deleted from *ime2-2* strains contains a completely functional *IME2* gene, as defined by our earlier studies (18).

Plasmid pAM414, containing the *GAL1,10* upstream activation site and *IME2* 5′ half, was constructed from plasmid pAM403 (18), containing the *ime2-1*::*LEU2* insertion allele, and plasmid pRY25 (provided by Roger Yocum), which contains the 365-bp *Dde*I-*Sau*3AI *GAL1,10* intergenic fragment inserted between the pBR322 *Hin*dIII and *Ava*I sites. pRY25 contains a *Sal*I linker at the *Dde*I-*Hin*dIII insert-vector junction and a *Bgl*II linker at the *Sau*3AI-*Ava*I insert-vector junction. pRY25 was modified by inserting a *Bam*HI linker at the filled-in *Eco*RI site derived from pBR322 sequences, yielding plasmid pRY25-Bam. To construct pAM414, plasmid pAM403 was digested with *Sal*I, rendered flush with Klenow fragment, and digested with *Bgl*II. The 4-kbp pAM403 fragment containing the 5′ *IME2* fragment and *LEU2* was inserted between the *Bgl*II and *Pvu*II sites of pRY25-Bam. Integration of pAM414 at the *IME2* locus was targeted by digestion with *Pvu*II and was confirmed by Southern analysis of Leu$^+$ transformants.

Sources of other mutations were as follows. *ime1-12*::*TRP1* is a deletion-substitution allele described previously (18); sequence analysis indicates that it removes 90% of the *IME1* coding region and 0.5 kbp of upstream DNA (H. E. Smith and A. P. Mitchell, manuscript in preparation). The

TABLE 1. Yeast strains

Strain	Genotype[a]
107	**a**
108	α
113	α *ime1-12*::*TRP1*
114	**a** *ime1-12*::*TRP1*
197	α *gal80*::*LEU2 ime1-12*::*TRP1 IME2-6*::*LEU2*
199	**a** *gal80*::*LEU2 ime1-12*::*TRP1 IME2-6*::*LEU2*
249	**a** *gal80*::*LEU2 spo13*::*hisG*
255	**a** *gal80*::*LEU2 arg6*
256	α *gal80*::*LEU2 arg6 ime1-12*::*TRP1 IME2-6*::*LEU2*
257	**a** *gal80*::*LEU2 arg6 IME2-6*::*LEU2*
258	**a** *gal80*::*LEU2 arg6 ime1-12*::*TRP1*
259	**a** *ime2-2*::*LEU2*
260	α *ime2-2*::*LEU2*
273	α *gal80*::*LEU2 his1*
274	α *gal80*::*LEU2 his1 IME2-6*::*LEU2*
275	**a** *gal80*::*LEU2 his1 ime1-12*::*TRP1 IME2-6*::*LEU2*
276	α *gal80*::*LEU2 his1 ime1-12*::*TRP1*
470	**a** *gal80*::*LEU2 his4-G ade3 ime1-12*::*TRP1 IME2-6*::*LEU2*
471	**a** *gal80*::*LEU2 his4-G ade3 ime1-12*::*TRP1*
473	**a** *gal80*::*LEU2 his4-G ade3 IME2-6*::*LEU2*
474	α *gal80*::*LEU2 his4-G ade3*
475	α *gal80*::*LEU2 his4-N arg6 IME2-6*::*LEU2*
476	α *gal80*::*LEU2 his4-N arg6 ime1-12*::*TRP1*
477	**a** *gal80*::*LEU2 his4-N arg6*
478	α *gal80*::*LEU2 his4-N arg6 ime1-12*::*TRP1 IME2-6*::*LEU2*

[a] All strains have the additional markers *ura3 leu2*::*hisG trp1*::*hisG lys2 ho*::*LYS2*

gal80 mutation is a deletion-substitution allele that renders SK1 strains Gal$^+$ (21). The *spo13*::*hisG* mutation was provided in an SK1 derivative by Eric Alani and Nancy Kleckner. The *ade3*, *arg6*, and *his1* mutations were isolated from an ethyl methanesulfonate-mutagenized SK1 derivative (S. Su and L. Neigeborn, unpublished data) and were crossed twice to unmutagenized strains prior to the present studies. The *his4-N* and *his4-G* alleles are fill-in mutations of the 5′ *Cla*I and 3′ *Bgl*II sites, respectively, in the *HIS4* coding region (6). They were constructed on YIp5 plasmids and transplaced into the genome with sequential Ura$^+$ and 5-fluoroorotic acid resistance selections (2).

Hybridization probes. Probes for *IME1*, *SPS1*, and *SPS2* were prepared by random-primed labeling of double-stranded DNA fragments excised from low-melting-point agarose gels. The *IME1* probe was the 0.6-kbp *Eco*RI-*Hin*dIII fragment, excised from plasmid pAM504 (18). The probe for *SPS1* and *SPS2* was a 3-kbp *Cla*I-*Cla*I fragment, excised from plasmid p18 (16).

Probes for *IME2*, *SPO11*, and *SPO13* were prepared by primer extension across single-stranded phage clones. The *IME2* clone is an internal 200-bp *Bam*HI-*Eco*RI fragment (Fig. 1A) inserted between the *Bam*HI and *Eco*RI sites of M13mp19 (18). The *SPO11* clone, in Bluescript plasmid derivative pGB426, was provided by C. N. Giroux; it is a 1.4-kbp *Acc*I-*Spe*I segment (containing the *SPO11* coding region [1]) inserted between the *Acc*I and *Spe*I sites of pBluescript KS+. For some experiments, the pGB436 primer extension mixture was cleaved with *Eco*RI and the ca. 340-base fragment was purified from a urea-acrylamide gel. The *SPO13* probe was prepared from a 1.1-kbp *Eco*RI-*Pst*I fragment inserted between the *Eco*RI and *Pst*I sites of M13mp18 (23).

The control probe, plasmid pC4, was prepared by random-primed labeling. Law and Segall (12) identified this plasmid by its hybridization to an RNA unaffected by starvation or by cell type.

General procedures. Medium composition, growth conditions, preparation of RNA, and manipulations for Northern (RNA) blots have been described previously (18).

RESULTS

Characterization of the *IME2-6* allele. We set out to construct an *IME1*- independent *IME2* gene by replacing *IME2* upstream sequences with those from the galactose-inducible *GAL1,10* locus. Plasmid pAM414 (Fig. 1C) contains a 365-bp *Sau*3AI-*Dde*I fragment from the *GAL1,10* intergenic region, which includes the upstream activation site (9), adjacent to a *Bgl*II site that lies ca. 250 bp upstream of the *IME2* initiation codon (S. Su and A. Mitchell, unpublished results). Our previous subcloning experiments indicated that the 1.4-kbp *Bgl*II-*Bam*HI *IME2* fragment in pAM414 does not possess *IME2* function (18). Homologous integration at the *IME2* locus of the entire pAM414 plasmid, after cleavage at its unique *Pvu*II site, yielded the *IME2-6* allele. The structure of *IME2-6* includes, from left to right in Fig. 1D, a nonfunctional 5′ fragment of the *IME2* gene (also present in the *ime2-1* insertion allele [18]), the *LEU2* gene, pBR322 sequences, and the *GAL1,10* intergenic region flanking an intact *IME2* coding region.

A functional test verified that *IME2-6* is negatively regulated by the *GAL80* product, which is a negative regulator of the *GAL1,10* locus (21). The *IME2-6* allele provided *IME2* activity in a *gal80* mutant strain: **a**/α diploids homozygous for a *gal80* deletion and *IME2-6* sporulated efficiently (>95% sporulation), and spore viability was normal (>95% viability). However, *IME2-6* led to a recessive sporulation defect (<0.1% sporulation) and failed to complement an *ime2-1* mutation, in strains with a wild-type *GAL80* allele. These results indicate that *IME2-6* is active only when the *GAL1,10* upstream activation site is active.

A second functional test indicated that *IME2-6* is not regulated by the mating-type locus, as expected for an *IME1*-independent allele. Normally, *IME2* is expressed only in starved **a**/α cells, which express *IME1* and can enter meiosis; *IME2* is not expressed in **a** and α cells, which do not express *IME1* and are unable to enter meiosis. Previous results suggested that expression of *IME2* would permit **a** and α cells to enter meiosis (18). To detect meiosis in haploid cells, we used a *spo13* mutation, which bypasses meiosis I, thus removing the mechanical barrier to sporulation of haploids (22). An α *IME2-6 gal80* strain (strain 475) was crossed to an **a** *spo13 gal80* strain (strain 249), and meiotic segregants from eight tetrads were analyzed. All eight *IME2-6 spo13* haploid segregants produced two-spored asci (20% ± 4% sporulation) after incubation on sporulation plates for 5 days, but the eight *IME2 spo13* segregants produced no asci (<0.1% sporulation). Similarly, *GAL80 IME2-6 spo13* segregants (from a different cross) were unable to sporulate. These observations suggest that *IME2-6* can express functional *IME2* activity in the absence of *IME1* induction and verify that functional *IME2-6* expression is inhibited by the *GAL80* product.

The transcripts produced by *IME2-6* were examined directly by Northern (RNA) analysis (Fig. 2). RNA was prepared from four isogenic **a**/α *gal80*/*gal80* diploids during vegetative growth in YEP plus acetate or after 4 h of incubation in starvation (sporulation) medium and probed with an *IME2* probe. The *IME1*/*IME1 IME2*/*IME2* diploid produced no detectable *IME2* RNA in vegetative medium

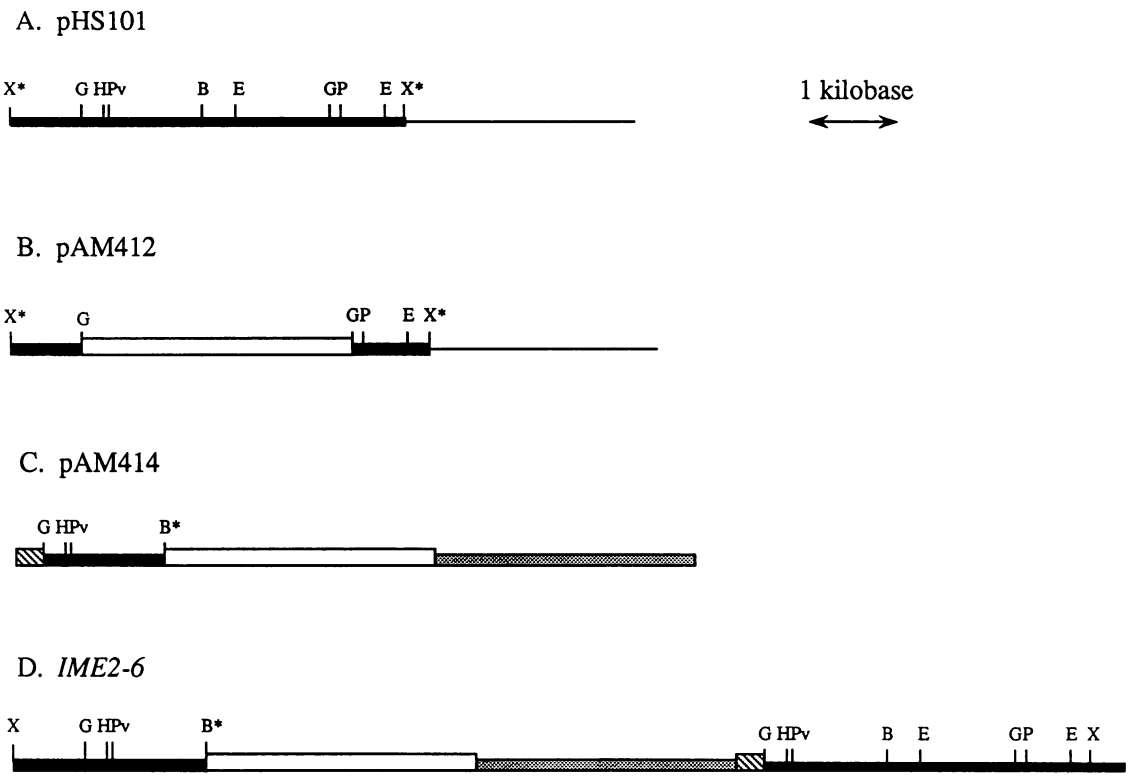

FIG. 1. Restriction maps. The plasmids pHS101 (A), pAM412 (B), and pAM414 (C) are represented by linear maps opened at a *Bam*HI site. The structure of the *IME2-6* allele is indicated (D). The direction of *IME2* transcription is from left to right; the *IME2* initiation codon lies between the *Hin*dIII and *Pvu*II sites in pHS101. Only restriction sites that derive from *IME2* sequences are indicated. Abbreviations for restriction sites: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; X, *Xho*I; B*, *Bam*HI-*Bgl*II hybrid site resulting from construction; X*, *Xho*I-*Sal*I hybrid site resulting from construction. Symbols: ■, *IME2*; ▩, pBR322; ▨, *GAL1,10*; □, *LEU2*; ——, pUC18.

and expressed the 2.6-kilobase (kb) *IME2* RNA after 4 h of starvation. As expected, an *ime1-12/ime1-12* diploid produced no *IME2* RNA. The *IME1/IME1 IME2-6/IME2-6* diploid produced three RNAs in vegetative medium, of lengths 2.4, 2.8 kb, and approximately 4 kb. RNase protection assays (results not shown) suggest that the 2.4-kb RNA encodes a functional product: the most 5′ AUG is the *IME2* initiation codon, preceded by a 30- to 100-base leader. (The wild-type *IME2* RNA has a ca. 300-base 5′ leader, the role of which is under investigation.) The 2.8-kb RNA may be functional; it initiates within the *GAL1,10* segment. The 4-kb RNA is probably nonfunctional, because it includes sequences from pBR322, which lies upstream of the *GAL1,10* segment and *IME2* coding region (Fig. 1D). During starvation, levels of the 2.4-kb RNA increased, levels of the 2.8-kb RNA were diminished slightly, and levels of the 4-kb RNA dropped precipitously. The same three RNAs were detected in the vegetative *ime1-12/ime1-12 IME2-6/IME2-6* diploid; each RNA responded to starvation just as it had in the *IME1/IME1 IME2-6/IME2-6* strain. We conclude that expression of the *IME2-6* RNAs is independent of *IME1* activity and that the bulk of *IME2-6* functional RNA is induced by starvation.

Partial suppression of an *ime1* deletion through *IME2-6* expression. We found previously that the sporulation defect of an *ime1* deletion mutant was partially suppressed by presence of a multicopy *IME2* plasmid (18). Suppression may have been incomplete because of inappropriate *IME2* expression from the plasmid or because the *IME1* product may have a second role, in addition to activation of *IME2*. We therefore examined suppression of an *ime1* deletion by the chromosomal, *IME1*-independent *IME2-6* allele. Because *IME2-6* activity is inhibited by the wild-type *GAL80* product, our experiments with *IME2-6* were conducted with *gal80* mutant strains.

Assays of both spore formation and meiotic recombination indicate that *IME2-6* can compensate for the absence of the *IME1* product (Table 2). An a/α diploid homozygous for the wild-type *IME1* allele sporulated efficiently (>95%); an isogenic diploid homozygous for an *ime1-12* deletion was unable to sporulate. Sporulation ability was partially restored (27%) in an isogenic strain that carried *IME2-6* along with the *ime1-12* deletion. Similarly, deletion of *IME1* abolished meiotic allelic recombination, but *IME2-6* permitted allelic recombination in the absence of the *IME1* product (Table 2). We verified that suppression of *ime1-12* was dominant and cosegregated with *IME2-6*.

Sporulation of *ime1-12 IME2-6* diploids displayed several qualitative defects (Table 2). For example, spore viability was poor (23%), and nonmating segregants were frequent (19%). Nonmating segregants presumably arose through chromosome 3 nondisjunction, because they occurred only in tetrads with two or three inviable spores. Similar errors in sporulation were observed when *ime1-12* was suppressed by a multicopy *IME2* plasmid (18). We attribute these flaws in the sporulation program to the absence of the *IME1* product, rather than to inappropriate expression of *IME2*, because the *IME1 IME2-6* diploid sporulated normally (Table 2). Therefore, *IME2* activity alone promotes defective sporulation; *IME1* and *IME2* together can promote efficient sporulation.

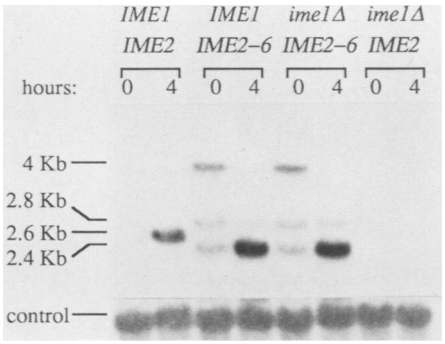

FIG. 2. RNA levels from *IME2* and *IME2-6*. RNA was prepared from four isogenic a/α *gal80/gal80* diploids, homozygous for the *IME1* and *IME2* alleles indicated at the top of the figure, at 0 or 4 h after a shift from YEP plus acetate to sporulation medium. The panels show a Northern blot of total RNA (10 μg per lane) from the time points indicated above each lane. The blot was probed separately for *IME2* and control RNAs. Haploid parents of each diploid were strains 474 and 477 (*IME1/IME1 IME2/IME2*), strains 473 and 475 (*IME1/IME1 IME2-6/IME2-6*), strains 470 and 478 (*ime1-12/ime1-12 IME2-6/IME2-6*), and strains 471 and 476 (*ime1-12/ime1-12 IME2/IME2*).

These observations suggest that the *IME1* product plays some role in sporulation in addition to activation of *IME2* expression.

Effects of altered *IME1* and *IME2* expression on sporulation-specific transcripts. A number of genes have been identified that are induced during sporulation. This group includes *SPO11*, which is required for meiotic recombination (1), *SPO13*, which is required for meiosis I division (23), and *SPS1*, which is required after meiosis II (16). Some sporulation-specific genes, such as *SPS2*, have no known role in meiosis or spore formation (16). We sought to determine whether *IME1* or *IME2* stimulates meiosis by stimulating the expression of these sporulation-specific genes.

We first examined the expression of several sporulation-specific genes in isogenic wild-type and *ime1* mutant strains (Fig. 3). In the wild-type diploid, one group of genes (*SPO11* and *SPO13*) was induced within 2 h after starvation; the other group (*SPS1* and *SPS2*) was induced between 4 and 6 h after starvation. The *ime1-12* deletion blocked detectable expression of all four genes until at least 14 h after starvation. In other experiments, we saw no expression of these genes after 26 h of starvation (not shown). We conclude that the *IME1* product is required for expression of these four sporulation-specific genes.

TABLE 2. Effects of *IME2-6* on sporulation in the presence and absence of the *IME1* product[a]

Genotype	% Sporulation	% Spore viability	% Nonmating segregants	His$^+$ recombinant frequency[b] in:	
				YEP + acetate	Sporulation medium
IME1 IME2	>95	>95	0 (n = 44)[c]	3.1×10^{-5}	3.6×10^{-3}
ime1-12 IME2	<0.02	NA[d]	NA	2.0×10^{-5}	2.2×10^{-5}
ime1-12 IME2-6	27	23	19 (n = 54)	2.5×10^{-5}	2.3×10^{-3}
IME1 IME2-6	>95	>95	0 (n = 83)	2.8×10^{-5}	6.2×10^{-3}

[a] Diploids were homozygous for the indicated alleles and for a *gal80* mutation. Determinations of sporulation, spore viability, and nonmating segregant frequency were conducted with strains 255 × 273, 258 × 276, 256 × 275, and 257 × 274. Determinations of His$^+$ recombinant frequency were conducted with strains 474 × 477, 471 × 476, 470 × 478, and 473 × 475. Complete genotypes are listed in Table 1.

[b] Samples of each diploid were analyzed during log-phase growth in YEP plus acetate or 24 h after a shift to sporulation medium. Numbers are the averages of two or three determinations. Twofold variation in these assays was typical.

[c] n, Number of viable spore clones tested.

[d] NA, Not applicable.

The *ime1* mutant may fail to express sporulation-specific genes because of the absence of the *IME2* product. In that case, expression of *IME2* in an *ime1* mutant should restore induction of sporulation-specific genes. Indeed, expression of *IME2-6* permitted the induction of all four sporulation-specific genes in an *ime1* mutant (Fig. 3). We note that the genes were induced more gradually in the *ime1 IME2-6* strain than in the wild type and that peak *SPO11* and *SPO13* RNA accumulation was reduced three- to fourfold. These aspects of induction may reflect either reduced expression per cell or greater asynchrony in the *ime1 IME2-6* population than in the wild type. Failure of the *ime1 IME2-6* strain to express *IME1* RNA was confirmed by Northern analysis (Fig. 3). Clearly, the *IME2* product is sufficient to permit sporulation-specific gene expression in the absence of the *IME1* product.

We tested whether the *IME2* product is essential for expression of these sporulation-specific genes by examining their expression in an *ime2* deletion mutant (Fig. 4). The deletion removes the entire 2.5-kbp *Bgl*II fragment that possesses *IME2* function (18). The two early genes (*SPO11* and *SPO13*) were induced more slowly in the *ime2* mutant than in the wild type, reaching peak levels at 10 to 12 h rather than at 4 h. The shutoff of these early genes was delayed until 20 to 26 h, compared with 8 to 10 h for the wild type. Induction of the later genes, *SPS1* and *SPS2*, was delayed in the *ime2* mutant until 14 to 20 h, compared with 4 to 6 h in the wild type. The peak accumulation of *SPS1* and *SPS2* RNAs was greatly reduced in the *ime2* mutant, but we note that the RNAs may accumulate further after 26 h. The *ime2* defect is not due to decreased *IME1* transcript levels; in fact, the *ime2* deletion mutant accumulated threefold-higher *IME1* RNA levels than the wild type. These results indicate that the *IME2* product is required for normal induction of sporulation-specific genes, but that it is not absolutely essential for their expression. These observation strengthens the conclusion that the *IME1* products activates sporulation-specific genes both through stimulation of *IME2* expression and through an *IME2*-independent mechanism.

DISCUSSION

IME1 and *IME2* products were originally considered activators of meiosis, because increased dosage of either gene stimulated meiotic events in the absence of normal nutritional and mating-type signals (11, 18). These signals govern meiosis, in part through transcriptional regulation of sporulation-specific genes (1, 4, 13, 15, 16, 20, 23). In this paper, we have provided direct evidence that *IME1* and *IME2* are positive regulators of four sporulation-specific genes.

Evidence for a positive role of the *IME1* product in sporulation-specific gene expression is straightforward: a deletion of *IME1* blocks the accumulation of *SPO11*, *SPO13*, *SPS1*, and *SPS2* RNAs. This result is consistent with our previous finding that *IME1* is required for *IME2* expression; *IME2* is itself a sporulation-specific gene. These observations support the notion that activation of *IME1* expression is part of the switch that activates sporulation, because induction of *IME2*, *SPO11*, and *SPO13* is among the earliest events in sporulation (1, 18, 23).

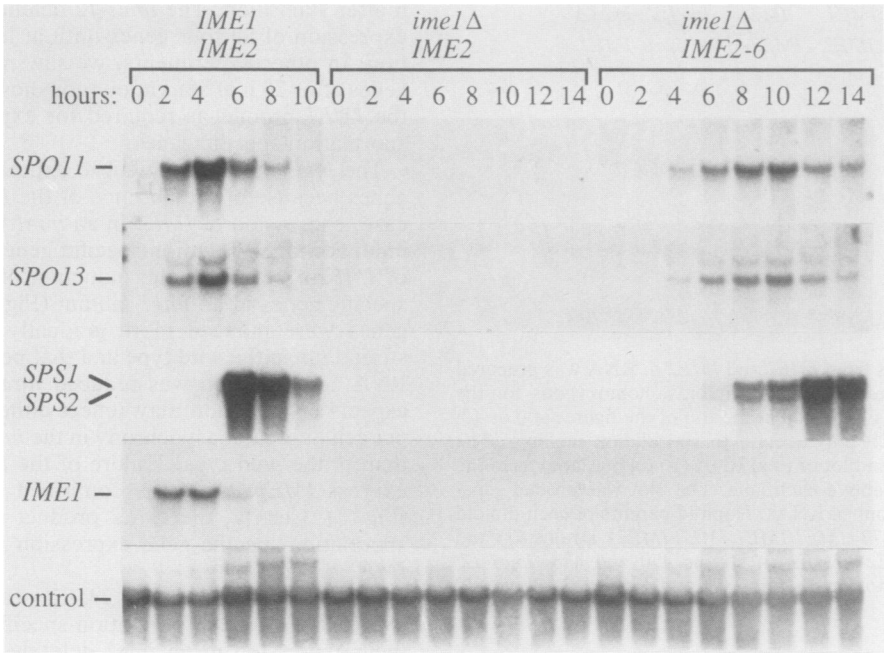

FIG. 3. Sporulation-specific RNA levels in diploids lacking *IME1* product. RNA was prepared from three isogenic a/α diploids, homozygous for the *IME1* and *IME2* alleles indicated at the top of the figure, at various times after a shift from YEP plus acetate to sporulation medium. The panels show a Northern blot of total RNA (10 μg per lane) from the time points indicated above each lane. The blot was probed separately for *SPO11*, *SPO13*, *SPS1* and *SPS2*, *IME1*, and control RNAs. Haploid parents of each diploid were 107 and 108 (*IME1*/*IME1 IME2*/*IME2*), 113 and 114 (*ime1-12*/*ime1-12 IME2*/*IME2*), and 197 and 199 (*ime1-12*/*ime1-12 IME2-6*/*IME2-6*).

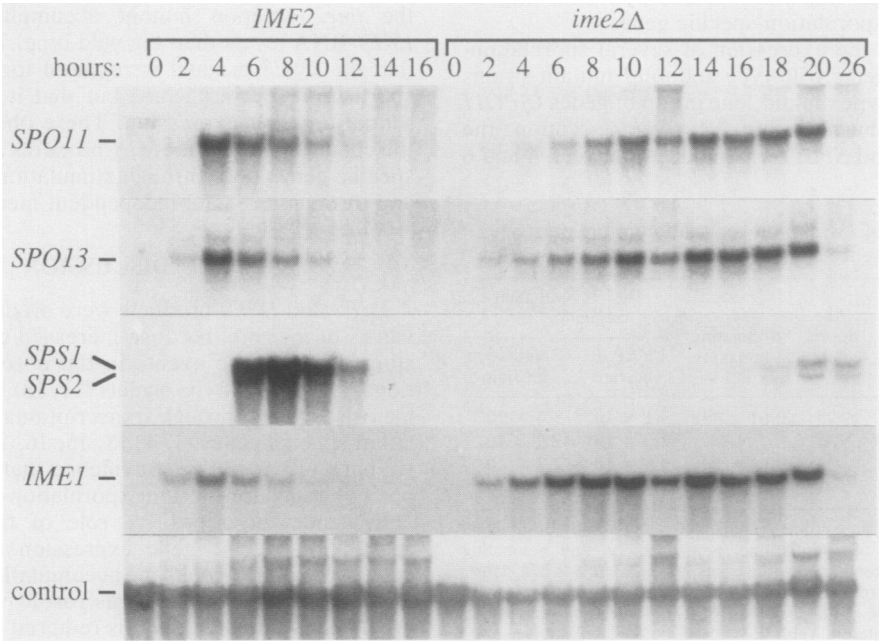

FIG. 4. Sporulation-specific RNA levels in a diploid lacking the *IME2* product. RNA was prepared from two isogenic a/α diploids, homozygous for either *IME2* or *ime2-2* as indicated at the top of the figure, at various times after a shift from YEP plus acetate to sporulation medium. The panels show a Northern blot of total RNA (10 μg per lane) from the time points indicated above each lane. The blot was probed separately for *SPO11*, *SPO13*, *SPS1* and *SPS2*, *IME1*, and control RNAs. Haploid parents of each diploid were 107 and 108 (*IME2*/*IME2*) and 259 and 260 (*ime2-2*/*ime2-2*).

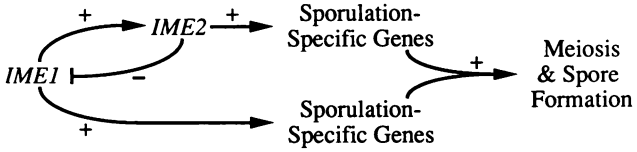

FIG. 5. Two pathways that lead to sporulation-specific gene expression in *S. cerevisiae*. The *IME1* product can activate sporulation-specific genes by activating *IME2* expression, or in the absence of *IME2*. The *IME2* product can activate sporulation-specific genes in the absence of *IME1*, but *IME1* is normally required for *IME2* expression. The *IME2* product is also a negative regulator of *IME1* expression. The *IME2* pathway alone leads to inefficient meiosis and spore formation; the two pathways act together to bring about efficient sporulation of wild-type cells.

Evidence for a positive role of the *IME2* product in sporulation-specific gene expression comes from two observations. First, an *ime2* deletion leads to delayed accumulation of the four sporulation-specific RNAs examined. The *ime2* defect is not a result of an *IME1* expression defect, because *IME1* RNA accumulates at a normal rate in the *ime2* mutant. (In fact, *ime2* defects lead to elevated levels of *IME1* RNA [18] [Fig. 4].) Second, expression of *IME2-6* in an *ime1* deletion mutant permits induction of the four sporulation-specific genes and permits sporulation. These observations indicate that the *IME2* product is required for normal induction of sporulation-specific genes and that it can activate sporulation-specific genes through an *IME1*-independent mechanism.

We infer that the *IME1* product can activate sporulation-specific genes through an *IME2*-independent mechanism, because an *ime1* deletion causes a more severe sporulation-specific gene expression defect than does an *ime2* deletion. The finding that expression of *IME2-6* does not completely bypass the need for *IME1* in sporulation suggests that the *IME2*-independent role of *IME1* product is functionally important.

To account for the ability of either *IME1* or *IME2* expression to activate *SPO11*, *SPO13*, *SPS1*, and *SPS2*, we suggest that two pathways lead to activation of sporulation-specific genes (Fig. 5). In one pathway, the *IME1* product activates *IME2* and the *IME2* product activates its target genes. This pathway operates alone in an *ime1* mutant that expresses *IME2-6* and results in a sporulation program with qualitative defects. In the second pathway, *IME1* product activates target genes independently of *IME2*. This pathway operates alone in an *ime2* deletion mutant and cannot lead to spore formation on its own, but does stimulate genetic recombination (18; A. P. Mitchell, unpublished results). Both pathways are required together for the efficient sporulation of wild-type cells.

Our results give no indication of how direct a role the *IME1* and *IME2* products play in sporulation-specific gene expression. Strich et al. have identified several negative regulators of *SPO11* and *SPO13*: the *UME* products (19). Their epistasis experiments suggest that *IME1* and *IME2* may activate *SPO11* and *SPO13* indirectly, through relieving repression by one or several *UME* products, or that *IME* and *UME* products act independently (19). Although the *IME1* and *IME2* products have similar physiological roles, we point out that their mechanisms of action may be quite different.

Our model leads us to ask why the cell uses two pathways to activate one set of genes. One possibility is that the *IME1* and *IME2* products play different roles in the kinetics of induction. *IME1*, which is expressed first, may initiate sporulation-specific gene expression, whereas *IME2* may amplify expression. Given that neither product alone promotes efficient sporulation, this model stipulates that proper expression kinetics are critical for execution of meiosis. A second possibility is that each pathway activates certain unique target genes that the other pathway cannot. For example, the *IME2* pathway may activate a negative regulator of *IME1* expression; thus, *ime2* mutants display prolonged accumulation of *IME1* RNA. Similarly, the *IME2*-independent pathway may permit the expression of a protein involved in the fidelity of chromosome segregation; thus, *ime1-12 IME2-6* homozygotes produce inviable spores and nonmating spores. This model may explain why so many sporulation-specific genes turn out to be dispensable for sporulation (8, 10, 13, 15, 16); perhaps they lie in one pathway and have functional counterparts in the other. Multiple activation pathways may exist in other cellular differentiation programs, such as myogenesis, for which several activators of the differentiation program have also been discovered (3, 5, 17, 24).

In addition to its effects on *SPO11* and *SPO13* induction, the *ime2* deletion causes a delay in the shutoff of *IME1*, *SPO11*, and *SPO13* RNAs. Parallel behavior of all three RNAs may reflect a common, *IME2*-dependent mechanism in the down regulation of early sporulation-specific genes. An alternative explanation is that the *IME2* product is required only for shutoff of *IME1*: high *IME1* product levels in the *ime2* mutant may mask down regulation of the other genes by maintaining elevated transcription rates. (This latter possibility is diagrammed in Fig. 5.) We find it intriguing that induction of *SPS1* and *SPS2* is delayed in the *ime2* mutant until the earlier genes are about to be shut off. Perhaps induction of later genes is coupled with down regulation of early genes. Regardless of mechanism, the prolonged duration of early sporulation-specific gene expression may make *ime2* mutants an ideal biochemical source for early meiotic proteins and protein complexes.

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