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Stimulation of Later Functions of the Yeast Meiotic Protein Kinase Ime2p by the *IDS2* Gene Product

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Ime2p is a protein kinase that is expressed only during meiosis in *Saccharomyces cerevisiae*. Ime2p stimulates early, middle, and late meiotic gene expression and down-regulates expression of *IME1*, which specifies an activator of early meiotic genes that acts independently of Ime2p. We have identified a new gene, *IDS2* (for IME2-dependent signaling), which has a functional relationship to Ime2p. An *ids2* null mutation delays down-regulation of *IME1* and expression of middle and late meiotic genes. In an *ime1* null mutant that expresses *IME2* from the *GAL1* promoter (*ime1*Δ P_{GAL1}-IME2 mutant), early meiotic gene expression depends only upon Ime2p. In such strains, Ids2p is dispensable for expression of the early genes *HOP1* and *SPO13* but is essential for expression of the middle and late genes *SPS1*, *SPS2*, and *SPS100*. Ids2p is also essential for the autoregulatory pathway through which Ime2p activates its own expression via the *IME2* upstream activation sequences (UAS). An P_{GAL1}-*IME2* derivative that produces a truncated Ime2p (lacking its C-terminal 174 residues) permits *IME2* UAS activation in the absence of Ids2p. This observation suggests that Ids2p acts upstream of Ime2p or that Ids2p and Ime2p act in independent, convergent pathways to stimulate *IME2* UAS activity. Accumulation of epitope-tagged Ids2p derivatives is greatest in growing cells and declines during meiosis. We propose that Ids2p acts indirectly to modify Ime2p activity, thus permitting Ime2p to carry out later meiotic functions.

The yeast sporulation program leads through meiosis to spore formation. The sequence of events includes DNA synthesis, recombination, meiosis I and II divisions, and packaging of meiotic products into spores (reviewed in references 5 and 28). These events are accompanied by the successive activation of early, middle, and late classes of meiotic or sporulation-specific genes (reviewed in references 27 and 29). Many early genes are involved in meiotic chromosome metabolism, such as *HOP1*, which is required for chromosome pairing (15), and *SPO13*, which is required for meiosis I division (44). Several middle and late genes are involved in spore wall formation, such as *SPS1*, which encodes a protein kinase related to Ste20p (7), *SMK1*, which encodes a mitogen-activated protein kinase homolog (24), and *SPS100* (25). The signals and gene products that coordinate expression of temporal classes of meiotic genes are not well understood.

Both genetic and environmental signals are required for sporulation. The genetic signal, presence of the *MAT***a***1* and *MAT*α*2* products, establishes the **a**/α cell type and generally indicates that cells are diploid (reviewed in reference 13). The environmental signal, starvation in the absence of a fermentable carbon source, likely indicates that continued growth is not possible. These signals converge to stimulate expression of *IME1* (21). The *IME1* product (Ime1p) is a positive regulator of many or all early meiotic genes (4, 31, 40). Ime1p is thought to act as a positive transcription factor (1, 29, 38).

Among the genes activated by Ime1p is *IME2*, which specifies a serine-threonine protein kinase (39, 46). Present evidence suggests that Ime2p has three roles in meiosis. (i) Ime2p is a positive regulator of early meiotic genes. *ime2* deletion mutants accumulate early meiotic transcripts slowly compared with isogenic wild-type strains. In addition, expression of Ime2p from an Ime1p-independent promoter permits expression of most early meiotic genes in an *ime1* deletion mutant (31, 34). Thus, Ime1p and Ime2p have partially overlapping functional roles, because they activate expression of many of the same genes. *IME2* itself is an early meiotic gene, and Ime2p activates expression from the *IME2* promoter (1). (ii) Ime2p is a positive regulator of middle meiotic gene expression. *ime2* deletion mutants are defective in expression of middle genes, and the presence of Ime2p can activate middle genes in an *ime1* deletion mutant (31). (iii) Ime2p is a negative regulator of *IME1*. *IME1* RNA is normally down-regulated at 6 h after starvation, but *ime2* mutants continue to express high levels of *IME1* RNA until 20 to 30 h (39). The relationship between these roles is unclear: they may all be indirect consequences of a single function, or they may each represent distinct functions of Ime2p. We describe here a new gene, *IDS2* (IME2-dependent signaling), that may function in an Ime2p signal transduction pathway or in a parallel pathway that converges with an Ime2p-dependent pathway. Our findings have implications with regard to interdependence of Ime2p functions and coordination of meiotic gene expression.

MATERIALS AND METHODS

Strains and media. Yeast strains are derivatives of SK-1 (20) and are listed in Table 1. Mutations have been described previously (2, 30) except for *ime1*Δ*20*, P_{GAL1}-*IME2-8*::*TRP1*, P_{GAL1}-*IME2-9*, and *ids* mutations. Genetic methods were standard (19). **a**/α diploids homozygous for all other markers were occasionally constructed by transformation of haploid strains with an *HO* plasmid (12).

The *ids2-1* mutation was monitored in crosses by its suppression of P_{GAL1}-*IME2* toxicity: P_{GAL1}-*IME2 IDS2* strains survive poorly after incubation on sporulation plates for 4 days, whereas P_{GAL1}-*IME2 ids2-1* strains survive well. The presence of *ids2-1* in strains lacking a P_{GAL1}-*IME2* allele was verified through meiotic analysis of crosses to P_{GAL1}-*IME2* strains.

Growth media and conditions have been described elsewhere (19, 30, 39). KAcXgal indicator plates were minimal sporulation plates buffered with 0.2 M KPO_4 at pH 7 and containing 50 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per liter (42). All experiments were conducted at 30°C.

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TABLE 1. Yeast strains used

Strain	Genotype[a]
AMP179	**a**/α
AMP474	α *his4 ade3*
AMP1212	α *ime2Δ2::LEU2 his4 ade3*
AMP1435	**a** *ime1Δ20* P_{GAL1}*-IME2-8::TRP1 rad52::LEU2 his1*
AMP1436	α *ime1Δ12::TRP1 ime2Δ2::LEU2 arg6*
AMP1437	α *ime1Δ20* P_{GAL1}*-IME2-8::TRP1 arg6*
AMP1438	**a** *ime1Δ20* P_{GAL1}*-IME2-8::TRP1 his1*
AMP1439	α P_{GAL1}*-IME2-9 his4*
AMP1440	**a** *ime1Δ20* P_{GAL1}*-IME2-9 ids2-1 his4*
AMP1441	**a**/α *ime1Δ20/ime1Δ20* P_{GAL1}*-IME2-8::TRP1/*P_{GAL1}*-IME2-8::TRP1 ids2-3::URA3/ids2-3::URA3*
AMP1442	**a**/α *ime1Δ20/ime1Δ20* P_{GAL1}*-IME2-8::TRP1/*P_{GAL1}*-IME2-8::TRP1*
AMP1443	**a**/α *ids2-3::URA3/ids2-3::URA3*
AMP1444	**a**/α *ime1Δ20/ime1Δ20* P_{GAL1}*-IME2-9/*P_{GAL1}*-IME2-9 his1/his1 his4/his4*
AMP1445	**a**/α *ime1Δ20/ime1Δ20* P_{GAL1}*-IME2-9/*P_{GAL1}*-IME2-9 ids2-3::URA3/ids2-3::URA3*
AMP1446	**a**/α *ime1Δ20/ime1Δ20* P_{GAL1}*-IME2-9/*P_{GAL1}*-IME2-9 ids2-1/ids2-1*
AMP1447	**a**/α *ids2-1/ids2-1 his1/his1 his4/his4*
AMP1452	**a**/α *ime1Δ20/ime1Δ20 ime2-7-HIS3::LEU2/ime2-7-HIS3::LEU2 his3/his3 arg6/+*
AMP1453	**a**/α *ids2Δ4/ids2Δ4 ime1Δ20/ime1Δ20 ime2-7-HIS3::LEU2/ime2-7-HIS3::LEU2 his3/his3 arg6/+*
AMP1454	**a** *ime1Δ12::TRP1 ime2Δ2::LEU2*
AMP1456	**a**/α *ids2Δ4/ids2Δ4*

[a] All haploid strains carried additional markers *ura3*, *leu2*, *trp1*, *lys2*, *ho*::*LYS2*, and *gal80*::*LEU2*, and all diploid strains were homozygous for these markers.

Genomic integration of DNA was confirmed by Southern analysis or by PCR assays (19).

Plasmid constructions and genomic mutations. The *ime1Δ20* mutation is a replacement of the *IME1* coding region with nonfunctional segment of the *HIS3* gene. Plasmid pAM510 (32), a YIp5 derivative carrying *URA3* and the *IME1* 5′ region fused to the *HIS3* coding region, was digested with *Bam*HI and *Hin*dIII (to release most of *HIS3*) and ligated to a 1.25-kbp *Bam*HI-*Sca*I fragment of plasmid pAM504 (40) containing *IME1* 3′ sequences. The resulting plasmid, pHY10-1, was digested within *IME1* 5′ sequences with *Cla*I and transformed into an *ime1Δ12*::*TRP1* strain. A Ura^+ transformant was streaked on 5-fluoro-orotic acid medium and then screened for Trp^- segregants.

The *ids2-3*::*URA3* mutation was constructed by insertion of a *Sal*I fragment carrying *URA3* into the *Sal*I site of *IDS2* in plasmid pREY150 to create plasmid pREY157 (Fig. 1). An *Eag*I-*Cla*I fragment from pREY157 was used to transform *ura3* recipients to uracil prototrophy.

The *ids2Δ4* mutation is a deletion of codons 70 to 334. A 2.2-kbp *Eag*I-*Hin*dIII fragment from plasmid pREY146 (Fig. 1) was inserted between the *Eag*I and *Hin*dIII sites of plasmid YIp5 to yield plasmid pREY160. pREY160 was digested with *Sal*I and *Bgl*II, and then the ends were filled in and ligated, to create pREY161. Plasmid pREY161 was digested with *Kpn*I to target integration to the *IDS2* locus. Segregants from 5-fluoro-orotic acid medium carrying the *ids2Δ4* deletion were identified by Southern analysis.

The P_{GAL1}*-IME2-8*::*TRP1* allele was derived from plasmid pREY125. First, plasmid pREY124 was constructed by ligation of a 614-bp *Xho*I-*Bgl*II fragment containing *IME2* nucleotides −883 to −269 and an 850-bp *Bam*HI-*Eco*RI fragment containing the *TRP1* gene into plasmid pBSIISK+ (Stratagene) digested with *Xho*I and *Eco*RI. A P_{GAL1}*-IME2* hybrid gene, in plasmid pSS33, was constructed by ligation of a 3.4-kbp *Hin*dIII-*Xho*I fragment from plasmid pAM400 (39), containing the *IME2* coding region (46), into *GAL1,10* promoter plasmid pBM272 (17, 40) digested with *Hin*dIII and *Sal*I. A 2.1-kbp *Eco*RI fragment from pSS33 containing P_{GAL1}*-IME2* sequences was inserted into the pREY124 *Eco*RI site to make plasmid pREY125. An *Xho*I-*Spe*I fragment from pREY125 containing *IME2* 5′ sequences, the *TRP1* gene, the *GAL1,10* promoter, and the *IME2* coding region was used to replace the chromosomal *IME2* gene.

The P_{GAL1}*-IME2-9* allele, which has no accompanying nutritional marker, was derived from plasmid pREY132. First, a 1.0-kbp *Hin*dIII-*Xho*I fragment containing *IME2* 5′ noncoding sequences was ligated into plasmid YIp5 digested with *Hin*dIII and *Sal*I to create plasmid pREY131. A 2.1-kbp *Eco*RI fragment from pSS33 containing P_{GAL1}*-IME2* sequences was inserted into the pREY131 *Eco*RI site to create pREY132. Plasmid pREY132 was digested within the *IME2* coding region with *Bam*HI and used to transform a P_{GAL1}*-IME2-8*::*TRP1* strain to Ura^+. Purified transformants were streaked on 5-fluoro-orotic acid medium and then screened for a Trp^- phenotype.

Plasmid pREY140 is our initial *IDS2* clone. Plasmids pREY140ΔSac, pREY140ΔXba, pREY140ΔCla, and pREY140ΔSal were constructed by digestion with *Sac*I, *Xba*I, *Cla*I, and *Sal*I, respectively, and ligation. Plasmids pREY141 and pREY142 were pRS316 derivatives (37) carrying the 6.0-kbp *Xba*I fragment from pREY140. Plasmids pREY143 and pREY144 were pRS316 derivatives carrying the 4.0-kbp *Xba*I fragment from pREY140. Plasmids pREY146 and pREY147 were pRS316 derivatives carrying the 6.0-kb *Eag*I fragment from pREY140. Plasmid pREY150 was constructed by deleting a 600-bp *Cla*I fragment from pREY141. Plasmid pREY151 was constructed by deleting a 1.0-kbp *Kpn*I fragment from pREY142.

Plasmid pREY138 carries an *IME2-CYC1-lacZ* reporter gene (with *IME2* nucleotides −584 to −443) and a *TRP1* selectable marker. It was constructed by replacing a 1.0-kbp *Stu*I fragment containing *URA3* sequences from plasmid pKB100 (1) with a *Bam*HI-*Bgl*II fragment containing the *TRP1* gene.

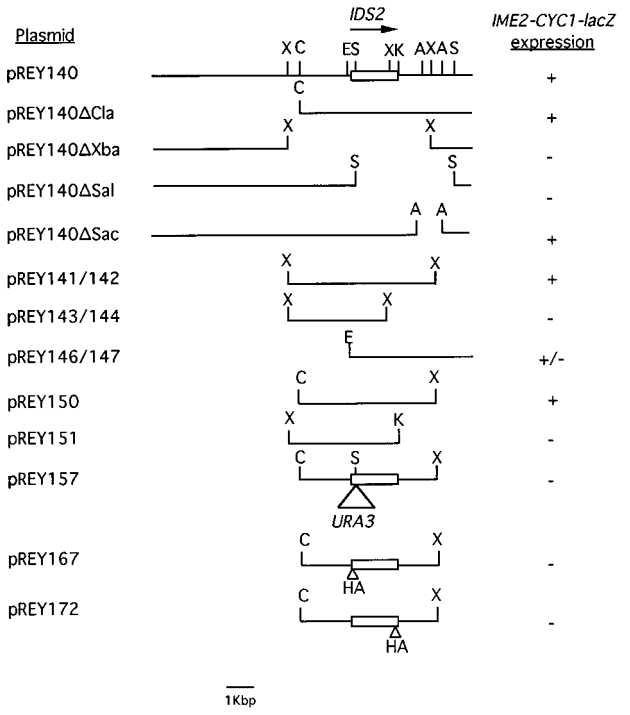

FIG. 1. *IDS2* plasmids and derivatives. Each bar represents the DNA insert in the plasmids designated. Pairs of plasmids on the same line (pREY141/142, pREY143/144, and pREY146/147) differ by orientation of the insert. The *IDS2* coding region is represented by the rectangle on the top line. *ids2-1* complementation was assessed by *IME2-CYC1-lacZ* expression in strain AMP1440 carrying pREY138: +, complementation; −, no complementation; +/−, orientation-dependent complementation. Restriction site abbreviations: A, *Sca*I; C, *Cla*I; E, *Eag*I; K, *Kpn*I; S, *Sal*I; X, *Xba*I.

Plasmids pHS256, pHS263, and pHS264 are all YCp50-based plasmids carrying hemagglutinin (HA) epitope-tagged (6) P_{GAL1}-*IME2* derivatives. A *Not*I fragment encoding a triple HA epitope (43) was inserted into a *Not*I site introduced 3′ to the *IME2* AUG codon in P_{GAL1}-*IME2* plasmid pSS33 to create plasmid pHS257. A derivative carrying an arginine substitution for lysine 97, plasmid pHS264, was created through oligonucleotide mutagenesis. A derivative carrying a nonsense codon at *IME2* codon 472, plasmid pHS263, was created by inserting an *Nhe*I linker (CTAGCTAGCTAG) into a filled-in *Eco*RI site within the *IME2* coding region in pHS257.

Plasmids pREY167 and pREY172 are YCp50-based plasmids carrying HA epitope-tagged (6) *IDS2* derivatives. They carry triple epitope insertions (43) into *Not*I sites between codons 3 and 4 (pREY167) or codons 441 and 442 (pREY172). *Not*I sites were introduced at these locations by insertion of nucleotides GGCGGCCGC through primer mutagenesis of plasmid pREY142 (Fig. 1), and then 6-kbp *Cla*I-*Bam*HI inserts were transferred into *Cla*I- and *Bam*HI-digested plasmid YCp50 to create pREY164 (codon 3 site) and pREY171 (codon 441 site). A ~300-bp *Not*I fragment specifying three HA epitopes was inserted from plasmid pGTEP (43) into each plasmid's *Not*I site. Presence of the insert and proper orientation were determined by sequencing. Both *IDS2* plasmids carrying the *Not*I site insertions were functional, and both *HA-IDS2* plasmids were nonfunctional, as determined by *ids2-1* complementation tests.

Isolation of P_{GAL1}-*IME2* toxicity suppressors. Single colonies of strain AMP1435 (**a** *ime1*Δ*20* P_{GAL1}-*IME2*) carrying *IME2-CYC1-lacZ* plasmid pKB102 (1) were grown as patches on a YPD plate overnight and then replicated to sporulation plates and incubated for 4 days. The sporulation plates were replica plated to YPD and incubated for 1 to 2 days. One papilla per patch was picked to a fresh YPD master plate for retesting. Survivors with rho$^-$ or Gal$^-$ defects were eliminated through growth tests as described previously (30). Mutants with defects in the P_{GAL1}-*IME2* gene were identified by their failure to complement *ime2*Δ mutants for sporulation (in crosses to strain AMP1212) or for *IME2-CYC1-lacZ* expression (in crosses to strain AMP1436 carrying reporter plasmid pKB102). (Although the selection began with a strain carrying plasmid pKB102, the mutants had lost the plasmid during nonselective growth.) Since each papilla derived from an individual single colony, all mutants from this selection were independent.

Cloning of *IDS2*. Strain AMP1440 (*ime1*Δ*20 ids2-1* P_{GAL1}-*IME2*) carrying *IME2-CYC1-lacZ* reporter plasmid pREY138 was transformed with a *URA3-CEN* plasmid library (35). Eight thousand Ura$^+$ transformants were replica plated to SC-Ura containing 0.5% glucose and, after overnight growth, to KAcXgal plates. Colonies that turned blue on the KAcXgal plates (which might arise through complementation of *ime1* or *ids2*) were tested for failure to survive after incubation on sporulation plates. Plasmids were retrieved in *Escherichia coli* and grouped through restriction digestion. The clones were then distinguished by map location through hybridization to a grid of ordered genomic lambda clones (American Type Culture Collection). We identified 12 *IME1* clones, which hybridized to lambda clones 70409 and 70616, and one *IDS2* clone, which hybridized to lambda clones 70547, 70374, and 70245. These clones derive from the left arm of chromosome X, approximately 70 kbp from the *IME2* locus (lambda clones 70334 and 70442). The *IME1* clones lacked 5′ sequences and were thus expressed in non-**a**/α cells (3, 9).

Immunological methods. Antibodies to an Ime2p fragment purified from *E. coli* were produced in rabbits. An internal 668-bp *Eco*RV fragment of *IME2* was ligated into the filled-in *Bam*HI site of plasmid pAR3039 (41). The resulting plasmid expresses 222 codons of *IME2* and 11 5′ codons of phage T7 gene *10*. Expression of the protein, purification, and antibody production were as described previously (30).

Crude extracts (2) were made from cells grown under conditions specified in the figure legends. One hundred fifty micrograms of extract protein was fractionated on an 8% polyacrylamide–sodium dodecyl sulfate gel and transferred to Immobilon-P. Immunoblots were developed with rabbit anti-Ime2p antiserum and goat anti-rabbit antiserum conjugated to horseradish peroxidase or with mouse 12CA5 (anti-HA) ascites fluid (6) and goat anti-mouse antiserum conjugated to horseradish peroxidase (11).

β-Galactosidase assays. Quantitative β-galactosidase assays were carried out as described previously (40). Cells were shifted from exponential growth in YPAc to sporulation medium for 13 to 16 h. This choice of incubation time was based on a time course of expression of *IME2-CYC1-lacZ* plasmid pKB100 (1) in an *ime1*ΔP_{GAL1}-*IME2* strain: peak expression was detected between 12 and 16 h (36). Expression depended upon Ime2p because it was reduced over 100-fold in an *ime1*Δ *ime2*Δ strain.

Miscellaneous methods. The *IDS2* nucleotide sequence was determined for both strands of the insert in plasmid pREY141 (Fig. 1) by dideoxy sequencing, using nested deletions and oligonucleotide primers. The data were analyzed with Intelligenetics Geneworks 2.3 software on a Power Macintosh 6100 computer. Homology searches were conducted through the NCBI BLAST electronic mail server.

Methods for preparation of RNA and Northern (RNA) blots have been described elsewhere (39). Probes for meiotic transcripts were the following: for *IME1*, m13mp18 derivative mpHS106 (39); for *IME2*, m13mp19 derivative mp19BE200 (39); for *SPO13*, a 1.1-kbp *Eco*RI-*Pst*I fragment from plasmid pSW1 (provided by M. Slater and R. E. Esposito); for *HOP1*, a 1-kbp *Bam*HI-*Sac*I fragment from plasmid pNH50-1 (15); for *SPS1* and *SPS2*, a 3-kbp *Cla*I fragment from plasmid p18 (33); and for *SPS100*, a 750-bp *Nco*I-*Bam*HI fragment from plasmid pE18-B8a (25). The control probe for RNA loading was plasmid pC4/2 (25, 42).

Nucleotide sequence accession number. The *IDS2* sequence has been deposited in GenBank under accession number U21326.

RESULTS

Characterization of suppressors of Ime2p toxicity. We set out to isolate mutations that block Ime2p biological activity through a selection for relief of Ime2p toxicity. Expression of *IME2* from the *GAL1* promoter caused haploid cells to lose viability in sporulation medium, and loss of viability was more extreme in *rad52* mutants (data not shown). Inviability may result from initiation of meiosis by haploid cells (16, 30). We isolated 392 independent survivors from a P_{GAL1}-*IME2 rad52* strain after incubation on sporulation medium. We eliminated false positives and trivial mutants, including 142 Gal$^-$ mutants, 46 petite mutants, and 117 mutants with defects in the P_{GAL1}-*IME2* gene (see Materials and Methods). Twenty remaining mutants were tested for a defect in Ime2p-dependent gene expression. We made use of an *IME2-CYC1-lacZ* reporter plasmid (pKB102), in which a 142-bp fragment from the *IME2* 5′ region serves as an upstream activation sequence (UAS) (1). Activation of this reporter gene in P_{GAL1}-*IME2* strains is an assay of the autoregulatory pathway through which Ime2p activates its own expression (1). Ten of the mutants displayed a reduction in *IME2-CYC1-lacZ* reporter expression, as determined by KAcXgal plate tests.

To establish that suppressors of P_{GAL1}-*IME2* toxicity were single-gene traits, we crossed three mutants to a P_{GAL1}-*IME2* strain (AMP1439) and analyzed meiotic tetrads. The three suppressors displayed 2:2 segregation in at least 15 of 16 tetrads. To establish that the mutations did not lie in the P_{GAL1}-*IME2* gene, we crossed the same three mutants to an *IME2* strain (AMP474) and analyzed meiotic tetrads. In each case, at least 7 of 15 tetrads were tetratypes in which toxicity of P_{GAL1}-*IME2* was apparent in one segregant. To determine whether the defect in *IME2-CYC1-lacZ* reporter expression cosegregated with the suppressor, we transformed two suppressor-bearing P_{GAL1}-*IME2* meiotic segregants with an *IME2-CYC1-lacZ* reporter plasmid (pKB102) and scored its expression through KAcXgal plate tests. For each of two mutants (representing each complementation group, as determined below), the segregants were defective in *IME2-CYC1-lacZ* reporter expression. We conclude that the lesions in these three mutants lie at single genetic loci. We designate these suppressors *ids* mutations.

Dominance and complementation were assessed through the *IME2-CYC1-lacZ* expression defect, as monitored on KAcXgal plates. The 10 *ids* mutations were recessive: diploids from crosses of each mutant to an *ime1*Δ P_{GAL1}-*IME2 IDS* strain expressed the *IME2-CYC1-lacZ* reporter at high levels. Matings of meiotic *ids* mutant segregants to the collection of *ids* mutants defined two complementation groups: *ids1*, with eight members, and *ids2*, with two members. Linkage analysis confirmed that different genetic loci were affected by these mutations (data not shown). Thus, the *ids* mutations lie in two genes.

We detected genetic linkage of *IDS2* to the *IME2* and *TPK1* loci, which lie on chromosome X. From crosses of the form *ids2-1* P_{GAL1}-*IME2-8*::*TRP1* × *IDS2* P_{GAL1}-*IME2-9*, we found 22 parental ditype, 0 nonparental ditype, and 13 tetratype tetrads for *IDS2* and *IME2*. From crosses of the form *TPK1 ids2-1* P_{GAL1}-*IME2-8*::*TRP1* × *TPK1*::*URA3 IDS2* P_{GAL1}-*IME2-9*, we found 16 parental ditype, 0 nonparental ditype, and 2 tetratype tetrads for *TPK1* and *IDS2*. Analysis of indi-

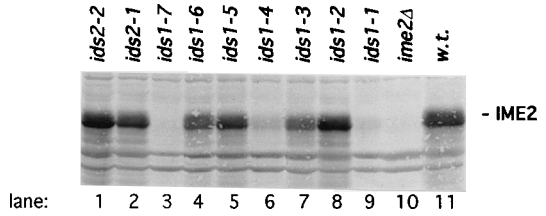

FIG. 2. Ime2p levels in *ids* mutants. Strains were grown to log phase in YPAc, and Ime2p was visualized on an immunoblot. Lanes 1 and 2, *ime1*Δ P_{GAL1}-*IME2 ids2* mutants; lanes 3 to 9, *ime1*Δ P_{GAL1}-*IME2 ids1* mutants; lane 10, *ime1*Δ *ime2*Δ control (wild-type [w.t.]) strain AMP1454; lane 11, *ime1*Δ P_{GAL1}-*IME2* strain AMP1435 (parent of the *ids* mutants).

vidual recombinant tetrads was most consistent with the map order *TPK1-IDS2-IME2.*

Ime2p levels in *ids* mutants. Among mutants defective in Ime2p-dependent gene expression, we expected that some may fail to accumulate the *IME2* protein product. Ime2p accumulation was assayed on immunoblots. Ime2p was identified as an 84-kDa protein, as first reported by Kominami et al. (23), on the basis of three lines of evidence. First, the protein is detected in a P_{GAL1}-*IME2* extract and not in an *ime2*Δ extract (Fig. 2, lanes 11 and 10, respectively). Second, the protein reacted with immune serum, not preimmune serum (data not shown). Third, mobility of the immunologically reactive protein was altered in strains expressing an Ime2p–β-galactosidase fusion protein (data not shown). The two *ids2* mutants had Ime2p levels comparable to that of the parent strain (Fig. 2; compare lanes 1 and 2 with lane 11). Five of the *ids1* mutants also had high Ime2p levels (including one not shown), whereas three of the *ids1* mutants had low Ime2p levels. These findings suggest that some *ids1* mutations may relieve Ime2p toxicity through a reduction in P_{GAL1}-*IME2* expression. On the other hand, the two *ids2* mutations do not affect P_{GAL1}-*IME2* expression and may affect Ime2p activity or the response to that activity.

Characterization of the *IDS* gene. We cloned *IDS2* from a genomic *CEN* plasmid library by complementation (see Materials and Methods). Identity of *IDS2* was indicated by its hybridization to DNA from the *IME2-TPK1* region of chromosome X (see Materials and Methods) and by studies of *ids2* mutants described below.

We determined the *IDS2* nucleotide sequence from the insert in plasmid pREY141 (Fig. 1). The sequence included a single large open reading frame that specified a 470-amino-acid polypeptide (Fig. 3). The polypeptide does not have extensive homology to any protein in current databases. It has a possible nuclear localization signal at residues 98 to 100 (8). We infer that this open reading frame is the *IDS2* coding region because it is included in all functional subclones (Fig. 1). In addition, it is adjacent to one end of the pREY146/147 insert, which displays orientation-dependent complementation and is presumably expressed from an adventitious promoter in plasmid sequences (Fig. 1). Finally, *IDS2* complementation activity is abolished by insertions at both ends of the open reading frame (after codon 3 or 441; Fig. 1).

Possible regulatory sites that govern sporulation-specific gene expression (summarized in reference 29) were identified in the *IDS2* 5′ flanking region (Fig. 3). These include sequences that resemble URS1 sites (at nucleotides −372 and −311), a UAS_H site (−166), and T_4C sites (−522 and −353). Both of the URS1 homology regions had deviations within the CGGCGG core nucleotides. No matches to the negative regulatory element found at later sporulation-specific genes (22) were identified. Whether these sites have a role in *IDS2* expression is uncertain.

Role of *IDS2* in sporulation. To determine the function of Ids2p, we created two *ids2* mutations: *ids2-3*::*URA3*, an insertion of the *URA3* gene at *IDS2* codon 70, and *ids2*Δ*4*, a deletion of *IDS2* codons 70 to 334. The *ids2-3*::*URA3* insertion abolished *IDS2* function in plasmid complementation tests (Fig. 1; compare pREY150 and pREY157). Haploid *ids2-3*::*URA3* and *ids2*Δ*4* strains displayed no obvious growth defects.

We tested the effects of *ids2* mutations on sporulation of diploids with wild-type *IME1* and *IME2* alleles (Table 2). The *IDS*/*IDS* control diploid sporulated efficiently. The *ids2-1*/*ids2-1* diploid displayed a 4-fold sporulation defect, and the *ids2-3*::*URA3*/*ids2-3*::*URA3* and *ids2*Δ*4*/*ids2*Δ*4* diploids displayed a 1.5-fold sporulation defect. Spore viability was high (>95%) from homozygous *ids2* mutant diploids (data not shown). These observations indicate that Ids2p is not essential for sporulation, though it improves sporulation efficiency of wild-type strains.

We examined expression of several meiotic genes on Northern blots as a more refined test of *IDS2* gene function. We monitored transcript accumulation from the early genes *IME1*, *IME2*, *HOP1*, and *SPO13*, the middle genes *SPS1* and *SPS2*, and the late gene *SPS100* (Fig. 4A). In the control wild-type diploid (lanes 1 to 8), early transcript levels increased at 2 to 4 h and declined by 6 to 8 h; middle transcript levels increased at 6 to 8 h; late transcript levels increased at 10 to 12 h. In a control *ime2*Δ/*ime2*Δ diploid (lanes 9 to 17), early transcript levels increased to approximately the same extent as for the wild-type diploid but had not declined by 14 h; middle transcript were undetectable until 14 h; the late transcript was not detected. *IME1* RNA accumulation was elevated compared with the peak wild-type levels. In the *ids2-3*::*URA3*/*ids2-3*::*URA3* diploid (lanes 18 to 26), early transcript levels increased to wild-type levels but declined after 10 to 12 h; middle transcript levels increased at 10 to 12 h; the late transcript was undetectable until after 14 h. *IME1* RNA accumulation was also elevated compared with the peak wild-type levels. An *ids2-1*/*ids2-1* diploid displayed a similar transcript accumulation pattern (though the late transcript was not tested; data not shown). These results indicate that *ids2* mutations cause a delay in the transition between early and middle gene expression. The *ids2-3*::*URA3* phenotype is similar to, but less severe than, the *ime2*Δ phenotype.

Sporulation and meiotic gene expression were also determined for *ime1*Δ/*ime1*Δ P_{GAL1}-*IME2*/P_{GAL1}-*IME2* diploids. In such strains, Ime2p is essential for expression of all meiotic genes (29, 31). An *IDS*/*IDS* diploid yielded 21% sporulation; *ids2-1*/*ids2-1*, *ids2-3*::*URA3*/*ids2-3*::*URA3*, and *ids2*Δ*4*/*ids2*Δ*2* diploids failed to sporulate (Table 2), even after prolonged incubation. All three *ids2* mutations caused a severe defect in *IME2* UAS activation as well (Tables 3 and 4). Northern blot analysis (Fig. 4B) indicated that the early *HOP1* and *SPO13* transcripts accumulated to comparable levels in *IDS2*/*IDS2* and *ids2-3*::*URA3*/*ids2-3*::*URA3* diploids. Both early transcripts were down-regulated at 4 to 8 h in the *IDS2*/*IDS2* diploid but not in the *ids2-3*::*URA3*/*ids2-3*::*URA3* diploid. The middle *SPS1* and *SPS2* transcripts were detectable from 4 to 12 h in the *IDS2*/*IDS2* diploid but were undetectable in the *ids2-3*::*URA3*/*ids2-3*::*URA3* diploid. The late *SPS100* transcript was detectable at 12 h in the *IDS2*/*IDS2* diploid but not in the *ids2-3*::*URA3*/*ids2-3*::*URA3* diploid. An *ime1*Δ/*ime1*Δ P_{GAL1}-*IME2*/P_{GAL1}-*IME2 ids2-1*/*ids2-1* diploid had a similar transcript accumulation defect (data not shown). The *ids2*::*URA3* defect did not result from reduced P_{GAL1}-*IME2* expression;

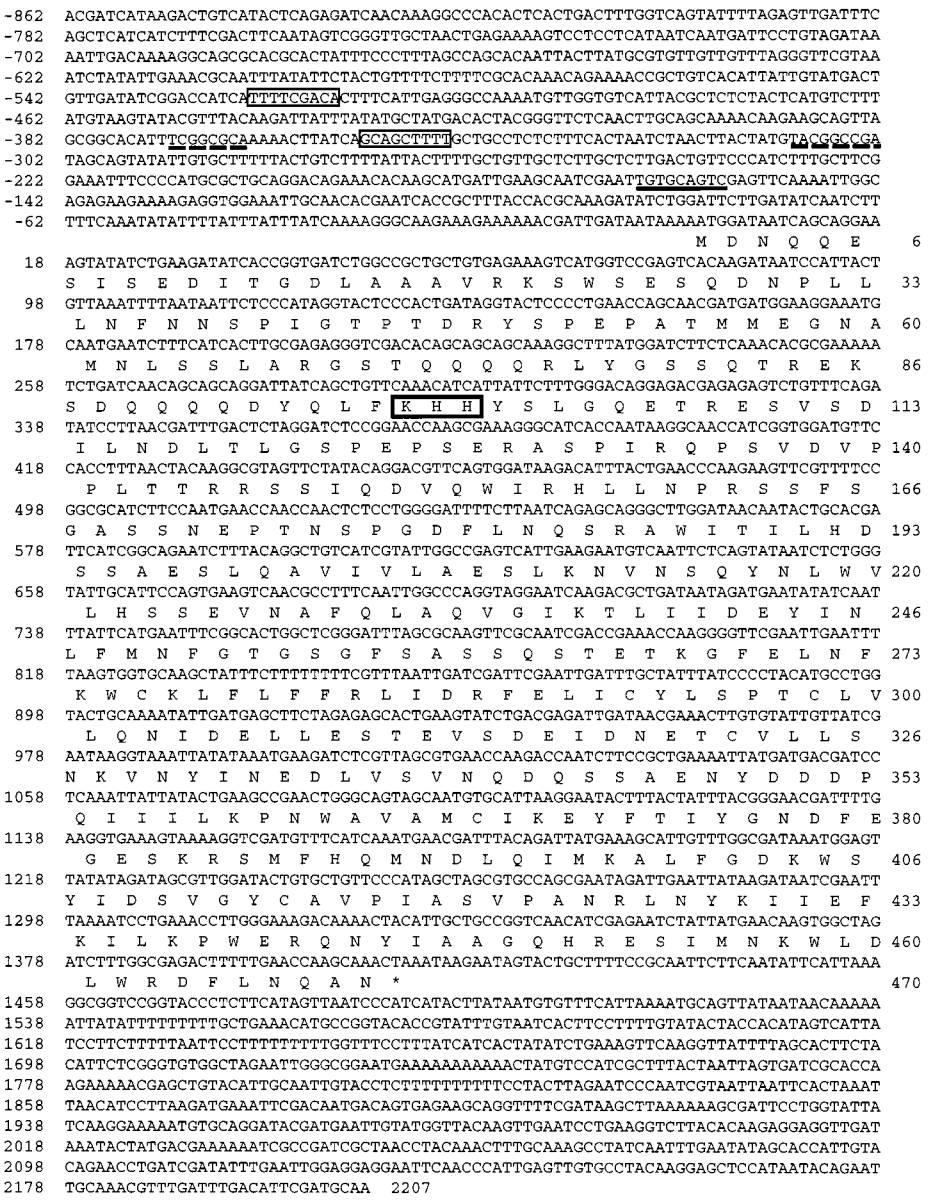

FIG. 3. Deduced sequence of *IDS2*. A possible nuclear localization signal is boxed (amino acid residues 98 to 100). DNA sequences homologous to URS1 (TCGGCGGCT; dashed underline), UAS_H (TGTGAAGTG; solid underline), and T_4C (TTTTCXXCG; boxed) sites are also indicated.

P_{GAL1}-IME2 was expressed for an extended period in the *ids2* mutant (Fig. 4B). (We note that down-regulation of the *GAL* promoter and early meiotic genes occurs at the same time during meiosis [18, 36].) We conclude that Ids2p is dispensable for Ime2p to stimulate expression of *HOP1* and *SPO13*. However, Ids2p is required for Ime2p to stimulate expression of *SPS1*, *SPS2*, and *SPS100*, activity of the *IME2* UAS, and sporulation in the *ime1*Δ *P_{GAL1}-IME2* background.

Partial suppression of an *ids2* defect by an altered form of Ime2p. We considered three simple relationships between Ime2p and Ids2p: Ids2p may act downstream of Ime2p, Ime2p may act downstream of Ids2p, or Ime2p and Ids2p may act in independent, convergent pathways. The first model predicts that overexpression or hyperactivity of Ids2p may bypass the need for Ime2p; the second model predicts that overexpression or hyperactivity of Ime2p may bypass the need for Ids2p; the

TABLE 2. Effects of *ids2* mutations on sporulation ability

Strain	Genotype[a]	% Sporulation[b]
AMP179	*IME1 IME2 IDS2*	93
AMP1447	*IME1 IME2 ids2-1*	23
AMP1443	*IME1 IME2 ids2-3::URA3*	61
AMP1456	*IME1 IME2 ids2Δ4*	72
AMP1442	*ime1*Δ *P_{GAL1}-IME2 IDS2*	21
AMP1446	*ime1*Δ *P_{GAL1}-IME2 ids2-1*	<0.1
AMP1441	*ime1*Δ *P_{GAL1}-IME2 ids2-3::URA3*	<0.1
AMP1452	*ime1*Δ *ime2*Δ *IDS2* [YCp-*P_{GAL1}-IME2*]	22
AMP1453	*ime1*Δ *ime2*Δ *ids2Δ4* [YCp-*P_{GAL1}-IME2*]	<0.1

[a] Strains were **a**/α diploids, homozygous for the alleles indicated. Strains AMP1452 and AMP1453 carried *P_{GAL1}-IME2* plasmid pHS257.

[b] Sporulation was determined after incubation for 26 h (strains AMP179, AMP1447, AMP1456, and AMP1443) or 48 h (strains AMP1442, AMP1446, AMP1441, AMP1452, and AMP1453) in liquid sporulation medium. Values are the means of triplicate determinations; standard deviations were less than 10% of the mean.

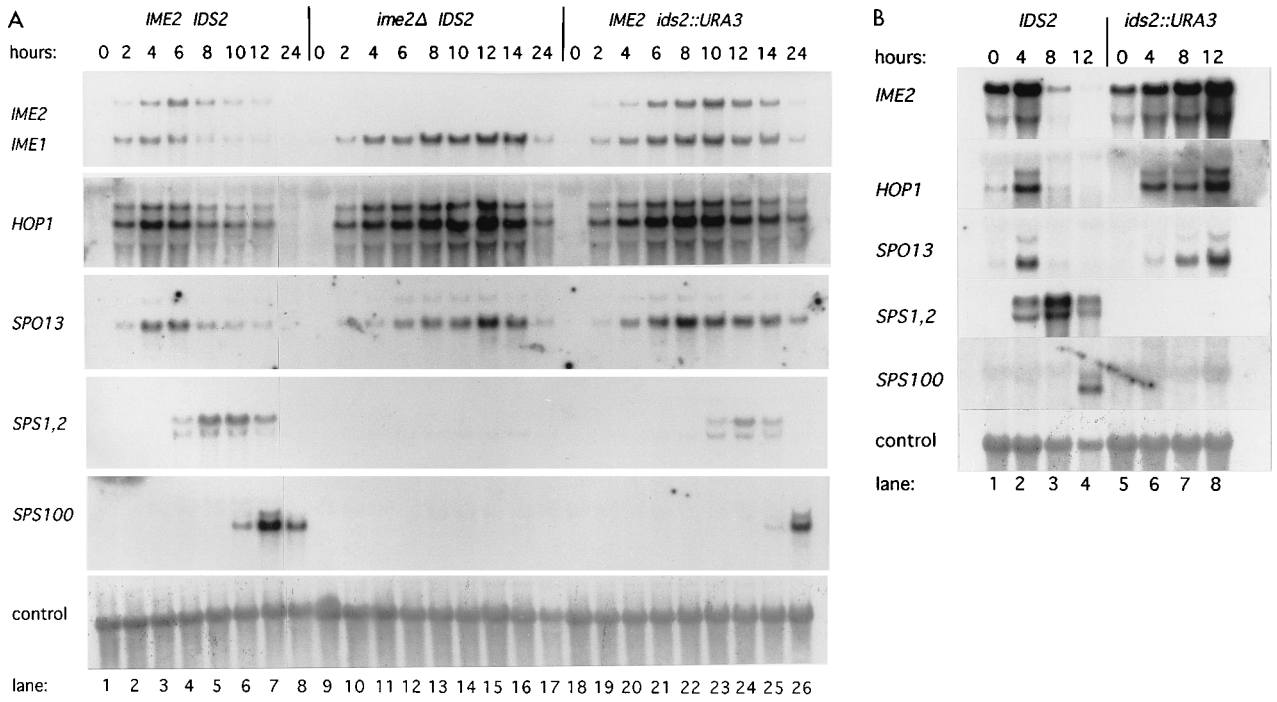

FIG. 4. Meiotic gene expression in *ids2* mutants. Cultures were grown to mid-exponential phase in YPAc and then transferred to sporulation medium. RNA was prepared from YPAc-grown cells (0 h) and from cells after the numbers of hours indicated in sporulation medium. Northern filters were probed for the indicated meiotic transcripts and with the control probe pC4/2. The exposure shown for each probe derives from the same film and Northern filter. (A) **a**/α *IME1*/*IME1* diploids. Strains: AMP179 (*IME2*/*IME2 IDS2*/*IDS2*; lanes 1 to 8); AMP245 (*ime2*Δ*2*/*ime2*Δ*2 IDS2*/*IDS2*; lanes 9 to 17); AMP1443 (*IME2*/*IME2 ids2-3*::*URA3*/*ids2-3*::*URA3*; lanes 18 to 26). (B) **a**/α *ime1*Δ/*ime1*Δ P_{GAL1}-*IME2*/P_{GAL1}-*IME2* diploids. Strains: AMP1442 (*IDS2*/*IDS2*; lanes 1 to 4); AMP1441 (*ids2-3*::*URA3*/*ids2-3*::*URA3*; lanes 5 to 8).

third model can accommodate either prediction. Preliminary experiments lent no support to the first model: we did not observe suppression of *ime2*Δ or temperature-sensitive *ime2* alleles by a high-copy *IDS2* plasmid (36). To test the second model, we made use of the report by Kominami et al. that some Ime2p C-terminal deletion derivatives have elevated activity (23). We created a truncated *IME2* derivative, P_{GAL1}-*IME2-472*, that has a nonsense codon at codon 472. We also constructed a negative control, P_{GAL1}-*ime2-K97R*, with a substitution for the lysine residue conserved among protein kinases that stabilizes ATP binding (10). Immunoblots indicated that the P_{GAL1}-*IME2*, P_{GAL1}-*IME2-472*, and P_{GAL1}-*ime2-K97R* products accumulated to comparable levels (data not shown). We assayed *IME2* UAS activation by these Ime2p derivatives in both *IDS2* and *ids2*Δ*4* backgrounds (Table 4). Expression of P_{GAL1}-*IME2* permitted detectable *IME2* UAS activity only in the *IDS2* background, not in the *ids2*Δ*4* background. Expression of P_{GAL1}-*ime2-K97R* did not yield detectable *IME2* UAS activity in either background. Expression of P_{GAL1}-*IME2-472* permitted *IME2* UAS activity in both *IDS2* and *ids2*Δ*4* backgrounds, though UAS activity was reduced fourfold in the *ids2*Δ*4* mutant. The *IME2* UAS is activated less well by P_{GAL1}-*IME2-472* than by P_{GAL1}-*IME2* in the *IDS2* background. This observation suggests that Ime2-472p is not hyperactive, but that it is partially Ids2p independent. The bypass of Ids2p by Ime2-472p indicates that Ime2p acts downstream of or in parallel with Ids2p to activate the *IME2* UAS.

Expression of *IDS2*. To determine the pattern of *IDS2* expression, we created two *IDS2* derivatives specifying HA epitope-tagged Ids2p products. One had an N-terminal epitope (inserted between codons 3 and 4); the other had a C-terminal epitope (inserted between codons 441 and 442). Both epitope insertions abolished *IDS2* complementation activity (Fig. 1, plasmids pREY167 and pREY172). We determined the levels of HA-Ids2p accumulation in sporulating cells on immunoblots

TABLE 3. Effects of *ids2* mutations on *IME2* UAS activation

Strain	Genotype[a]	*IME2-CYC1-lacZ* expression[b]
AMP1444	*ime1*Δ P_{GAL1}-*IME2 IDS2*	42 ± 5
AMP1446	*ime1*Δ P_{GAL1}-*IME2 ids2-1*	<0.4
AMP1445	*ime1*Δ P_{GAL1}-*IME2 ids2-3*::*URA3*	0.8 ± 0.2

[a] Strains were **a**/α diploids, homozygous for the alleles indicated.
[b] Strains carried *IME2-CYC1-lacZ* plasmid pREY138 and were incubated in sporulation medium for 13.5 h before β-galactosidase was assayed. Values (in Miller units) are the means and standard deviations of triplicate determinations.

TABLE 4. Suppression of *ids2*Δ*4* by Ime2-472p

Strain	Genotype[a]	P_{GAL1}-*IME2* allele[b]	*IME2-CYC1-lacZ* expression[c]
AMP1452	*IDS2*	*IME2*	127 ± 8
		ime2-K97R	<0.4
		IME2-472	46 ± 4
AMP1453	*ids2*Δ*4*	*IME2*	<0.4
		ime2-K97R	<0.4
		IME2-472	13 ± 3

[a] Strains were **a**/α diploids, homozygous for *ime1*Δ*20*, *ime2-7-HIS3*::*LEU2*, and the *IDS2* alleles indicated.
[b] P_{GAL1}-*IME2* derivatives were expressed from plasmids pHS257, pHS263, and pHS264.
[c] Strains carried *IME2-CYC1-lacZ* plasmid pREY138 and were incubated in sporulation medium for 16 h before β-galactosidase was assayed. Values (in Miller units) are the means and standard deviations of triplicate determinations.

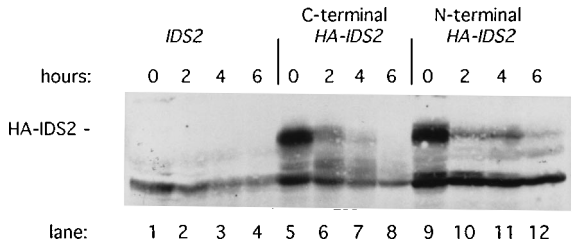

FIG. 5. HA-Ids2p accumulation during sporulation. HA-Ids2p was detected in crude extracts on an immunoblot. **a**/α diploid strain AMP179 carrying plasmid pREY171 (untagged Ids2p; lanes 1 to 4), pREY172 (HA-Ids2p C-terminal epitope; lanes 5 to 8), or pREY167 (HA-Ids2p N-terminal epitope; lanes 9 to 12) was grown to log phase in YPAc medium (lanes 1, 5, and 9) and shifted to sporulation medium for 2, 4, or 6 h before extracts were prepared (as indicated above each lane).

(Fig. 5). HA-Ids2p was identified as a 66-kDa immunologically reactive polypeptide present only in strains carrying an *HA-IDS2* plasmid (compare lanes 5 and 9 with lane 1). HA-Ids2p was much more abundant in growing cells (lanes 5 and 9) than in cells transferred to sporulation medium for 2 or 4 h (lanes 6, 7, 10, and 11). By 6 h in sporulation medium, HA-Ids2p was barely detectable (lanes 8 and 12). We conclude that Ids2p accumulates in growing cells and is degraded during sporulation.

DISCUSSION

Ime2p has three functions: it contributes to activation of early meiotic genes, it is required for activation of middle and late genes, and it is required for down-regulation of *IME1*. Early genes are expressed from about 2 to 6 h after **a**/α cells are starved (44) and are activated independently by both Ime1p and Ime2p (31). *IME1* and early genes are then shut off, and middle genes are expressed. It has been unclear how Ime2p carries out different functions at different times. The phenotype of *ids2* mutants indicates that these functions can be separated genetically. Therefore, *IDS2* provides a genetic basis for the temporal change in Ime2p-dependent functions.

IDS2 is dispensable for sporulation of homozygous *IME1 IME2* diploids. In these strains, Ime2p is essential for activation of middle and late genes and for down-regulation of *IME1* RNA; Ime1p activates *IME2* and other early genes (31, 39). *ids2* mutations cause a delay in Ime2p-dependent events. One explanation for these observations is that *ids2* mutations impair Ime2p-dependent functions specifically. *ids2* mutations must not abolish these functions, because the *ids2* phenotype is less severe than the *ime2* null phenotype. We note that strains with reduced *IME2* expression can sporulate at high levels if they express *IME1* (30, 38); therefore, it is plausible that *ids2* mutations impair Ime2p-dependent functions but have little effect on sporulation efficiency. An alternative explanation is that *ids2* mutations delay meiosis for reasons unrelated to Ime2p activity. (Such a nonspecific delay might result from slow acetate metabolism or slow DNA synthesis, for example.) Our studies in the *IME1 IME2* background are consistent with either explanation.

IDS2 is essential for sporulation of homozygous *ime1*Δ P_{GAL1}-*IME2* diploids. Ime2p activity is probably limiting for sporulation of these strains, because high-copy *IME2* plasmids promote only low-level sporulation in *ime1*Δ strains (39, 46). The idea that *ids2* mutations specifically impair Ime2p-dependent functions accounts for the sporulation defect of *ids2 ime1*Δ P_{GAL1}-*IME2* homozygotes and for partial suppression of *ids2*Δ*4* by Ime2-472p. The idea that *ids2* mutations cause a nonspecific delay in meiosis does not predict either their sporulation defect or their partial suppression by Ime2-472p. Studies in the *ime1*Δ P_{GAL1}-*IME2* background thus favor a more specific role of Ids2p in Ime2p-dependent functions.

In strains that lack Ime1p, meiotic gene expression depends solely upon Ime2p (31). Analysis in the *ime1*Δ P_{GAL1}-*IME2* background thus permits several Ime2p-dependent functions to be distinguished. *ids2* mutations permitted expression of the early genes *HOP1* and *SPO13*, but severely impaired expression of later genes *SPS1*, *SPS2*, and *SPS100*, and activation of the *IME2* UAS. (We note that the defect in *SPS100* expression may be a consequence of low *SPS1* expression [7].) Therefore, Ids2p is required for a subset of Ime2p-dependent functions.

Examination of sporulation efficiencies in *IME1 IME2* strains suggests that *ids2-1* causes a more severe defect than either *ids2-3*::*URA3* or *ids2*Δ*4* (Table 2). In principle, *ids2-1* might be a true null allele, and *ids2-3*::*URA3* and *ids2*Δ*4* might cause only partial defects. However, Northern analysis in both *IME1 IME2* and *ime1*Δ P_{GAL1}-*IME2* strains indicates that *ids2-1* causes a milder defect than *ids2-3*::*URA3* (36). *ids2-1* fails to complement *ids2-3*::*URA3* and *ids2*Δ*4* for activation of the *IME2* UAS, and the mutations map to the same locus; therefore, they are clearly allelic. Among many possible explanations of these results, the simplest is that the *ids2-1* strains carry a second mutation that causes a slight decrease in sporulation efficiency.

With one exception, Ids2p is required only for Ime2p-dependent functions that occur later in meiosis. The exception is *IME2* UAS activation. *IME2* is considered an early gene because it is first expressed at the same time as other early genes (31). In addition, several regulatory mutations affect expression of *IME2* and *SPO13* in parallel (1). Therefore, the distinction between Ids2p-independent and Ids2p-dependent genes may not precisely correspond to early and later meiotic genes. However, *IME2* RNA reaches peak accumulation later than *HOP1* and *SPO13* RNA (as seen in Fig. 4A, for example). It is possible that Ids2p and Ime2p contribute to *IME2* UAS activity only at later times during meiosis; Ime1p may be primarily responsible for *IME2* UAS activity early in meiosis.

Suppression of *ids2*Δ*4* by the Ime2-472p truncated product indicates that Ids2p does not act downstream of IME2. Ids2p may act directly or indirectly as an effector of Ime2p activity; Ime2-472p may have effector-independent protein kinase activity. Alternatively, Ime2p and Ids2p may act in independent, convergent pathways to stimulate *IME2* UAS activity; hyperactivity of Ime2-472p may bypass the need for the Ids2p pathway. We favor the first interpretation because Ime2-472p seems less active than wild-type Ime2p, as judged from *IME2* UAS activity in the *IDS2*/*IDS2* strain. However, our results do not rule out either interpretation.

The finding that Ids2p accumulation is greatest prior to sporulation suggests that Ids2p acts in the premeiotic cell. This proposal is tentative because we have not established the time of Ids2p action, but we note that it is consistent with evidence that Ime2p acts downstream of Ids2p. Premeiotic events that may be essential for meiosis include proper completion of the cell division cycle (14) and homologous chromosome association (26, 45).

Our observations are consistent with a simple model for the relationship between Ids2p, Ime2p, and temporal changes in meiotic gene expression. We suggest that Ime2p can exist in two functional states (Fig. 6). Ime2p is initially expressed in an early state, in which it can activate early meiotic genes such as *SPO13* and *HOP1*. This state of Ime2p is independent of Ids2p and is functionally redundant with Ime1p. Ime2p is then modified to a late state, in which it acts to shut *IME1* off, to activate middle and late genes, and to stimulate its own expression.

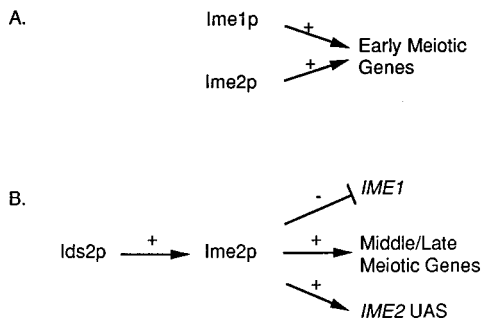

FIG. 6. Model for relationship between Ids2p and Ime2p. (A) Ime2p stimulates early meiotic gene expression in the absence of an Ids2p-dependent signal or event. Ime1p independently stimulates early genes. (B) Subsequently, an Ids2p-dependent signal is relayed or an Ids2p-dependent event occurs. Ime2p is then modified to permit stimulation of the *IME2* UAS, expression of middle and late genes, and down-regulation of *IME1* expression.

These activities are stimulated by Ids2p. Partial suppression of *ids2Δ4* by P_{GAL1}-*IME2-472* may be explained if Ime2-472p exists exclusively in the late state, regardless of an Ids2p-dependent signal. If Ids2p acts prior to meiosis, then it must act indirectly to effect a change in Ime2p. Ids2p may generate a stable signal, modify a downstream gene product that acts upon Ime2p, or facilitate a meiotic event to which Ime2p responds more directly. The late state of Ime2p may simply result from elevated kinase activity or may result from a qualitative change, for example, in subcellular distribution or substrate specificity. Our model predicts that the two postulated states of Ime2p may be defined biochemically.

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