Genetic Evidence for Transcriptional Activation by the Yeast *IME1* Gene Product

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ABSTRACT

IME1 is required in yeast for meiosis and for expression of *IME2* and other early meiotic genes. IME1 is a 360-amino acid polypeptide with central and C-terminal tyrosine-rich regions. We report here that a fusion protein composed of the lexA DNA-binding domain and IME1 activates transcription *in vivo* of a reporter gene containing upstream lexA binding sites. Activation by the fusion protein shares several features with natural IME1 activity: both are dependent on the *RIM11* gene product; both are impaired by the same *ime1* missense mutations; both are restored by intragenic suppressors. The central tyrosine-rich region is sufficient to activate transcription when fused to lexA. Deletion of this putative activation domain results in a defective IME1 derivative. Function of the deletion derivative is restored by fusion to the acidic Herpesvirus VP16 activation domain. The C-terminal tyrosine-rich region is dispensable for transcriptional activation; rather it renders activation dependent upon starvation and RIM11. Immunofluorescence studies indicate that an IME1-lacZ fusion protein is concentrated in the nucleus. These observations are consistent with a model in which IME1 normally stimulates *IME2* expression by providing a transcriptional activation domain at the *IME2* 5′ regulatory region.

THE sporulation program of the yeast *Saccharomyces cerevisiae* leads through meiosis to spore formation. Meiosis is initiated in response to starvation, typically for nitrogen, and is restricted to one type of cell, the **a**/α cell. Entry into meiosis is accompanied by increased expression of many genes that are essential for genetic recombination and other meiotic processes. Activation of these early meiotic genes is thus critical to the decision to enter meiosis [for reviews, see ESPOSITO and KLAPHOLZ (1981), DAWES (1983) and MAGEE (1987)].

The *IME1* (*I*nducer of *Me*iosis) product plays a central role in meiotic gene expression. *IME1* is expressed at high levels only in meiotic cells (KASSIR, GRANOT and SIMCHEN 1988), such as starved **a**/α cells. An *ime1* deletion blocks meiotic gene expression (SMITH and MITCHELL 1989; MITCHELL, DRISCOLL and SMITH 1990; ENGEBRECHT and ROEDER 1990; KIHARA *et al.* 1991) and expression of IME1 from a constitutive promoter leads to expression of several early meiotic genes in the absence of starvation or **a**/α cell type signals (SMITH *et al.* 1990). IME1 activates meiotic genes through two pathways: in one, IME1 activates *IME2* expression and IME2 stimulates meiotic gene expression; in the other, IME1 acts independently of *IME2* [MITCHELL, DRISCOLL and SMITH (1990); reviewed in MALONE (1990)]. Therefore, IME1 is formally a positive regulator of *IME2* and other meiotic genes.

Despite an understanding of IME1 function, the mechanism of IME1 action has remained unclear. IME1 is a 360-residue, acidic polypeptide (Figure 1) with two tyrosine-rich segments, Y1 (residues 197–291; 15% tyrosine) and Y2 (residues 328–360; 18% tyrosine). The tyrosine-rich segments are separated by a segment we call B ("bridge"). We report here a set of structure-function studies that suggest that IME1 may participate directly in activation of *IME2* expression.

MATERIALS AND METHODS

Yeast strains and genetic methods: Yeast strains used in these studies (Table 1) were derived from the rapidly sporulating strain SK-1 (KANE and ROTH 1974). Most mutations have been described [see SMITH *et al.* (1990) and MITCHELL and BOWDISH (1992)]. The genotypes of all SK-1 derivatives include markers *ura3 leu2::hisG trp1::hisG lys2 ho::LYS2 gal80::LEU2*.

To duplicate the *GAL4* gene in strain 1070, a *Bam*HI-*Hin*dIII fragment containing *GAL4* (LAUGHON and GESTELAND 1984) was inserted between the *Bam*HI and *Hin*dIII sites of the integrating plasmid YIp5 (ROSE and BROACH 1991). Integration of the resulting YIp5-*GAL4* plasmid was directed to the *URA3* locus by cleaving the plasmid at a unique *Stu*I site within *URA3*. Integration was carried out in a *RAD52* strain; the integrated YIp5-*GAL4* was then crossed into a *rad52::LEU2* background.

Genetic methods, including transformation, matings, tetrad dissection and segregation analysis, followed standard procedures (ROSE, WINSTON and HIETER 1990). Growth media were as previously described (SMITH *et al.* 1990; ROSE, WINSTON and HIETER 1990). Liquid sporulation medium contained 2% potassium acetate plus auxotrophic supplements at 20 mg/liter. The β-galactosidase indicator medium KAcXgal has been described (MITCHELL and BOWDISH

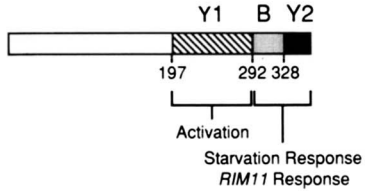

FIGURE 1.—Structure and function of IME1. IME1 is drawn to indicate tyrosine-rich regions Y1 (striped; residues 197–291) and Y2 (black, residues 328–360) and bridge region B (gray, residues 292–327), based on the sequence of SMITH *et al.* (1990). The main findings of the present study are summarized below the diagram. Residues 197–293 can function as a transcriptional activation domain. Residues 294–360 are required for RIM11 and starvation to regulate transcriptional activation and, we suggest, to direct IME1 to the *IME2* upstream regulatory region.

1992). SAcXgal was KAcXgal to which 0.67% yeast nitrogen base and 0.1% glucose were added.

Immunoblots: Cell extracts were prepared from log phase YEP Acetate cultures (MITCHELL and BOWDISH 1992). A 50-μg aliquot of soluble protein was fractionated on a 9% polyacrylamide sodium dodecyl sulfate gel and processed as previously described (COVITZ, HERSKOWITZ and MITCHELL 1991; MITCHELL and BOWDISH 1992). Rabbit antiserum against lexA protein was provided by ROGER BRENT.

IME1 was expressed in bacteria after cloning the IME1 coding region of pHS165A (modified to create an *Nde*I site at the initiation codon) between the *Nde*I and *Bam*HI sites of expression vector pAR3039 (STUDIER and MOFFAT 1986); induction and purification were essentially as described elsewhere (MITCHELL and BOWDISH 1992).

Sources of the P_{GAL1}-*IME1* hybrid gene: P_{GAL1}-*IME1* is a hybrid gene in which the *IME1* coding region is fused to the *GAL1* promoter and regulatory region (SMITH *et al.* 1990). YCpP_{GAL1}-*IME1* is P_{GAL1}-*IME1* in the low copy *CEN* plasmid, YCp50 (ROSE and BROACH 1991). YCpP_{GAL1} is the *GAL1* promoter in YCp50 without any *IME1* sequences. All *ime1* mutations were carried in YCp50 derivatives except *E294**, which was carried in the *CEN* plasmid pRS316 (SIKORSKI and HIETER 1989). The chromosomal P_{GAL1}-*IME1* allele, *IME1–14::TRP1*, is a replacement of much of the chromosomal *IME1* promoter with the *GAL1* promoter; the selectable marker *TRP1* is inserted upstream of *GAL1* sequences.

We have used a description of amino acid sequence changes rather than conventional nomenclature for *ime1* mutations in order to simplify interpretation. Thus an *ime1* allele encoding the missense product with a lysine substituted for arginine at residue 347 is designated *ime1-R347K* or P_{GAL1}-*ime1-R347K*. The corresponding protein product is designated IME1-R347K. An asterisk denotes a premature stop codon.

Plasmid mutagenesis: Hydroxylamine mutagenesis was carried out by incubating plasmid DNA (0.5 mg/ml) in a 200-μl volume of 1 M hydroxylamine HCl, 4 mM EDTA, 50 mM sodium pyrophosphate, and 100 mM NaCl, pH 7.0, at 75°. Samples of 20 μl were removed at various times between 15 and 120 min, cooled on ice, and DNA was purified through an Elutip (Schliecher and Schuell) followed by ethanol precipitation. Mutagenesis in an *Escherichia coli* mutator strain was conducted by transforming plasmid YCp-P_{GAL1}-*IME1* into *mutD5* strain HS3117 (provided by H. SHUMAN) in minimal medium (low mutagenesis rate), then growing transformants through up to 4 daily passages in rich LB + ampicillin medium (high mutagenesis rate).

Isolation of *ime1* mutations: *Mutations that block ime2-lacZ expression:* Mutagenized YCpP_{GAL1}-*IME1* was transformed into strain 918 and Ura$^+$ transformants were replica-plated to SAcXgal or KAcXgal indicator plates. Most colonies turned blue within 2–4 days, indicating presence of a functional P_{GAL1}-*IME1* gene. White colonies were purified, retested, screened on immunoblots and used for isolation of plasmid DNA. All point mutations except *L321F*, *E294** and *D166AILVTQ** were isolated through this screen. Allele *Q340** was recovered in a double mutant; the second mutation caused an S48F substitution. The separated S48F mutation was silent (based on assays of sporulation and *ime2-lacZ* expression), but was present in allele *Q340** when *ime2-lacZ* levels were quantitated.

Suppressors of IME1 toxicity: Expression of P_{GAL1}-*IME1* is toxic to starved *rad52* haploids, presumably because they enter meiosis and initiate recombination (MITCHELL and BOWDISH 1992). Spontaneous survivors of strain 1070 were isolated as previously described (MITCHELL and BOWDISH 1992). Briefly, patches derived from independent colonies of strain 1070 were incubated on Sporulation plates for four days, then replica-plated to YEPD growth medium. One surviving colony was picked from each patch, purified, retested, and further characterized on galactose indicator plates and by complementation tests (MITCHELL and BOWDISH 1992). The strain had two copies of the *GAL4* gene, which reduced the frequency of Gal$^-$ mutations isolated from 70% (MITCHELL and BOWDISH 1992) to 10%. Among 47 independent Gal$^+$ survivors, 33 *ime1* mutations were identified by complementation analysis. Only one mutation was associated with full-length IME1; this missense allele (P_{GAL1}-*ime1-L321F*) was crossed into a *RAD52* strain to permit transfer of the mutation to *Sac*I-digested YCpP_{GAL1}-*IME1* by gap rescue (ROTHSTEIN 1991).

In vitro constructed YCpP_{GAL1}-IME1 mutations: The *E294** mutation derived from oligonucleotide-directed mutagenesis (COVITZ, HERSKOWITZ and MITCHELL 1991). Internal deletions of *IME1* were generated in plasmid pHS159, a pBLUESCRIPT II derivative that contains *IME1* on a 1.3-kbp *Hin*dIII-*Nru*I fragment. Deletions were constructed by digestion with the indicated restriction enzymes followed by ligation: Δ6–68, *Nsi*I and *Pst*I; Δ68–124, *Pst*I and *Sac*I (both rendered flush); Δ123–182, *Sac*I (rendered flush) and *Eco*RV; Δ68–182, *Pvu*II and *Eco*RV; Δ165–182, *Eco*RV; Δ192–316, *Eco*RI. Deletion endpoints were confirmed by sequencing. The deletions were then cloned as *Hin*dIII-*Xho*I

TABLE 1

Yeast strains

Strain	Genotype[a]
113	α *ime1Δ12::TRP1 GAL80*
271	**a** *ime1Δ12::TRP1 his1*
476	α *ime1Δ12::TRP1 his4-N arg6*
480	α *ime1Δ12::TRP1 ime2Δ4-lacZ::LEU2 met4 his1*
918	**a** *ime1Δ12::TRP1 ime2Δ4-lacZ::LEU2 met4*
919	**a** *ime1Δ12::TRP1 ime2-HIS3::LEU2 his3 arg6*
1070	α P_{GAL1}-*IME1-14::TRP1 rad52::LEU2 ura3::GAL4::URA3 his3*
1184	α *ime1Δ12::TRP1 his3 met4*
1185	**a** *ime1Δ12::TRP1 his3 arg6*
1187	**a** *ime1Δ12::TRP1 rim11-6 his3 met4*
1188	α *ime1Δ12::TRP1 rim11-6 his3 arg6*
1243	α *ime1Δ12::TRP1 rim16-12 his3*
1245	**a** *ime1Δ12::TRP1 rim16-12 met4*
1246	α *ime1Δ12::TRP1 rim15-1 his3*
1247	**a** *ime1Δ12::TRP1 rim15-1*

[a] All strains have mutations *gal80::LEU2 ura3 leu2::hisG trp1::hisG lys2 ho::LYS2* unless otherwise noted.

fragments into the YCpP_{GAL1} plasmid digested with *Hin*dIII and *Sal*I.

Nucleotide sequences were determined for the entire coding region of each *ime1* point mutant with primers that hybridize within *IME1* or to the *GAL1* promoter.

***ime2-HIS3* construction and expression assays:** The *ime2-HIS3* fusion consists of the *IME2* promoter, the *HIS3* coding region, the *LEU2* gene (as a selectable marker), and *IME2* 3′ coding and downstream sequences. It was constructed in two steps. First, a flush-ended *Hin*dIII fragment from the pUC18-*IME2* plasmid pHS101 (SMITH and MITCHELL 1989) containing the *IME2* promoter was ligated with a flush-ended *Eco*RI-*Sma*I fragment from YIp5-SC3354 containing the *HIS3* coding region and 3′ sequences as well as the *URA3* gene [see NEIGEBORN and MITCHELL (1991)]. Second, a *Sma*I-*Xho*I fragment, which included the *IME2* promoter and *HIS3* coding region, was excised from that plasmid and ligated into *Sma*I-*Xho*I-digested plasmid pAM403, which contains the *ime2-1::LEU2* insertion (SMITH and MITCHELL 1989). The resulting plasmid, pAM416, was digested with *Sma*I and *Sph*I to permit *ime2-HIS3::LEU2* integration at the chromosomal *IME2* locus after selection of Leu$^+$ transformants. This fusion creates an *ime2* defect.

Assays of *ime2-HIS3* expression were conducted in strain 919 carrying YCpP_{GAL1}-*IME1* wild-type and mutant plasmids. Growth of patches was scored after 2 days on synthetic acetate medium lacking histidine (MSAc-His).

***ime2-lacZ* assays and sporulation determinations:** Overnight cultures of strain 918 (*ime1 ime2-lacZ*) or of 476x918 (*ime1/ime1 ime2-lacZ/IME2*) carrying each YCpP_{GAL1}-*IME1* plasmid were grown in media that selected for plasmid markers (SC-Ura) and diluted 1/30 to 1/50 into YEP Acetate. Vegetative cell samples were removed for assays after 8–12 hr (2–3 doublings), when the density reached 10^7 cells/ml. The remainder of the culture was transferred to sporulation medium at a density of 10^7 cells/ml. Sporulating cell samples were removed for assays after 8 hr. Sporulation was quantitated after 24 or 48 hr with strain 476x918. β-Galactosidase was measured in permeabilized cells as previously described (SMITH *et al.* 1990) with either 918 or 476x918; the two strains yielded comparable levels of *ime2-lacZ* expression when they carried YCpP_{GAL1}-*IME1*. The frequency of plasmid cures was 10–30% in a typical experiment. Each value for β-galactosidase activity or sporulation frequency is the average of values for three independent transformants which fell within a range of 30% or less.

lexA-IME1 fusions and reporter plasmids: The plasmid for expression of lexA-IME1 from the *GAL1* promoter was constructed in two steps. First, a lexA-IME1 fusion was constructed by inserting a 2.2-kbp fragment containing *IME1* with *Sal*I and flush-ended *Nsi*I ends [from plasmid pAM504 (SMITH and MITCHELL 1989)] into the lexA plasmid pSH2-1 (HANES and BRENT 1989) with *Sal*I and flush-ended *Eco*RI ends. A 2.25-kbp *Hin*dIII-*Sal*I fragment encoding the lexA-IME1 fusion was excised from that plasmid and inserted between *Hin*dIII and *Sal*I sites of plamid YCpP_{GAL1} to yield YCpP_{GAL1}-*lexA-IME1*. Mutant derivatives were constructed by inserting the 0.55-kbp *Hin*dIII-*Pvu*II fragment of YCpP_{GAL1}-*lexA-IME1* into the appropriate YCpP_{GAL1}-*IME1* plasmid. Presence of each mutation was confirmed by sequence determination.

The plasmids YCpP_{GAL1}-*lexA* and YCpP_{GAL1}-*lexA-Y1* were constructed in two steps. A 0.38-kbp *Eco*RI fragment from YCpP_{GAL1}-*ime1-E294** was inserted into the *Eco*RI site of plasmid LexA(1-202)+PL (RUDEN *et al.* 1991). Insertion in one orientation joins codons 1–202 of *lexA* to codons 197–293 of *IME1*; insertion in the opposite orientation generates a stop codon immediately after the *Eco*RI site. The 1.0-kbp *Hin*dIII-*Sal*I fragment from each plasmid was then cloned into YCpP_{GAL1} to create YCpP_{GAL1}-*lexA-Y1* and YCpP_{GAL1}-*lexA*, respectively.

The lexA-B-Y2 fusion was constructed by replacing a *Hin*dIII-*Eco*RI fragment (which includes IME1 codons 1–316) from YCpP_{GAL1}-*IME1* with a 0.65-kb *Hin*dIII-*Eco*RI fragment (which includes lexA codons 1–202) from pLexA(1–202)PL+ (RUDEN *et al.* 1991).

The lexA-operator-*gal1-lacZ* reporter plasmids were constructed from pLR1Δ1 (containing no lexA operators), p1840 (containing one lexA operator), and pSH18-18 [containing six lexA operators (HANES and BRENT 1989)] by replacing the 2.7-kbp *Sma*I-*Xba*I fragment (containing *URA3*) in each with the 4.1-kbp *Sma*I-*Xba*I fragment from pSH2-1 (containing *HIS3*) to create pHS178, pHS179 and pHS180, respectively.

The YCpP_{GAL1}-*lexA-VP16-IME1* plasmids were constructed in two steps. First, a 3.5-kb *Eco*RI-*Xba*I fragment from pMA540 (SADOWSKI *et al.* 1988), containing the C-terminal 78 codons of VP16, was inserted between the *Eco*RI and *Xba*I sites of plasmid plexA(1–202)PL+ (RUDEN *et al.* 1991) to create the lexA-VP16 fusion plasmid pHS217. Polymerase chain reaction (PCR) was used to amplify a 0.4-kb *Sna*BI-*Nde*I fragment containing the lexA and VP16 coding regions and to convert the stop codon of VP16 to an *Nde*I site. This *Sna*BI-*Nde*I fragment, along with a 2.0-kb fragment of pHS165A (containing *IME1* codons 1–360), was ligated between the *Sna*BI and *Sal*I sites of pHS202 (the P_{GAL1}-*lexA-IME1* plasmid) to create P_{GAL1}-*lexA-VP16-IME1* plasmid pHS230. The derivative of this plasmid containing *ime1-Δ192–316* was constructed by inserting the 1.1-kb *Hin*dIII-*Pvu*II fragment of pHS230 into the *Hin*dII and *Pvu*II sites of YCpP_{GAL1}-*lexA-ime1-Δ192–316* to create pHS235. None of these VP16-containing fusions caused a detectable growth defect.

β-Galactosidase assays were conducted on strains 1184x1185 (*RIM11/RIM11*), 1187x1188 (*rim11-6/rim11-6*), 1246x1247 (*rim15-1/rim15-1*), and 1243x1245 (*rim16-12/rim16-12*) carrying both lexA-IME1 and lacZ reporter plasmids. Assays were conducted after 8 hr in sporulation medium unless otherwise stated. All values are the average of determinations with three independent transformants except in the case of 1243x1245, where only two transformants were used for assays.

Isolation of lexA-IME1 revertants: YCpP_{GAL1}-*lexA-IME1* derivatives containing each mutant allele were mutagenized with hydroxylamine and amplified in *Escherichia coli*, then transformed into *ime1Δ12 ime2-HIS3* strain AMP919. Approximately 2×10^5 transformants with each mutagenized plasmid were selected on SC-Ura, replica-plated to SAG-His-Ura (acetate-glycerol synthetic medium lacking histidine and uracil), and His$^+$ derivatives were collected. Transformants with a plasmid-independent His$^+$ phenotype were discarded, as were transformants that grew on SC-His, in which the glucose should repress the *GAL1* promoter. Plasmids were then isolated and partially sequenced. For *ime1-R347K*, 92 plasmid-dependent revertants were identified; 8 plasmid inserts were partially sequenced; one had a back mutation and the rest had S360F substitutions. For *ime1-L325R*, 2 plasmid-dependent revertants were identified; both inserts had back mutations. For *ime1-L321F*, 62 plasmid-dependent revertants were identified; 11 plasmid inserts were partially sequenced; one had a back mutation; one had an A329V substitution, and nine had S360F substitutions. The inserts of the particular plasmids characterized in detail were sequenced from the *GAL1* promoter to the end of the *IME1* coding region.

IME1-lacZ fusions and immunofluorescence: The *IME1-lacZ* plasmid contained 1.1 kbp of IME1 5′-flanking sequences plus the N-terminal 340 codons of *IME1* in a

multicopy *lacZ*-bearing, *URA3* plasmid. The plasmid, YEpIME1(340)-lacZ, was constructed by inserting the 2.15-kbp *Sph*I-*Bcl*I fragment of pAM504 (SMITH and MITCHELL 1989) into *Sph*I-*Bam*HI-digested YEp356R (MYERS *et al.* 1986).

Indirect immunofluorescence experiments employed strain 113x271 (**a**/α *ime1Δ12/ime1Δ12*) carrying the IME1-lacZ plasmid or a control CYC1-lacZ fusion plasmid (pLGΔ312S). Cultures were grown overnight in medium selecting the plasmids, diluted 1/200 in YEP acetate, grown for 16 hr (to log phase), and shifted to sporulation medium for 5 hr. Cells were fixed and prepared for immunofluorescence (ROSE, WINSTON and HIETER 1990) with 1/250-diluted monoclonal mouse anti-β-galactosidase (Promega) and 1/100-diluted fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG (Boehringer). Cells were visualized through a 100× objective with a Nikon Optiphot microscope.

RESULTS

Isolation of *ime1* mutations: We have used mutational analysis to identify functional regions of IME1. Twenty-six *ime1* mutations were identified through a screen for failure to activate an *ime2-lacZ* fusion gene; 33 *ime1* mutations were identified as suppressors of IME1 toxicity (see MATERIALS AND METHODS). Extracts of all mutants were screened on immunoblots for presence of IME1 polypeptide. Four mutants made normal levels of full-length IME1, 16 made truncated products, and 39 made no detectable product. The immunoblot in Figure 2A, presented as an example, shows extracts of the first ten mutants identified through failure to activate *ime2-lacZ* expression. Other *ime1* mutations were produced through *in vitro* manipulations (see MATERIALS AND METHODS). Immunoblots established that these mutant products accumulated to detectable levels (Figure 2B).

Immunoblots indicate that IME1 is a heterogeneous protein in yeast extracts. Anti-IME1 antiserum reacted with a family of proteins of molecular mass 44–54 kD (Figure 2C, lane 2) that were altered or abolished by *ime1* mutations (see Figure 2, A and B). Expression of IME1 in *E. coli* yielded a single 44 Kd polypeptide (Figure 2C, lane 1), as predicted by the *IME1* DNA sequence (SMITH *et al.* 1990). Therefore, IME1 must be modified in yeast. This modification is unlikely to be phosphorylation because phosphatase treatment does not affect IME1 electrophoretic mobility and because we have been unable to label IME1 with ^{32}P-phosphate (our unpublished results). No single region is common to all IME1 derivatives that appear to be modified. We infer that IME1 can be modified at multiple sites. Because no mutant protein appears to be unmodified, our present results do not indicate whether modification affects IME1 activity.

Functional activity of *ime1* products: We used functional tests to determine activity of IME1 mutant products. IME1 derivatives were expressed from the *GAL1* promoter; a *gal80* mutation permitted P_{GAL1}-*IME1* expression in growth and sporulation media

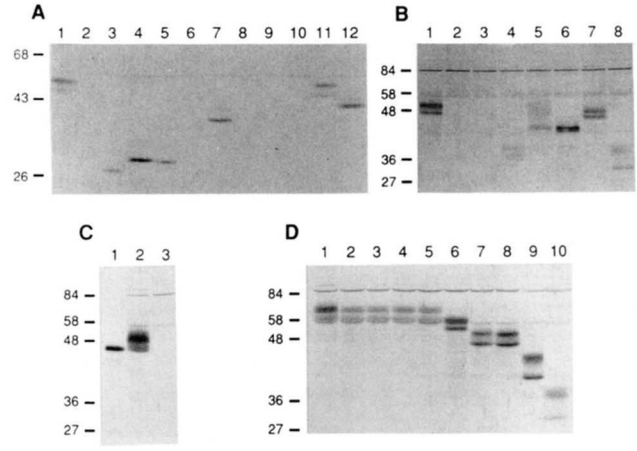

FIGURE 2.—Detection of IME1 and derivatives on immunoblots. (A) Hydroxylamine-induced YCpP_{GAL1}-*IME1* mutations. Extracts were prepared from *ime1* strain 918 carrying plasmids YCpP_{GAL1}-*IME1* (lane 1), YCpP_{GAL1} (lane 2) and the first ten YCpP_{GAL1}-*ime1* mutants (lanes 3–12). Mutants *ime1-230** (lane 5), *ime1-R347K* (lane 11) and *ime1-Q340* (lane 12) are analyzed in detail in this study. Other mutants shown are *ime1-110* (lane 3), *111* (lane 4), *Q38** (lane 6), *112* (lane 7), *W55** (lane 8), *Q24** (lane 9) and *Q25ATTT** (lane 10). The *110*, *111* and *112* alleles resulted from plasmid rearrangements; other alleles are indicated by the wild-type amino acid (in one-letter code), residue number, and the mutant substitution (in one-letter code or with "*" to indicate a termination codon). (B) YCpP_{GAL1}-*IME1* deletion mutants. Extracts were prepared from *ime1* strain 480 carrying plasmids YCpP_{GAL1}-*IME1* (lane 1), YCpP_{GAL1} (lane 2) and YCpP_{GAL1}-*IME1* deletion derivatives *Δ6–68* (lane 3), *Δ68–182* (lane 4), *Δ68–124* (lane 5), *Δ123–182* (lane 6), *Δ165–182* (lane 7) and *Δ192–316* (lane 8). (C) Comparison of IME1 from *E. coli* and yeast. Samples contained *ca.* 10 ng of IME1 partially purified from *E. coli* (lane 1), 50 μg of crude extract from yeast strain 918 carrying YCpP_{GAL1}-*IME1* (lane 2) or YCpP_{GAL1} (lane 3). (D) lexA-IME1 fusion proteins. Extracts were prepared from *ime1* strain 1184 carrying YCpP_{GAL1}-*lexA-IME1* (lane 1) or YCpP_{GAL1}-*lexA-IME1* fusion derivatives with *ime1* mutations *R347K* (lane 2), *L325R* (lane 3), *Y328H* (lane 4), *L321F* (lane 5), *Q340** (lane 6), *E294** (lane 7), *V286GPPTITH** (lane 8), *Q230** (lane 9) and *D166AILVTQ** (lane 10).

without galactose (SMITH *et al.* 1990). The first test employed an *ime2-HIS3* hybrid gene. In *ime2-HIS3* strains that have a deletion of the chromosomal *HIS3* gene, histidine biosynthesis depends on IME1 activity: an *ime1 ime2-HIS3* strain is His$^-$; introduction of the low copy YCpP_{GAL1}-*IME1* plasmid makes cells His$^+$ (Figure 3). We found that YCpP_{GAL1}-*ime1* point mutant alleles *Q340**, *Y328H* and *L321F* allowed weak His$^+$ growth of an *ime1 ime2-HIS3* strain; no other YCpP_{GAL1}-*ime1* point mutation permitted His$^+$ growth. All of the internal YCpP_{GAL1}-*ime1* deletions allowed His$^+$ growth, although *Δ192–316* had weaker activity than the others.

To quantitate IME1 defects, we measured β-galactosidase expression from an *ime2-lacZ* fusion (Figure 3). Assays were conducted on vegetative and starved cells; our prior studies indicated that *IME2* RNA levels and *ime2-lacZ* expression increase in starved cells that express P_{GAL1}-*IME1* (SMITH *et al.* 1990). In vegetative cells, all of the YCpP_{GAL1}-*ime1* mutations except dele-

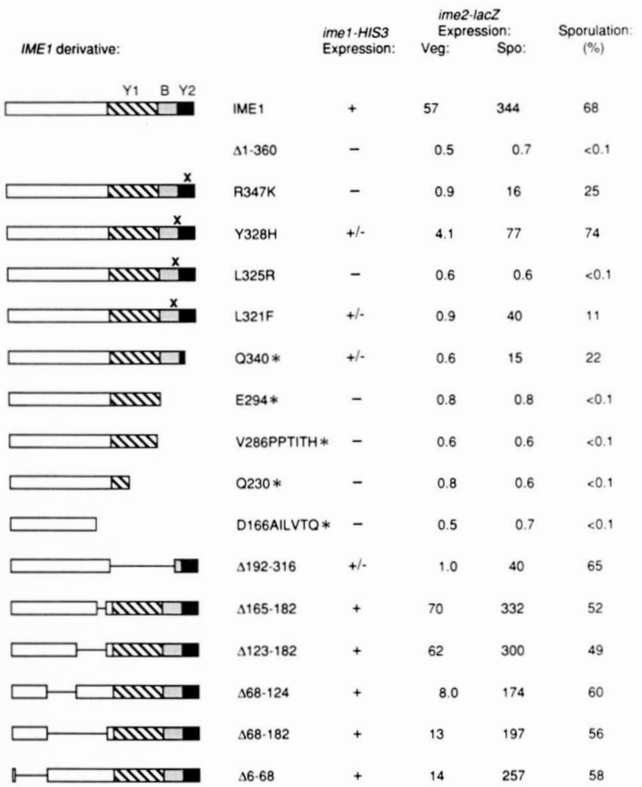

FIGURE 3.—Properties of mutant IME1 derivatives. The phenotypes of strains expressing plasmid-borne YCpP_{GAL1}-*ime1* mutant alleles are summarized. *ime1* missense mutations are indicated by an "X" at the position of the substitution; truncated and deleted products are drawn to scale. Mutations are designated by wild-type amino acid, codon number and substituted amino acid(s); "*" represents a termination codon. Deletions are indicated by Δ followed by the codon numbers that have been deleted. Δ1–360 refers to the control plasmid YCpP_{GAL1}; similar results were obtained with YCpP_{GAL1}-*ime1-Q24**, *Q38**, *W55** and *Q25ATTT**. The phenotypic tests included *ime2-HIS3* expression (activation of the *IME2* promoter as measured by a *HIS3* reporter in strain 919), *ime2-lacZ* expression (activation of the *IME2* promoter as measured by a *lacZ* reporter in vegetative and sporulation media; strains 918 and 476x918 were used in assays of *ime1* point mutations and deletions, respectively), and sporulation [percent of *ime1/ime1* diploids (strain 476x918) that sporulate when carrying each plasmid].

tion alleles *Δ123–182* and *Δ165–182* led to reduced expression of *ime2-lacZ*. In starved cells, one group of YCpP_{GAL1}-*ime1* alleles could not stimulate *ime2-lacZ* expression; this group included the missense allele *L325R* and all chain termination mutations except *Q340**. A second group stimulated low levels of *ime2-lacZ* expression (5–25% of wild type); this group included nonsense allele *Q340**, missense alleles *R347K*, *Y328H* and *L321F*, and deletion allele *Δ192–316*. A third group stimulated *ime2-lacZ* levels in the range of 50–100% of wild type; this group included all remaining internal deletions. Although mutant *R347K* had detectable activity only in the *ime2-lacZ* assay, all other IME1 derivatives that stimulated *ime2-lacZ* also stimulated *ime2-HIS3*.

We also tested the ability of each YCpP_{GAL1}-*ime1* allele to promote sporulation of an *ime1/ime1* mutant diploid (Figure 3). All YCpP_{GAL1}-*ime1* mutants that

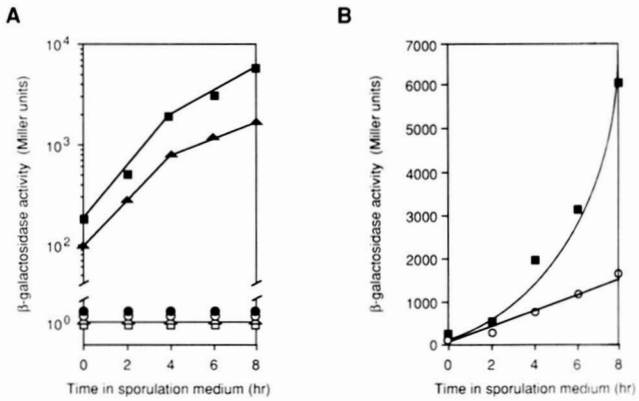

FIGURE 4.—Transcriptional activation by lexA-IME1. β-Galactosidase levels were determined during growth in YEP acetate (0 hr) or at indicated times after transfer to sporulation medium. (A) Cells carried reporter plasmids with zero (circles), one (triangles), or six (squares) upstream lexA operators and a second plasmid expressing lexA-IME1 (filled symbols) or lexA codons 1–87 (open symbols). (B) Cells carried reporter plasmids with six upstream lexA operators and a second plasmid expressing lexA-IME1 (filled squares) or lexA-IME1-E294* (open circles).

failed to stimulate *ime2-lacZ* expression also failed to promote sporulation. Conversely, all YCpP_{GAL1}-*ime1* mutants capable of stimulating *ime2-lacZ* expression under starvation conditions did promote sporulation. Alleles *R347K*, *Q340** and *L321F* stimulated low levels of sporulation compared to the wild type; other alleles stimulated sporulation to wild-type levels.

Transcriptional activation by lexA-IME1 fusions: Given that IME1 is a positive regulator of meiotic genes, we tested the possibility that IME1 may function directly as a transcriptional activator. To monitor activation by IME1 bound to an upstream DNA target site, we used the approach of HANES and BRENT (1989). The lexA DNA-binding domain (codons 1–87) was fused to IME1 (codons 6–360). Activity was assessed by β-galactosidase expression from a promoter with 0, 1 or 6 upstream lexA operators (Figure 4A). We examined activity in both vegetative and starved cells. lexA-IME1 had no effect on the promoter lacking lexA operators, but stimulated β-galactosidase expression over 50-fold from a single operator and over 200-fold from six operators, compared to lexA alone, in vegetative cells. Stimulation by lexA-IME1 increased a further 10–30-fold within 8 hr after starvation. This increase in lexA-IME1 activity was accompanied by little increase in lexA-IME1 protein levels, as detected with anti-IME1 antiserum (data not shown). Thus lexA-IME1, like IME1 itself (SMITH *et al.* 1990), has greater intrinsic activity in starved cells. We conclude that IME1 can provide a transcriptional activation domain.

Activation by lexA-IME1 may reflect the natural function of IME1. For example, IME1 may stimulate expression of some meiotic genes by direct stimulation of transcription. Alternatively, activation by lexA-IME1 might reflect a coincidental similarity between

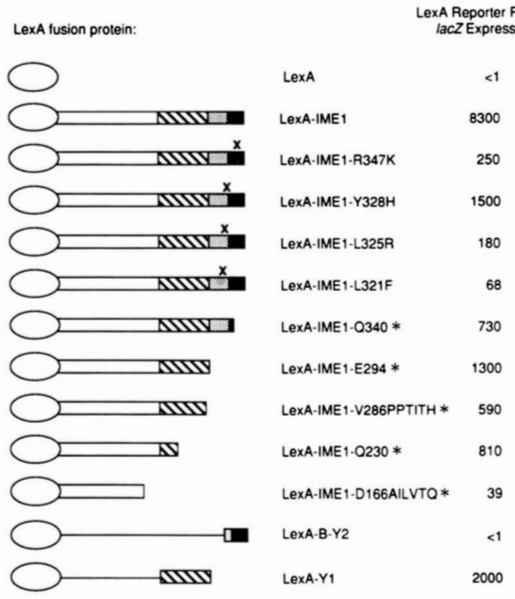

FIGURE 5.—Transcriptional activation by lexA-IME1 fusion derivatives. The lexA DNA-binding domain (or all of lexA in the lexA-Y1 and lexA-B-Y2 fusions) was expressed as a fusion protein to the indicated IME1 derivatives in strain 1184 × 1185, carrying lexA reporter plasmid pHS180 (with six lexA operators). β-galactosidase activity from plasmid pHS180 was determined after 8 hours in sporulation medium.

IME1 and activation domains. For example, IME1 is slightly acidic, as are many activation domains (MITCHELL and TJIAN 1989). The first explanation predicts that mutations that interfere with natural IME1 function may also interfere with activation by lexA-IME1. The second explanation predicts no such correlation. Thus we determined the activation abilities of lexA fusions to IME1 missense mutant derivatives (Figure 5). Fusion of lexA to IME1-Y328H, which has a 5-fold defect in activation of *ime2-lacZ*, produced a 5-fold less potent activator than lexA-IME1. Fusion of lexA to IME1-R347K, which has a 20-fold defect in activation of *ime2-lacZ*, produced a 30-fold less potent activator. Fusion of lexA to IME1-L325R, which has a >100-fold defect in activation of *ime2-lacZ*, produced a 50-fold less potent activator. Thus the effects of these mutations on IME1 activity are roughly proportional to their activation defects. The last missense mutation caused disproportionate defects: IME1-L321F has a 10-fold *ime2-lacZ* expression defect; lexA-IME1-L321F has a 120-fold activation defect. Clearly, though, all lexA-IME1 missense derivatives are less potent activators than the lexA-IME1 fusion derived from wild-type sequences. We point out that the L321F, Y328H and R347K substitutions are fairly conservative and that none of the mutations affects the IME1 net charge severely. We conclude that transcriptional activation by lexA-IME1 is impaired by the same missense substitutions that impair IME1 activity.

We considered two indirect mechanisms through which *ime1* mutations might reduce lexA-IME1 activity. First, the mutations might destabilize the fusion protein. However, immunoblots indicated that the mutations caused only a slight defect in fusion protein accumulation (Figure 2D, compare lanes 2–5 with lane 1). Thus instability may account for much of the 5-fold lexA-IME1-Y328H defect but cannot account for the 30-fold or greater defects of the other lexA-IME1 mutant fusions. Second, the mutations might cause defects in lexA-IME1 dimerization, as the lexA segment alone lacks the dimerization domain necessary for high affinity operator binding (KIM and LITTLE 1992). However, we observed comparable activation defects with fusions of IME1 mutants to full-length lexA, which has its own dimerization domain (data not shown). Therefore, these mutations reduce activation ability more directly.

TABLE 2

Properties of lexA-IME1 revertant products

lexA fusion protein	*ime2-HIS3* expression	Sporulation (%)	lexA reporter expression[a]
lexA	−	<0.2	<1
lexA-IME1	+	68	8300
lexA-IME1-R347K	−	1.0	250
lexA-IME1-R347K, S360F	+	70	3000
lexA-IME1-L321F	−	2.5	68
lexA-IME1-L321F, A329V	+	67	2100
lexA-IME1-L321F, S360F	+	67	3600

[a] Assays of strain 1184x1185 carrying lexA reporter plasmid pHS180, with six lexA operators, and the YCpP_{GAL1}-*lexA-IME1* plasmids indicated, were conducted after 8 hr in sporulation medium.

We used suppressor analysis to further test the correlation between IME1 and lexA-IME1 activities. We selected suppressors that restored IME1 activity to lexA-IME1 mutant fusions and then determined whether transcriptional activation ability was simultaneously restored. For these experiments, we made use of the observation that lexA-IME1 can complement an *ime1* defect to permit both *ime2-HIS3* expression and sporulation (Table 2). lexA-IME1-R347K, -L325R and -L321F were associated with *ime2-HIS3* expression defects severe enough to select His^+ revertants. Revertants derived from lexA-IME1-R347K included back mutations and a suppressor causing an S360F substitution. Revertants derived from lexA-IME1-L325R included only back mutations. Revertants derived from lexA-IME1-L321F included back mutations and suppressors causing either A329V or S360F substitutions. All second-site suppressors also restored ability of lexA-IME1 fusions to complement the sporulation defect of an *ime1/ime1* diploid (Table 2). Therefore, these intragenic suppressors restore natural IME1 function. We then determined the ability of each lexA-IME1 suppressor derivative to activate expression of the lexA reporter. Activation by the R347K mutant was improved 12-fold by the S360F suppressor. Activation by the L321F was improved 30- and 50-fold by the A329V and S360F

TABLE 3

Replacement of Y1 region function by the Herpesvirus VP16 activation domain

lexA fusion protein	lexA reporter expression[a]	*ime2-HIS3* expression	*ime2-lacZ* expression[b]
lexA-IME1-Δ192-316	40	+/−	5.2
lexA-VP16-IME1-Δ192-316	4200	+	150
lexA-IME1	8300	+	150
lexA-VP16-IME1	5700	+	250

[a] Assays were conducted with strain 1184x1185, carrying lexA reporter plasmid pHS180 (with six lexA operators) and the indicated YCpP_{GAL1}-*lexA-IME1* plasmids, after 8 hr in sporulation medium.

[b] Assays of *ime2-lacZ* expression were conducted with strain 476x918, carrying the indicated YCpP_{GAL1}-*lexA-IME1* plasmids, after 8 hr in sporulation medium.

suppressors, respectively (Table 2). Immunoblots indicated that thse suppressors do not simply cause elevated lexA-IME1 accumulation (data not shown). We conclude that intragenic IME1 suppressors restore transcriptional activation ability to lexA-IME1.

Identification of an IME1 activation region: We set out to map the region of IME1 responsible for transcriptional activation with fusions of lexA to truncated IME1 derivatives (Figure 5). Truncations affecting the C-terminal B-Y2 region caused a 5–10-fold decrease in activation (lexA-IME1-Q340* and -E294*). Further truncations had little effect (V286PPTITH* and Q230*) until the entire Y1 region was removed (D166AILVTQ*). Immunoblots indicated that all active truncated derivatives were heterogeneous and accumulated to levels comparable to full-length lexA-IME1 (Figure 2D, lanes 6–9 compared to lane 1). The inactive product (D166AILVTG*) was less detectable on immunoblots (Figure 2D, lane 10), perhaps due to instability or to loss of epitopes. These observations suggested that the B-Y2 and Y1 regions may both play positive roles in activation. However, a fusion of the B-Y2 region to lexA was unable to activate lexA reporter expression. On the other hand, a fusion of the Y1 region to lexA activated lexA reporter expression to 25% the level observed with full-length lexA-IME1 (Figure 5). Thus the Y1 region has properties expected of a transcriptional activation domain. The B-Y2 region appears to improve activation by the Y1 region, although we cannot rule out the possibility that the B-Y2 region is an independent activation domain that suffers conformational disruption when fused to lexA.

Results above indicated that deletion of the Y1 region impaired IME1 activity: the *IME1-Δ192–316* allele caused defects in *ime2-HIS3* and *ime2-lacZ* expression. We confirmed that lexA-IME1-Δ192–316 shared these properties and that it was defective in lexA reporter activation (Table 3). If the Y1 domain functions in transcriptional activation, then a heterologous activation domain may be able to substitute

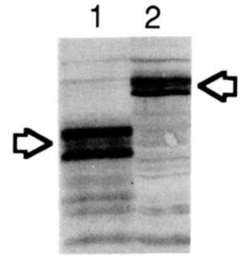

FIGURE 6.—Detection of IME1-Δ192–316 derivatives on immunoblots. A 50-μg sample of extract protein was analyzed by immunoblotting with anti-lexA antiserum. Extracts were prepared from strain 919 carrying plasmids YCpP_{GAL1}-*lexA-IME1Δ192–316* (lane 1) or YCpP_{GAL1}-*lexA-VP1G-IME1Δ192–316* (lane 2). Arrows indicate the regions with immunologically reactive proteins unique to each strain.

for Y1. We tested this prediction with the C-terminal 78 residue activation domain of Herpesvirus VP16 (SADOWSKI *et al.* 1988), which has little sequence homology with the tyrosine-rich Y1 region. The VP16 region was inserted between lexA and IME1-Δ192–316. We confirmed that lexA-VP16-IME1-Δ192–316 was capable of activating the lexA reporter efficiently (Table 3). IME1 activity of the fusion protein was then tested with assays of *ime2-HIS3* and *ime2-lacZ* expression. We found that lexA-VP16-IME1-Δ192–316 had greater IME1 activity than lexA-IME1-Δ192–316 in both assays (Table 3). In fact, lexA-VP16-IME1-Δ192–316 had activity comparable to lexA-IME1 or lexA-VP16-IME1. Immunoblots indicated no effect of the VP16 domain on levels or apparent modification of the fusion protein (Figure 6). We conclude that the the VP16 activation domain can substitute for the Y1 region in stimulating the natural activity of IME1.

Regulatory role of the B-Y2 region: Studies above indicate that the B-Y2 region is absolutely essential for natural IME1 activity but is not vital for transcriptional activation. The finding that missense mutations in the B-Y2 region impair activation more than removal of B-Y2 argues that B-Y2 might function in regulation of transcriptional activation. We compared activation by lexA-IME1 and by lexA-IME1-E294*, which lacks the B-Y2 region, to see whether any physiological or genetic signals act through this region.

We first compared the effect of starvation on activation. We had found that lexA-IME1 activates lexA reporter expression at least 10-fold more efficiently in starved cells than in vegetative cells (Figure 4B). In contrast, lexA-IME1-E294* does not activate more efficiently in starved cells; β-galactosidase reporter activity accumulated linearly after starvation, as expected for a constant rate of synthesis (Figure 4B). Activation by lexA-IME1-V286PPTITH* and -Q230* was also insensitive to starvation (data not shown). We conclude that the B-Y2 region is essential for starvation-responsiveness of lexA-IME1.

TABLE 4

Effects of rim mutations on lexA-IME1 activity

Activator	lexA reporter β-galactosidase expression[a]			
	RIM+	*rim11-6*	*rim15-1*	*rim16-12*[b]
lexA-IME1	8300	20	3300	970
lexA-IME1-E294*	1300	1000	540	730
lexA	<1	<1	ND[c]	ND

[a] Assays were conducted with rim mutant strains, carrying lexA reporter plasmid pHS180 (with six lexA operators) and the indicated YCpP_{GAL1}-*lexA-IME1* plasmids, after 8 hr in sporulation medium.
[b] These values are averages of two determinations.
[c] Abbreviation: ND, not determined.

We also compared the requirement for three genes functionally related to *IME1: RIM11*, *RIM15* and *RIM16*. The *rim11-6*, *rim15-1* and *rim16-12* mutations cause 20–100-fold defects in *IME2* expression but have little effect on IME1 accumulation or heterogeneity (MITCHELL and BOWDISH 1992; **Su** and MITCHELL 1993; **A.** P. MITCHELL, unpublished results). We tested the effects of these mutations on activation of the lexA reporter (Table 4). Activation by lexA-IME1 was reduced 400-fold in a *rim11-6* mutant, 2-fold in a *rim15-1* mutant and 8-fold in a *rim16-12* mutant. Activation by lexA-IME1-E294* was reduced slightly in the *rim11-6* mutant, 2-fold in the *rim15-1* mutant, and 2-fold in the *rim16-12* mutant. We conclude that the IME1 B-Y2 region confers RIM11-dependence and, to a lesser extent, RIM16-dependence, on activation. The fact that, in a *rim11-6* mutant, lexA-IME1-E294* is a 50-fold better activator than lexA-IME1 indicates that the B-Y2 region interferes with activation in the absence of *RIM11* activity.

Subcellular distribution of an IME1-lacZ fusion protein: The idea that IME1 is a transcriptional activator predicts that IME1 should be a nuclear protein. We used indirect immunofluorescence to examine the subcellular distribution of an IME1-lacZ fusion protein containing IME1 residues 1–340. The fusion permitted sporulation of an *ime1/ime1* diploid, indicating that it had IME1 function. The fusion protein was concentrated in the nucleus (Figure 7). A control lacZ fusion to the first two *CYC1* codons was distributed throughout the cells (data not shown). These results suggest that IME1 is a nuclear protein.

DISCUSSION

Prior studies have shown that IME1 is a positive regulator of meiotic gene expression but have not provided insight into its mechanism of action. The breadth of possible mechanisms is illustrated by the fact that IME2, which can substitute for IME1 to activate many meiotic genes (MITCHELL, DRISCOLL and SMITH 1990). is a protein kinase homolog (YOSHIDA *et al.* 1990). One simple model is that IME1 stimulates early meiotic genes by interacting with their

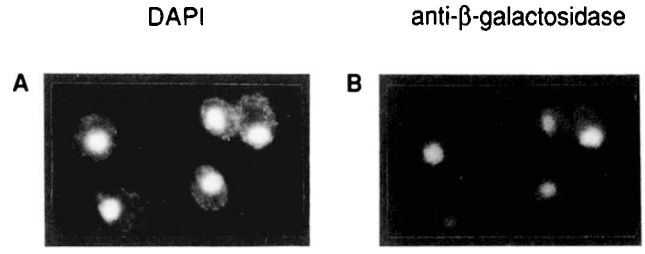

FIGURE 7.—Subcellular localization of an IME1-β-galactosidase fusion protein. 4′,6-diamidino-2-phenylindole (DAPI) and FITC fields (panels A and B, respectively) are shown of strain I 13x271 (*ime1/ime1*) expressing a *lacZ* fusion to IME1 codon 340.

cis-acting regulatory regions and providing a transcriptional activation domain. In this study, we established a correlation between the requirements for natural IME1 activity and for transcriptional activation by a lexA-IME1 fusion protein.

IME1 structure-function relationships: We used two different methods to isolate random *ime1* mutations. One was a screen for failure to activate *ime2-lacZ* expression. The second was a selection for **loss** of IME1 toxicity (MITCHELL and BOWDISH 1992). Because *ime2* mutations suppress IME1 toxicity, it seems reasonable that the selection demands failure to activate *IME2*. Very few missense mutants were detected from either isolation. It seems unlikely that many missense mutants would produce unstable products, and thus go undetected, because several truncated products were readily detected. In addition, sequence determination of six alleles associated with no detectable product revealed chain-termination defects within the first 100 codons (S. E. DRISCOLL, unpublished results). Two factors may have biased our isolations in favor of nonsense mutations. First, both isolations employed P_{GAL1}-*IME1* genes, which express *IME1* RNA at 5-fold higher levels than the native *IME1* gene (SMITH *et al.* 1990). Thus we may have overlooked many less severely defective mutations. Second, our deletion analysis indicated that the N-terminal half of IME1 is dispensable, *so* the target size for nonsense mutations is larger than for missense mutations.

The main conclusion from mutational analysis is that the B and Y2 regions of IME1 are critical for its activity. All truncated IME1 products that lack B-Y2 are nonfunctional and one nonsense mutation within the Y2 region (*Q340**) has reduced activity. In addition, the only missense mutations recovered lie in the B-Y2 region. Our finding that one intragenic suppressor (*S360F*) restores function to either of two missense alleles (*L321F*, *R347K*) is consistent with the idea that these missense alleles cause similar types of defects. Analysis of lexA-IME1 fusions indicates that the B-Y2 region is required for response to RIM11 and RIM16. Thus the missense substitutions may result in failure to interact with or respond to RIM11, RIM16 or a product that transmits a related signal.

Our results also suggest that the Y1 region plays a positive role in IME1 activity. This conclusion is more tenuous because it is based on a single deletion which may affect the B-Y2 region. In addition, the deletion product, IME1-Δ192–316, reacts weakly on immunoblots (Figure 2B, lane 8). Thus the deletion may reduce IME1 activity by reducing protein accumulation; alternatively, the deletion may remove a major IME1 epitope. However, our studies of VP16 fusions indicate that reduced IME1 accumulation cannot be the cause of the IME1-Δ192–316 defect. Addition of the VP16 activation domain to lexA-IME1-Δ192–316 restores IME1 function without affecting the amount of reactive material on immunoblots. We conclude that residues 192–316, which include the Y1 region, play a qualitative role in IME1 activity.

Finally, we have examined the subcellular localization of IME1 through the use of an IME1-lacZ fusion protein. The fact that the fusion protein retains IME1 function provides some assurance that its localization accurately reflects that of IME1. The finding that the IME1-lacZ fusion protein is concentrated in the nucleus is consistent with, but does not prove, a more direct role for IME1 in the regulation of meiotic genes.

Transcriptional activation by IME1: We have found four correlations between the requirements for natural IME1 activity and transcriptional activation by a lexA-IME1 fusion protein: (1) both IME1 and lexA-IME1 activities are reduced by four *ime1* missense mutations and are restored by intragenic suppressors; (2) both IME1 and lexA-IME1 activities are reduced by a deletion of the tyrosine-rich Y1 region which, by itself, functions as transcriptional activation domain; (3) the acidic VP16 activation region can suppress the Y1 deletion to restore the natural activity of IME1 and (4) both IME1 and lexA-IME1 depend on *RIM11* for their respective activities. These observations, along with the nuclear concentration of an IME1-lacZ fusion protein, are consistent with a model in which IME1 activates *IME2* by providing an activation domain at the *IME2* 5′ regulatory region.

We acknowledge that our proposal is based on internal consistency rather than direct studies demonstrating contact between IME1 and the *IME2* UAS region. In principle, IME1 might carry out some function with the same structural requirements as transcriptional activation. For example, the transcriptional activator GAL4 can also stimulate replication origin activity (MARAHRENS and STILLMAN 1992). However, the finding that *IME1* expression is necessary and sufficient for expression of *IME2* and other meiotic genes (MITCHELL, DRISCOLL and SMITH 1990; SMITH *et al.* 1990) is simply explained by a more direct role for IME1 in transcriptional activation.

Although the Y1 region functions as an activation domain, it does not resemble previously identified activation regions (reviewed by MITCHELL and TJIAN 1989). The abundance of glutamine (5%) and proline (7%) is not as high as in glutamine-rich or proline-rich activation domains. It is acidic (charge −6) but has little predicted α-helical content. Its most striking feature is an abundance of tyrosine residues (15%). Why might IME1 have such an unusual activation domain? One possibility is that tyrosine-rich regions are a newly discovered class of activation domain. We note that one small activation domain of hepatocyte nuclear factor 3β has tandem tyrosine residues, both of which are essential for activation (PANI *et al.* 1992). A second possibility is that tyrosine-rich regions can present acidic residues in an appropriate configuration for activation. Studies of CRESS and TREISENBERG (1991) have demonstrated the importance of bulky aromatic residues in acidic activation domains and questioned the necessity of an amphipathic α-helical structure. RUDEN (1992) described short functional acidic activation regions that included tyrosines at 2 of 6 variable positions. Thus the Y1 region may adopt a structure that resembles that of more familiar acidic activation domains. A third possibility, which does not exclude the other two, is that the high Y1 region tyrosine content is vital for interaction with the B-Y2 region. For example, B-Y2 may regulate activation by Y1 through interactions between tyrosine residues. Binding of B-Y2 to Y1 may be examined through structural studies or more refined genetic tests (FIELDS and SONG 1989).

One function of the B-Y2 region is to regulate activation ability. Based on a comparison of lexA-IME1 and lexA-IME1-E294*, we infer that regulation through B-Y2 responds both to starvation and to *RIM11* activity. Prior studies indicated that starvation and *RIM11* are both required for maximal *IME2* expression in strains that express *IME1* constitutively (SMITH *et al.* 1990, MITCHELL and BOWDISH 1992). We have recently determined that effects of *RIM11* and of starvation are exerted at an IME1-dependent *IME2* UAS (BOWDISH and MITCHELL 1993). Our present results indicate that these signals are transmitted to the UAS by effects on IME1 itself. Our findings are consistent with the suggestion that *RIM11* may transmit a starvation signal (MITCHELL and BOWDISH 1992).

The properties of IME1-E294* indicate that regulation of activation cannot be the only role of B-Y2. IME1-E294*, like other derivatives that lack B-Y2, has no detectable IME1 activity but displays considerable activation ability when fused to lexA. Thus its failure to provide IME1 activity cannot simply be a consequence of failure to activate transcription. The second function of B-Y2 may be to provide specificity to direct IME1 to the *IME2* regulatory region. This function would be dispensable for lexA-IME1 fusions to activate through lexA binding sites. We suggest

that the B-Y2 region binds to a protein situated at the *IME2* UAS; binding would deliver the Y1 activation region to the UAS and stimulate *IME2* transcription. *RIM15* and *RIM16*, which are essential for *IME2* UAS activity but have only 2–10-fold effects on lexA-IME1 activation, may specify proteins that are required for IME1 to interact with the *IME2* UAS.

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