MOLECULAR AND CELLULAR BIOLOGY, May 1994, p. 3446–3458
0270-7306/94/$04.00+0

Vol. 14, No. 5

The Yeast *UME5* Gene Regulates the Stability of Meiotic mRNAs in Response to Glucose

RICHARD T. SUROSKY,[1] RANDY STRICH,[2] AND ROCHELLE EASTON ESPOSITO[1]*

Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637,[1] and Fox Chase Cancer Center, Institute for Cancer Research, Philadelphia, Pennsylvania 19111[2]

Received 1 November 1993/Returned for modification 9 December 1993/Accepted 7 February 1994

We reported previously that early meiotic transcripts are highly unstable. These mRNAs exhibit half-lives of approximately 3 min when expressed during vegetative growth in glucose medium and are stabilized twofold during sporulation in acetate medium. Two genes, *UME2* and *UME5*, that regulate the stability of meiosis-specific transcripts have been identified. The wild-type *UME5* gene, which has been analyzed in detail, decreases the stability of all meiotic mRNAs tested approximately twofold when expressed during vegetative growth but has no effect on the half-lives of a number of vegetative mRNAs examined. The *UME5* gene is dispensable for mitotic and meiotic development. Cells in which the entire *UME5* gene has been deleted are viable, although the generation time is slightly longer and sporulation is less efficient. The *UME5* transcript is constitutively expressed, and its stability is not autoregulated. The *UME5* gene encodes a predicted 63-kDa protein with homology to the family of CDC28 serine/threonine-specific protein kinases. The kinase activity appears to be central to the function of the UME5 protein, since alteration of a highly conserved amino acid in the kinase domain results in a phenotype identical to that of a *ume5* deletion. Genetic epistasis studies suggest that the *UME2* and *UME5* gene products act in the same pathway to regulate meiotic transcript stability. This pathway is independent of deadenylation and translation, two factors known to be important in regulating mRNA turnover. Significantly, the *UME5*-mediated destabilization of meiotic mRNAs occurs in glucose- but not in acetate-containing medium. Thus, the *UME5* gene appears to participate in a glucose signal transduction pathway governing message stability.

Both transcription and mRNA stability play important roles in the regulation of gene expression. While many transcriptional control pathways have been elucidated, only recently have the mechanisms that regulate mRNA stability been examined (55, 58). Studies have identified several factors that regulate mRNA stability, including growth conditions and cell metabolism. For example, levels of β-tubulin monomers, free histones, and iron regulate the stability of β-tubulin (50), histone (20), and transferrin receptor (12) mRNAs, respectively. These factors act through *cis*-acting sequences within the 3′ untranslated region or the coding region of the mRNA (48, 51, 63). Some *cis*-acting determinants alter mRNA stability by affecting translation (1, 52, 80) or polyadenylation (47). Transcripts such as those of c-*fos* (63) and yeast *STE3* (24) contain multiple stability determinants, and the turnover of these transcripts is influenced by several factors. These studies demonstrate that many pathways have developed for regulating mRNA stability, although few of the *trans*-acting genes that mediate these pathways have been identified.

In *Saccharomyces cerevisiae*, many genes that are required for meiosis are expressed only in *MAT***a**/*MAT*α cells in medium lacking nitrogen with acetate as a carbon source (5, 17). At specific times in meiosis, these transcripts accumulate as much as 70- to 100-fold over vegetative levels (3, 27, 77). The meiosis-specific genes are classified as early, middle, or late by their time of peak induction (3, 56). Recent studies have identified several *trans*-acting transcriptional regulators of genes that are expressed early in meiosis (33, 46, 66, 67, 67a, 75). These regulators are organized into a complex system that may be necessary to maintain precise control of expression during vegetative and meiotic development (8, 60). The absence of this control, such as the unscheduled expression of early meiotic genes during vegetative growth, may alter generation times and sporulation efficiency (67, 67a).

We previously initiated a study of meiotic transcript stability to understand the role of mRNA turnover in regulating the expression of meiosis-specific genes (69). Although this investigation demonstrated that changes in transcription were primarily responsible for the large induction of these mRNAs, it was also observed that changes in the rate of turnover contribute to their accumulation. Early meiotic transcripts were highly unstable when expressed during vegetative growth (half-lives of approximately 3 min) and were stabilized twofold during meiosis. Subsequent experiments showed that this difference in half-lives, which is specific to meiotic mRNAs, depends upon the carbon source (glucose versus acetate) and not on the developmental stage of the cell (mitosis versus meiosis). A mutational analysis of one early meiotic gene, *SPO13*, revealed the presence of determinants within the coding region that destabilize the mRNA. Normal translation of the *SPO13* mRNA was also shown to be required for maximum instability. The investigation described below was undertaken to identify *trans*-acting genes that modulate these effects and are essential for the rapid turnover of the meiotic transcripts.

MATERIALS AND METHODS

Strains and media. *Escherichia coli* LE392 was used as the host for plasmid amplification (22). The yeast strains used in this study are listed in Table 1. The Luria-Bertani medium for growth of *E. coli* (44) and the YPD, YPA, and complete synthetic media for growth and SPII medium for sporulation of yeast cells (62) have been described previously. Transforma-

* Corresponding author. Mailing address: Department of Molecular Genetics and Cell Biology, University of Chicago, 920 East 58th St., Chicago, IL 60637. Phone: (312) 702-8046.

TABLE 1. Yeast strains

Strain	Genotype	Source or reference
D36	*MATα ade6 his3-11,15 leu2-3,112 trp1-1 ume5::TRP1 ura3-1*	This study
D39	*MATα his4-c leu2-3,112 ume5::LEU2 ura3-1*	Derivative of RSY75
D44	*MATα can1-100 his4-c leu2-3,112 trp1-1 ume2-2 ume5::LEU2 ura3-1*	Derivative of RSY94
D46	*MATa can1-100 his4-519 leu2-3,112 rpb1-1 ume5::LEU2 ura3-1*	Derivative of S86
F106	*MATa his3 leu2 lys2 pep4Δ::URA3 trp1 ura3*	S. Judd
F113	*MATa ade2-1 his3-200 lys2-801 rad1Δ::TRP1 trp1Δ ura3-52*	L. Prakash
RSY75	*MATα his4-c leu2-3,112 ura3-1*	67a
RSY94	*MATα can1-100 his4-c leu2-3,112 trp1-1 ume2-2 ura3-1*	67a
RSY107	*MATα can1-100 his4-c leu2-3,112 ume5-2 ura3-1*	67a
S86	*MATa can1-100 his4-519 leu2-3,112 rpb1-1 ura3-1*	*rbp1-1* (49)
C170	*MATα leu2-3,112 his4-c ADE2 HIS3 ADE6 CAN1 ura3-1 TRP1* *MATa leu2-3,112 HIS4 ade2 his3-11 ade6 can1-100 ura3-1 trp1-1*	This study
C173	*MATα leu2-3,112 his4-c ADE2 HIS3 ADE6 CAN1 ura3-1 TRP1 ume5::LEU2* *MATa leu2-3,112 HIS4 ade2 his3-11 ade6 can1-100 ura3-1 trp1-1 ume5::LEU2*	This study
C193	*MATα leu2-3,112 his4-519 can1-100 ura3-1 rpb1-1* *MATa leu2-3,112 his4-519 can1-100 ura3-1 rpb1-1*	*rbp1-1* (49)
C194	*MATα leu2-3,112 his4-519 can1-100 ura3-1 rpb1-1 ume5::LEU2* *MATa leu2-3,112 his4-519 can1-100 ura3-1 rpb1-1 ume5::LEU2*	*rbp1-1* (49)
W303α/**a**	*MATα leu2-3,112 ade2 his3-11,15 can1-100 ura3-1 trp1-1* *MATa leu2-3,112 ade2 his3-11,15 can1-100 ura3-1 trp1-1*	R. Rothstein
W303α/α	*MATα leu2-3,112 ade2 his3-11,15 can1-100 ura3-1 trp1-1* *MATα leu2-3,112 ade2 his3-11,15 can1-100 ura3-1 trp1-1*	R. Rothstein

tions of *E. coli* (15) and yeast cells (6) were performed by electroporation.

Plasmids and probes. The parental vector for the *GAL1* fusions (with the exception of the *GAL1-DIT2* fusion) was pBM150, a derivative of YCp50 containing the *GAL1-GAL10* promoter region (30). The structures of the *GAL1-SPO13* fusion (F24), the *GAL1-SPO11* fusion (F29; provided by C. Atcheson, University of Chicago), the *GAL1-SPO12* fusion (F44), the *GAL1-IME1* fusion (F136; provided by A. Mitchell, Columbia University), and the *GAL1-HIS3* fusion (F165) have been described elsewhere (69). The *GAL1-DIT2* fusion, provided by M. Eckerstorfer, Vienna Biocenter, was constructed by inserting a *Bam*HI fragment containing the *DIT2* gene into the *Bam*HI site of plasmid YEplac195-Gal1/10 (19). The *GAL1-UME5* fusion (F144-22) was constructed by converting the *Afl*II restriction site in the 5′ untranslated region of *UME5* to a *Bam*HI site by the addition of linkers (39). The 3.7-kb *Bam*HI-*Cla*I fragment containing *UME5* was inserted into the *Bam*HI and *Sal*I sites of plasmid pBM150. The *GAL1-ume5-4* fusion was constructed in a similar manner by using the *ume5-4* allele. Plasmid pPL144-13, used in constructing the *UME5* deletions, was made by replacing the 3.2-kb *Sac*I-*Hpa*I fragment from the *UME5* region with a *Bgl*II-*Sal*I fragment containing the *LEU2* gene. The *UME5* gene was cloned into a high-copy-number vector by inserting the 4.2-kb *Cla*I-*Sac*I fragment into plasmid pRS426 (14). The *GAL1-SPO13* fusion containing a nonsense mutation (F127) (69) and the *spo13-lacZ* (67) fusion have been described previously.

All RNA probes were synthesized in SP6, T3, or T7 in vitro transcription reactions (43). The structures of the *SPO13* 3′, *SPO11*, *SPO12*, *IME1*, *GAL1*, *HIS3*, and 18S rRNA probes have been described (69). Other probes were made from the following templates: *FUS1*, 0.55-kb *Pvu*II-*Nhe*I fragment; *STE2*, 0.31-kb *Hin*dIII-*Hpa*I fragment; *ACT1*, 0.55-kb *Bam*HI-*Xho*I fragment; *DIT2*, 0.57-kb *Sph*I-*Bam*HI fragment; *UME5* 5′, 1.0-kb *Sac*I-*Stu*I fragment; *UME5* 3′, 0.72-kb *Eco*RI-*Eco*RI fragment; and *GAL1-UME5*, 0.9-kb *Eco*RI-*Stu*I fragment. This last probe contains, in addition to the *UME5* sequence, 64 bases of *GAL1* sequence and can distinguish between the *GAL1-UME5* and the wild-type *UME5* mRNAs.

Cloning and sequencing of the *UME5* gene. A plasmid library containing random yeast DNA inserted into centromeric plasmid p366, provided by P. Hieter (Johns Hopkins University), was used to transform strain RSY107 carrying a multicopy plasmid containing a *spo13-lacZ* fusion. Transformants (approximately 2,100) were grown in microtiter wells and then spotted onto Whatmann 3MM filter disks. After 36 h of growth, the filters were frozen in liquid nitrogen and overlaid with top agar containing 140 μg of 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside per ml (67).

Physical mapping was done with yeast chromosomes fractionated in a contour-clamped homogeneous electric field (CHEF) gel system (18). Further mapping was done by L. Riles and M. Olson (Washington University) with a gridded set of yeast genomic contig clones. Genetic mapping was performed by crossing strain D36 with F106 and strain D36 with F113. Genetic distances were calculated by the formula of Perkins (57).

For sequencing, a series of nested deletions were generated for both strands by using exonuclease III as described by Henikoff (25). Double-stranded templates were sequenced by the dideoxynucleotide chain termination procedure (59) with the Sequenase sequencing kit (United States Biochemicals, Cleveland, Ohio). Protein homologies were identified with the BLASTP search algorithm (2). Alignments between homologous sequences were made with the Megalign program (DNASTAR, Madison, Wis.).

RNA analysis. The procedure for measuring the half-life of *GAL1* fusion mRNAs has been described elsewhere (69). Briefly, cells bearing the fusion construct were grown in galactose-containing medium for 6 h to induce expression and then shifted to glucose-containing medium to repress transcription, and aliquots of cells were harvested and frozen at various times after the shift. In the *rpb1-1* strains (49), mRNA half-lives were measured by growing the cells in YPD medium at the permissive temperature of 25°C, shifting them to YPD medium at the nonpermissive temperature of 37°C, and removing aliquots at various times after the shift (53). Isolation of total RNA (16), analysis by S1 protection (7), and quantitation of RNA levels (69) have been described previously.

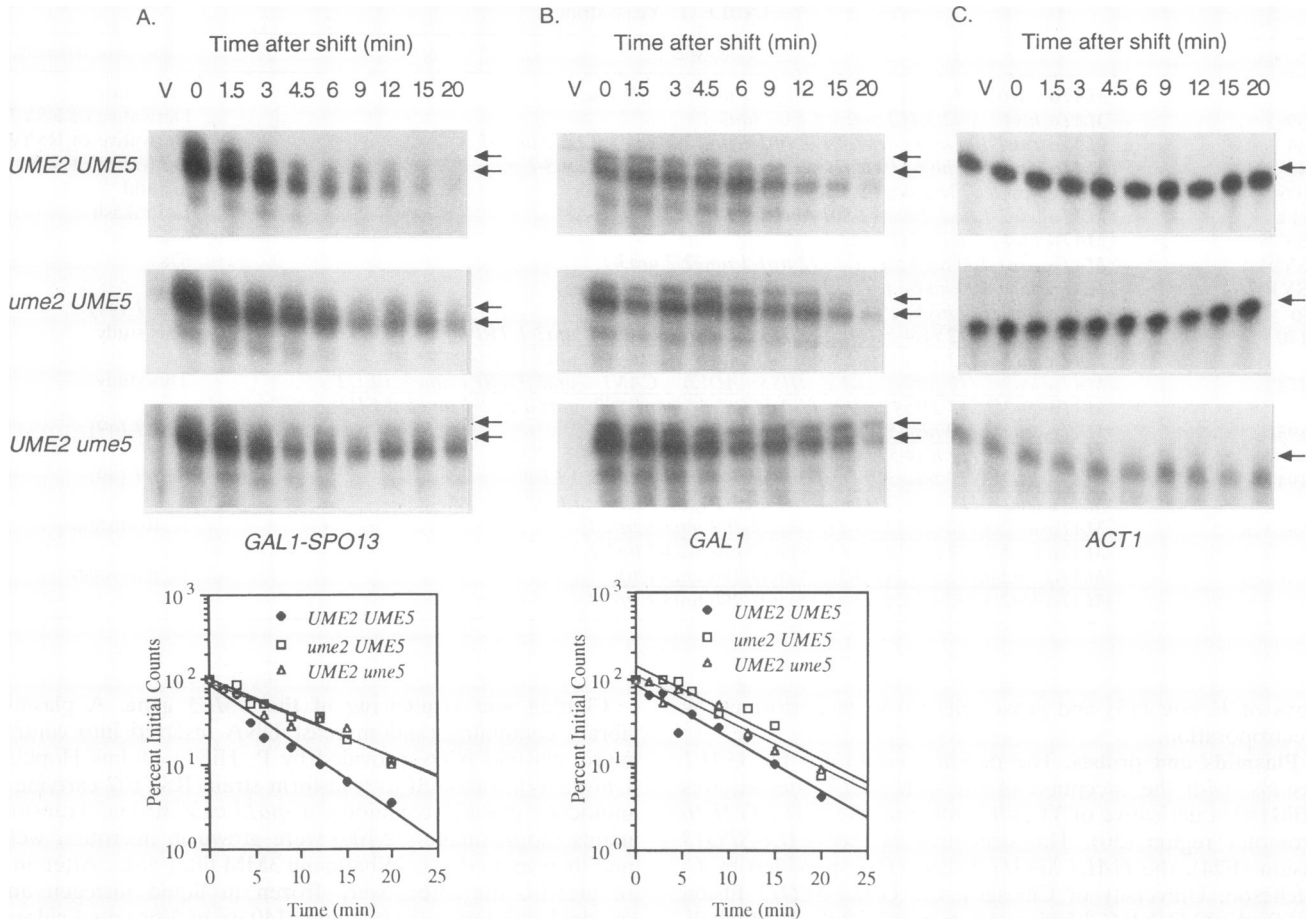

FIG. 1. Stability of *GAL1-SPO13* and *GAL1* mRNAs in wild-type, *ume2*, and *ume5* strains. (A) Strains RSY75 (*UME2 UME5*), RSY94 (*ume2 UME5*), and RSY107 (*UME2 ume5*) containing the *GAL1-SPO13* fusion (F24) were grown in galactose-containing synthetic medium and transferred to a 2% glucose solution, and aliquots were removed at the times indicated. S1 protection analysis was performed with 20 μg of total RNA and radiolabeled *SPO13* probe. The multiple bands result from multiple transcription termination sites. V, RNA prepared from log-phase cells grown in YPD medium. The *GAL1-SPO13* bands (arrows) were excised, and their radioactivities were counted. RNA levels were normalized to levels of the *ACT1* mRNA at each time point. The percentage of the fusion mRNA relative to the zero time point was determined and then plotted against time of the shift on a semi-log graph. (B) *GAL1* mRNA was analyzed as described for panel A except that a *GAL1* probe was used. The multiple bands result from multiple transcription termination sites. (C) *ACT1* mRNA was analyzed as described for panel A except that an *ACT1* probe was used.

mRNA levels were normalized against *ACT1* mRNA or 18S rRNA levels. Half-lives were determined by linear regression analysis of the data from at least two independent time courses. Any set of data with a correlation coefficient of less than 0.90 was disregarded. For any experiment, the half-lives calculated from the independent time courses differed by no more than 16%.

Northern (RNA) blot analysis was carried out with 20 μg of total RNA or 2 μg of polyadenylated RNA and 2×10^6 cpm of ^{32}P-labeled probe (71). Primer extension reaction mixes each contained 20 μg of total RNA and 4×10^4 cpm of the ^{32}P-end-labeled primer (42).

Levels of *UME5* mRNA throughout the cell cycle were determined after synchronization with synthetic α-factor (41). *UME5* mRNA levels during meiosis were examined in the isogenic diploids W303 α/**a** and W303 α/α, which were grown in YPA medium to the mid-log phase and resuspended in one-fifth volume of SPII medium.

Polyadenylation of the *GAL1-SPO13* mRNA was examined by RNase H protection analysis (29). Total mRNA (20 μg) was annealed to 5 μg of an oligonucleotide (5′-GGCCAA CAAAATCTCCGTTATTAGAAATCG). As a control, 500 μg of oligo(dT)$_{12-17}$ was added to remove the entire poly(A) tail during RNase H treatment. After hybridization, 1 U of RNase H was added, and the tubes were incubated at 37°C for 1 h. RNA samples were treated with glyoxal and fractionated on a 5% NuSieve 3:1 agarose gel (FMC, Rockland, Maine).

Site-directed mutagenesis. A mutation was inserted in the kinase domain of the *UME5* gene by PCR with the oligomers PR5 (5′-GCCATTAGAAAGTTCAAGAC) and PR6 (5′-CTTTCTAATGGCATAAAATACCG) (4). The *Sac*I-*Sph*I fragment from *UME5* was inserted into a plasmid pUC19 derivative (79), and two reactions were carried out with this plasmid as the template, one with the PR5 and T7 primers and a second with the PR6 and T3 primers. The two PCR products were gel purified and used in a third reaction with the T3 and T7 primers. The single PCR product was gel purified and inserted into the *Sac*I and *Sph*I sites of *UME5*. The gene was sequenced to verify the presence of the mutation.

Nucleotide sequence accession number. The GenBank accession number for *UME5* is L27151.

RESULTS

Meiotic mRNAs are stabilized in *ume2* and *ume5* mutants. The identification and preliminary characterization of six *ume* (for unscheduled mitotic expression) mutant genes have been described previously (67, 67a). The mutations were detected by increased expression of a *SPO13-lacZ* fusion during vegetative growth. It was subsequently shown that these alleles affect expression of the wild-type chromosomal *SPO13* gene as well.

To determine whether the increased expression results from changes in transcription or mRNA turnover, the stability of the *SPO13* mRNA was examined in each of the *ume* mutants. A transcriptional fusion between the *GAL1* promoter and the *SPO13* gene (F24), used in previous studies to measure mRNA half-life, was used in these experiments (69). Transcription of the fusion mRNA was induced to high levels in galactose medium and repressed by the addition of glucose, and the rate of decay of the mRNA was determined by quantitative S1 protection analysis and normalized to *ACT1* mRNA levels (Fig. 1C). In a congenic wild-type strain, RSY75, a half-life of 3.2 min was observed for the fusion mRNA (Fig. 1A), in good agreement with the value determined previously (69). The *ume1*, *ume3*, *ume4*, and *ume6* mutants also exhibited an mRNA half-life of approximately 3 min for the F24 transcript (data not shown). In contrast, in the *ume2* (RSY94) and *ume5* (RSY107) mutants, the fusion mRNA displayed a half-life that was twice as long (6.4 and 6.5 min, respectively) (Fig. 1A).

Two experiments ruled out the possibility that the difference in half-life observed for the *ume2* and *ume5* mutant mRNAs results from changes in repression of transcription from the *GAL1* promoter by glucose. First, the half-life of the wild-type *GAL1* transcript was similar in the wild-type, *ume2*, and *ume5* strains (5.0, 5.2, and 4.7 min, respectively) (Fig. 1B). Second, the stability of the *GAL1-SPO13* mRNA was measured in strains containing the *rpb1-1* mutation, a temperature-sensitive lesion in the RNA polymerase II gene (49). The half-life of the *GAL1-SPO13* mRNA, determined by measuring mRNA levels at various times after cells were shifted to the nonpermissive temperature for transcription, was twofold longer in the *ume2* and *ume5* mutants than in the wild-type strain (data not shown). We therefore conclude that the observed differences in the half-life of the fusion transcript result from changes in mRNA stability and not from differences in transcription from the *GAL1* promoter.

Isolation of the *UME5* gene. The remainder of this study focused on characterization of the *UME5* gene. The wild-type *UME5* gene was cloned by virtue of its ability to complement the increased mitotic expression of a *SPO13-lacZ* fusion gene in a *ume5* recipient strain. Transformants (approximately 2,100) from a yeast DNA library were screened for β-galactosidase expression, and four colonies with wild-type levels were obtained. Restriction analysis of the library plasmid in each strain revealed that they contained overlapping yeast sequences. Reintroduction of one of the plasmids into a *ume5* mutant strain decreased the half-life of the *GAL1-SPO13* fusion mRNA from approximately 6 min to 3 min (data not shown). To determine whether the wild-type *UME5* gene or a second-site suppressor had been isolated, the *LEU2* gene was integrated at the chromosomal locus of the complementing sequence in a *ume5* mutant strain. Cosegregation of the *LEU2* and *ume5* markers in all 20 tetrads analyzed demonstrated that the wild-type *UME5* gene had been isolated (data not shown).

The *UME5* gene product is similar to the family of CDC28 protein kinases. Hybridization of labeled sequences from the *UME5* region to a filter containing fractionated yeast chromosomes mapped the *UME5* gene to chromosome XVI (data not shown). By using a set of yeast genomic contig clones, the gene was further localized to the left arm of chromosome XVI, approximately 50 kb distal to the *RAD1* locus. Genetic analysis of the *ume5* locus verified its location on the left arm of chromosome XVI, 19.6 centimorgans (cM) distal to the *rad1* locus and 42.1 cM proximal to the *pep4* locus (Table 2).

TABLE 2. *UME5* linkage analysis[a]

Map interval	No. of spores			Distance (cM)
	Parental ditype	Nonparental ditype	Tetratype	
ume5-rad1	51	0	33	19.6
ume5-pep4	17	1	59	42.2

[a] Linkage for the interval from *ume5* to *rad1* was determined by crossing strains D36 and F113. Actual markers scored were *TRP1* integrated at *ume5* and *URA3* integrated at *rad1*. Linkage for the interval from *ume5* to *pep4* was determined by crossing strains D36 and F106. Actual markers scored were *TRP1* integrated at *ume5* and *URA3* integrated at *pep4*.

A 3.2-kb fragment containing the functional *UME5* gene was sequenced and found to contain a single large open reading frame of 1,665 nucleotides, encoding a predicted 62.8-kDa protein of 555 amino acids (Fig. 2 and 3). This predicted protein is basic (pI, 9.6) and has a codon adaptation index of 0.120, indicative of a poorly expressed protein (61). The sequence homology search algorithm BLASTP (2) revealed significant homology between the predicted UME5 protein and the family of serine/threonine-specific protein kinases, which includes the *S. cerevisiae* CDC28 protein (Fig. 4) (23). The predicted UME5 protein displays between 30 and 40% homology to other kinases in this family, and this homology extends to all kinase subdomains. The highly conserved amino acids present in other kinases are also found in UME5 (23). Portions of the UME5 protein outside of the kinase domain show no significant similarity to other proteins in the data base.

***UME5* is required for optimal growth rate and sporulation efficiency.** A deletion of the *UME5* gene was constructed by using plasmid pPL144-13, in which the entire *UME5* coding region was replaced by the *LEU2* gene. Transformation of diploid strain C170 to Leu prototrophy with this plasmid yielded transformants with one *UME5* and one *ume5*::*LEU2* chromosome. Among 20 tetrads analyzed from this diploid, 17 contained four viable spores and 3 contained three viable spores. In those tetrads with four viable spores, the Leu^+ phenotype segregated 2+:2−, indicating that the *UME5* gene

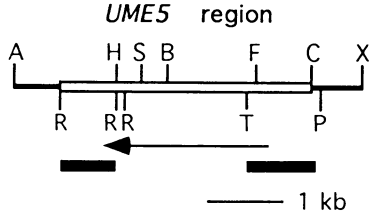

FIG. 2. Restriction map of the *UME5* region. The open box indicates the portion that was sequenced. The arrow below indicates the length and direction of the *UME5* transcript. The two solid boxes represent the probes used in the S1 protection analysis. Restriction sites: A, *Aat*II; B, *Bst*EII; C, *Sac*I; F, *Afl*II; H, *Hpa*I; P, *Pst*I; R, *Eco*RI; S, *Sph*I; X, *Xba*I. Not all restriction sites are indicated.

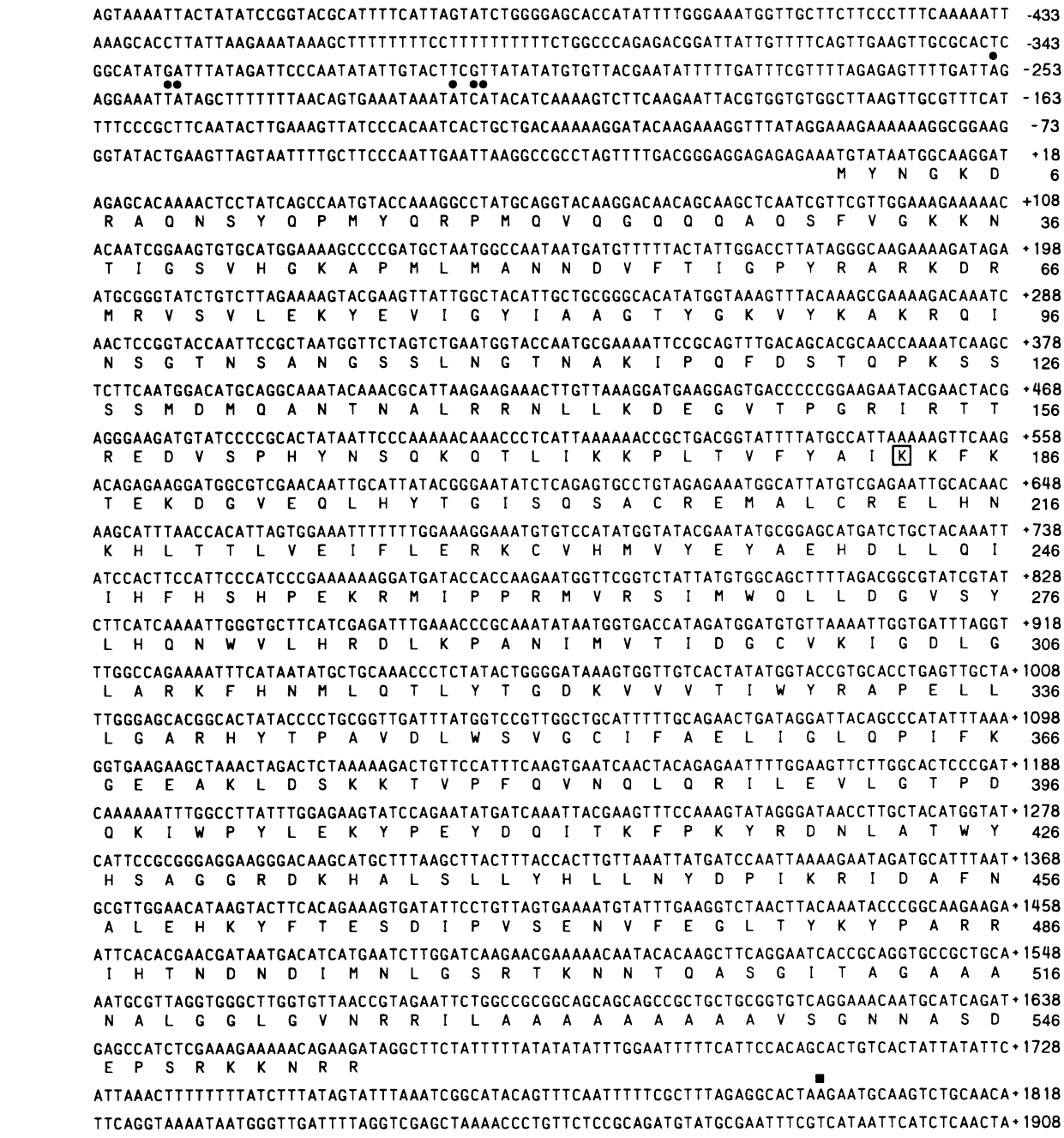

FIG. 3. Nucleotide sequence of the *UME5* gene and the predicted amino acid sequence. The top numbers in the right margin indicate the positions of the nucleotides, with the first nucleotide of the predicted translation initiation codon numbered +1. The bottom numbers in the right margin indicate the positions of the amino acids relative to this initiation codon. The solid circles above the nucleotides indicate transcription start sites determined by primer extension experiments. The solid square indicates the approximate transcription termination site determined by S1 protection analysis. The boxed lysine residue (position 183) was replaced by an arginine residue. The GenBank accession number for this sequence is L27151.

is not essential for spore germination or growth. Among those tetrads, however, two large Leu$^-$ spore colonies and two small Leu$^+$ spore colonies per tetrad were observed, suggesting that the *ume5* deletion alters growth rate.

The growth rates of a wild-type *UME5* strain (RSY75) and a *ume5*::*LEU2* deletion derivative (D39) were examined. In rich, glucose-containing (YPD) medium, during mid-log phase, the generation time of the *ume5* mutant was 1.5-fold longer than that of the wild-type strain (1.46 h for RSY75 versus 1.94 h for D39) (Fig. 5A). In rich, acetate-containing (YPA) medium, the two strains displayed no difference in generation times (data not shown). Comparison of the rate of turnover of *GAL1-SPO13* mRNA in the two strains showed that the transcript is twofold more stable in the *ume5* deletion strain (3.3 min in RSY75 versus 6.9 min in D39), similar to the effect of the original *ume5-2* mutation (Fig. 5B). Finally, a diploid homozy-

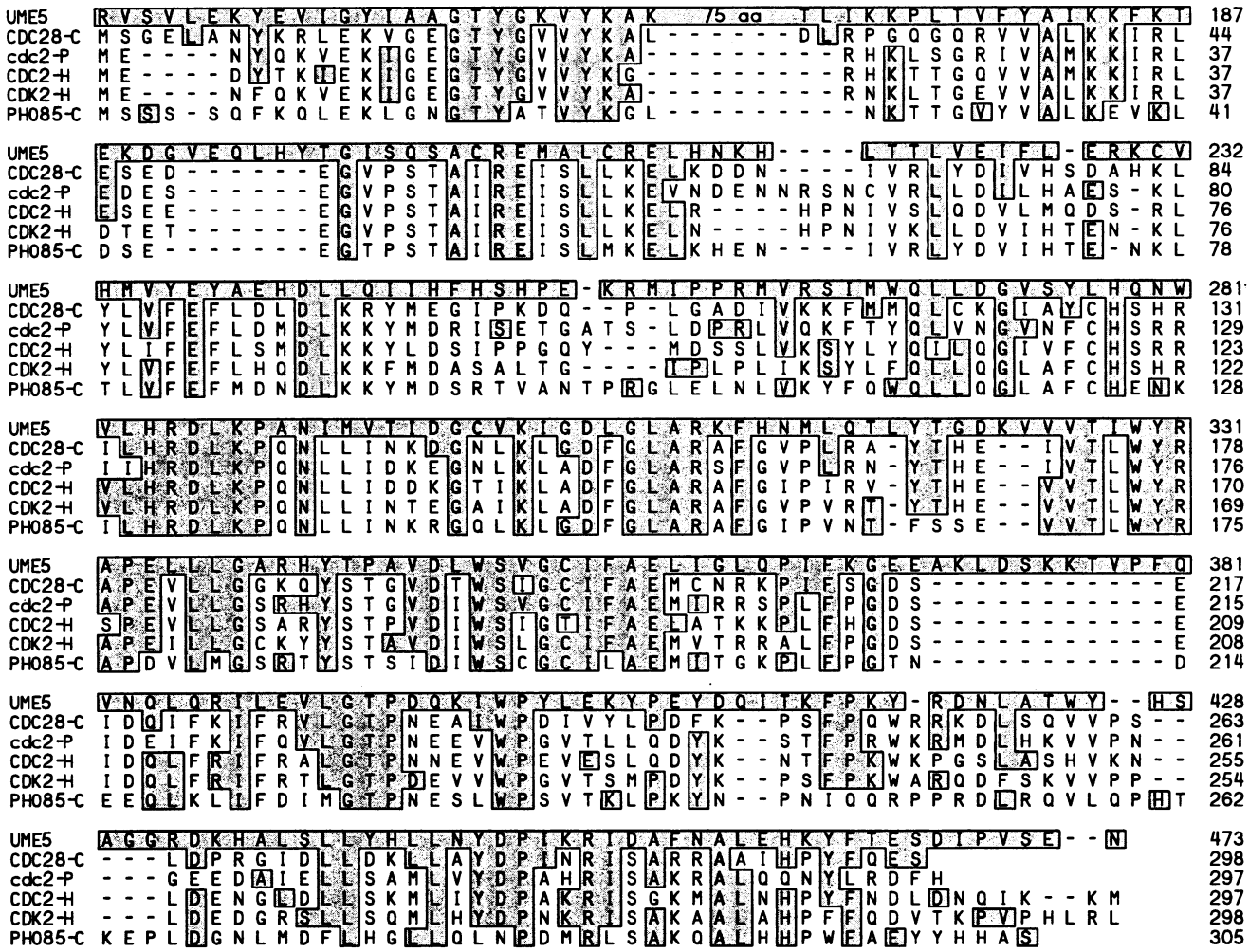

FIG. 4. Comparison of the UME5 protein with members of the CDC28 family of serine/threonine-specific kinases. Amino acids identical to those in the *UME5* sequence are boxed and shaded. The predicted UME5 protein shows 38% identity to the *S. cerevisiae* CDC28 protein (CDC28-C) over 298 amino acids (36), 36% identity to the *Schizosaccharomyces pombe* cdc2 protein (cdc2-P) over 297 amino acids (64), 37% identity to the human CDC2 protein (CDC2-H) over 297 amino acids (34), 37% identity to the human CDK2 protein (CDK2-H) over 298 amino acids (74), and 32% identity to the *S. cerevisiae* PHO85 protein (PHO85-C) over 305 amino acids (72). Spacing between kinase subdomains in UME5 is similar to that found in other kinases except for the large insertion (75 amino acids [aa]) between the first and second subdomain, as noted in the figure (23).

gous for the *ume5* deletion (C173) sporulated less efficiently than a wild-type diploid (C170) (30.5% versus 69.0%, respectively). However, among the diploids that do sporulate, no changes in sporulation kinetics or spore germination were observed.

Growth, sporulation, and mRNA turnover were also monitored in strains that overexpress the *UME5* gene. At least 20-fold overexpression was achieved by either inserting the gene on a high-copy-number plasmid containing a 2μm origin of replication or fusing the coding portion of the gene to the *GAL1* promoter. Strains carrying the *UME5* overexpression plasmids showed no changes in generation time, sporulation efficiency, or stability of the *GAL1-SPO13* fusion mRNA, suggesting that *UME5* activity is not limited by transcript level (data not shown).

***UME5* specifically decreases the stability of meiotic transcripts.** The specificity of the *UME5* gene in controlling mRNA stability was determined by comparing the half-lives of a variety of vegetative and meiotic transcripts in wild-type and *ume5::LEU2* mutant strains, by using either *GAL1* fusions or the temperature-sensitive *rpb1-1* RNA polymerase II allele (Table 3). The results demonstrate that the *ume5* mutation stabilizes all meiotic transcripts 1.6- to 2.2-fold, whether they are expressed in early (*SPO13* [78], *SPO11* [3], and *IME1* [33]), middle (*SPO12* [38]), or middle-late (*DIT2* [10]) meiosis, but does not affect any of the vegetative mRNAs tested (*GAL1*, *HIS3*, *ACT1*, *STE2*, and *FUS1*).

Mutation of a highly conserved amino acid in the kinase domain. The role of the predicted kinase domain in the function of the UME5 protein was determined by altering one of the highly conserved amino acids, a lysine residue, in this domain (Fig. 3) (23). Alteration of the lysine residue at the homologous position in the $p60^{src}$ protein inactivated the protein (32). A conservative substitution of arginine for lysine was made at position 183 in the predicted UME5 protein sequence, and plasmids bearing this mutant allele (*ume5-4*) on the wild-type allele (*UME5*) were introduced into *ume5::LEU2* mutant strains (haploid D39 and diploid C173). We observed

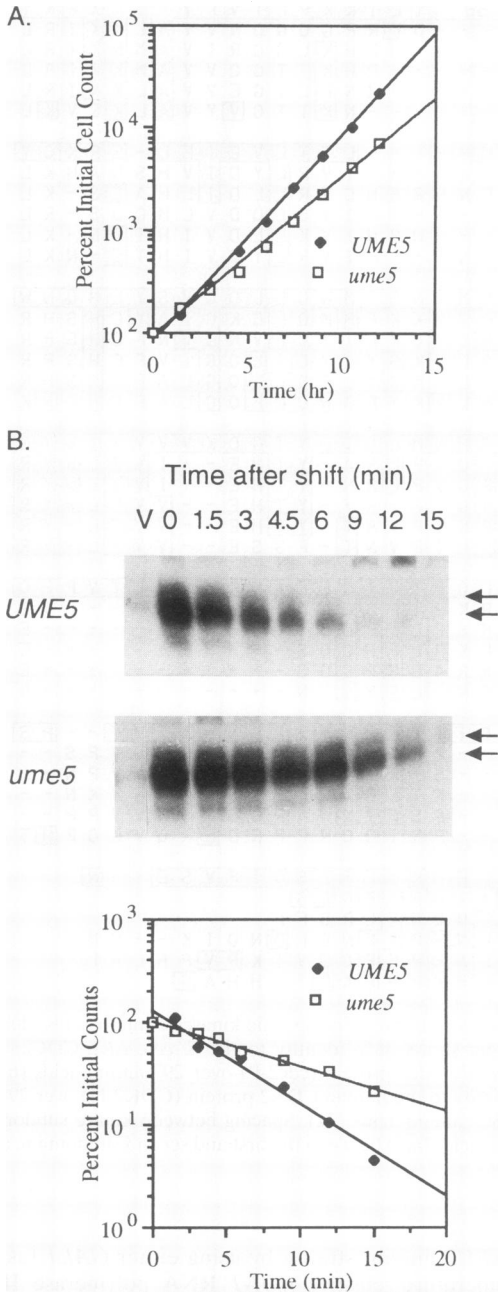

FIG. 5. Growth and mRNA stability in a *ume5* deletion strain. (A) Strains RSY75 (*UME5*) and D39 (*ume5*) were grown at 30°C in YPD medium, and cell densities were measured at 1.5-h intervals with a hemacytometer. Densities relative to that at the zero time point were calculated and plotted against time on a semi-log graph. (B) mRNA turnover in strains RSY75 and D39 containing the *GAL1-SPO13* fusion (F24) was analyzed as described in the legend to Fig. 1A with a *SPO13* probe. V, RNA prepared from log-phase cells grown in YPD medium. Multiple bands from the *GAL1-SPO13* mRNA (arrows) result from multiple transcription termination sites.

an increase in generation time (3.4 h for the *ume5-4* allele and 2.4 h for the *UME5* allele) (Fig. 6A), a decrease in sporulation efficiency (42% for the *ume5-4* and 86.5% for the *UME5* allele), and a twofold increase in the stability of the *GAL1-SPO13* mRNA (5.8 min for the *ume5-4* and 2.9 min for the

TABLE 3. Specificity of the *UME5* gene in regulating mRNA stability

Transcript[a]	mRNA half-life[b] (min)		mRNA half-life ratio, *ume5* mutant/wild type
	Wild type	*ume5* mutant	
Meiotic			
SPO13	3.3	6.9	2.1
SPO11	3.1	5.4	1.7
IME1	3.2	7.0	2.2
SPO12[c]	7.7	14.3	1.8
DIT2	9.1	14.9	1.6
Mitotic			
GAL1	4.7	5.2	1.1
HIS3	5.7	6.6	1.2
ACT1[d]	25.5	22.2	0.88
STE2[d]	3.1	3.5	1.1
FUS1[d]	4.4	4.0	0.91

[a] Half-lives were measured by using a *GAL1* fusion unless stated otherwise.
[b] The wild-type strain was RSY75 for measurements with a *GAL1* fusion and S86 for measurements with the *rbp1-1* mutant. The *ume5* mutant strain was D39 for measurements with a *GAL1* fusion and D46 for measurements with the *rbp1-1* mutant.
[c] Our previous report that *SPO12* expression was unaffected by *UME5*, based on steady-state mRNA levels, was irreproducible (63).
[d] Half-life measured by using the *rbp1-1* mutant.

UME5 allele) (Fig. 6B) compared with strains bearing the wild-type *UME5* allele. Since the phenotypes produced by this mutant allele and the *ume5* deletion allele are identical, we conclude that kinase activity plays an important role in the function of the UME5 protein.

Transcription and stability of *UME5* mRNA. The *UME5* mRNA was identified as a 2.0-kb transcript in polyadenylated RNA by Northern blot analysis (data not shown). Further characterization by S1 protection analysis revealed a single transcription termination site and three initiation sites (Fig. 2 and 3). Primer extension studies defined three clusters of major initiation sites plus a number of minor initiation sites (Fig. 3). In cells grown in YPD medium, the *UME5* transcript is present at similar steady-state levels in mid-log- and stationary-phase cells, and its levels remain constant at all stages of the cell cycle (data not shown). After a shift to sporulation medium, a *MAT*α/*MAT***a** diploid (W303 α/**a**) shows a 2.6-fold increase in *UME5* mRNA levels, but a similar induction is also observed in a *MAT*α/*MAT*α diploid (W303 α/α) (Fig. 7A). Therefore, this induction is not meiosis specific but results from growth under starvation conditions. This response has been observed for a number of vegetative genes (31).

The stability of the *UME5* mRNA was examined during vegetative growth by using a *GAL1-ume5-4* transcriptional fusion (F144-27). The *ume5-4* allele, containing the kinase domain mutation, is transcribed and translated normally, although the resulting protein is nonfunctional. A probe capable of distinguishing the fusion and wild-type *UME5* mRNAs was used. The results in Fig. 7B show that the fusion mRNA is highly unstable, with a half-life of 2.2 min in a wild-type strain and 2.0 min in a *ume5*::*LEU2* strain. These data provide evidence that the *UME5* gene does not regulate the stability of its own transcript.

***UME5* does not affect polyadenylation.** Studies with *S. cerevisiae* have demonstrated that mutations that alter poly(A) tail length or the rate of deadenylation may also alter mRNA half-life (45, 47). The rate of deadenylation of the *GAL1-SPO13* mRNA was therefore examined in wild-type (RSY75) and *ume5* deletion (D39) strains. RNA was annealed to a deoxyoligonucleotide complementary to a sequence near the 3′

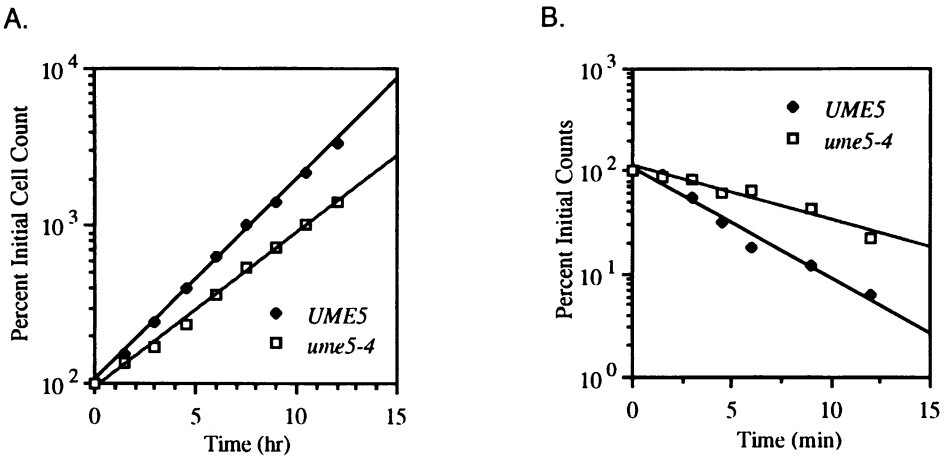

FIG. 6. Growth and transcript stability in a strain with a mutation in the kinase domain of *ume5*. (A) Generation times for strain D39 containing a centromeric plasmid with either the *UME5* or *ume5-4* allele were determined as described in the legend to Fig. 5A except that the cells were grown in synthetic medium to maintain selection for the plasmids. (B) mRNA turnover in strain D39 (*ume5*) containing the *GAL1-SPO13* fusion (F24) and a centromeric plasmid with either the *UME5* or *ume5-4* allele was analyzed as described in the legend to Fig. 1A with the *SPO13* probe.

end (positions 716 to 744) of the *GAL1-SPO13* mRNA. The RNA was then treated with RNase H, which degrades the RNA strand of RNA-DNA hybrids. Treatment with RNase H in the presence of oligo(dT) results in removal of the entire poly(A) tail. In both strains, the length of the completely deadenylated 3′ fragment was approximately 200 nucleotides, in good agreement with the major transcription termination site of the *SPO13* gene (Fig. 8, lanes 1 and 5). The polyadenylated transcripts are represented by a diffuse signal trailing the 200-nucleotide band. During transcription of the fusion mRNA (in galactose medium), the maximum length of this polyadenylated fragment was approximately 275 nucleotides, indicating a poly(A) tail length of 75 nucleotides in both the wild-type and mutant strains (Fig. 8, lanes 2 and 6). At subsequent time points after the cessation of transcription (in glucose medium), the length of the poly(A) tract decreased, but at the same rate in the wild-type and mutant strains (Fig. 8, lanes 3, 4, 7, and 8). Thus, the *UME5* gene does not exert its effects on stability through changes in poly(A) tail length or rate of deadenylation.

***UME5* and *UME2* act in the same pathway.** Genetic epistasis analysis was used to determine whether *UME5* and *UME2* act in the same or independent pathways. We reasoned that if the mutant alleles act in the same pathway, their combined effects on mRNA stability should be similar to their individual effects, whereas if they act in independent pathways, their combined effects should be greater. A double mutant (D44) was created by introducing the *ume5*::*LEU2* mutation into a *ume2* mutant strain (RSY94). The half-life of the *GAL1-SPO13* fusion in the *ume2* single mutant and *ume2 ume5* double mutant was 6.2 and 5.9 min, respectively, indicating that the two mutations act in the same pathway (Fig. 9A).

Previously, we reported that drugs or mutations that disrupt the normal translation of the *SPO13* mRNA stabilize the transcript (69). The same rationale outlined for the epistasis study above was used to determine whether destabilization of the *GAL1-SPO13* transcript by the *UME5* gene requires translation of this mRNA. In this experiment, a *GAL1-SPO13* fusion bearing a nonsense mutation at position +19 (F127) was introduced into a wild-type strain (RSY75) and a *ume5* mutant strain (D39). As demonstrated previously, the half-life of the nontranslated *GAL1-SPO13* mRNA is 5.9 min in the wild-type strain, approximately twofold longer than that observed for the wild-type *SPO13* fusion mRNA (F24) (compare Fig. 9B with Fig. 1A). In the *ume5* strain, this half-life increased to 13.4 min. These results provide evidence for two independent turnover pathways, one dependent on translation and the other dependent on the *UME5* gene.

***UME5* destabilizes the chromosomal *SPO13* mRNA in glucose-containing medium.** Previous studies showed that in wild-type strains, the *SPO13* mRNA is twofold less stable in glucose (vegetative) than in acetate (sporulation) medium (69). To determine whether the *UME5* gene mediates degradation of the *SPO13* mRNA specifically in glucose medium, mRNA turnover was assayed in wild-type (C193) and *ume5* mutant (C194) diploids homozygous for the *rpb1-1* mutation. RNA stability was examined after the diploids were shifted from acetate medium at the permissive temperature for transcription to glucose or acetate medium at the nonpermissive temperature for transcription. In agreement with previous results, in the wild-type diploid, the chromosomal *SPO13* mRNA displayed a half-life of 3.3 min in glucose medium and 6.2 min in acetate medium (Fig. 10A). In the *ume5* mutant diploid in glucose medium, the chromosomal *SPO13* mRNA showed a half-life of 6.6 min, very similar to the stability observed for the *GAL1-SPO13* fusion transcript (Fig. 10B). In contrast to the wild-type strain, the *ume5* mutant displayed little change in *SPO13* mRNA stability in acetate medium, which had a half-life of 5.9 min. Similar results were obtained when the chromosomal *SPO11* mRNA was analyzed under identical conditions (data not shown). These results demonstrate that the *UME5* gene regulates the chromosomal mRNAs and *GAL1* fusion mRNAs similarly. More importantly, the twofold destabilization of meiotic transcripts in glucose medium requires the *UME5* gene product.

DISCUSSION

In this report, we describe the isolation and characterization of the *UME5* gene. This gene (i) destabilizes meiotic transcripts approximately twofold but has no effect on vegetative transcripts, (ii) is not essential for cell viability but is required for optimum growth rate in glucose-containing medium, (iii) encodes a predicted protein with homology to the CDC28

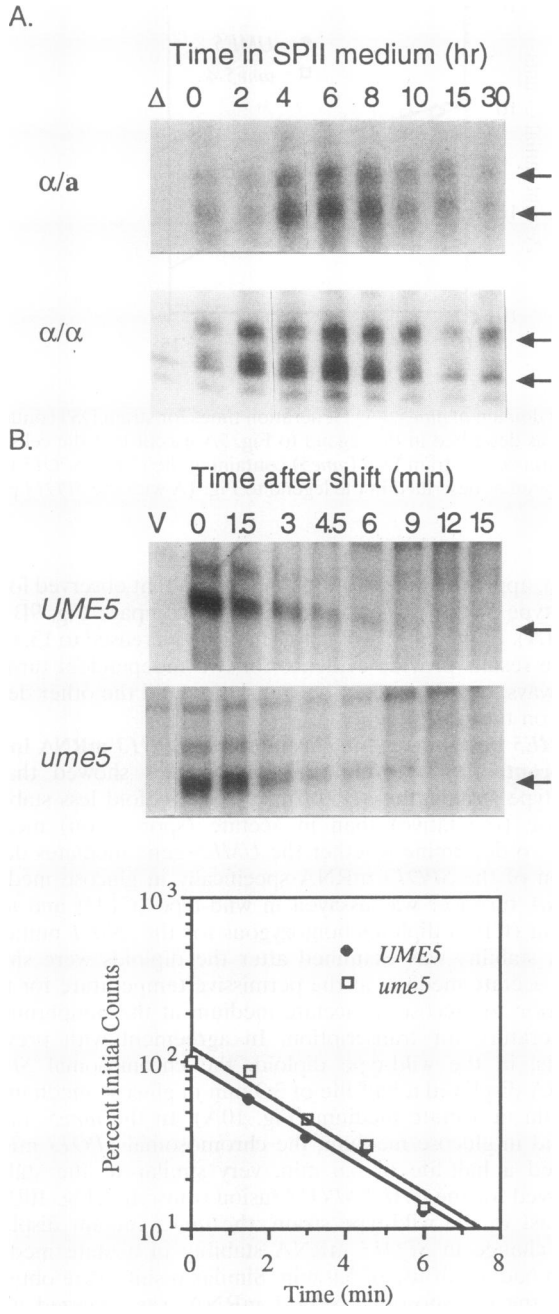

FIG. 7. Expression and stability of *UME5* mRNA. (A) Diploids W303 α/**a** and W303 α/α were grown to mid-log phase in YPA medium and shifted to SPII sporulation medium, and aliquots were removed at the times indicated. RNA was examined by S1 protection analysis with the *UME5* 5′ probe. Δ, RNA prepared from *ume5* deletion strain D39; α/**a**, strain W303 α/**a**; α/α, W303 α/α. The multiple bands result from multiple *UME5* transcription initiation sites. (B) mRNA turnover in strains RSY75 (*UME5*) and D39 (*ume5*) containing the *GAL1-ume5-4* fusion (F144-22) was analyzed as described in the legend to Fig. 1A with the *GAL1-UME5* probe. V, RNA prepared from log-phase cells grown in vegetative medium. Arrows indicate the *GAL1-ume5-4* fusion mRNA.

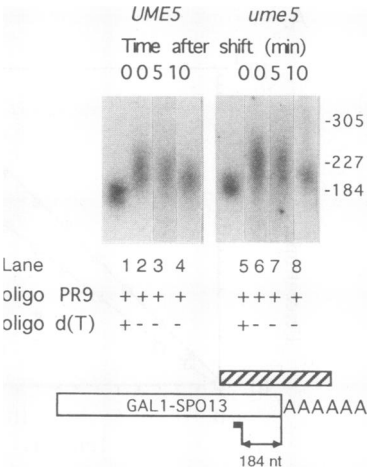

FIG. 8. Polyadenylation in a *ume5* mutant strain. Strains RSY75 (*UME5*) and D39 (*ume5*) containing *GAL1-SPO13* fusion (F24) were induced in galactose-containing synthetic medium and shifted to a 2% glucose solution; at the times indicated, aliquots were removed and RNA was prepared. Total RNA (20 μg) was hybridized with the oligonucleotides indicated and then digested with RNase H. Products were fractionated on an agarose gel, transferred to a nylon membrane, and detected by hybridization with the *SPO13* 3′ probe. +, oligonucleotide added; −, oligonucleotide not added. Numbers at the right indicate the positions of size markers (in nucleotides). The diagram below shows the *SPO13* mRNA (open rectangle), the site of annealing of the PR9 deoxyoligonucleotide (solid rectangle), and the *SPO13* probe used in detection (hatched rectangle).

family of serine/threonine-specific protein kinases and is inactivated by a mutation in this kinase domain, and (iv) is required to destabilize meiotic transcripts in response to glucose. These results suggest that glucose, presumably acting through a signal transduction pathway, activates the UME5 protein, which phosphorylates a substrate that (directly or indirectly) increases the rate of turnover of meiotic mRNAs (Fig. 11). Accordingly, in the absence of glucose, the UME5 protein is inactive, no phosphorylation occurs, and meiotic mRNAs are stabilized. Below, we discuss evidence for this model, considering first how the *UME5* gene might control turnover of meiotic transcripts and then how *UME5* activity itself is regulated.

The *ume5* mutation was identified by a twofold increase in the steady-state levels of meiotic mRNAs in vegetative cells (67). In this study, we found that during vegetative growth, the *ume5* mutation stabilizes meiotic transcripts by as much as 2.2-fold. Thus, the increases in the steady-state levels of meiotic mRNAs can be completely explained by changes in mRNA stability. The *UME5* gene regulates the stability of all meiotic transcripts tested, irrespective of their time of peak induction. In contrast, all of the vegetative mRNAs tested are unaffected by the gene regardless of their stability or whether they are expressed constitutively or conditionally. We specifically examined the stabilities of mRNAs encoded by genes under cell type control (*FUS1*) and mating type control (*STE2*), since these transcripts display short half-lives, similar to early meiotic transcripts (26). The results suggest that meiotic mRNAs possess some feature that distinguishes them from vegetative transcripts and places them specifically under *UME5* regulation.

At least two components that are required for rapid turnover of the *SPO13* mRNA, translation and *cis*-acting sequences, have been identified (69). The *SPO13* transcript is

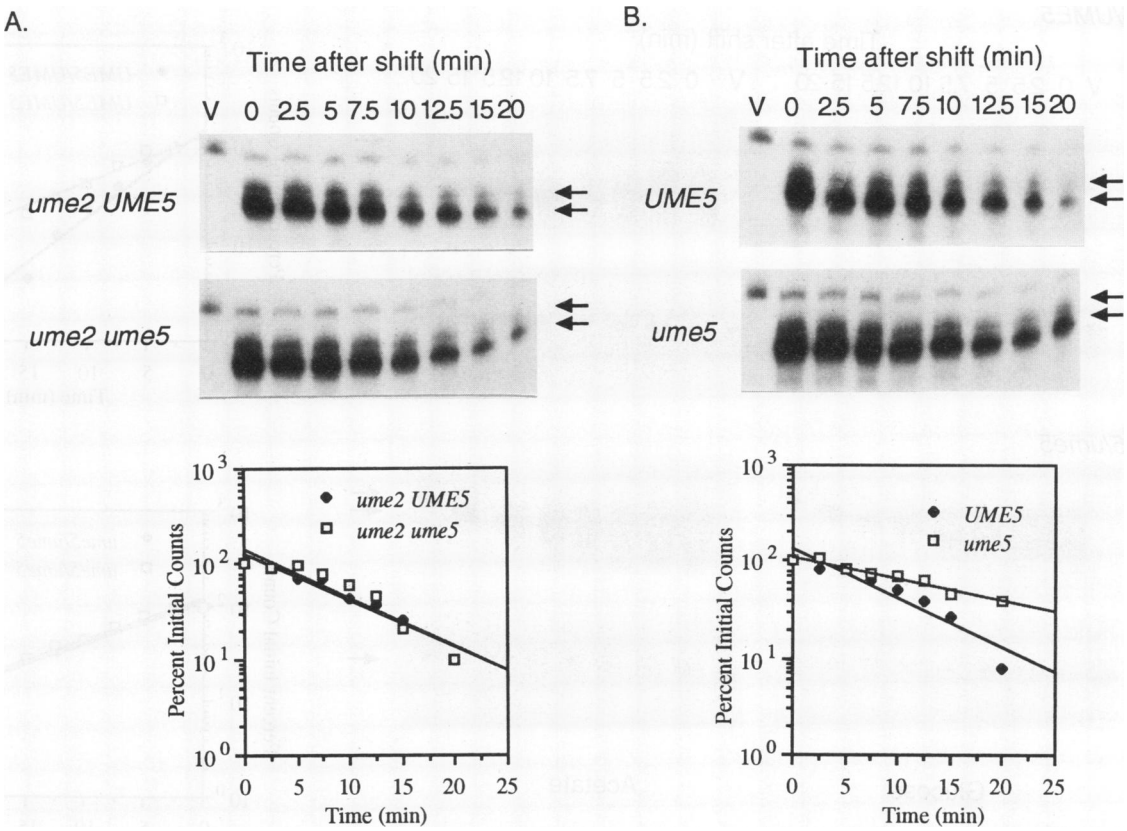

FIG. 9. Turnover in *ume5* double mutants. (A) Strains RSY94 (*ume2*) and D44 (*ume2 ume5*) containing the *GAL1-SPO13* fusion (F24) were analyzed with the *SPO13* probe as described in the legend to Fig. 1A. V, RNA prepared from log-phase cells grown in YPD medium. (B) Strains RSY75 (*UME5*) and D39 (*ume5*) containing a *GAL1-SPO13* fusion with a nonsense mutation (F127) were analyzed as in panel A.

stabilized by a translation inhibitor or a nonsense mutation, indicating that normal translation destabilizes the mRNA. Other transcripts, including the yeast *MATα1* mRNA, are also destabilized by translation, which represents an important pathway for regulating transcript turnover (52). Since the stability of a *SPO13* transcript containing a nonsense mutation is still regulated by the *UME5* gene, we conclude that the *UME5* gene does not require translation for its activity and that the two pathways act independently. Two *cis*-acting determinants within the coding region of the *SPO13* mRNA have been identified, and preliminary data indicate that the *SPO11* mRNA contains a different stability determinant in the 5′ untranslated region (68, 69). Since deletion of any of these sequences increases transcript stability twofold, one or more of these determinants may be recognized by the *UME5* pathway.

In vivo and in vitro analyses of several transcripts has demonstrated that mRNA degradation is a multistep pathway (9, 47, 76). The first step is deadenylation of the transcript, during which all or most of the poly(A) tail is removed. The transcript is then subjected to nucleolytic degradation. For some yeast transcripts, such as *MFA2*, deadenylation is a rate-limiting step, and mutations that alter the rate of deadenylation also change the half-life of the mRNA (47). In contrast, the degradation of the *PGK1* transcript is not limited by deadenylation, and the half-life of this mRNA is determined by the rate of endonucleolytic digestion (76). The role of polyadenylation in regulating the stability of meiotic transcripts in *S. cerevisiae* is not known, but the experiments here rule out any effects of the *UME5* gene on deadenylation. In comparing the *GAL1-SPO13* mRNA in wild-type and *ume5* deletion strains, the length of the poly(A) tract and the rates of deadenylation of the mRNA are identical. Alternatively, the *UME5* pathway may alter the activity of a nuclease that specifically degrades meiotic mRNAs or may modify the transcripts to make them more susceptible to nucleolytic attack.

The factors that regulate the activity of the *UME5* gene are not known. Two lines of evidence from this study indicate that the activity of the *UME5* gene is not limited by the abundance of its mRNA. First, although *UME5* transcript levels increase more than twofold during sporulation, rates of meiotic mRNA turnover actually decrease. Second, 20-fold overexpression of the *UME5* mRNA has no effect on the stability of meiotic transcripts, generation time, or sporulation efficiency. The data also indicate that the *UME5* gene does not regulate the stability of its own mRNA. The activity of the UME5 protein may be modulated in some other way, perhaps by phosphorylation. Multiple kinases are involved in the pathway that mediates glucose repression of transcription (see below), including one capable of autophosphorylation (13, 37). The predicted UME5 protein does contain several possible phosphorylation sites, including one for tyrosine kinases (54). One important component in the *UME5* pathway is the *UME2* gene product, as indicated by genetic epistasis studies, although the relationship between the two genes is not known. Overexpression of the *UME5* gene does not suppress a *ume2* mutation, suggesting that their activities are not complementary (70). Further experiments are required to determine whether the

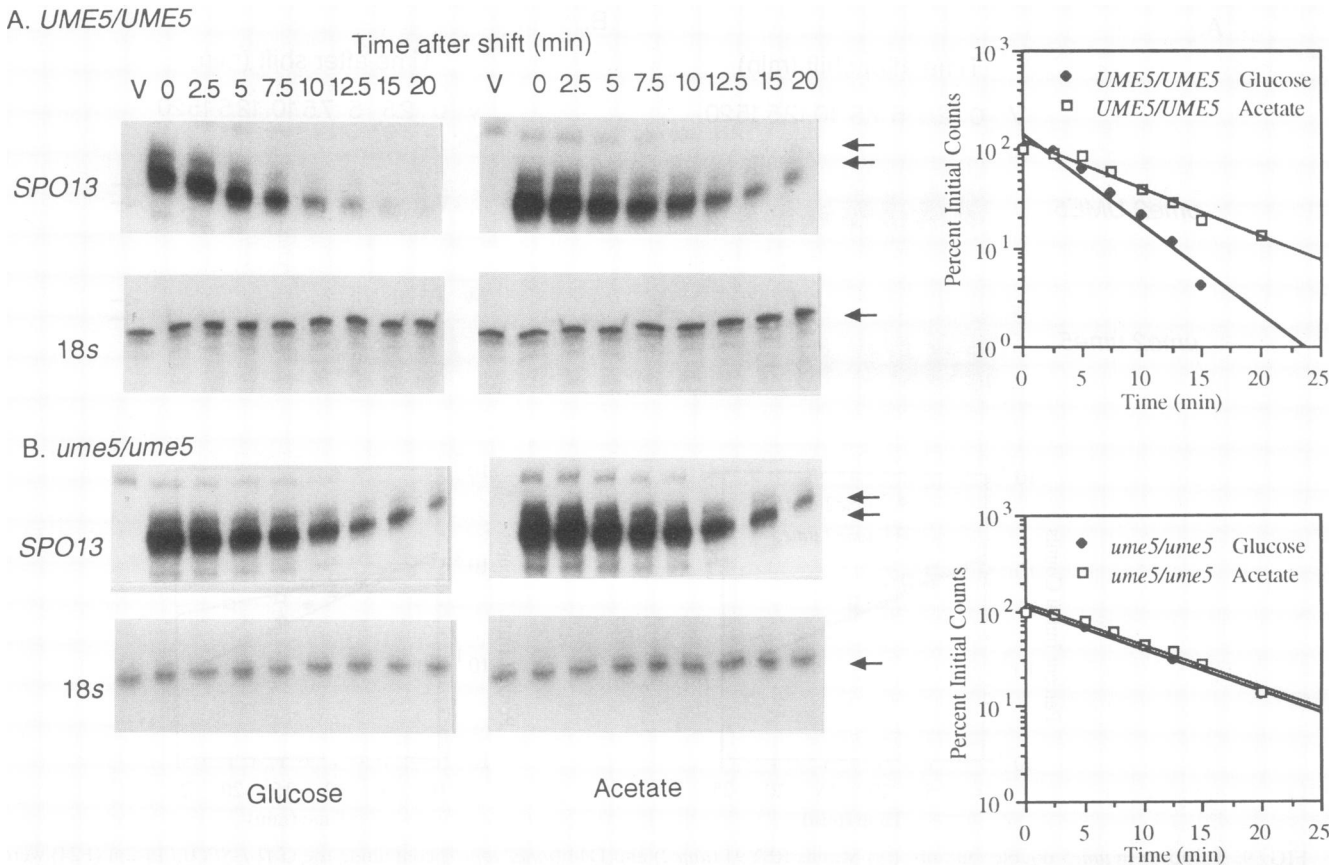

FIG. 10. Stability of *SPO13* mRNA in the wild-type and *ume5* mutant diploids in glucose and acetate media. (A) Diploid C193 (*UME5*), homozygous for the *rpb1-1* mutation, was incubated in acetate (SPII) medium at the permissive temperature for 8 h to induce expression of the *SPO13* gene and shifted to glucose or acetate medium at the nonpermissive temperature, and aliquots were removed at the times indicated. Total RNA (20 μg for the *SPO13* probe, 0.2 μg for the 18S rRNA probe) was analyzed with the *SPO13* and 18S rRNA probes. V, RNA prepared from log-phase cells grown in YPD medium. The results for the *SPO13* mRNA were quantitated and graphed as described in the legend to Fig. 1A except that RNA levels were normalized to 18S rRNA levels (which are unaffected by the *rpb1-1* mutation) at each time point. (B) Diploid C194 (*ume5*) was treated and analyzed as described in the legend to panel A.

UME2 gene is also required for destabilizing meiotic transcripts in response to glucose.

In yeast cells, glucose or catabolite repression controls the expression of many genes in different metabolic pathways (11, 73). These genes are repressed in glucose-containing medium and induced when glucose is replaced by a nonfermentable carbon source, such as glycerol or acetate. For most genes, this regulation appears to occur at the level of transcription, although recent studies indicate that glucose may have post-transcriptional effects as well (21, 35). For example, the iron-binding (Ip) (and perhaps the flavoprotein) subunit of succinate dehydrogenase in *S. cerevisiae* has been shown to be repressed by glucose at the level of both transcription and mRNA stability (35). The half-life of the Ip mRNA decreases 12-fold after a shift from glycerol to glucose medium. It is not known whether other glucose-repressible genes are also regulated by mRNA turnover, but these studies raise the interesting possibility that the *UME5* gene may control the stability of a broader class of transcripts. It must be noted that all glucose-repressible genes cannot be controlled in this manner, since the *GAL1* mRNA is not regulated by *UME5*.

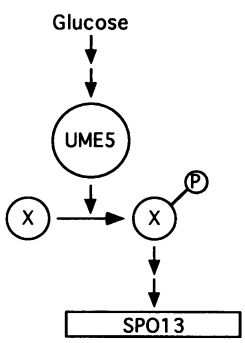

FIG. 11. Simple model summarizing the role of the *UME5* gene in regulating meiotic mRNA stability in response to glucose. A circled X indicates a substrate of UME5 kinase.

Meiotic genes are induced as much as 70- to 100-fold during the course of sporulation. Compared with the effects of transcription, the contribution to this induction made by changes in mRNA stability is small. Here we have identified at least two pathways, one *UME5* dependent and one translation dependent, that destabilize the *SPO13* mRNA. In this case, if one pathway is inactivated, the presence of another pathway(s) ensures the rapid turnover of the *SPO13* mRNA. The evolution of multiple degradation pathways may be especially

important to keep meiotic gene expression to a minimum during vegetative growth, when meiotic gene products can be detrimental to the cell. For example, high-level mitotic expression of the *SPO13* gene results in cell cycle arrest (40). It is tempting to speculate that mechanisms that rapidly degrade mRNA may also be critical for cells to accommodate rapid transitions from one developmental state to another. For example, yeast cells in the early stages of meiotic development can re-enter the cell division cycle if shifted to vegetative medium (28, 65). Immediately after the shift, transcription of the early meiotic genes is rapidly repressed, and the existing early meiotic transcripts are quickly degraded, in part by the action of the *UME5* pathway (69). The rapid degradation of the early meiotic transcripts thus may allow cells to resume vegetative growth rather than proceed unneccessarily through meiosis and spore formation.

ACKNOWLEDGMENTS

We thank members of the Esposito laboratory for helpful discussions; C. Atcheson, C. Fritze, L. Henninger, and B. Washburn for critical reading of the manuscript; and J. Ceccatti for preparation of the figures.

This work was supported by National Research Service Training Award postdoctoral training grant GM12358 (to R.T.S.) and Public Health Service grant GM29182 (to R.E.E.).

REFERENCES

1. **Aharon, T., and R. J. Schneider.** 1993. Selective destabilization of short-lived mRNAs with the granulocyte-macrophage colony-stimulating factor AU-rich 3′ noncoding region is mediated by a cotranslational mechanism. Mol. Cell. Biol. **13:**1971–1980.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Atcheson, C. L., B. DiDomenico, S. Frackman, R. E. Esposito, and R. T. Elder.** 1987. Isolation, DNA sequence, and regulation of a meiosis-specific eukaryotic recombination gene. Proc. Natl. Acad. Sci. USA **84:**8035–8039.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. (ed.)** 1991. Current protocols in molecular biology. Greene Publishing Associates, New York.
5. **Baker, B. S., A. T. C. Carpenter, M. S. Esposito, R. E. Esposito, and L. Sandler.** 1976. The genetic control of meiosis. Annu. Rev. Genet. **10:**53–134.
6. **Becker, D. M., and L. Guarente.** 1991. High-efficiency transformation of yeast by electroporation. Methods Enzymol. **194:**182–187.
7. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12:**721–732.
8. **Bowdish, K. S., and A. P. Mitchell.** 1993. Bipartite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**2172–2181.
9. **Brewer, G., and J. Ross.** 1988. Poly(A) shortening and degradation of the 3′ A+U-rich sequences of human c-*myc* mRNA in a cell-free system. Mol. Cell. Biol. **8:**1697–1708.
10. **Briza, P., M. Breitenbach, A. Ellinger, and J. Segall.** 1990. Isolation of two developmentally regulated genes involved in spore wall maturation in *Saccharomyces cerevisiae*. Genes Dev. **4:**1775–1789.
11. **Carlson, M.** 1987. Regulation of sugar utilization in *Saccharomyces* species. J. Bacteriol. **169:**4873–4877.
12. **Casey, J. L., M. W. Hentze, D. M. Koeller, S. W. Caughman, T. D. Rouault, R. D. Klausner, and J. B. Harford.** 1988. Iron-responsive elements: regulatory RNA sequences that control mRNA levels and translation. Science **240:**924–928.
13. **Celenza, J. L., and M. Carlson.** 1986. A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science **233:**1175–1180.
14. **Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter.** 1992. Multifunctional yeast high-copy-number shuttle vectors. Gene **110:**119–122.
15. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6143.
16. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from yeast transposable element Ty*1* has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80:**2432–2436.
17. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Gerring, S. L., C. Connelly, and P. Hieter.** 1991. Positional mapping of genes by chromosome blotting and chromosome fragmentation. Methods Enzymol. **194:**57–77.
19. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
20. **Graves, R. A., N. B. Pandey, N. Chodchoy, and W. F. Marzluff.** 1987. Translation is required for regulation of histone mRNA degradation. Cell **48:**615–626.
21. **Hahn, S., and L. Guarente.** 1988. Yeast HAP2 and HAP3: transcriptional activators in a heteromeric complex. Science **240:**317–321.
22. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
23. **Hanks, S. K., A. M. Quinn, and T. Hunter.** 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science **241:**42–52.
24. **Heaton, B., C. Decker, D. Muhlrad, J. Donahue, A. Jacobson, and R. Parker.** 1992. Analysis of chimeric mRNAs derived from the *STE3* mRNA identifies multiple regions within yeast mRNAs that modulate mRNA decay. Nucleic Acids Res. **20:**5365–5373.
25. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:**351–359.
26. **Herrick, D., R. Parker, and A. Jacobson.** 1990. Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2269–2284.
27. **Hollingsworth, N. M., L. Goetsch, and B. Byers.** 1990. The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61:**73–84.
28. **Honigberg, S. M., C. Conicella, and R. E. Esposito.** 1992. Commitment to meiosis in *Saccharomyces cerevisiae*: involvement of the *SPO14* gene. Genetics **130:**703–716.
29. **Hwang, S.-P., M. Eisenberg, R. Binder, G. S. Shelness, and D. L. Williams.** 1989. Predicted structures of apolipoprotein II mRNA constrained by nuclease and dimethyl sulfate reactivity: stable secondary structures occur predominantly in local domains via intraexonic base pairing. J. Biol. Chem. **264:**8410–8418.
30. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**1440–1448.
31. **Kaback, D. B., and R. L. Feldberg.** 1985. *Saccharomyces cerevisiae* exhibits a sporulation-specific temporal pattern of transcript accumulation. Mol. Cell. Biol. **5:**751–761.
32. **Kamps, M. P., and B. M. Sefton.** 1986. Neither arginine nor histidine can carry out the function of lysine 295 in the ATP-binding site of $p60^{src}$. Mol. Cell. Biol. **6:**751–757.
33. **Kassir, Y., D. Granot, and G. Simchen.** 1988. *IME1*: a positive regulator gene of meiosis in *S. cerevisiae*. Cell **52:**853–862.
34. **Lee, M. G., and P. Nurse.** 1987. Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. Nature (London) **327:**31–35.
35. **Lombardo, A., G. P. Cereghino, and I. E. Scheffler.** 1992. Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**2941–2948.
36. **Lorincz, A. T., and S. I. Reed.** 1984. Primary structure homology between the product of the yeast cell division gene *CDC28* and vertebrate oncogenes. Nature (London) **307:**183–185.
37. **Ma, H., L. M. Bloom, C. T. Walsh, and D. Botstein.** 1989. The residual enzymatic phosphorylation activity of hexokinase II mutants is correlated with glucose repression in *Saccharomyces*

cerevisiae. Mol. Cell. Biol. **9**:5643–5649.
38. **Malavasic, M. J., and R. T. Elder.** 1990. Complementary transcripts from two genes necessary for normal meiosis in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:2809–2819.
39. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **McCarroll, R. M.** Unpublished data.
41. **McCarroll, R. M., and W. L. Fangman.** 1988. Time of replication of yeast centromeres and telomeres. Cell **54**:505–513.
42. **McKnight, S. L., and R. Kingsbury.** 1982. Transcription control signals of a eukaryotic protein-coding gene. Science **217**:316–324.
43. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
44. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Minvielle-Sebastia, L., B. Winsor, N. Bonneaud, and F. Laccroute.** 1991. Mutations in the yeast *RNA14* and *RNA15* genes result in an abnormal mRNA decay rate: sequence analysis reveals an RNA-binding domain in the RNA15 protein. Mol. Cell. Biol. **11**:3075–3087.
46. **Mitchell, A. P., S. E. Driscoll, and H. E. Smith.** 1990. Positive control of sporulation-specific genes by the *IME1* and *IME2* products in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:2104–2110.
47. **Muhlrad, D., and R. Parker.** 1992. Mutations affecting stability and deadenylation of the yeast *MFA2* transcript. Genes Dev. **6**:2100–2111.
48. **Mullner, E. W., and L. C. Kuhn.** 1988. A stem-loop in the 3′ untranslated region mediates iron-dependent regulation of transferrin receptor mRNA stability in the cytoplasm. Cell **53**:815–825.
49. **Nonet, M., C. Scafe, J. Sexton, and R. Young.** 1987. Eukaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol. Cell. Biol. **7**:1602–1611.
50. **Pachter, J. S., T. J. Yen, and D. W. Cleveland.** 1987. Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. Cell **51**:283–292.
51. **Pandey, N. J., and W. F. Marzluff.** 1987. The stem-loop structure at the 3′ end of the histone mRNA is necessary and sufficient for regulation of histone mRNA stability. Mol. Cell. Biol. **7**:4557–4559.
52. **Parker, R., and A. Jacobson.** 1990. Translation and a 42-nucleotide segment within the coding region of the mRNA encoded by the *MATα1* gene are involved in promoting rapid mRNA decay in yeast. Proc. Natl. Acad. Sci. USA **87**:2780–2784.
53. **Parker, R., S. Peltz, D. Herrick, and A. Jacobson.** 1991. Measurement of mRNA decay rates in *Saccharomyces cerevisiae*. Methods Enzymol. **194**:415–422.
54. **Patschinsky, T., T. Hunter, F. S. Esch, J. A. Cooper, and B. M. Sefton.** 1982. Analysis of the sequence of amino acids surrounding sites of tyrosine phosphorylation. Proc. Natl. Acad. Sci. USA **79**:973–977.
55. **Peltz, S. W., G. Brewer, P. Bernstein, P. A. Hart, and J. Ross.** 1991. Regulation of mRNA turnover in eukaryotic cells. Crit. Rev. Eukaryotic Gene Expression **1**:99–126.
56. **Percival-Smith, A., and J. Segall.** 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:142–150.
57. **Perkins, D. D.** 1949. Biochemical mutants in the smut fungus *Usilago maydis*. Genetics **34**:607–626.
58. **Sachs, A. B.** 1993. Messenger RNA degradation in eukaryotes. Cell **74**:413–421.
59. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
60. **Shah, J. C., and M. J. Clancy.** 1992. *IME4*, a gene that mediates *MAT* and nutritional control of meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12**:1078–1086.
61. **Sharp, P. M., and W.-H. Li.** 1987. The codon adaptation index—a measure of directional synonymous codon usage bias and its potential applications. Nucleic Acids Res. **15**:1281–1295.
62. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1974. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
63. **Shyu, A.-B., J. G. Belasco, and M. E. Greenberg.** 1991. Two distinct destabilizing elements in the c-*fos* message trigger deadenylation as a first step in rapid mRNA decay. Genes Dev. **5**:221–231.
64. **Simanis, V., and P. Nurse.** 1986. The cell cycle control gene $cdc2^+$ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. Cell **45**:261–268.
65. **Simchen, G., R. Pinon, and Y. Salts.** 1972. Sporulation in *Saccharomyces cerevisiae*: premeiotic DNA synthesis, readiness and commitment. Exp. Cell Res. **75**:207–218.
66. **Smith, H. E., and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**:2142–2152.
67. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86**:10018–10022.
67a. **Strich, R., R. T. Surosky, C. Steber, E. Dubois, F. Messenguy, and R. E. Esposito.** *UME6* is a key regulator of nitrogen repression and meiotic development. Genes Dev., in press.
68. **Surosky, R. T., C. L. Atcheson, and R. E. Esposito.** Unpublished data.
69. **Surosky, R. T., and R. E. Esposito.** 1992. Early meiotic transcripts are highly unstable in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12**:3948–3958.
70. **Surosky, R. T., and R. E. Esposito.** Unpublished data.
71. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
72. **Toh-e, A., K. Tanaka, Y. Uesono, and R. B. Wickner.** 1988. *PHO85*, a negative regulator of the PHO system, is a homolog of the protein kinase gene, *CDC28*, of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **214**:162–164.
73. **Trumbly, R. J.** 1992. Glucose repression in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. **6**:15–21.
74. **Tsai, L.-H., E. Harlow, and M. Meyerson.** 1991. Isolation of the human *cdk2* gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase. Nature (London) **353**:174–177.
75. **Vidal, M., R. Strich, R. E. Esposito, and R. F. Gaber.** 1991. *RPD1* (*SIN3/UME4*) is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11**:6306–6316.
76. **Vreken, P., and H. A. Raue.** 1992. The rate-limiting step in yeast *PGK1* mRNA degradation is an endonucleolytic cleavage in the 3′-terminal part of the coding region. Mol. Cell. Biol. **12**:2986–2996.
77. **Wang, H., I. Clark, P. R. Nicholson, I. Herskowitz, and D. J. Stillman.** 1990. The *Saccharomyces cerevisiae SIN3* gene, a negative regulator of HO, contains four paired amphipathic helix motifs. Mol. Cell. Biol. **10**:5927–5936.
78. **Wang, H. T., S. Frackman, J. Kowalisyn, R. E. Esposito, and R. Elder.** 1987. Developmental regulation of *SPO13*, a gene required for separation of homologous chromosomes at meiosis I. Mol. Cell. Biol. **7**:1425–1435.
79. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
80. **Yen, T. J., D. A. Gay, J. S. Pachter, and D. W. Cleveland.** 1988. Autoregulated changes in stability of polyribosome-bound β-tubulin mRNAs are specified by the first 13 translated nucleotides. Mol. Cell. Biol. **8**:1224–1235.