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Early Meiotic Transcripts Are Highly Unstable in *Saccharomyces cerevisiae*

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Meiosis in *Saccharomyces cerevisiae* requires the induction of a large number of genes whose mRNAs accumulate at specific times during meiotic development. This study addresses the role of mRNA stability in the regulation of meiosis-specific gene expression. Evidence is provided below demonstrating that the levels of meiotic mRNAs are exquisitely regulated by both transcriptional control and RNA turnover. The data show that (i) early meiotic transcripts are extremely unstable when expressed during either vegetative growth or sporulation, and (ii) transcriptional induction, rather than RNA turnover, is the predominant mechanism responsible for meiosis-specific transcript accumulation. When genes encoding the early meiotic mRNAs are fused to other promoters and expressed during vegetative growth, their mRNA half-lives, of under 3 min, are among the shortest known in *S. cerevisiae*. Since these mRNAs are only twofold more stable when expressed during sporulation, we conclude that developmental regulation of mRNA turnover can be eliminated as a major contributor to meiosis-specific mRNA accumulation. The rapid degradation of the early mRNAs at all stages of the yeast life cycle, however, suggests that a specific RNA degradation system operates to maintain very low basal levels of these transcripts during vegetative growth and after their transient transcriptional induction in meiosis. Studies to identify specific *cis*-acting elements required for the rapid degradation of early meiotic transcripts support this idea. A series of deletion derivatives of one early meiosis-specific gene, *SPO13*, indicate that its mRNA contains determinants, located within the coding region, which contribute to the high instability of this transcript. Translation is another component of the degradation mechanism since frameshift and nonsense mutations within the *SPO13* mRNA stabilize the transcript.

In *Saccharomyces cerevisiae*, diploid cells expressing *MAT***a** and *MAT*α information undergo meiosis and spore formation (sporulation) when starved for glucose and nitrogen. The successful execution of meiosis requires the integration of a number of events including DNA synthesis, recombination, and two meiotic divisions (2, 10). Mutant analysis and differential hybridization studies have identified a number of genes required for each of the meiotic events (7, 10). At specific times in meiosis, the mRNAs for many of these genes accumulate 70- to 100-fold over mitotic levels. Based on their time of peak induction, sporulation-specific genes are classified as early, middle, or late. Two early genes, *SPO11*, required for meiotic recombination, and *SPO13*, required for meiosis I, are induced within 1.5 h after transfer to sporulation medium and reach maximum induction 6 h into sporulation (1, 40). The mRNAs from middle meiotic genes, such as *SPO12* required for meiosis I, reach peak levels at 8 to 10 h (23), and the mRNAs for late meiotic genes, such as *SPS2*, reach maximum levels after 10 h (31). Studies thus far have not distinguished whether the induction of these genes is achieved by changes in transcription, changes in mRNA stability, or a combination of both. The present analysis was initiated to answer this question.

The role of mRNA stability in regulating gene expression in yeast cells is generally not well understood. Studies to determine vegetative mRNA stabilities using either drugs that inhibit transcription or a temperature-sensitive mutation in RNA polymerase II have produced mRNA half-lives ranging from under 3 min to over 1 h (14, 33). Efforts to define the factors responsible for these differences have shown that there is no apparent correlation between mRNA stability and transcript size (14), length of poly(A) tail (14, 32), or efficiency of ribosome loading onto the mRNA (32). Even less is known about mRNA turnover during meiosis, primarily because of poor uptake of labeled precursors into cells during sporulation and reincorporation of radioactive isotopes from cells prelabeled during growth (due to RNA degradation during sporulation) (27, 36). Nevertheless, using both labeling procedures and an in vitro translation system, it was demonstrated that many vegetative mRNAs as well as meiosis-specific mRNAs are degraded during sporulation (16, 21), while other selected mRNAs are packaged into the mature ascospores (21). These changes in specific RNAs are not obvious in comparisons of total mRNA populations in mitotic and meiotic cells in which the average mRNA half-life (20 min) and the percentage of poly(A)$^+$ RNA appear to be similar (19).

In the experiments described below, RNA stability was measured by procedures designed to eliminate the precursor labeling problems noted above. The contribution of mRNA degradation to meiotic transcript accumulation was evaluated as well as the effects of developmental program, ploidy, and growth medium on turnover. To identify *cis*-acting stability determinants, we constructed an extensive set of alterations in one meiotic gene, *SPO13*, and measured the stabilities of the modified transcripts. The effect of translation of the *SPO13* mRNA on stability was also investigated.

MATERIALS AND METHODS

Strains and media. Yeast strains used in this study are shown in Table 1. Plasmids were maintained in *Escherichia coli* LE392 (12). Transformations in *E. coli* (8) and *S.*

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TABLE 1. Yeast strains

Strain	Genotype	Source
C18	*MATα can1-100 leu2-3,112 LYS2 trp1 ura3-52 HO::LYS2* *MATa CAN1 LEU2 lys2 TRP1 ura3-52 ho*	This study
F83	*MATa his4 leu2-3,112 ura3-1*	RSY75 (35)
S71	*MATa ade2 ade6 his3-200 rpb1-1 ura3-52*	*rpb1-1* from reference 25
C146	*MATα ade2 his3-200 his4 leu2 LYS2 rpb1-1 trp1 ura3-52* *MATa ADE2 his3-200 HIS4 LEU2 lys2 rpb1-1 trp1 ura2-52*	*rpb1-1* from reference 25
S65	*MATa his3-1 his4 leu2-3,112 trp1-289 ura3-52*	This study

cerevisiae (13) were performed by electroporation with the Gene Pulsar (Bio-Rad Laboratories, Richmond, Calif.). For yeast electroporation, the cells were grown in yeast extract-peptone-dextrose (YPD) to the mid-log phase, washed twice, and resuspended in 0.01 volume of 10% glycerol. One hundred microliters of cells was mixed with 1 μg of DNA, and current was applied at settings of 2.5 kV and 25 μFD with the pulse controller set at 1,000 Ω. Luria-Bertani medium for growth of *E. coli* (26), YPD, and complete synthetic medium for growth and SPII (acetate) medium for sporulation of yeast cells have been described previously (35). Yeast complete synthetic medium lacking uracil (Com-ura) and containing 2% glucose, galactose, or potassium acetate is indicated by Com-ura+glu, Com-ura+gal, or Com-ura+ace, respectively.

Plasmids. In plasmid constructions involving DNA fragments with noncompatible ends, sticky ends were filled in with Klenow enzyme before the addition of linkers (24). Junctions resulting from end filling and/or the addition of linkers were sequenced to verify that no deletions or insertions had occurred.

All fusions were constructed in *CEN-ARS* vectors. The parental vector for the *GAL1* fusions was pBM150 (17). Oligo-directed mutagenesis (20) was used to create an *Xho*I site at position −13 in the *SPO13* gene, and the 998-bp *Xho*I-*Pst*I fragment was inserted into the *Bam*HI site of plasmid pBM150 to construct the original *GAL1-SPO13* fusion (F24). The *ACT1-SPO13* fusion (F99) was constructed by inserting the same *SPO13* fragment into a derivative of plasmid YCp50 containing the *ACT1* 415-bp *Bam*HI-*Alu*I fragment at the *Bam*HI-*Sal*I sites. The *SPO13-HIS3* fusion (pN2) provided by M. Slater (University of Chicago) consists of the 157-bp *Eco*RI-*Xho*I *SPO13* fragment fused to the 882-bp *Eco*RI-*Xho*I *HIS3* fragment. The *GAL1-SPO11* fusion (pCA29) containing the 1,579-bp *Sau*3A *SPO11* fragment was provided by C. Atcheson (University of Chicago). The *GAL1-SPO12* fusion (F44) contains the 1,056-bp *Spe*I-*Bgl*II fragment. The *GAL1-HIS3* fusion (F75) contains the 882-bp *Eco*RI-*Xho*I fragment. The *GAL1-IME1* fusion (HS136) was provided by A. Mitchell (Columbia University) and contains a 2.4-kb *IME1* fragment.

In the *SPO13* 5′ deletion series, the deleted *SPO13* gene was fused directly to the *GAL1* sequences. Fusion F82 contains the 983-bp *Ban*I-*Pst*I *SPO13* fragment; F32 contains the 948-bp *Bst*EII-*Pst*I fragment; F30 contains the 848-bp *Dra*I fragment; F28 contains the 374-bp *Hpa*I-*Pst*I fragment. All *SPO13* 3′ deletion fusions were derived from fusion F78, consisting of *GAL1* sequences joined to the 401-bp *Bgl*II-*Xho*I *HIS3* fragment by a *Bam*HI linker. *SPO13* sequences were inserted at this *Bam*HI site. Linkers of 8, 10, and 12 bp were used to maintain the translational reading frame in the 5′ deletion series. Fusion F155 contains the 770-bp *Xho*I-*Sty*I *SPO13* fragment; F81 contains the 624-bp *Xho*I-*Hpa*I fragment; F106 contains the 420-bp *Xho*I-*Hpa*II fragment; F90 contains the 275-bp *Xho*I-*Sca*I fragment; F105 contains the 243-bp *Xho*I-*Bst*NI fragment; F105 contains the 187-bp *Xho*I-*Hin*fI fragment; F80 contains the 132-bp *Xho*I-*Dra*I fragment; F79 contains the 56-bp *Xho*I-*Bst*EII fragment; F85 contains the 19-bp *Xho*I-*Ban*I fragment. All *SPO13* internal deletions were derived from fusion F24, and in all constructs, the translational reading frame was maintained. Fusion F102 lacks the 258-bp *Ban*I-*Sca*I *SPO13* fragment; F113 lacks the 35-bp *Ban*I-*Bst*EII fragment; F115 lacks the 77-bp *Bst*EII-*Dra*I fragment; F116 lacks the 141-bp *Dra*I-*Sca*I fragment. Fusion F138 contains an oligo-directed deletion mutation of 72 bp; F153 contains a deletion of 87 bp; F154 contains a deletion of 65 bp.

Frameshift mutations were constructed by the insertion of *Bam*HI 8-, 10-, and 12-bp linkers into the *Bst*EII site of fusion F24. Nonsense mutations were constructed by the insertion of *Xba*I 14-bp linkers containing stop codons in all reading frames (New England Biolabs, Beverly, Mass.) into the +19 site (an artificially created *Bam*HI site) of fusion F24 or the *Bam*HI site of the 3′ deletion plasmids at the *SPO13-HIS3* junction.

Probes. All RNA probes used in the S1 nuclease analysis were synthesized in SP6, T3, or T7 in vitro transcription reactions (25). The probes were made from the following templates: *SPO13* 3′ probe, 0.50-kb *Xho*I-*Xba*I fragment; *SPO13* 5′ probe, 0.82-kb *Eco*RI-*Dra*I fragment; *SPO11* probe, 0.64-kb *Hin*dIII-*Eco*RI fragment; *SPO12* probe, 0.42-kb *Eco*RI-*Bam*HI fragment; *SPO16* probe, 0.68-kb *Bgl*II-*Hin*dIII fragment; *SPS2* probe, 0.76-kb *Bgl*II fragment; *GAL1* probe, 1.1-kb *Eco*RI-*Dra*I fragment; 18*s* rRNA probe, 0.75-kb *Hin*dIII-*Sac*II fragment; *ACT1* probe, 1.0-kb *Bam*HI-*Bgl*II fragment; *HIS3* probe, 0.40-kb *Bgl*II-*Xho*I fragment; *IME1* probe, 1.1-kb *Eco*RI-*Sac*I fragment.

Time course for turnover of mRNAs. Strain C18, F83, or S65, containing a plasmid bearing a fusion gene, was grown in Com-ura+glu medium at 30°C to the mid-log phase, and 10 ml was removed and frozen. The remainder was washed and resuspended in an equal volume of Com-ura+gal or SPII medium. After 6 h (2×10^7 cells per ml), the cells were harvested and resuspended in an equal volume of YPD medium or 2% glucose. At the appropriate times, 10-ml samples were removed, and cells were collected by filtration through 1.2-μm-pore-size filters (Micron Separations, Westboro, Mass.) and frozen in liquid nitrogen. mRNA stability in the *rpb1-1* strains, S71 and C146, was measured after 6 h in SPII medium at 25°C. Half of the culture was shifted to SPII medium at 37°C, and the other half was shifted to a 2% glucose solution at 37°C. Aliquots of cells were removed at 0, 1.5, 3, 4.5, 6, 9, 12, and 15 min after the shift. In wild-type strains, RNA half-lives measured at 30 and 37°C were nearly identical. Steady-state levels of RNA were measured after 8

h of growth in Com-ura+glu or Com-ura+ace medium to 10^7 cells per ml.

RNA analysis. Total RNA was isolated by the glass bead disruption method (9). RNAs were detected by S1 nuclease reactions (3) with 20 μg of total RNA and 20,000 cpm of probe. Samples were fractionated on a 5% acrylamide (19:1 acrylamide-bisacrylamide)–8.3 M urea gel. RNAs were quantitated by excising bands from the gel and subjecting them to liquid scintillation spectrometry with Econoflour scintillation solution (Dupont, NEN Research Products, Boston, Mass.). Levels of the mRNA were normalized against *ACT1* mRNA levels or, in the case of mRNA derived from strains S71 and C146, against 18*s* rRNA levels. Half-lives were determined by linear regression analysis 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 differ by no more than 18%.

In vivo RNA labeling. Cells were grown at 30°C in 200 ml of Com-ura+gal medium to the mid-log phase. The culture was split, harvested, and resuspended in 10 ml of Com-ura+gal or 10 ml of Com-ura+glu medium. After 5 min, 0.5 mCi of $^{32}P_i$ was added to each culture. After another 5 min, the cells were harvested by filtration, and RNA was isolated. The yeast sequences probed were the same as those used to prepare the SP6 labeled probes (described in a previous section). Twenty micrograms of plasmid DNA was denatured and dot blotted onto Nytran filter membranes. The filters were probed with 2×10^7 cpm of labeled RNA. Each spot was cut out, and its radioactivity was counted to quantitate RNA levels.

Inhibition of protein synthesis by verrucarin A. Two 100-ml cultures were grown in Com-ura+gal to the mid-log phase and harvested, and each was resuspended in 50 ml of water. Verrucarin A (Sigma Chemical Co., St. Louis, Mo.) dissolved in dimethyl sulfoxide was added to a final concentration of 25 μg/ml to one flask, and dimethyl sulfoxide alone was added to the other (6). After 2 min, 5 ml of cells was removed for polysome profiles. Forty-five milliliters of a 4% glucose solution was added to each flask, and an RNA turnover time course was performed. Polysomes were prepared by the glass bead procedure and fractionated on a 7 to 47% (wt/wt) sucrose gradient (41).

RESULTS

Early meiosis-specific transcripts decay rapidly after return to growth. The first indication that meiotic transcripts were unusually unstable came from experiments in which cells were interrupted during normal meiotic development and cultured under conditions that permitted them to resume mitotic cell division. Meiotic yeast cells can resume mitotic division up until a point designated commitment to meiosis if they are resupplied with rich growth medium (e.g., containing glucose and nitrogen) (36). Since return to growth occurs even after a number of meiosis-specific genes have been induced, we inquired about the fate of these meiotic transcripts after a shift of meiotic cells from sporulation medium to vegetative medium (YPD). The levels of specific mRNAs that accumulate in meiosis were determined by quantitative S1 analysis (see Materials and Methods). The results indicated that the levels of those mRNAs that accumulated early in meiosis underwent an extremely rapid decrease, while those that accumulated later in meiosis declined at a slower rate. In the experiment shown in Fig. 1, diploid strain C18 was shifted after 6 h in sporulation medium to examine the decay of the mRNA from the early meiotic gene *SPO13*. The transcript levels began to decline shortly after the shift and by 15 min were barely detectable (Fig. 1A). Normalization relative to *ACT1* mRNA levels, which remain constant under all conditions, indicated that the decay rate (apparent half-life) of the *SPO13* mRNA was 2.9 min (Table 2). Transcripts from two other early meiosis-specific genes, *SPO11* and *SPO16*, also displayed short apparent half-lives of 2.7 and 3.0 min, respectively, when shifted after 6 h in sporulation medium. Decay of the *SPO12* mRNA, a middle-expression transcript, was examined after 9 h in sporulation medium. In contrast to the early transcripts, this mRNA was detectable 30 min after the shift and showed an apparent half-life of 10.0 min (Fig. 1B and Table 2). Transcripts from the late gene *SPS2* also displayed a longer apparent half-life of 11.9 min.

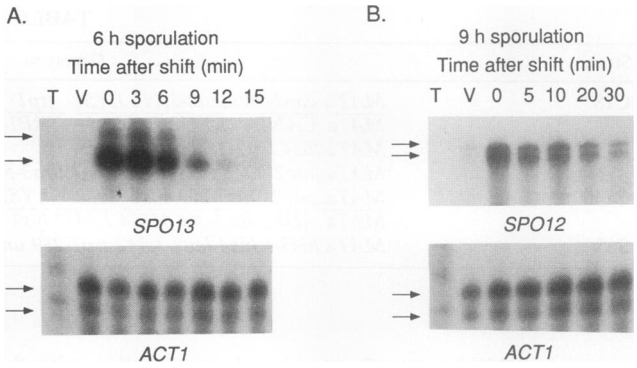

FIG. 1. Decay in levels of meiosis-specific transcripts after return to growth. Diploid C18 was shifted from SPII sporulation medium to YPD vegetative growth medium at times of peak induction for each mRNA, and aliquots were removed at the indicated times after the shift. T, tRNA (20 μg) control; V, RNA prepared from log-phase cells in vegetative growth medium. (A) Cells were shifted after 6 h in SPII medium, and RNA was hybridized with *SPO13* 3′ and *ACT1* probes. The two bands produced by the *SPO13* probe result from the two transcription stop sites, and the two bands produced by the *ACT1* probe result from two transcription start sites. In each case, both bands were counted. (B) Cells were shifted after 9 h in SPII medium, and RNA was hybridized with *SPO12* and *ACT1* probes. The two bands produced by the *SPO12* probe, resulting from two transcription termination sites, were counted.

The rate of decay of the meiosis-specific transcripts analyzed was not altered significantly by the length of time that cells remained in sporulation medium before the shift. The *SPO13* mRNA displayed apparent half-lives of 2.8, 2.9, and

TABLE 2. Decay of meiosis-specific transcripts after shift to vegetative medium

mRNA	mRNA apparent half-life (min)[a] with the following no. of hours of sporulation before shift:			
	4	6	9	11
SPO13	2.8	2.9	3.2	ND[b]
SPO11	2.9	2.7	2.9	ND
SPO16	3.3	3.0	2.8	ND
SPO12	ND	9.1	10.0	ND
SPS2	ND	ND	ND	11.9

[a] Apparent half-lives were determined from gels such as those shown in Fig. 1. As with all half-lives presented in this report, they represent the average of at least two independent time courses. The results from the time courses differ by no more than 18%.

[b] ND, not determined.

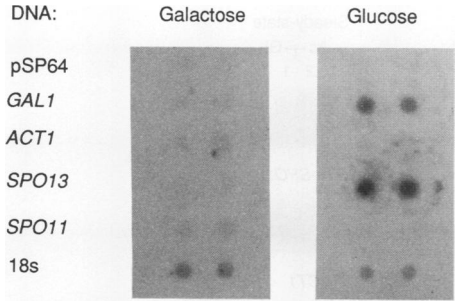

FIG. 2. RNA synthesis from the *GAL1* promoter in glucose and galactose media. Haploid strain F83 containing the *GAL1-SPO13* fusion, F24, was grown in galactose-containing medium to the mid-log phase, and half of the culture was shifted to glucose-containing medium. After 5 min, ^{32}P was added to both cultures, and after an additional 5 min, the cells were harvested. Total RNA (2×10^7 cpm) prepared from each culture was used to probe filters containing 20 μg of denatured DNAs. Relative RNA levels were determined by cutting out each spot and counting its radioactivity.

3.2 min if shifted to vegetative medium after 4, 6, and 9 h into meiosis, respectively (Table 2). Apparent half-lives of the *SPO11* and *SPO16* transcripts also were similar when transferred after different lengths of meiotic development. The slower-decaying *SPO12* transcript exhibited apparent half-lives of 9.1 and 10.0 min if shifted after 6 and 9 h, respectively, into meiosis (Table 2). In addition, nearly identical rates of decay were obtained when the cells were shifted from sporulation medium to a simple 2% glucose solution rather than YPD (data not shown). Since glucose alone was sufficient to trigger the rapid decay response, all subsequent experiments, unless otherwise noted, utilized a 2% glucose solution.

Instability of *GAL1-SPO13* fusion transcripts is independent of return to growth and cell ploidy. Although the return-to-growth experiments suggested that the early meiotic transcripts were unstable, true half-lives could not be determined because the rates of transcript synthesis after the shift were not known. Therefore, to measure the turnover of the meiosis-specific transcripts more precisely, we substituted the *GAL1* promoter, whose activity could be experimentally controlled (37), for specific *SPO* gene promoters. The transcribed portions of the meiotic genes were fused to a sequence containing the promoter and a portion of the 5′ untranslated region of the *GAL1* gene. Transcription of these fusion mRNAs was then induced to high levels in acetate or galactose medium and shut off by repression in glucose medium, and the rate of decay of the fusion transcripts was determined.

Since accurate measurement of mRNA half-life from the *GAL1-SPO13* fusion (F24) depended on a rapid cessation of transcription from the *GAL1* promoter after the shift to glucose, the following control study was performed to determine the efficiency and kinetics of transcriptional repression after the shift. The experiment monitored in vivo incorporation of ^{32}P into newly synthesized RNA in galactose-grown cells and cells shifted from galactose- to glucose-containing medium. The RNA from the galactose-grown culture hybridized to the *GAL1*, *SPO13*, and control 18*s* rDNA sequences (Fig. 2). The RNA from the culture shifted to glucose medium hybridized only to the rDNA. The *ACT1* mRNA, although constitutively synthesized, is not transcribed at a sufficient rate to be detected in this assay. After 5 min in glucose medium, synthesis of *GAL1* or *GAL1-SPO13* mRNA decreased by at least 15-fold compared with that in galactose medium. This study demonstrated that glucose repression of transcription from the *GAL1* promoter occurs rapidly enough to allow accurate determination of RNA decay rates in such fusions.

The stability of the *SPO13* fusion mRNA was subsequently examined after shifting diploid C18 from sporulation medium to glucose, conditions identical to those in the return-to-growth experiments. The *GAL1-SPO13* fusion was induced to high levels in sporulation medium (18). A 5′ *GAL1-SPO13* probe was used to distinguish the wild-type *SPO13* and fusion transcripts. The fusion mRNA was rapidly degraded with a half-life of 2.8 min (data not shown), nearly identical to the apparent half-life of the wild-type *SPO13* mRNA measured in the return-to-growth experiments. These results indicate that transcription from the wild-type *SPO13* promoter is shut off as rapidly as that from the *GAL1* promoter upon shifting cells to glucose-containing medium.

To assess the importance of the shift from meiotic development to mitotic division in the rapid turnover of the *SPO13* transcript, we induced the fusion in vegetative galactose-containing medium rather than sporulation medium before the shift to glucose. Under these conditions, the fusion transcript displayed a half-life of 3.0 min (data not shown). The identical shift performed in haploid strain F83 yielded a transcript half-life of 2.7 min (data not shown). These results demonstrate that neither the shift from meiotic to mitotic development nor the ploidy of the cell is important in generating the rapid turnover of the *SPO13* mRNA.

Carbon source affects stability of *GAL1-SPO13* fusion. In the experiments described above, glucose was used to inhibit transcription from the *GAL1* promoter of the fusion gene. To examine the effect of glucose itself on transcript stability, we used haploid S71, containing the *rpb1-1* mutation, a temperature-sensitive mutation in RNA polymerase II (28). In this strain, all RNA polymerase II transcription can be rapidly blocked by a shift to the nonpermissive temperature of 37°C. Turnover of the *SPO13* mRNA was measured by using a fusion between the *SPO13* sequence and the actin gene (*ACT1*) promoter (F99) to provide constitutive synthesis of the transcript independent of carbon source. The *ACT1-SPO13* fusion was expressed in sporulation (acetate) medium at the permissive temperature and then shifted to acetate or glucose medium at the nonpermissive temperature, and decay of *ACT1-SPO13*, *ACT1*, and 18*s* RNAs was measured. The *ACT1-SPO13* fusion transcript was approximately twofold more stable in acetate than in glucose medium (half-life of 4.9 min in glucose medium versus 10.9 min in acetate medium) (Fig. 3A and Table 3). In contrast, the actin transcript displayed similar stabilities in acetate and glucose media, indicating that the difference observed in the stability of the fusion mRNA does not result from differential transcription from the actin promoter in the two media.

Changes in transcript stability are expected to produce changes in the steady-state transcript levels. To determine the effect of the medium on the steady-state levels of the *ACT1-SPO13* transcript, we measured the abundance of this mRNA in cells grown in vegetative medium with glucose or acetate as a carbon source. To eliminate any effects of differential transcription on the amounts of the fusion mRNA, we normalized levels to those of the wild-type *ACT1* mRNA. The turnover of actin mRNA is similar in glucose and acetate media (see above). Therefore, any differences in the level of the *ACT1-SPO13* fusion mRNA must result from

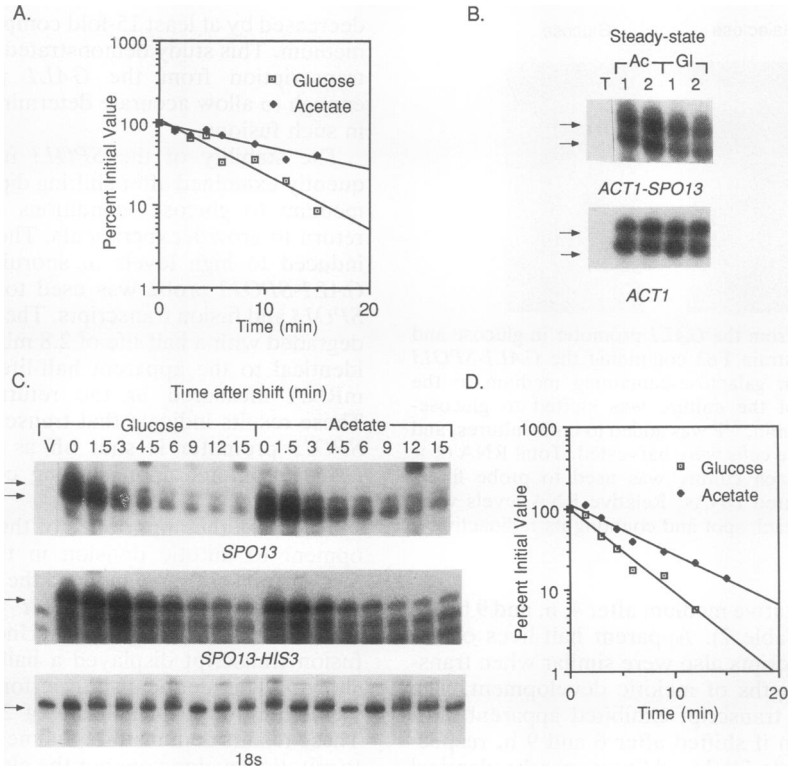

FIG. 3. Turnover and steady-state levels of mRNA in glucose and acetate media. (A) mRNA stability was measured in haploid strain S71 containing the *rpb1-1* mutation, a *his3* deletion, and the *ACT1-SPO13* fusion (F99). Cells were grown at 25°C and shifted to SPII medium at 25°C. After 6 h, half of the culture was shifted to SPII medium at 37°C and the other half was shifted to a 2% glucose solution at 37°C. Cells were removed at intervals after the shift, and total RNA was prepared. Total RNA (20 μg for the *SPO13* and *ACT1* probes, 0.2 μg for the 18*s* probe) was hybridized with *SPO13* 3′, *ACT1*, and 18*s* ribosomal probes. After quantitation of mRNA levels, the percentage of *SPO13* fusion mRNA relative to the zero time point was determined and then plotted against the time after the shift on a semi-log graph. (B) Steady-state mRNA levels were measured in haploid strain S71 containing fusion F99. The cells were grown in Com-ura+ace or Com-ura+glu medium at 25°C to the mid-log phase. Total RNA prepared from the cells was hybridized with *SPO13* 3′ and *ACT1* probes. Ac and Gl indicate acetate- and glucose-grown cultures, respectively; 1 and 2 indicate the two independent cultures analyzed; T indicates tRNA control. The two bands produced by each probe result from multiple transcription stop (*ACT1-SPO13*) or start (*ACT1*) sites. Both bands were excised, and their radioactivities were counted. (C) Turnover of the wild-type *SPO13* mRNA was measured in diploid C146 homozygous for the *rpb1-1* mutation and a *his3* deletion and containing the *SPO13-HIS3* fusion (N2). The analysis was done as described for panel A, except the mRNA was hybridized to *SPO13* 3′, *HIS3*, and 18*s* probes. In analyzing *SPO13-HIS3* mRNA, only the band indicated by the arrow was counted. Other bands are artifacts of the probe and are also present in the V lane when the mRNA is not expressed. V indicates RNA prepared from log-phase cells grown in vegetative medium. (D) Results for the *SPO13* mRNA from panel C were quantitated and graphed as described for panel A.

differences in stability of the transcript. The level of the fusion mRNA was 1.9-fold higher in acetate-containing medium than in glucose-containing medium (Fig. 3B), in agreement with the 2-fold increase in transcript stability in acetate medium. Comparable differences in half-lives and steady-state levels were also obtained with a *LYS2-SPO13* fusion (data not shown).

TABLE 3. Stability of mRNA in glucose and acetate media

mRNA	mRNA half-life (min)[a]	
	Glucose[b]	Acetate[c]
ACT1-SPO13	4.9	10.9
ACT1	24.3	23.4
SPO13	2.9	4.9
SPO13-HIS3	6.8	7.2
SPO11	2.3	5.4

[a] Half-lives for the *ACT1-SPO13*, *SPO13*, and *SPO13-HIS3* mRNAs were calculated from the results shown in Fig. 3. For others, data not shown.
[b] 2% glucose solution.
[c] SPII medium.

Wild-type *SPO13* mRNA behaves like its *GAL1* fusion counterpart. To examine the behavior of the wild-type *SPO11* and *SPO13* mRNAs, we used diploid C146, homozygous for the *rpb1-1* mutation. The diploid contained a *SPO13-HIS3* fusion to control against differences in transcription from the *SPO13* promoter. The half-life of the *SPO13* transcript was 2.9 min in glucose medium and 4.9 min in acetate medium (Fig. 3C and D and Table 3). The *SPO13-HIS3* fusion mRNA displayed half-lives of 6.8 and 7.2 min in glucose and acetate media, respectively. The similar half-lives of the *SPO13-HIS3* RNA indicate that the difference observed in the wild-type transcript does not result from differences in transcription from the *SPO13* promoter. Using the same RNA preparations, turnover of the *SPO11* transcript was measured and found to be 2.3 min in glucose medium and 5.4 min in acetate medium (Table 3). The wild-type *SPO13* and *SPO11* transcripts are unstable and are approximately twofold more stable in acetate-con-

TABLE 4. Stability of *GAL1* fusion mRNAs

Gene fusion	mRNA half-life (min)[a]
GAL1-SPO13	2.7
GAL1-SPO11	2.4
GAL1-IME1	3.1
GAL1-SPO12	7.6
GAL1	8.0

[a] Half-lives were determined as described in Materials and Methods. Samples were collected at 0, 1.5, 3, 4.5, 6, 9, 12, and 15 min after the shift.

taining than in glucose-containing medium. The fact that the *ACT1* and *SPO13-HIS3* mRNAs display similar stabilities in the two media indicates that the difference is specific and does not result from general changes in cell metabolism or growth rate.

Stabilities of other meiotic transcripts. Fusions between the *GAL1* gene and other meiotic and vegetative genes were constructed, and the stabilities of the chimeric transcripts were analyzed. Like the wild-type *SPO11* mRNA, the *SPO11* fusion transcript was highly unstable, with a half-life of 2.4 min (Table 4). The half-lives of *SPO11* and *SPO13* mRNAs are among the shortest measured for any eukaryotic transcript. A fusion to another early meiotic gene, *IME1*, produced a fusion mRNA with a half-life of 3.1 min. However, not all meiotic transcripts were as rapidly degraded; the *SPO12* fusion mRNA displayed a half-life of 7.6 min. The wild-type *GAL1* mRNA exhibited a half-life of 8.0 min.

Identification of *cis*-acting regions that determine transcript stability. After establishing that the early meiotic transcripts are generally very unstable, we sought to identify the determinants within the transcripts responsible for the rapid turnover. A series of 5′, 3′, and internal deletion derivatives of the transcribed region of the *SPO13* gene were constructed, and the stabilities of these transcripts were determined in haploid strain F83 or S65. The *SPO13* genes with 5′ deletions were fused directly to the *GAL1* promoter sequence (Fig. 4A). The initial fusion, F24, yielded an mRNA with a half-life of 2.9 min. The smallest deletion, which removes only 15 bp including the translation initiation codon, increased the mRNA stability twofold to a half-life of 5.8 min (compare F24 and F82 in Fig. 4B and C). In this deletion, the first AUG is located at +200 (+1 is the normal translation start) but is out of frame with respect to the *SPO13* reading frame. Deletion of additional 5′ sequences (F32, F30, F28) produced transcripts that were only slightly more stable. One possible explanation for this result is that sequences at or adjacent to the translation initiation site are important for the rapid turnover of the transcript. Alterna-

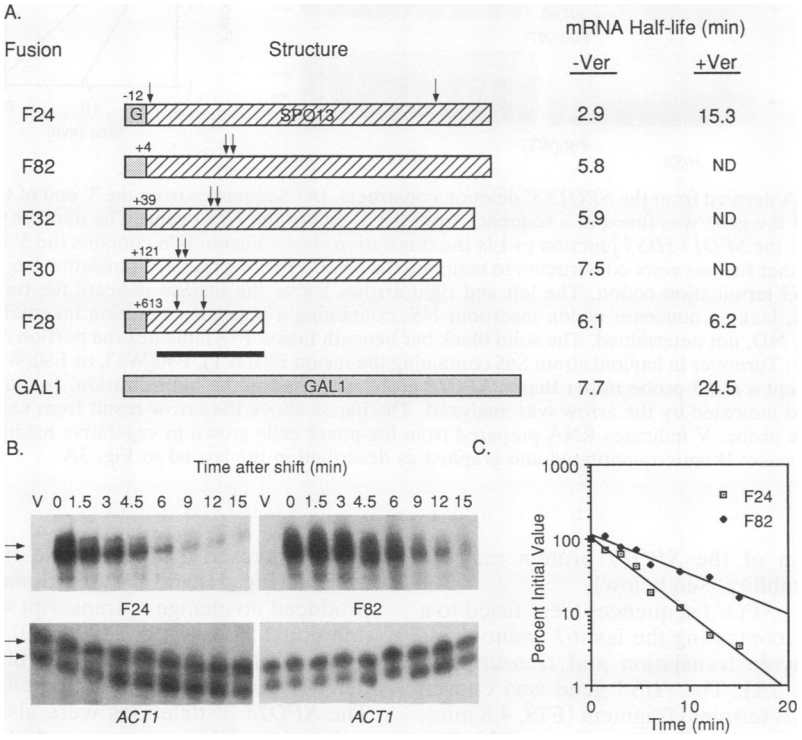

FIG. 4. Stability of mRNAs derived from *SPO13* 5′-deletion constructs. (A) Sequences from the 5′ end of the *SPO13* gene were deleted, and the remainder of the gene was fused directly to *GAL1* sequences. The number above each fusion is the first base from the *SPO13* gene at the *GAL1-SPO13* junction (+1 is the translation start). The left arrow above each fusion indicates the 5′-most AUG present. The right arrow indicates the first termination codon in frame with that AUG. −Ver, turnover measured in the absence of translation inhibitor verrucarin A; +Ver, turnover in the presence of verrucarin A; ND, not determined. The solid black bar beneath fusion F28 indicates the portion of the mRNA protected in the S1 hybridization analysis. (B) Haploid strain F83 containing the F24 or F82 fusion was grown in Com-ura+gal medium and shifted to a 2% glucose solution, and aliquots were removed at the times indicated. Total RNA was hybridized with *SPO13* 3′ and *ACT1* probes. The two bands result from multiple transcription termination (*SPO13*) or initiation (*ACT1*) sites. Both bands were excised, and their radioactivities were counted. V indicates RNA prepared from log-phase cells grown in vegetative medium. (C) Analyses of the F24 and F82 mRNAs from panel B were quantitated and graphed as described in the legend to Fig. 3A.

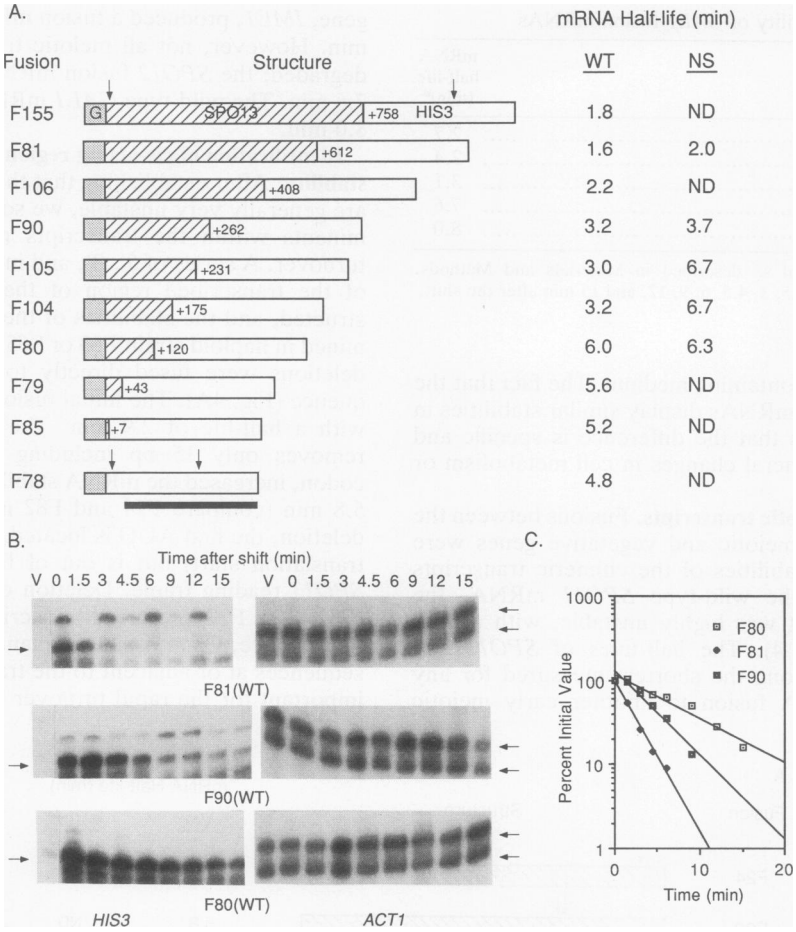

FIG. 5. Stability of mRNA derived from the *SPO13* 3′ deletion constructs. (A) Sequences from the 3′ end of the *SPO13* gene were deleted, and the remaining portion of the gene was fused to a sequence from the 3′ end of the *HIS3* gene. The number within each fusion is the first base from the *SPO13* gene at the *SPO13-HIS3* junction (+1 is the translation start). Fusion F78 contains the 3′ *HIS3* fragment fused directly to the *GAL1* sequence. All other fusions were constructed to maintain the *SPO13* reading frame. Translation begins at the normal *SPO13* start and ends at the normal *HIS3* termination codon. The left and right arrows above the fusions indicate the translation start and stop sites, respectively. WT, wild type, lacking nonsense codon insertion; NS, containing a nonsense mutation inserted at the junction between the *SPO13* and *HIS3* sequences; ND, not determined. The solid black bar beneath fusion F78 indicates the portion of the mRNA protected in the S1 hybridization analysis. (B) Turnover in haploid strain S65 containing the fusion F81(WT), F90(WT), or F80(WT) was analyzed as described in the legend to Fig. 4B except a *HIS3* probe rather than a *SPO13* probe was used in the hybridization. In the analysis of the *SPO13-HIS3* fusion mRNA, only the band indicated by the arrow was analyzed. The bands above the arrow result from undigested probe. Bands below the arrow are artifacts of the probe. V indicates RNA prepared from log-phase cells grown in vegetative medium. (C) Analyses of the F81, F90, and F80 mRNAs from panel B were quantitated and graphed as described in the legend to Fig. 3A.

tively, normal translation of the *SPO13* protein may be required for the high instability (see below).

In the 3′ deletion series, *SPO13* sequences were fused to a portion of the *HIS3* gene containing the last 67 amino acids and the 3′ UTR to provide translation and transcription termination signals (Fig. 5A). The *HIS3* gene was chosen because the stability of this terminal fragment (F78, 4.8 min), was similar to the stability of the *SPO13* fragment (F28, Fig. 4) of comparable size. In the construction of the fusions, the translational reading frame was maintained. This deletion series defined the 3′ boundaries of at least two distinct sequences within the coding region of the *SPO13* gene that determine mRNA stability. The removal of both of these regions increased transcript stability by more than threefold. The most terminal deletion (F155), which removed 171 bp (38 amino acids), yielded a fusion mRNA with a half-life of 1.8 min (Fig. 5A). A deletion of *SPO13* sequences to +262 (F90) increased the stability nearly twofold to a half-life of 3.2 min (Fig. 5B and C). Additional deletions to +175 (F104) produced no change in transcript stability, but deletion of an additional 55 bases to +120 (F80) again increased the stability twofold to a half life of 6.0 min (Fig. 5B and C). Further deletions to +7 (F85) had little effect on transcript stability. The *SPO13* 3′ deletions were also fused to terminal *LYS2* rather than *HIS3* sequences. Although the absolute stabilities of these fusions were approximately 30% greater than those of the *HIS3* fusions, the relative stabilities of these fusions were similar to those of their *HIS3* counterparts (data not shown).

A series of internal deletions was next constructed within the *SPO13* gene to further characterize the stability determinant whose 3′ boundary was defined by fusions F80 and F104 to lie between +120 and +175. This set of internal deletions, which consists of one large deletion and several

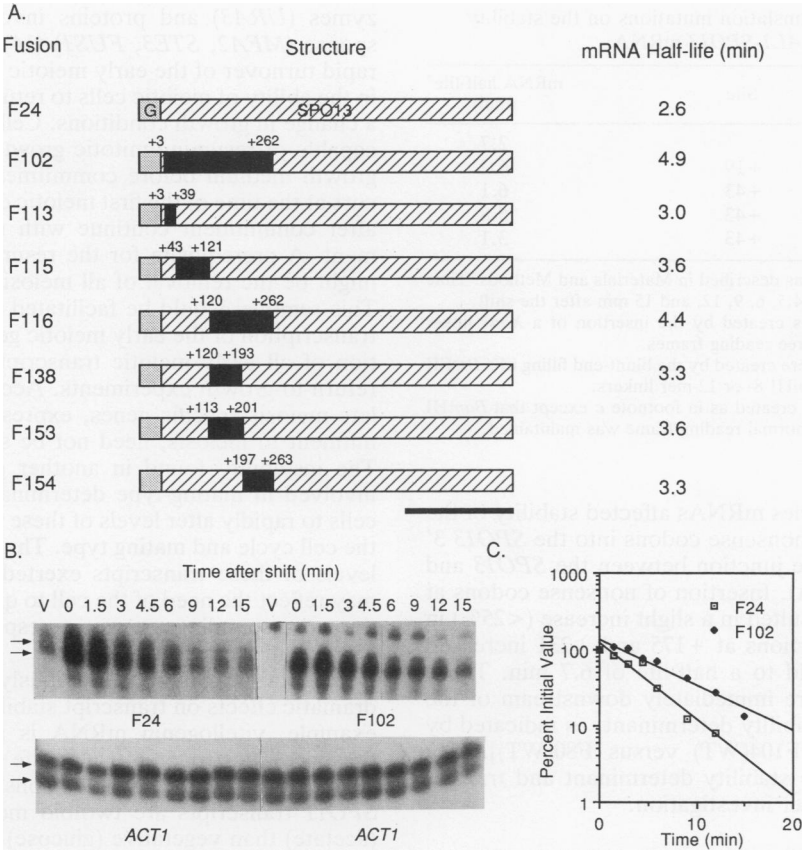

FIG. 6. Stability of mRNAs derived from *SPO13* internal deletions. (A) Solid black regions indicate the sequences deleted. Above each fusion, the number to the left is the first base from the *SPO13* gene at the 5′ end of the deletion, and the number to the right is the first base from the *SPO13* gene at the 3′ end of the deletion (+1 is the translation start). All deletions are contained within the *SPO13* coding region, and all maintain the normal reading frame. Translation of these deletion constructs initiates and terminates at the same sites as that of the wild-type *SPO13* protein (Fig. 4A). The solid black bar beneath fusion F154 indicates the portion of the mRNA protected in the S1 hybridization analysis. (B) Turnover in strain F83 containing the fusion F24 or F102 was analyzed as described in the legend to Fig. 4B. The two bands result from multiple transcription termination (*SPO13*) or initiation (*ACT1*) sites. Both bands were excised and counted. V indicates RNA prepared from log-phase cells grown in vegetative medium. (C) Analyses of the F24 and F102 mRNAs from panel B were quantitated and graphed as described in the legend to Fig. 3A.

subdeletions, is contained within the coding region and maintains the *SPO13* reading frame. The large deletion produced a transcript with a stability comparable to those obtained from the 3′ deletion series, but attempts to further localize the determinant by using smaller internal deletions produced mRNAs with intermediate stabilities. The largest deletion, from +3 to +262 (F102), increased the half-life of the mRNA from 2.6 to 4.9 min (Fig. 6). A subdeletion of this region from +120 to +262 (F116) yielded an mRNA with a half-life of 4.4 min. The mRNAs produced from further subdeletions of this region (F138, F153, and F154) displayed intermediate stabilities of approximately 3.5 min.

Normal translation of the *SPO13* transcript is required for rapid turnover. One possible explanation for the results from the 5′ deletion analysis is that translation of the *SPO13* transcript plays some role in the rapid turnover of the mRNA. Two approaches were used to further examine the relationship between translation and *SPO13* transcript stability. First, the effect of the translation inhibitor verrucarin A on the turnover of *GAL1-SPO13* transcripts was examined. This drug inhibits the first few steps of peptide bond synthesis prior to polysome formation and produces polysome breakdown since it allows polysome runoff while preventing new polysome formation (39). A polysome profile verified that in the verrucarin A-treated cells, no polysomes were present (data not shown). In the presence of the drug, the stability of the original fusion mRNA, F24, was increased fivefold (Fig. 4A). In contrast, the stability of the deletion fusion lacking much of the *SPO13* coding sequence, F28, was not affected by the drug. The half-life of the *GAL1* transcript also increased threefold in the presence of the drug, suggesting that the turnover of this transcript also has a translational component.

The second approach used to examine the relationship between translation and mRNA stability was to specifically alter the translation of the *SPO13* protein. A nonsense codon inserted at position +19 of fusion F24 increased the stability of the *SPO13* mRNA over twofold to a half-life of 6.2 min (Table 5). Linker insertions resulting in +1 and +2 frameshifts at position +43 of fusion F24 also increased the transcript half-life. In contrast, the linker insertion that maintained the normal reading frame yielded a transcript with a half-life similar to that of the original fusion. Both of these studies clearly demonstrate that normal translation of the *SPO13* transcript is required for its rapid turnover.

To determine whether translation through the *HIS3* por-

TABLE 5. Effect of translation mutations on the stability of the *GAL1-SPO13* mRNA

Mutation	Site	mRNA half-life[a] (min)
None		2.7
Nonsense[b]	+19	6.2
Frameshift +1[c]	+43	6.1
Frameshift +2[c]	+43	5.8
Insertion[d]	+43	3.1

[a] Half-lives were determined as described in Materials and Methods. Time points analyzed were 0, 1.5, 3, 4.5, 6, 9, 12, and 15 min after the shift.

[b] The nonsense mutation was created by the insertion of a *Xba*I linker containing stop codons in all three reading frames.

[c] The frameshift mutations were created by the blunt-end filling of a *Bst*EII site and then the ligation of *Bam*HI 8- or 12-mer linkers.

[d] The insertion mutation was created as in footnote *c* except that *Bam*HI 10-mer linkers were used. The normal reading frame was maintained.

tion of the 3′ deletion series mRNAs affected stability of the transcripts, we inserted nonsense codons into the *SPO13* 3′ deletion constructs at the junction between the *SPO13* and *HIS3* sequences (Fig. 5A). Insertion of nonsense codons at +120, +262, or +612 resulted in a slight increase (<25%) in transcript stability. Insertions at +175 and +231 increased the stability over twofold to a half-life of 6.7 min. These latter two insertions were immediately downstream of the region that contains a stability determinant, as indicated by the 3′ deletion series [F104(WT) versus F80(WT)]. The relationship between the stability determinant and translation of this region is under investigation.

DISCUSSION

In this study, we investigated the stability of meiosis-specific transcripts and the role of mRNA turnover in the expression of meiotic genes. Several factors that regulate the stability of the early meiotic transcripts were identified. These include growth medium, *cis*-acting sequences, and translation. The stabilities of the meiotic transcripts were examined by using (i) return to growth, (ii) *GAL1* fusions, and (iii) a temperature-sensitive RNA polymerase II mutation. The latter method has been used successfully to measure the stabilities of a variety of yeast transcripts (14). The *GAL1* fusion method utilized in this study has the advantage that turnover can be measured for a specific mRNA, while possible pleiotropic effects resulting from general inhibition of RNA polymerase II are eliminated. Although heterologous genes were analyzed, the similarities in the stabilities of the *GAL1* fusion and wild-type transcripts argue that the chimeric mRNAs yield true half-lives. Moreover, the similarity between the apparent half-lives calculated for the early meiotic transcripts in return-to-growth experiments and the actual half-lives determined by using the *GAL1* fusions indicates that the wild-type *SPO* promoters and the *GAL1* promoter shut off transcription just as rapidly in glucose-containing medium. A rapid shutoff of transcription has also been observed for the *CYC1* gene after a shift to glucose (43) and may be a general characteristic of promoters under glucose repression.

Measurements on a number of transcripts in *S. cerevisiae* have produced mRNA half-lives ranging from 2.5 min to over 1 h (14, 33). In vegetative growth medium, the early meiosis-specific mRNAs lie at one end of this spectrum among the least stable transcripts with half-lives under 3 min. Other highly unstable RNAs encode metabolic enzymes (*URA3*) and proteins involved in mating-type response (*MFA2*, *STE3*, *FUS1*) (14). A possible role for the rapid turnover of the early meiotic transcripts may be found in the ability of meiotic cells to return to mitotic growth after a change in growth conditions. Cells undergoing meiosis are capable of resuming mitotic growth if shifted to vegetative growth medium before commitment to meiosis, which occurs at the time of the first meiotic division (36). Cells shifted after commitment continue with normal meiotic development. A prerequisite for the resumption of mitotic growth might be the removal of all meiosis-specific gene products. This removal would be facilitated by a rapid shutoff of the transcription of the early meiotic genes and a rapid degradation of all early meiotic transcripts, both observed in the return-to-growth experiments. Accordingly, the middle and late meiosis-specific genes, expressed primarily after commitment to meiosis, need not be subjected to this control. The instability found in another group of yeast mRNAs, involved in mating-type determination, presumably allows cells to rapidly alter levels of these transcripts in response to the cell cycle and mating type. Thus, the tight control on the levels of these transcripts exerted by their rapid turnover may reflect the need of the cell to quickly alter expression of the corresponding genes in response to intracellular or extracellular changes.

External factors have previously been shown to produce dramatic effects on transcript stability in other systems. For example, vitellogenin mRNA is stabilized 30-fold in the presence of estrogen (4). Half-life measurements as well as steady-state mRNA levels demonstrate that the *SPO13* and *SPO11* transcripts are twofold more stable in sporulation (acetate) than vegetative (glucose) medium. This difference is observed in haploid cells during mitotic growth, indicating that it results from environmental signals and not the developmental program of the cell. This modest twofold change in transcript stability would aid in the accumulation of *SPO13* and *SPO11* transcripts in meiosis, but is clearly insufficient to account for the 70- to 100-fold induction that is observed (1, 40). Increases in rates of transcription must therefore be primarily responsible for the accumulation of these transcripts in meiosis. This view is supported by the recent identification of both *cis*-acting sequences and *trans*-acting factors that developmentally control the transcription of these genes (5, 38).

In the analysis of the chimeric transcripts, the fact that a few of the *HIS3* fusion mRNAs (F155 and F81) have shorter half-lives than the wild-type *SPO13* mRNA raises the possibility that the *HIS3* sequence itself alters the stability of the fusion. In view of this, two points must be emphasized: (i) all the fusions were constructed with the same *HIS3* fragment; (ii) the stabilities of the 3′ fusions were compared only to those of other 3′ fusions. Although the presence of the *HIS3* sequences may be affecting mRNA stability, the direct comparison of the 3′ deletion mRNAs demonstrates that removal of specific portions of the *SPO13* coding region alters the stability of the transcript. Two observations support the importance of the *SPO13* sequences in the determination of transcript stability. First, when the *LYS2* 3′ fragment was substituted for the *HIS3* sequences, similar results were obtained. Second, in the analysis of the internal *SPO13* deletions in which no heterologous sequences are present, when the 5′ coding region is removed, a similar increase in stability is observed.

The combination of 3′ and internal deletions demonstrates that sequences within the coding region destabilize the *SPO13* transcript. To define these destabilizing sequences,

we analyzed turnover of transcripts containing small internal deletions, but only intermediate stabilities were obtained. The intermediate stabilities may result from the fact that this region contains multiple determinants such that removal of part of the region produces only a partial effect. In these deletions, the secondary structure as well as the primary sequence is being altered, and this may also affect transcript stability. However, analysis of the 5′ region containing a stability determinant showed it to be devoid of any striking secondary structure, homology to any previously identified destabilizing sequence, or homology to the other unstable early meiotic mRNAs. The *SPO13* 3′ untranslated region was also examined for the presence of RNA stability determinants by the construction of chimeric transcripts, and no such determinants could be detected (38a).

Alterations in the translation of a transcript may stabilize or destabilize the transcript. Nonsense mutations at the 5′ end of the transcript decrease the stability of the yeast *URA3* (22), *URA1* (30), and *PGK1* (15) mRNAs. This instability is attributed to an increased susceptibility of the transcript to nuclease attack as a result of premature ribosome loss. In contrast, some mRNAs with specialized systems controlling their stability couple regulated turnover with transcript translation. Translation of the histone transcript must proceed within 300 bases of the stem-loop structure in the 3′ untranslated region for this structure to destabilize the transcript (11). Turnover of the β-tubulin transcript in response to cellular tubulin levels requires the translation of the first four amino acids, although translation must proceed beyond codon 41, presumably to allow this peptide recognition signal to emerge from the ribosome (42). The features of *SPO13* mRNA turnover more closely resemble those of mRNAs with specialized systems regulating their stability. Experiments with the translation inhibitor verrucarin A and frameshift and nonsense mutations indicate that normal translation of the *SPO13* transcript is required for the rapid turnover. Like the β-tubulin mRNA, *SPO13* contains stability determinants within the coding region, and the results from the insertion of nonsense mutations into the 3′ deletion series suggest that translation must proceed beyond a region containing one of these determinants for maximum instability. The relationship between this stability determinant and translation is not known.

Recent studies have suggested a relationship between codon bias and transcript stability. *PGK1* mRNA stability is decreased by threefold when the *PGK1* coding region, which contains mostly optimal codons, is changed to rare codons without changing the protein sequence (15). Herrick and coworkers (14) observed a correlation between unstable transcripts and high percentages of rare codons within the transcript. Parker and Jacobson (29) identified a 42-base region within the *MATa1* transcript encoding mostly rare codons capable of destabilizing heterologous transcripts. The unstable *SPO11*, *SPO13*, and *SPO16* transcripts all contain high percentages of rare codons (>35%) (34). However, the codon bias in the more stable *SPO12* and *SPS2* mRNAs is similar to that found in the early meiotic transcripts. The 5′ region in the *SPO13* gene, which contains a stability determinant, actually contains a lower proportion of rare codons than the overall transcript. While codon bias may play some role in determining the stability of the early meiosis-specific transcripts, other mechanisms must also be operating.

This study identified a number of factors that are capable of destabilizing the *SPO13* transcript, including growth medium, internal sequences, and translation. Significantly, no single factor had more than a two- to threefold effect on mRNA stability. Thus, we believe that the high instability of the *SPO13* transcript may result from the additive effects of several pathways, in addition to the general turnover pathway. We have recently identified two *trans*-acting genes that alter meiotic transcript stability (38b). These genes act independently, and mutations in either of these genes increase the stability of the meiotic transcripts twofold. To elucidate the pathways regulating meiotic mRNA stability, we are presently looking for interactions between these *trans*-acting genes and the factors described in this study.

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