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Saccharomyces cerevisiae Exhibits a Sporulation-Specific Temporal Pattern of Transcript Accumulation

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Cultures of the yeast *Saccharomyces cerevisiae* that are heterozygous for the mating type (*MAT*a/*MAT*α) undergo synchronous meiosis and spore formation when starved for nitrogen and supplied with a nonfermentable carbon source such as acetate. Haploid and homozygous *MAT*α/*MAT*α and *MAT*a/*MAT*a diploid cells incubated under the same conditions fail to undergo meiosis and are asporogenous. It has not yet been firmly established that gene expression during sporulation is controlled at the level of transcript accumulation. To examine this question, we used cloned genes that encode a variety of "housekeeping" functions to probe Northern blots to assay the appearance of specific transcripts in both sporulating and asporogenous *S. cerevisiae*. In sporulating cells, each transcript showed a characteristic pattern of accumulation, reaching a maximum relative abundance at one of several different periods. In contrast, in both asporogenous haploid *MAT*a and diploid *MAT*α/*MAT*α cells, all transcripts accumulated with similar kinetics. These results suggest a sporulation-specific pattern for transcript appearance. During these studies, high levels of several different transcripts were observed at unexpected times in sporulating cells. Histone (H)2A and (H)2B1 transcripts, although most abundant during premeiotic DNA synthesis, remained at one-third to one-half maximal levels after its end and were found in mature ascospores. Their appearance at this time is in sharp contrast to vegetative cells in which these histone transcripts are only found just before and during the period of DNA synthesis. Furthermore, transcripts from *GAL10* and *CDC10* genes, which are believed to be dispensable for sporulation, were much more abundant in sporulating cells than in asporogenous cells and vegetative cells grown on glucose or acetate. The presence of these transcripts did not appear to be due to a general activation of transcription because each accumulated with different kinetics. In addition, the transcript for at least one gene, *HO*, that is also dispensable for sporulation was not detected. The increased abundance of transcripts from some genes not required for sporulation leads us to propose that genes preferentially expressed during sporulation need not be essential for this differentiation.

Sporulation of the yeast *Saccharomyces cerevisiae* provides an excellent model system for studies on both meiosis and cell differentiation. Cultures of *S. cerevisiae* that are heterozygous for the mating type locus (*MAT*a/*MAT*α) undergo synchronous meiosis and spore formation when starved for nitrogen and supplied with a nonfermentable carbon source such as potassium acetate (for a review, see reference 12). A total of 93 to 98% of the cells from a culture of strain SK1 or its close relatives are capable of sporulating with a high degree of synchrony (24, 32). Approximately 80% of the cells complete sporulation in 12 h, whereas the remaining cells complete it in 15 to 16 h. In contrast to *MAT*a/*MAT*α cells, haploid and homozygous *MAT*a/*MAT*a or *MAT*α/*MAT*α cells are asporogenous and undergo neither meiosis nor spore formation when incubated in sporulation medium.

Our interest was to understand the function and regulation of transcripts that are present in sporulating cells. A total of 95% of the RNA species and almost all of the abundant protein species synthesized during sporulation are also synthesized during mitotic growth and in asporogenous haploid and diploid cells incubated in sporulation medium. Only a small number of transcripts and abundant protein species appear to be sporulation specific (26, 43, 45; D. Mills, personal communication). Sequences complementary to several sporulation-specific transcripts have been isolated on recombinant DNA plasmids (6, 30; E. G. Ninfa and D. B. Kaback, unpublished data). These sequences have been assumed to be important for the differentiation of vegetative cells into ascospores. However, it has not yet been determined whether these genes are required for sporulation.

Control of gene expression during sporulation could occur at the level of transcript accumulation. Many different developing systems are characterized by the appearance of specific transcripts that are directly involved in the cytodifferentiation under examination (e.g., globin transcripts in reticulocytes, muscle actin transcripts in myocytes, chorion protein transcripts in chorion cells, fibroin transcripts in silk glands, etc.). By analogy, we predict that each sporulation-specific expressed sequence has a specific function in sporulating cells. However, it is also conceivable that things are not this simple and the sporulation-specific transcripts are the result of a generalized relaxation of control in which all transcribable DNA sequences are expressed. In this case, specific transcripts would not always have distinct or even definable functions. A generalized relaxation of transcriptional control during sporulation of *S. cerevisiae* might be compared with the meiotic diplotene lampbrush-chromosome stage found in oocytes of amphibians and other higher organisms. During this stage of oocyte development, an intensive period of RNA synthesis coincides with the appearance of all chromosomes as if they were being actively transcribed (10). If a relaxed state of transcriptional control exists during meiosis and sporulation of *S. cerevisiae*, it could imply that gene expression during this differentiation

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TABLE 1. *S. cerevisiae* strains

Strains	Genotype	Source or reference
SK1	*MATa HO* *MATα HO*	24
g716-5A	*MATa ho his1-7 hom3-10 can1*	J. Game[a]
AP1	*MATα ade1 ade2 ura1 his7 lys2 trp1 gal1 + + + +* *MATα + ade2 + + + + + ura3 cyh2 can1 leu1*	19
Y185	*MATa ade2 his8 lys2 +* *MATα + + + his2*	27

[a] A strain closely related to SK1 constructed by repeated backcrosses of *ho* haploid segregants to haploid spores from SK1 (J. Game, personal communication).

was controlled post-transcriptionally. In fact, meiotic cells (oocytes) of higher organisms are thought to regulate some gene expression post-transcriptionally. Transcripts synthesized in oocytes are often stored and translated in the embryo after fertilization (10, 39). In view of these findings, there has been a need to demonstrate that transcript accumulation is controlled by a developmental program in sporulating *S. cerevisiae* cells.

MATERIALS AND METHODS

Growth and sporulation of *S. cerevisiae*. Strains used in this study are listed in Table 1. Cells were routinely grown on 1.0% (wt/vol) yeast extract (Difco Laboratories)–2.0% (wt/vol) Bacto peptone (Difco)–2.0% (wt/vol) glucose (YEPD [13]). Acetate vegetative medium was 0.67% (wt/vol) yeast nitrogen base without amino acids (Difco), 0.1% (wt/vol) yeast extract, 1.0% (wt/vol) potassium acetate, and 0.05 M potassium pthalate (35). Exponential phase (vegetative) cells were harvested when their density reached 2×10^7 to 3×10^7 cells per ml (100 Klett Units; Klett-Summerson, Inc.).

Sporulation was carried out as described by Petersen et al. (31). Cells were first grown on 1.0 liter of YEPD medium in 2.0-liter Erlenmeyer flasks at 30°C in a New Brunswick gyratory shaker, shaking at 250 rpm. Approximately 12 h after the end of exponential growth (12 to 14 h after reaching 200 Klett Units), cells were harvested by centrifugation (4000 rpm for 5 min at 15°C), washed once at room temperature with supplemented sporulation medium (SUSP; 1.0% [wt/vol] potassium acetate, 0.05% [wt/vol] glucose, and 0.1% [wt/vol] yeast extract), suspended in a small volume of SUSP, and distributed into 2.0-liter Erlenmeyer flasks, each containing 200 ml of SUSP. Cells from one volume of YEPD growth medium were sporulated in two volumes of SUSP. The resulting concentration of cells gave the most rapid and synchronous sporulation (31). Harvesting and transfer to sporulation medium was carried out as rapidly as possible. Sporulating cells were vigorously shaken (250 to 300 rpm) at 30°C in a New Brunswick gyratory shaker to ensure maximum aeration.

Cells were harvested at the intervals noted in the text by adding cycloheximide to a concentration of 200 μg/ml, chilling rapidly, and centrifuging at 4000 rpm for 5 min at 4°C.

The mixtures of sporulating and asporogenous cells were pools of equal volumes of cells harvested at 1-h intervals up until 12 h.

Preparation of RNA. Total yeast RNA was prepared by adapting the guanidinium thiocyante procedure (5, 18) for use with *S. cerevisiae*. One volume of chilled, packed cells was suspended in two volumes of ice-cold buffer containing 4.0 M guanidinium thiocyanate (EM reagents), 1.0 M β-mercaptoethanol, 0.05 M sodium acetate (pH 4.5), and 1.0 mM EDTA (GTC buffer). The components of the buffer were first dissolved and filtered through a 0.45-μm pore-size nitrocellulose filter to remove particulate impurities. The cell slurry (15 ml) was added to a prechilled, 70-ml Braun homogenizer bottle containing 35 g of acid-washed, siliconized, baked 0.5-mm glass beads. Cells were homogenized for 1 min in a CO_2-cooled Braun homogenizer. The breakage of vegetative cells, sporulating cells, asci, and spores was routinely greater than 99%. The homogenized cells were permitted to thaw in an ice bath and removed with a Pasteur pipette. The glass beads were washed three to four times with 2 to 3 ml of ice-cold GTC buffer. All washes were combined with the extracted material, and the mixture was centrifuged in a Sorvall HB4 rotor (10,000 rpm, 10 min, 4°C) to clear it of glass beads and cell debris. One gram of solid CsCl was dissolved for each milliliter of supernatant. 30 ml of the extract was added per centrifuge tube (8.9 by 2.5 cm [3.5 in by 1 in]; Quick-Seal; Beckman Instruments, Inc.). Each tube was then underlayed with 10 ml of CsCl (19 volumes of saturated CsCl in distilled water and 1 volume of 1.0 M sodium acetate[pH 4.5]–20 mM EDTA) with a Pasteur pipette inserted through the extract to the bottom of the tube. Tubes were sealed and centrifuged in a Beckman 50.2 Ti rotor (40,000 rpm, 20 to 50 h, 20°C). All opalescent bands were extracted from the gradient with a syringe. To prevent precipitation of CsCl, the volume of the extract was increased threefold with ice-cold distilled water. Two volumes of ice-cold ethanol were added to precipitate macromolecules. After at least 1 h at −20°C, the RNA-containing precipitate was centrifuged (10,000 rpm, 60 min, −20°C) and suspended in water or 10 mM Tris (pH 7.4)–1 mM EDTA for use.

Polyadenylate [poly(A)]-containing RNA was separated by two passages over an oligodeoxythymidylate cellulose column as described previously (4).

Polysomes from spores and asci were isolated as described by Harper et al. (14). RNA was isolated from polysomes by three phenol extractions and ethanol precipitation.

Analysis of RNA on Northern blots. Total RNA (20 μg) or poly(A)-containing RNA (2.0 μg) was electrophoresed on a denaturing 5.0 mM CH_3HgOH–1.0% agarose gel, as described by Bailey and Davidson (3). All lanes on a single gel contained the same amount of RNA. To allow for the normalization of data, one or more of the lanes on each gel being compared contained RNA from YEPD-grown SK1 cells, SK1 sporulating mixtures, or SK1 asci. Transfer to diazotized paper (Transa-bind; supplied by Schleicher & Schuell, Inc.), hybridization to [^{32}P]DNA probes, washes, and autoradiography were carried out essentially as described by Alwine et al. (1), except that dextran sulfate was added to the hybridization reaction at a concentration of 100 mg/ml. The relative abundance of the different transcripts was obtained from densitometric scans of the Northern blot

autoradiograms performed on a Zeineh Scanning densitometer with an automatic integrator. All quantitations were carried out on autoradiographs that were clearly exposed well below saturation. Relative abundances of like transcripts from different blots were compared by normalizing absorbances to the ratio of the intensities of the band in the common lanes containing the same RNA preparations. For example, if the absorbance of a given transcript was 100 U in the vegetative RNA lane of one blot and 150 U in the vegetative RNA lane of another blot (containing the same RNA preparation), the absorbances for that transcript in all lanes of the first blot were multiplied by 1.5.

Recombinant DNA techniques. The plasmids described in Table 2, generously provided by the person(s) cited, were amplified and isolated by previously described methods (9).

Restriction endonuclease digestions were carried out by the manufacturer's specifications. When necessary, restriction fragments were isolated from agarose gels by the method of Tabak and Flavell (42). ^{32}P-labeling of DNA was done by nick-translation by previously described procedures (33).

Electron microscopy of R loop-containing DNA and quantitation of transcript abundance were carried out as described previously (22, 23).

RESULTS

***S. cerevisiae* cells appear to exhibit a sporulation-specific pattern for RNA accumulation.** To determine whether there is a sporulation-specific developmental program for transcript accumulation, we examined the kinetics of transcript appearance in sporulating cells for several cloned genes that are normally expressed during vegetative growth. Vegetative genes are important to investigate because many of them are needed for sporulation (38). Transcripts complementary to *TDH* (encodes glyceraldehyde-3-phosphate dehydrogenase [28]) and *URA3* (encodes orotodine-5'-phosphate decarboxylase [2]), two genes involved in the "housekeeping" functions of glycolysis and pyrimidine biosynthesis, respectively, were examined, as were the histone genes H2A and H2B1 (16). These genes are all assumed to play some role in meiosis and sporulation because glycogen is catabolized, presumably through glycolysis, and nucleic acids and chromatin are synthesized (12). We also investigated a vegetatively transcribed region adjacent to the H2A1 gene, designated *TRT1-transcript 1* (16) or *TRT1-1*, and a vegetatively transcribed region we found near the *CDC10* gene (see below), designated *pYE98F4T-1*. The functions of these two genes are unknown at present. Finally, we examined several vegetatively expressed genes thought to be dispensable for sporulation (38). Transcription of genes that are nonessential for differentiation might be expected if there were a generalized relaxation of transcriptional control during sporulation. Alternatively, if only the genes needed for sporulation are expressed, these genes make good candidates for ones that may be be transcriptionally repressed.

TABLE 2. Recombinant plasmids

Plasmid	Relevant genes	Vector	Reference
TRT1	H2A1-H2A1, *TRT1-1*	pMB9	16
pYE98F4T	*CDC10*, *pYE98F4T-1*, *pYE98F4T-2*, *TRP1*	pLC544	7,25
pNN 76	*GAL10*	pBR322	41
YIp5-HO(BH2)	*HO*, *URA3*	YIp5	21
pGAPDH492	*TDH*	pBR322	28

The genes thought to be dispensable for sporulation that were available for these studies were the *HO* gene involved in homothallic switching (21), the *CDC10* gene involved in cell separation (15), and the *GAL10* gene which encodes UDP-glucose-4-epimerase (11, 40, 41).

First, we had to determine the precise location of the *CDC10*-transcribed region on its recombinant plasmid pYE98F4T (7). Using election microscopy of R-loop-containing hybrids, we mapped four transcripts to this plasmid (Fig. 1). Three transcripts (transcript 1, transcript 2, and *CDC10*) mapped to the *cdc10* complementing insert. The fourth transcript was the *S. cerevisiae TRP1* gene from the vector pLC544 (25). The noted transcribed region is the *CDC10* gene because it maps over a 0.9-kilobase (kb) *Eco*RI fragment needed for complementation of *cdc10* mutations. Cleavage of this region with *Bam*HI both inactivates complementation activity (7, 8) and bisects the transcribed region (see Fig. 1b). This 0.9-kb *Eco*RI fragment hybridized to a single 1.0-kb RNA species on Northern blots and was used as the *CDC10* gene-specific probe. Transcript 1 (designated *pYE98F4T-1*) partially overlapped a unique 2.1-kb *Hpa*I-*Pvu*II fragment which hybridized to a 1.3-kb RNA species on Northern blots (data not shown). These two fragments were used in the experiments described below.

Northern blots were used to analyze poly(A)-containing RNA from both sporulating SK1 *MAT***a**/*MAT*α and asporogenous g716-5A *MAT***a** and AP1 *MAT*α/*MAT*α cells harvested at hourly intervals after being suspended in sporulation medium. RNA from glucose- and acetate-grown vegetative SK1 cells was also investigated. Analysis of densitometric tracings of autoradiographs from Northern blots hybridized to all the genes mentioned above suggests the existence of a sporulation-specific temporal program of transcript accumulation. Different transcripts increased and then decreased in relative abundance during specific periods (Fig. 2). The *URA3*, *TDH*, *TRT1-1*, and *pYE98F4T-1* transcripts reached a maximum early (1 to 3 h) and then disappeared or were present at reduced levels for most of the remaining period examined. Transcripts for H2A, H2B1, and *GAL10* reached their maximum levels at intermediate times (4 to 6 h), and *CDC10* reached a maximum level at late times (after 7 h). The relative abundance of the H2A, H2B1, *GAL10*, *TDH*, and *URA3* transcripts also increased again at later times. Furthermore, these transcripts were found in the mature asci harvested at 24 h. Several uncharacterized high-molecular-weight (1.9 and 4.6 kb) transcripts complementary to plasmid TRT1 (Fig. 3) and the 4.2-kb transcript complementary to the *GAL10* gene first characterized by St. John and Davis (41) also accumulated at late times. These transcripts appeared to be most abundant in mature asci.

In contrast to sporulating cells, all the transcripts appeared to have similar kinetics in asporogenous g716-5A and AP1 *MAT*α/*MAT*α cells incubated in sporulation medium. The results for only one of the asporogenous strains is shown. However, identical results were obtained with both AP1 *MAT*α/*MAT*α and g716-5A. In the asporogenous cells, all transcripts were most abundant during the first 3 h. The relative levels then decreased or stayed the same for the remaining period examined (Fig. 2).

Quantitative differences in transcript abundance were also observed when RNA extracted from mixtures of sporulating cells harvested at different times was compared with RNA from similar mixtures from asporogenous cells and with RNA from exponential-phase vegetative cells (Fig. 2). Surprisingly, the average relative abundance of two of the sporulation-dispensable transcripts, *CDC10* and *GAL10*, and

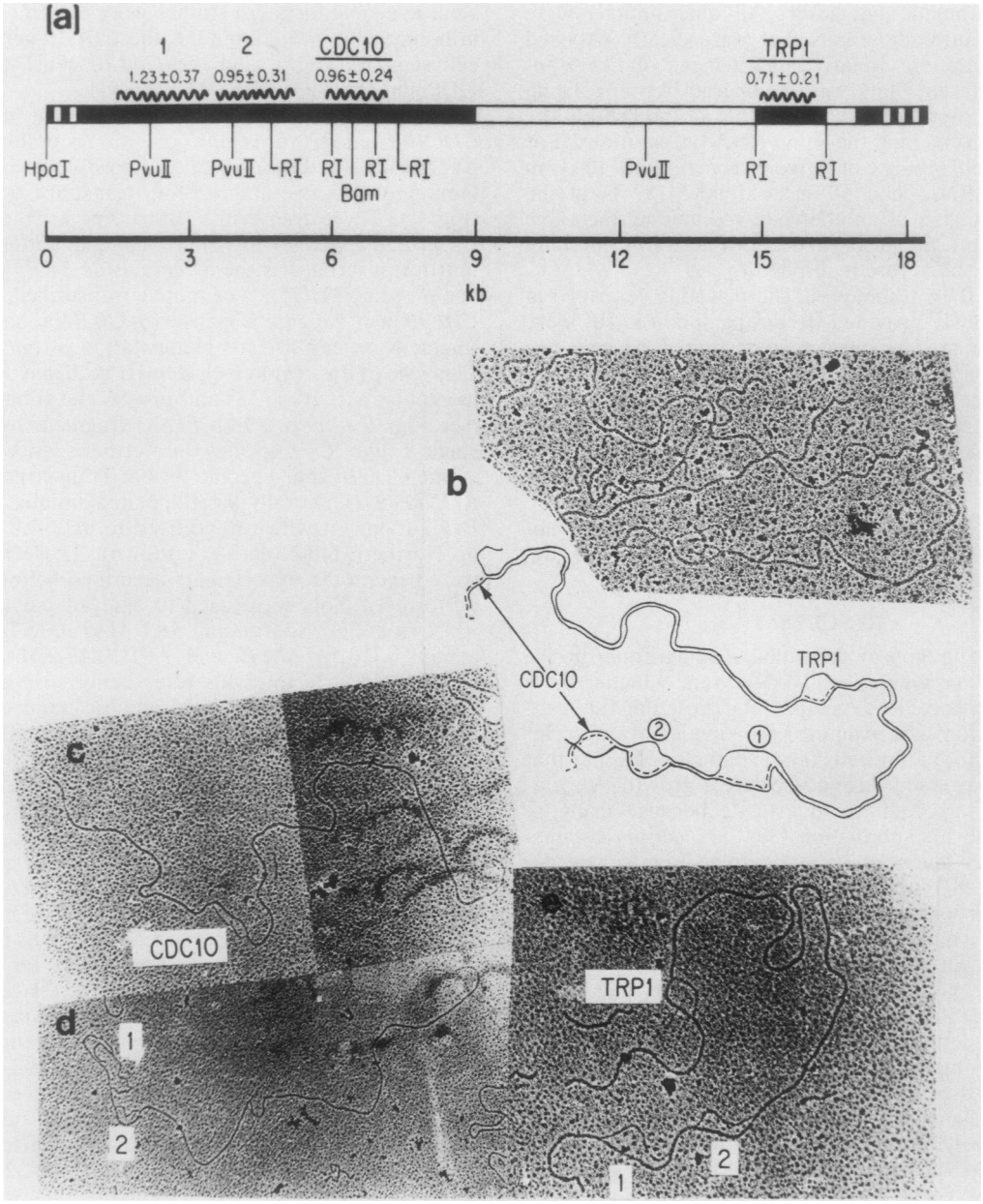

FIG. 1. Location of transcripts on the plasmid carrying the *CDC10* gene, pYE98F4T. Transcripts were localized by previously published techniques for R-loop mapping of nonabundant transcripts (22). To facilitate the R-loop mapping, we found a unique *Hpa*I site 5.6 kb from the single *Bam*HI site in the *S. cerevisiae* insert. This isolate of the plasmid contained a 2-kb deletion near the left vector-*S. cerevisiae* junction. However, this deletion was outside the region of interest on the *S. cerevisiae* DNA insert. To permit the unambiguous orientation of the R-loop-containing regions, overlapping R-loops maps were constructed with both *Bam*HI- and *Hpa*I-linearized plasmids. R loops were formed at 48°C in 70% formamide–0.1 M piperazine-*N*-*N*′-bis(2-ethanesulfonic acid (pH 7.2)–0.01 M EDTA, with 100 to 400 mg of poly(A)-containing RNA per ml and 5 μg of trioxsalen-cross-linked (one cross-link per 2 to 5 kb) plasmid DNA per ml. Incubation lasted for 16 to 20 h and were followed by 1.0 M glyoxal treatment for 2 h at 12°C to stabilize the R loops. *Bam*HI-cleaved DNA showed at least four transcribed regions but only transcripts 1, *CDC10*, and *TRP1* were unambiguously oriented in the *S. cerevisiae* DNA segments. In *Bam*HI-cleaved DNA, transcript 2 could have mapped to either side of the *Bam*HI site. Molecules that contained Y-shaped R-loop hybrids on both ends indicated that *Bam*HI cleaved the *CDC10* transcribed region (see b). *Hpa*I-linearized DNA also gave four R loops (c, d, and e). Transcripts 1 and *CDC10* wre unambiguously orientated on the *S. cerevisiae* DNA insert. However, transcripts 2 and *TRP1* could only be differentiated on molecules that contained an R loop at position 1 or the *CDC10* position. The map generated with *Hpa*I-cut DNA was consistent with only a single orientation for transcript 2 in the *Bam*HI cleaved sample because the two independently obtained maps were only superimposable if transcript 2 was to the left of the *Bam*HI site. Alternative orientations had *S. cerevisiae* transcripts mapping in the bacterial vector sequences. Finally, R-loop maps were identical to the RNA extracted from both sporulating and vegetatively growing SK1 cells and with RNA extracted from vegetatively growing Y185 cells (an unrelated strain). *Saccharomyces cerevisiae* sequences are shown by the unshaded line and bacterial DNA is shown by the solid line (a). The region of ambiguity because of the deletion is denoted by the hatched area. Measurements in kb ± the standard deviation are shown above each transcript in (a).

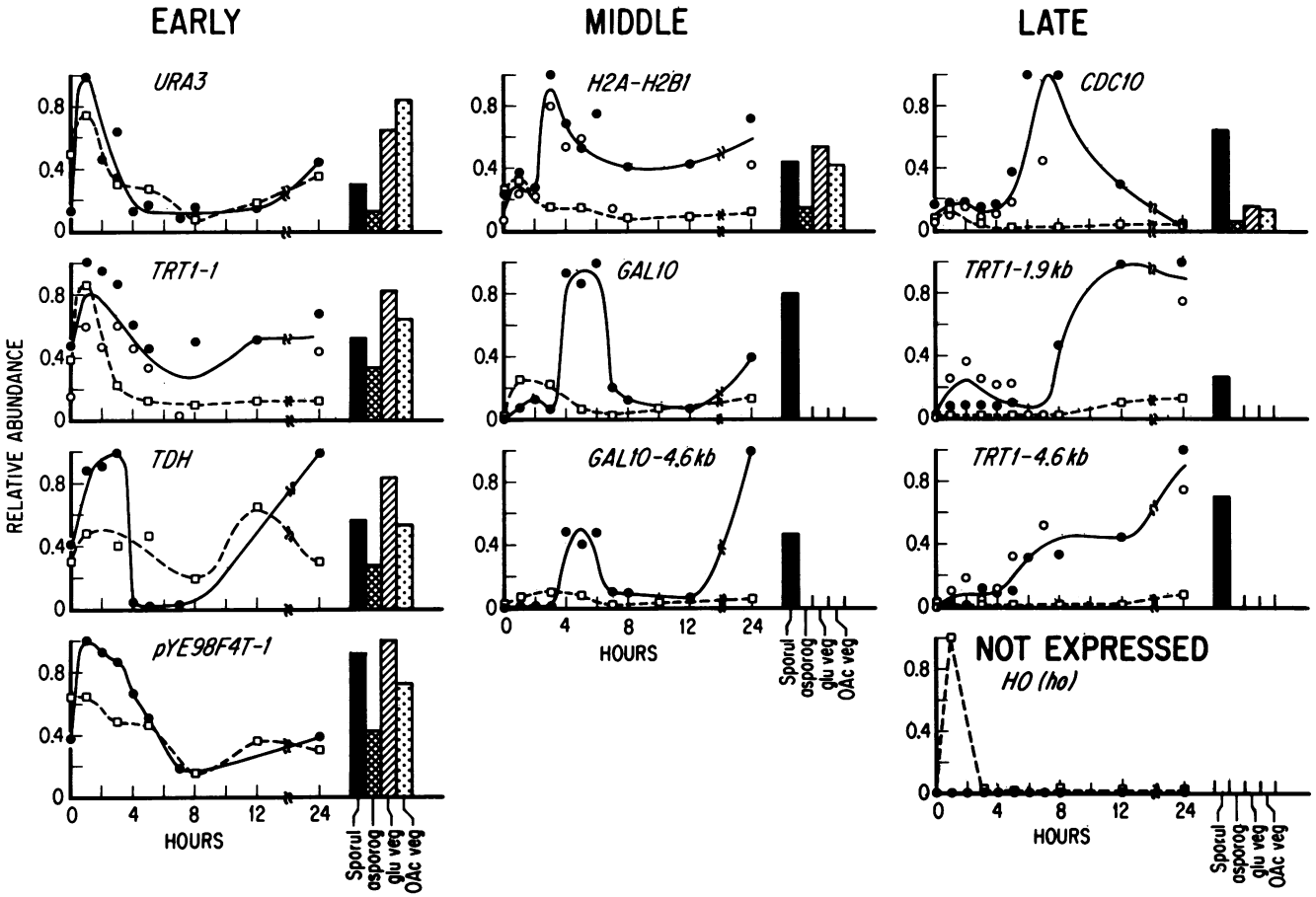

FIG. 2. Appearance of early, middle, and late transcripts in sporulating cells. Scanning densitometry of Northern blot autoradiographs was used to analyze the kinetics of appearance of the transcripts noted below. Equal amounts of poly(A)-containing RNA isolated from sporulating SK1 *MAT***a**/*MAT*α g716-5A *MAT***a** or AP1 *MAT*α/*MAT*α cells incubated for the noted times in sporulation medium was electrophoresed on 5.0 mM CH_3HgOH, (1.0% [wt/vol]) agarose gels and transferred to DBM paper. Blots were hybridized to ^{32}P-nick-translation-labeled DNA complementary to one or more of the noted transcripts and autoradiographed as described previously (1). Band intensities were quantitated and normalized as decribed in the text. Units of relative abundance are plotted as the fraction of maximal abundance. Probes were YIp5-HO(BH2), complementary to the *URA3* and the *HO* or *ho* transcripts; TRT1, complementary to H2A transcripts, the H2B1 transcript (17), *TRT1-1* (16), and the 1.9- and 4.6-kb high-molecular-weight transcripts; pGAPDH492, complementary to the*TDH* transcript; the 2.1-kb *Hpa*I-*Pvu*II fragment from pYE98F4T, complementary to *pYE98F4T-1*; pNN76, complementary to the *GAL10* and the 4.2-kb high-molecular-weight transcripts; the 0.9-kb *Eco*RI fragment from pYE98F4T, complementary to the *CDC10* transcript. The H2A and H2B1 transcripts were quantitated together (see text). Sporulating SK1 cells, experiment 1 (●), experiment 2 (○); asporogenous g716-5a or AP1 *MAT*α/*MAT*α cells incubated in sporulation medium (□). The bar graphs show relative transcript levels from 1 h time-point mixtures of sporulating or asporogenous cells incubated in sporulation medium and from YEPD- or acetate-grown vegetative SK1 cells. The absence of a bar indicates that the transcript was not present at a measurable level.

of the high-molecular-weight transcripts were many-fold greater in sporulating cells, whereas the abundance of H2A, H2B1, *URA3*, *TDH*, *URA3*, *TRT1-1*, and *pYE98F4T-1* was the same to 2.5-fold higher in sporulating cells compared with the asporogenous and vegetative controls. In only one case, *HO*, were we unable to detect a transcript in which one was detectable in the asporogenous cells. These and other interesting observations on the behavior of individual transcripts in sporulating and asporogenous cells will be discussed in more detail below.

Histone 2A and 2B transcripts are present both during and long after premeiotic DNA synthesis. The appearance of histone transcripts has not been examined previously throughout all stages of meiosis and sporulation. An autoradiograph showing the kinetics of appearance of H2A and H2B1 transcripts is shown (Fig. 3). Densitometric tracings of this and other Northern blot autoradiographs indicated that the H2A and H2B1 transcripts were controlled in parallel under all conditions examined. As a result, only the sums of the amounts of the H2A and H2B1 transcripts were plotted (Fig. 2). SK1 cells harvested from stationary phase (T = 0) and after 1 and 2 h in sporulation medium contained small amounts of H2A and H2B1 transcripts. At 3 to 6 h, the period coincident with premeiotic DNA synthesis (24; D. Kaback and L. Feldberg, unpublished data) H2A and H2B1 transcripts were most abundant. Unexpectedly, the H2A and H2B21 transcripts were present at approximately one-third to one-half the maximal level long after the cessation of premeiotic DNA synthesis. Indeed, even mature asci contained these high transcript levels. Equivalent amounts of H2A and H2B1 RNA were also found in purified ascospores prepared by the method of Rousseau and Halvorson (37) (data not shown).

Total RNA isolated from asci harvested over a 4-day

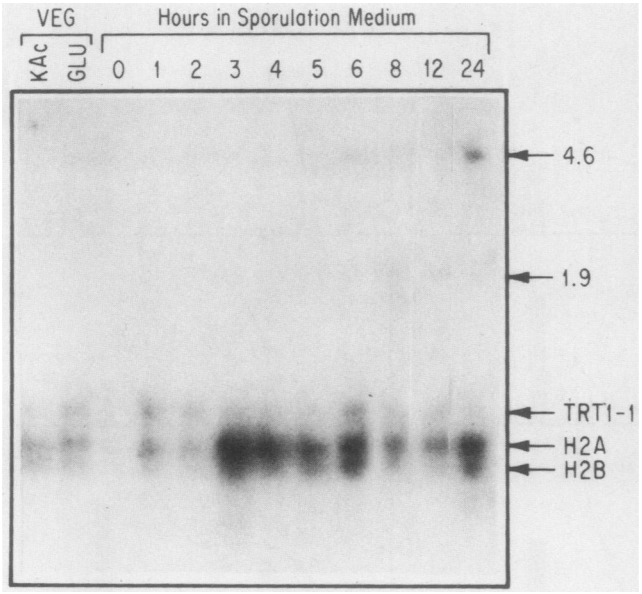

FIG. 3. Appearance of H2A, H2B1, *TRT1-1*, and the 1.9- and 4.6-kb high-molecular-weight transcripts throughout sporulation. Poly-(A)-containing RNA was isolated from glucose-grown vegetative SK1 cells (GLU VEG), acetate-grown vegetative SK1 cells (KAc VEG), and sporulating SK1 cells harvested at the noted times after being suspended in sporulation medium. The RNA was fixed to Northern blots. The blot was then hybridized to ^{32}P-nick-translation-labeled TRT1 DNA and autoradiographed, as described in the text.

period was then assayed on Northern blots to examine the persistence of these transcripts (Fig. 4). Sporulation was 90 to 95% complete at 16 h, when sampling was started. The histone transcript level was highest at this time and then slowly decreased over the next 2 to 3 days, so little remained at 96 h.

Asporogenous cells incubated in sporulation medium contained, on the average, two- to threefold less histone transcript than did sporulating cells (Fig. 2). As mentioned above, the kinetics of accumulation for asporogenous cells were different from that of sporulating cells and resembled the kinetics of all the other RNA species in the asporogenous cells. Transcripts were detected almost immediately after the cells were suspended in sporulation medium. Their appearance paralleled a short period of incorporation of [^{3}H]adenine into DNA (data not shown). After 1 h, the level of the H2A and H2B1 transcripts decreased two- to threefold. However, the transcripts remained detectable throughout the entire 24-h period examined, even though there was no detectable incorporation of [^{3}H]adenine into DNA after 4 h.

The persistence of *S. cerevisiae* histone RNA after DNA synthesis led us to examine mature asci and purified spores to determine whether the H2A-H2B1 RNA could be found in ribonucleoprotein particles or bound to polysomes. This experiment was performed because stored mRNA has been found in particles in the postribosomal supernatant of surf clam oocytes (34). Nucleic acid extracted from the polysome fraction from asci (fraction 3) contained almost all hybridizable H2A-H2B1 RNA (Fig. 5). Little, if any, histone transcript was detected in the monosome and ribonucleoprotein fraction (lane 2). The high-molecular-weight band found in fraction 1 (lane 1) is sheared *S. cerevisiae* DNA hybridized to the entire length of the TRT1 *S. cerevisiae* DNA insert. Similar results were obtained with polysomes extracted from purified ascospores (data not shown). These results suggest that the H2A and H2B1 transcripts are capable of being translated. However, the function of these late-appearing transcripts and whether they are actually translated is currently unknown.

Expression of genes nonessential for sporulation. To determine whether sporulation-dispensable genes were expressed during differentiation, RNA preparations from sporulating cells were examined for transcripts complementary to the *CDC10*, *GAL10*, and *HO* genes.

Diploids homozygous for thermosensitive mutations in the *CDC10* gene fail to complete mitotic cytokinesis and bud separation but can sporulate at the restrictive temperature (38). In addition, we found that spores from *cdc10/cdc10* homozygotes that had developed at the restrictive temperature were completely viable when grown at permissive temperatures. Accordingly, the *CDC10* gene product is thought to be dispensable for sporulation. Surprisingly, Northern blots hybridized to the 0.9-kb *Eco*RI fragment containing the *CDC10* gene indicated that mixtures of spor-

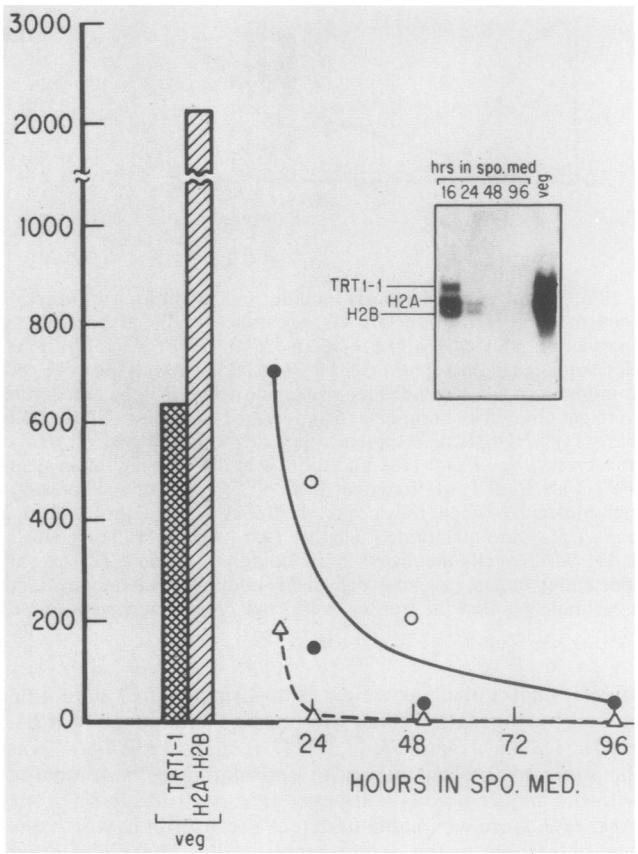

FIG. 4. Occurrence of H2A, H2B1, and *TRT1-1* transcripts in mature asci. Northern blot autoradiographs and densitometric quantitation of total RNA from asci harvested at the noted times from sporulation medium and from exponential phase glucose-grown cells hybridized with ^{32}P-labeled TRT1 DNA. Hybridizations and quantitations were carried out as described in the text. Relative absorbance units are arbitrary. H2A, H2B1, experiment 1 (shown in autoradiograph) (●), experiment 2 (○); TRT1-1 experiment 1 (△), *TRT1-1* was not detected in experiment 2. The bar graph shows the relative abundance of these RNA species in glucose-grown vegetative cells (veg).

ulating cells contained five- to sevenfold more *CDC10* transcript than did vegetative, g716-5A (Fig. 2 and 6), or AP1 *MATα/MATα* (data not shown) cells incubated in sporulation medium. As mentioned above, the *CDC10* transcript also showed sporulation-specific kinetics, appearing most abundant at late times (5 to 12 h) in sporulating cells. In contrast, in asporogenous cells the *CDC10* transcript appeared at early times, i.e., 1 to 3 h (Fig. 2).

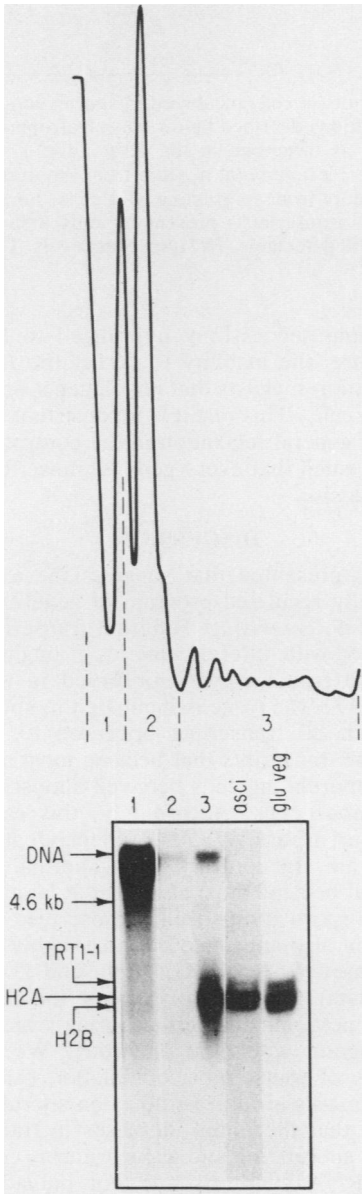

FIG. 5. Late-appearing H2A and H2B1 transcripts sediment with polysomes. Total RNA was prepared from spores and asci as described in the text. Cells were lysed and polysome gradients run as described by Harper et al. (14). RNA was prepared as described in the text from each of the pooled fractions noted in the tracing done at an absorbance of 260 nm. Equal amounts of nucleic acids from all preparations were electrophoresed, transferred to DBM paper, hybridized to [^{32}P]TRT1 DNA, and autoradiographed as described in the text. Lane 1, fraction 1, sheared yeast DNA; lane 2, fraction 2, ribonucleoproteins, ribosomal subunits, and monosomes; lane 3, fraction 3, polysomes. As a control, total RNA extracted from mature asci (asci) and glucose-grown vegetative cells (glu veg) was analyzed.

We confirmed these results by quantitating the transcripts on pYE98F4T using electron microscopy of R-loop-containing DNA. Poly(A)-containing RNA from sporulating SK1 cells, acetate-grown vegetative SK1 cells, glucose-grown vegetative SK1 cells, and asporogenous g716-5A cells was hybridized to excess plasmid DNA so that all complementary RNA was driven into R loop hybrids (23). The fraction of plasmid DNA containing each of the four different R loops was then determined and used to calculate the relative abundance of each transcript under each condition (Table 3). All four RNA species were found in sporulating and vegetative cells. All transcripts were also found in g716-5A cells incubated in sporulation medium, although transcript 2 and the *TRP1* transcript were not quantitated. By these methods, the *CDC10* transcript was at least 3.5-fold more abundant in sporulating cells than in vegetative cells and 7-fold more abundant than in asporogenous g716-5A cells. These results are in good agreement with the Northern blot data described above. Thus, the *CDC10* transcript has all the qualities of a sporulation-specific induced transcript except that it appears to encode a function that is not essential for sporulation.

Similar results were obtained when the *GAL10* gene was examined using Northern blots. Enzymes required for the catabolism of galactose are encoded by the inducible *GAL7*, *GAL10*, and *GAL1* gene cluster (11). Homozygous galactose-negative *S. cerevisiae* sporulate (D. Hawthorne, personal communication; D. Kaback, unpublished data), so the genes involved in galactose catabolism can also be considered dispensable for sporulation. High levels of transcripts complementary to the *GAL7*, *GAL10*, *GAL1* gene cluster are found in galactose-grown cells. However, these transcripts are not found in cells grown on glucose and are found at low or undetectable levels in cells grown on acetate (20, 40, 41).

Surprisingly, the *GAL10* transcript was more abundant in sporulating cells than in either vegetative acetate- or glucose-grown exponential cells in which it was almost undetectable. Sporulating cells also contained more of this transcript than both asporogenous AP1 *MATα/MATα* (Fig. 6) and g716-5A *MAT***a** (data not shown) cells incubated in sporulation medium. Furthermore, the *GAL10* transcript appeared with sporulation-specific kinetics. Notably, the kinetics were different from that of *CDC10* (Fig. 2). Thus, the *GAL10* gene is another example in which a gene with no apparent essential function in sporulation is preferentially expressed during this process.

The *HO* gene was the third gene examined that is nonessential for sporulation. *MAT***a** and *MATα* cells that carry *HO* switch mating types at high frequencies. However, *MAT***a**/*MATα* *HO* diploid cells do not switch. The *HO* transcript is only detected in switching *MAT***a** or *MATα* vegetative cells and is not detected in vegetative *MAT***a**/*MATα* diploid cells (21). Heterothallic (*ho*) *MAT***a** and *MATα* strains transcribe the cross-hybridizing *ho* allele which does not cause high-frequency switching. The *HO* gene is thought to be dispensable for sporulation, and sporulating cells rarely, if ever, switch mating types (Amar Klar, personal communication). Because the *GAL10* and *CDC10* transcripts were present at higher relative abundances, it seemed plausible that all transcripts, including the one from *HO* or *ho*, might be induced to higher levels during sporulation. To test this possibility, YIp5-*HO*(BH2), a plasmid containing most of the *HO* gene (21), was used to probe the Northern blots from the previous experiments. In these experiments, the *URA3* gene, which is also contained

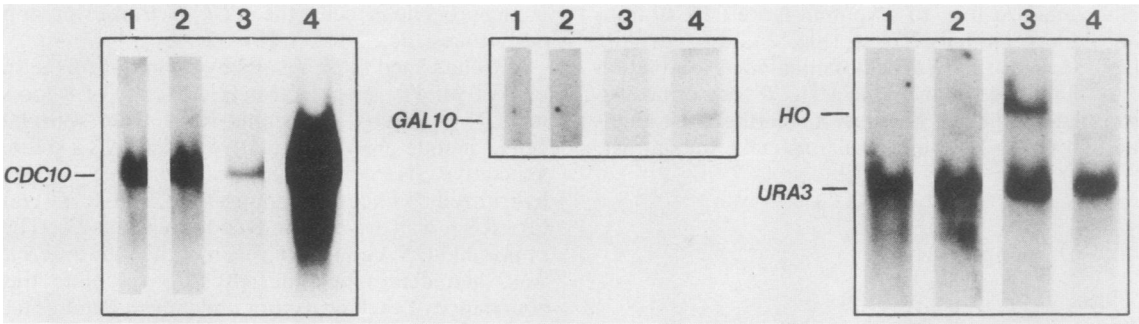

FIG. 6. Appearance of the sporulation-dispensable *CDC10*, *GAL10*, and *HO* transcripts in cells incubated in sporulation and vegetative growth medium. Poly(A)-containing RNA isolated from cells incubated under the conditions decribed below was electrophoresed; blotted; hybridized to the *CDC10*, *GAL10*, or *HO* ^{32}P-labeled probes and autoradiographed as described in the text. Lane 1, glucose-grown exponential-phase SK1 cells; lane 2, acetate-grown exponential-phase SK1 cells; lane 3, 1 h time-point mixture from asporogenous g716-5A or AP1 *MATα/MATα* cells incubated in sporulation medium; lane 4, 1 h time-point mixture from sporulating SK1 cells. For *HO*, lane 3, in which this transcript was most abundant, contains RNA isolated at 1 h. Because this transcript is present for only a short time in the asporogenous cells (Fig. 1), the time-point mixtures contained extremely low, although still detectable, *HO* transcript levels. These low levels could not be reproduced photographically.

on this plasmid, was present as a control. In contrast to all the other genes examined, the *HO* transcripts was not detected in either poly(A)-containing (Fig. 6) or total RNA (data not shown) isolated from sporulating cells, In contrast, the cross-hybridizing *ho* transcript was indeed found in the asporogenous *MAT***a** and *MATα/MATα* cells incubated in sporulation medium (Fig. 6). Accordingly, sporulation medium does not repress the appearance of this transcript in cells that are normally expected to express this gene. In agreement with Jensen et al. (21), *ho* RNA was also detected in vegetative *MAT***a** and *MATα/MATα* cells (data not shown). *URA3* RNA was detected in all samples examined from sporulating, asporogenous, and vegetative cells, indicating that the plasmid successfully hybridized to the Northern blots. Therefore, the inability to detect the *HO* transcript during sporulation suggests that not all genes are transcribed in sporulating cells. This result is inconsistent with the idea that there is a general relaxation in the control of transcript accumulation, such that every gene is transcribed during *S. cerevisiae* meiosis.

TABLE 3. Abundance of the *CDC10* transcript as determined by quantitative R-loop hybridizations[a]

Cell type	Weight fraction of poly(A)-containing RNA ($\times 10^{-4}$) of the following transcripts			
	pYE98F4T-1	*pYE98F4T-2*	*CDC10*	*TRP1*
SK1 glucose-grown vegetative	5.30	1.28	1.70	0.37
SK1 acetate-grown vegetative	4.53	0.91	0.93	0.38
SK1 pooled sporulating	13.10	1.01	5.70	0.54
g716-5A pooled asporogenous in sporulation media	3.65	—[b]	0.78	—

[a] pYE98F4T DNA containing R loops were prepared for electron microscopy as described in the legend to Fig. 4. The relative amount of each transcript was determined for each RNA preparation by counting the fraction of *HpaI*-cleaved plasmid DNA molecules containing each R loop. The weight fraction of each transcript in the total poly(A)-containing RNA population was calculated as previously described (22,23). Quantities shown are the average of two separate hybridization experiments with two different RNA concentrations and the same amount of DNA. For each R loop, an approximately linear relationship between the number of R loops and the concentration of poly(A)-containing RNA was observed. Transcript 2 and the *TRP1* transcript which could not be unambiguously differentiated in DNA molecules containing a single R loop were quantitated by first determining the ratio of these two transcripts on plasmid molecules with R loops from either transcript 1 or *CDC10*. The molecules could then be unambiguously oriented so that the two transcripts could be differentiated. The actual fraction of plasmid molecules containing each of these two R loops was then calculated by multiplying the combined number of *TRP1* and transcript 2 R loops by the relative fractions of each determined in the orientatable plasmids.

[b] —, Transcripts were present but not quantitated.

DISCUSSION

Evidence is presented that suggests the existence of a developmentally regulated program of gene expression for sporulation of *S. cerevisiae*. Different transcripts appeared and disappeared with different kinetics throughout this process. These patterns were not observed in asporogenous *MAT***a** and *MATα/MATα* cells incubated in sporulation medium in which all transcripts appeared to have similar kinetics. Those transcripts that became most abundant at 1 to 3 h in the sporulating cells behaved almost identically in the asporogenous cells. Accordingly, this early behavior may not be part of a *MAT***a***/MATα*-dependent sporulation-specific program. In contrast, only sporulating cells had transcripts that became most abundant at later times. Notably, all cloned sporulation-specific transcribed sequences so far investigated also appear to be most highly expressed at these later times (6, 30; L. G. Ninfa and D. B. Kaback, unpublished data). Consequently, the period after 3 h would represent the predominant period during which any developmental program would be operating. We suggest that these patterns of transcript accumulation can be used as temporal landmarks in future studies on sporulation.

We believe that the initial increases in transcript abundance in both sporulating and asporogenesis cells were due to de novo RNA synthesis and not polyadenylation of preexisting transcripts. First, unfractionated total cellular RNA extracted from T = 0 cells contained extremely low levels of transcripts complementary to many of the genes examined (unpublished data). Accordingly, transcript appearance was probably not due to polyadenylation. Second, the early increases in the relative abundance of specific transcripts paralleled an increase in the overall yield of poly(A)-containing RNA; i.e., yields of poly(A)-containing RNA (oligodeoxythymidylate-binding material) were almost negligible at T = 0, but increased 5- to 10-fold at T = 1 h. Consequently, all early increases must have been caused by transcript synthesis.

Between T = 1 and 7 h, the per cell yields of poly(A)-containing RNA were relatively constant to within a factor of two (unpublished data). Therefore, changes in relative transcript abundance during this period would have to parallel the actual per cell changes. In contrast, after 7 h the yields of poly(A)-containing RNA were usually 2- to 10-fold lower. Accordingly, late increases in transcript abundance need not be the result of net increases in abundance but could also be caused by the preferential stabilization of preexisting RNA.

Sporulating cells are known to be rich in ribonucleases (44). In spite of this fact, when we observed low or undetectable transcript levels (such as at T = 0), we do not believe it was due to the degradation of RNA during isolation. Our procedure for isolating RNA has allowed purification of intact RNA from other sources extremely rich in ribonucleases (5). It substantially reduces the risk of degradation because cells are broken rapidly in ice-cold, protein-denaturing guanidinium thiocyanate buffer and quickly frozen. By using this procedure, the yields of total RNA were approximately equal at most times, including T = 0, in both sporulating and asporogenous cells, and the purified RNA consisted mostly of intact rRNA, as assessed by its behavior on denaturing agarose gels. In addition, all specific transcripts detected in these samples appeared as discrete bands on Northern blots, further indicating that the RNA was not degraded.

In this study, we compared relative transcript levels between the different cell types, not absolute levels (transcripts per cell). Although transcript per cell values are the most useful for comparing gene expression, they are difficult to determine accurately. However, we can make a rough comparison of the per cell levels by taking into account the approximate yields of poly(A)-containing RNA from a fixed number of cells. We assume that these yields are proportional to the actual poly(A)-containing RNA content for each cell type. For example, the yields for poly(A)-containing RNA were approximately twofold greater for vegetative cells than for the sporulating cell mixtures. Accordingly, on our blots, transcripts that appear to be twofold more abundant in sporulating cells than in vegetative cells probably are equally abundant in absolute levels in both cell types. In contrast, the yield of poly(A)-containing RNA isolated from mixtures of asporogenous cells incubated in sporulation medium were usually several-fold lower than from mixtures of sporulating cells (L. Feldberg and D. Kaback, unpublished data). Therefore, relative increases in transcript levels in sporulating cells compared with asporogenous cells are probably an underestimate of the absolute differences that exist. For example, the *CDC10* and *GAL10* transcripts are probably several-fold more abundant per sporulating cell than per asporogenous cell than is evident from the autoradiographs.

Finally, we believe that all our results are caused by changes that take place in the population of cells that undergo sporulation. Several previous studies utilized strains in which only 60 to 80% of the cells sporulate (19, 26). In these other studies, physiological changes that are detected may be attributed to the population of cells that fail to sporulate. It is unlikely that this is a problem in our studies, because we utilized SK1, a strain that sporulated to greater than 95% during the period examined. Accordingly, we assume all transcripts that we observed appeared in the sporulating cells.

Histone transcripts. Histone transcripts increased during the period of premeiotic DNA synthesis. This increase was sporulation specific, although this was expected because only sporulating cells underwent premeiotic DNA replication. Unexpectedly, H2A and H2B1 transcripts were present long after the end of premeiotic DNA synthesis and may even have increased in abundance during this late period. This result is in sharp contrast to vegetative cells, in which histone transcripts only appeared coincidentally with DNA synthesis (17). As mentioned, the appearance of histone transcripts at this late time during sporulation could have been due to either de novo synthesis or long-term stabilization of preexisting RNA species. Making a distinction between these alternatives is not possible at the present time because it is difficult to determine if and when transcription stops in sporulating cells.

Histone transcripts were also found in *MAT***a** and *MATα*/*MATα* cells long after DNA synthesis stopped. The relative abundance at T = 24 was equal to, or slightly less, than at T = 0. Consequently, these transcripts could represent a basal level for starved cells. Alternatively, it is possible that these transcripts were induced to this low level by the same nutritional conditions which cause sporulation.

The function of the late histone transcripts in sporulating cells is unknown. These transcripts appear to be present on polysomes. However, it is not known whether these polysomes are functioning to synthesize histones. Stable histone mRNA species have been reported in postmeiotic cells (eggs) of some higher organisms (39). However, the histone transcripts of these higher organisms are usually stable for several months, whereas the *S. cerevisiae* transcripts were present for only 2 to 3 days after sporulation. Therefore, the two situations may not be analogous.

Transcript accumulation for sporulation-dispensable genes. The transcripts from the *CDC10* and *GAL10* genes, which appear to be nonessential for sporulation, were more abundant in sporulating cells than in nonsporulating cells. These results, combined with the presence of the late-appearing histone transcripts, initially suggested that there might be a general derepression of transcription during sporulation. These events might be compared with the lampbrush-chromosome stage of meiosis in amphibians and other higher organisms. However, the available evidence does not support this idea. The *CDC10* and *GAL10* transcripts each accumulated with different kinetics suggesting their appearances were controlled independently. In addition, with the exception of H2A and H2B1, which are normally coordinately regulated, transcribed regions on the same plasmid inserts showed no evidence for being expressed at the same times during sporulation. *TRT1-1* appeared earlier than the histone transcripts, whereas the *pYE98F4T-1* transcript became most abundant earlier than the closely linked *CDC10* transcript. Thus, there is no evidence for large chromosomal regions becoming transcriptionally active in a stage-specific manner. Finally, the *HO* transcript was not found in sporulating cells, suggesting that the expression of at least one gene is prevented during sporulation. In total, these results make it unlikely that there is a general or stage-specific relaxation of transcriptional control during sporulation.

Because *CDC10* and *GAL10* appear to be regulated, we need to consider the possibility that these genes have a role in sporulating cells. An essential role for *CDC10* seems unlikely, because this gene appears to be required to complete bud separation, and sporulation occurs in the absence of budding. In fact, all the genes of the budding pathway of the cell division cycle appear to be dispensable for sporulation (38). It is also conceivable that the *CDC10* gene product has an essential sporulation function that is not thermolabile

in the mutant strains examined or is only required under different conditions. Finally, the mutant *cdc10* gene could be providing sufficient activity for sporulation, but not for vegetative growth (38).

As with *CDC10*, we cannot eliminate the possibility of some role for the *GAL10* gene in sporulation. Increased levels of *GAL7* and *GAL1* transcripts were found in mature spores and asci (L. Feldberg and D. Kaback, unpublished data), whereas increased levels of the *GAL1* gene product galactokinase have been reported in sporulating cells (29). Thus, the whole *GAL* gene cluster may be expressed, giving sporulating cells the capacity to catabolize galactose. This ability could be useful for some facet of cell wall synthesis or when spores germinate.

Much is known about the normal regulation of the *GAL* gene cluster in vegetative cells (11, 20, 41). However, the mechanism for the appearance of the sporulation-induced *GAL10* transcript is not understood. Expression is in the absence of any exogenously added galactose and therefore appears gratuitous. It is possible that the inducer is being produced endogenously. In this case induction could occur by the normal control mechanism which utilizes the *GAL4* and *GAL80* gene products (20). Alternatively, induction might be controlled by a completely different mechanism from that which normally controls the expression of these genes (29).

In summary, the mechanism and reasons for the sporulation-specific appearance of both the *CDC10* and *GAL10* transcripts remain unclear. Their presence appears to be regulated because they all accumulate with different kinetics. We emphasize the expression "appears regulated" because it is still conceivable that the increased abundance is fortuitous, caused by mechanisms that are only incidentally activated by sporulation. Similarly, the sporulation-specific appearance of any transcript may be incidental. Irrespective of interpreting what really is developmentally specific, our results suggest that some genes that are not essential for sporulation can produce higher relative levels of RNA during sporulation than during vegetative growth. Conversely, genes that appear to be preferentially expressed (6, 30) during sporulation may neither be essential for nor involved in the differentiation of a vegetative cell into an ascospore. Accordingly, the ability to determine a role for the preferentially expressed sequences in *S. cerevisiae* sporulation by in vitro mutagenesis and gene replacement (36) will be important.

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ADDENDUM IN PROOF

Recently, Kurtz and Lindquist (Proc. Natl. Acad. Sci. U.S.A. **81**:7323–7327, 1984) reported complementing evidence for a sporulation-specific pattern of transcript accumulation based on in vitro translation experiments.

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