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Isolation of DNA Sequences Preferentially Expressed During Sporulation in *Saccharomyces cerevisiae*

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A differential hybridization screen has been used to identify genes cloned from the yeast *Saccharomyces cerevisiae* that are expressed preferentially during sporulation. Duplicate copies of a partial *Sau*3A yeast DNA library prepared in the vector pBR322 were hybridized with radioactive cDNA probes representing the mRNA populations of sporulating **a**α cells and asporogenous αα cells at various times after transfer to sporulation medium. Thirty-eight clones showed an enhanced hybridization signal with the **a**α sporulation probe relative to the αα control cDNA probe. A comparison of the array of fragments produced by restriction endonuclease digestion of these plasmids suggested that 15 different sequences had been cloned. An RNA blot analysis using these cloned DNAs to probe RNAs purified from **aa**, **a**α, and αα cells harvested either during vegetative growth or at 10 h after transfer to sporulation medium indicated that 14 different sporulation-specific genes had been identified. Transcripts complementary to these genes are present only in **a**α cells after transfer to sporulation medium. Three of these clones contain two sporulation-specific genes. Three genes have been identified that are expressed in all cell types during vegetative growth and only in **a**α cells in sporulation medium.

Sporulation in the yeast *Saccharomyces cerevisiae* can be viewed as a simple developmental process that includes meiosis and the formation of an ascus containing four haploid nuclei. This differentiation process is under the control of the mating type locus (*MAT*) that consists of two alleles (*MAT***a** and *MAT*α) controlling the two cell types **a** and α (for a review, see reference 11). Cells of opposite mating types can fuse to form a diploid cell (*MAT***a**/*MAT*α), which, if deprived of nitrogen and in the presence of an oxidative carbon source, will sporulate. However, haploid cells or diploid cells homozygous for the mating type locus cannot be induced to sporulate.

The increasing ease with which genetic manipulations can be carried out in *S. cerevisiae* (2) makes sporulation an appealing system for investigating the regulation of eucaryotic gene expression during differentiation. However, several attempts to identify sporulation-specific proteins with a two-dimensional gel electrophoretic analysis have failed to reveal any unique *MAT***a**/*MAT*α sporulation proteins (14, 22, 26). All proteins made in sporulating cells, but not made in vegetatively growing cells, were also found in asporogenous *MAT*α/*MAT*α cells upon nutrient starvation. On the other hand, it has been shown that protein synthesis is essential for sporulation (13, 15), and genetic studies suggest that approximately 50 genes are uniquely required for sporulation (6). More recently, several protein modifications specific to sporulation have been observed (27), and one sporulation-specific enzyme, an α-glucosidase involved in glycogen catabolism, has been described (4).

It seemed possible that the failure to detect sporulation-specific proteins by a two-dimensional polyacrylamide gel electrophoretic analysis was due to the limited sensitivity of this technique; only the most abundant 400 of approximately 4,000 different proteins (10) are visualized. Hence, we have reexamined the extent of the expression of sporulation-specific genes taking advantage of the increased sensitivity provided by recombinant DNA technology. We have screened a yeast DNA library for genes expressed preferentially in *MAT***a**/*MAT*α cells during sporulation. In this communication we report the identification of 14 sporulation-specific genes.

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MATERIALS AND METHODS

Strains and culture conditions. Isogenic diploids of the *S. cerevisiae* strain AP-3 differing only at the *MAT* loci (*MAT***a**/*MAT***a**, *MAT***a**/*MAT*α, *MAT*α/*MAT*α) were used throughout this study. The AP-3 genotype is *ade1*/+ *ade2*/*ade2* *gal1*/+ *tyr1*/+ *lys2*/+ *his7*/+ *ura1*/+ +/*ura3* +/*can1* +/*cyh2* +/*leu1* *CSP*$^+$/*CSP*$^+$. These strains were derived by Hopper et al. (12, 13) and provided by J. Haber. Cells were grown vegetatively in YEPA (1% yeast extract, 2% peptone, 1% potassium acetate, pH 5.5) at 30°C. For sporulation, cells were harvested from YEPA at 2×10^7 cells per ml, washed twice with sporulation medium (1% potassium acetate [pH 7] supplemented with 40 μg of adenine per ml) and suspended at 0.5×10^7 cells per ml in sporulation medium. As determined by light microscopy, asci were first visible at 13 h, and by 24 h greater than 65% of the cells had formed asci.

Materials. T4 ligase, bacterial alkaline phosphatase, DNA polymerase I (large fragment), *Sau*3A, and *Hin*fI were purchased from Bethesda Research Laboratories, Inc.; *Bam*HI was purchased from New England Biolabs; *Escherichia coli* DNA polymerase I was purchased from Boehringer Mannheim, and placental RNase inhibitor was from Bolton Biologicals. Avian myeloblastosis virus reverse transcriptase was a gift of J. Beard (Life Science, Inc.). Nitrocellulose was from Schleicher & Schuell, and [α-^{32}P]dGTP and [α-^{32}P]dATP were from New England Nuclear Corp. and Amersham Corp. pBR322 DNA was a gift from D. Pulleyblank. The plasmid containing the histone H2A and H2B genes (TRT1.1) was provided by L. Hereford (9) and contains a 1.1-kilobase (kb) yeast DNA fragment that spans a portion of the 5′ coding sequences of the divergently transcribed H2A and H2B genes and the spacer region between the genes. The plasmid containing the actin gene as well as a second uncharacterized gene was isolated by Ng and Abelson (21) and provided by J. Friesen.

Preparation of nucleic acids. Yeast DNA was extracted from strain LL-20 (α *leu2-3 leu2-112 his3-11 his3-15 can1*) essentially as described by Cryer et al. (5), with the omission of the RNase digestion steps, and was further purified by centrifugation to equilibrium in a CsCl-ethidium bromide density gradient. Plasmid DNA was extracted form *E. coli* by a sodium dodecyl sulfate (SDS) lysis procedure (7) as described previously (18) and purified by centrifugation to equilibrium in a CsCl-ethidium bromide density gradient.

RNA was prepared from yeast by a method similar to that described by Kraig and Haber (14). Cells harvested from 250- to 500-ml cultures were suspended in 0.5 ml of lysis buffer (50 mM Tris-hydrochloride [pH 6.8], 10 mM EDTA, 100 mM NaCl). After the addition of 1 ml of buffer-saturated phenol and 1.5 ml of acid-washed 0.5-mm glass beads, the suspension was vortexed for 6.5 min in 20-s bursts interrupted by 20-s cooling in an ice-salt slurry. After the addition of 0.5 ml of phenol, 0.5 ml of lysis buffer, and 0.1 ml of a 25% SDS solution to the cell lysate, the aqueous and organic phases were separated by centrifugation. Both phases were reextracted, and the pooled aqueous phases were then extracted with a 1:1 mixture of phenol-chloroform. RNA was precipitated from the aqueous phase by the addition of 0.1 volume of 3 M sodium acetate (pH 5.5) and 2.5 volumes of ethanol. After cooling at −20°C, the RNA was collected by centrifugation and suspended in 2 ml of water. The RNA was reprecipitated once by the addition of an equal volume of 5 M LiCl and twice with ethanol. Polyadenylated [poly(A$^+$)] RNA was purified by one passage of RNA through an oligodeoxythymidylic acid-cellulose (type II; Collaborative Research, Inc.) column as described by Aviv and Leder (1). [Based on absorbance at 260 nm, approximately 2 to 5% of the RNA was recovered as poly(A$^+$) RNA.]

Construction of a yeast DNA library. A partial *Sau*3A yeast library was constructed essentially as described by Nasmyth and Reed (20). Equivalent amounts of yeast DNA were partially digested with 1, 2, 3, or 4 relative units of *Sau*3A (1 relative unit resulted in extremely limited digestion, and 4 relative units resulted in almost complete digestion of the DNA). The pooled DNA fragments were then size fractionated by centrifugation through a gradient of 5 to 20% sucrose. Fractions from the gradient containing DNA fragments from 4 to 8 kb were pooled, and the DNA was concentrated by precipitation with ethanol. Vector DNA was prepared by treating pBR322 DNA that had been digested with *Bam*HI with bacterial alkaline phosphatase. Yeast DNA fragments (3 μg) were incubated with 3 μg of vector DNA (50 μg/ml) in the presence of T4 ligase, and the DNA mixture was used to transform calcium chloride-treated cells of *E. coli* HB101 (16). This yielded a library containing 24,000 ampicillin-resistant transformants of which 80% had inserts (as determined by an Amp^r Tet^s phenotype). The average insert size was found to be 5.6 kb (varying from approximately 1.1 to 13 kb) by an analysis of the fragments obtained on digestion of plasmid DNA purified from 20 colonies with *Eco*RI and *Hin*dIII. To store the library, the colonies were scraped off the plates, concentrated by centrifugation, and suspended in LB (0.5% yeast extract, 1% tryptone, 0.5% NaCl) containing 15% glycerol. Samples were stored at −70°C.

Preparation of ^{32}P-labeled cDNA probes. Radioactively labeled cDNAs were synthesized as described by St. John and Davis (25) in a 25-μl reaction containing 50 mM Tris-hydrochloride (pH 8.3), 8 mM $MgCl_2$, 50 mM KCl, 3 mM dithiothreitol, 0.5 mM dTTP, 0.5 mM dCTP, 0.5 mM dATP, 0.5 μg of oligo(dT)$_{12-18}$, 1 μg of poly(A$^+$) RNA, 7.5 U of placental RNase inhibitor, 16 U of reverse transcriptase, and 300 μCi of [α-^{32}P]dGTP (3,000 Ci/mmol). After a 1-h incubation at 37°C, NaOH and SDS were added to a concentration of 0.1 N and 0.04%, respectively. After hydrolysis of the RNA at 65°C for 10 min, the cDNA was purified by chromatography on Sephadex G-50. The cDNA probes used in the dot-blot hybridizations were prepared as described above, except that the reverse transcription reaction contained 0.5 μg of poly(A$^+$) RNA and 100 μCi of [α-^{32}P]dGTP (3,000 Ci/mmol). The reaction products were recovered by precipitation with polyethylene glycol (28) and resuspended in water. After hydrolysis of the RNA as described above, the solution containing the cDNA probe was neutralized.

Preparation of ^{32}P-labeled restriction fragments. Plasmid DNAs were digested with *Hin*fI and end labeled as follows. Plasmid DNA (0.5 μg) was incubated with an excess of *Hin*fI in a 10-μl reaction containing 50 mM Tris-hydrochloride (pH 8.0), 10 mM $MgCl_2$, and 50 mM NaCl. After 2.5 h at 37°C, 5 μCi of [α-^{32}P]dATP and 1 U of DNA polymerase I (large fragment) were added. The reaction was incubated for an additional 30 min at room temperature, and 5 μl of sample buffer (40% sucrose, 25 mM EDTA [pH 7.4], 0.02% bromophenol blue) was then added.

Differential hybridization screen. Four pairs of probes were used in the differential hybridization screen. Each pair consisted of radioactively labeled cDNAs prepared with RNA purified from **a**α and αα cells harvested at either 3, 7, 10, or 15 h after transfer to sporulation medium. For each pair of probes to be compared in the screen, approximately 600 transformants were plated on each of eighteen 6- by 6-cm nitrocellulose filters placed on LB plates. After the colonies had grown up, two replicates were made, in situ chloramphenicol amplification of the plasmid DNA was carried out, the colonies were lysed, and the DNA was fixed to the filters as described by Hanahan and Meselson (8). After prehybridization of the filters for 12 h at 65°C in a solution containing 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]), 1× Denhardt solution (0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrolidone), and 0.2% SDS, one set of the replicate filters was hybridized with the cDNA probe prepared with poly(A$^+$) RNA from **a**α cells, and the other set was hybridized with the cDNA probe prepared with poly(A$^+$) RNA from αα cells. The hybridization buffer contained 5× SSC, 1× Denhardt solution, 0.2% SDS, 50 μg of *E. coli* DNA per ml, and the probe (10^6 Cerenkov cpm per ml of hybridization buffer). After incubation at 65°C with gentle shaking for 48 h, the filters were washed three times in 5× SSC–0.2% SDS at 65°C, twice in 5× SSC–0.2% SDS at room temperature, and twice in 2× SSC–0.2% SDS at room temperature for 1 h each followed by a 7-min wash at room temperature in 0.2× SSC–0.2% SDS.

DNA dot-blot and RNA filter hybridizations. A Hybri-Dot manifold (Bethesda Research Laboratories) was used to prepare filters for the DNA dot-blot hybridizations. A filter that had been wetted in water and soaked in 20× SSC for 30 min was placed into the manifold, and 1 μg of the appropriate plasmid DNA that had been denatured in 0.3 N NaOH for 30 min at room temperature and then neutralized was placed in each well. The wells were then washed three times with 20× SSC, and the filter was baked for 3 h in vacuo at 80°C. The filters were prehybridized in a solution containing 5× SSC, 3× Denhardt solution, 0.2% SDS, and 50 μg of *E. coli* DNA per ml for 12 h at 65°C and then hybridized with radioactively labeled cDNA probes (10^6 Cerenkov cpm per

ml) in the same buffer for 48 h at 65°C. The filters were washed 3 times in 2× SSC–0.2% SDS for 15 min each at room temperature, once in 1× SSC–0.2% SDS, and once in 0.2× SSC–0.2% SDS for 1 h each at 65°C.

For the preparation of the RNA filters denatured poly(A^+) RNA was fractionated on 1.5% agarose gels containing 6% formaldehyde as described by Rave et al. (23). The RNA was then transferred to nitrocellulose paper as described by Mangiarotti et al. (17). The filters were hybridized as described for the DNA dot-blot filters with denatured plasmid DNA that had been radioactively labeled by nick translation (10^6 Cerenkov cpm per ml of hybridization buffer; 8×10^6 Cerenkov cpm per μg of DNA) (19, 24). One lane of each gel contained molecular weight markers (mouse 28S and 18S rRNAs and MS2 RNA; gifts of D. Lowe). This lane was cut out before the transfer and stained with ethidium bromide (17).

RESULTS

Differential hybridization screen of a yeast library. To identify genes of *S. cerevisiae* expressed preferentially during sporulation of *MAT***a**/*MAT*α cells, we performed a differential hybridization screen (25) of a partial *Sau*3A yeast genomic DNA library with radioactively labeled cDNA probes representing the poly(A^+) RNA of **a**α and αα cells at various times after transfer to sporulation medium. Since only the **a**α cells undergo sporulation, clones showing enhanced hybridization with the **a**α probe relative to the αα probe contain potential sporulation-specific genes. We initially used RNA from αα cells in sporulation medium to prepare the control probe, rather than RNA from **a**α cells growing vegetatively, since the former takes into consideration the transcriptional changes resulting from the metabolic adjustments upon transfer of cells to sporulation medium irrespective of their competence to sporulate.

Duplicate sets of nitrocellulose filters containing the DNA print of approximately 10,000 colonies from the yeast library were hybridized with **a**α and αα cDNA probes prepared with RNA from cells harvested at either 3, 7, 10, or 15 h after transfer to sporulation medium (see above); 350 colonies gave an apparent enhanced hybridization signal with the **a**α probe relative to the αα probe (Fig. 1). Upon rescreening, 94 of these clones again appeared to hybridize preferentially with the **a**α cDNA probe. To assess whether these clones do indeed contain sporulation-specific genes, a dot-blot analysis was performed as follows: purified plasmids DNAs were adsorbed onto nitrocellulose paper in a dotted array, and each DNA was then hybridized with radioactive cDNA probes prepared with poly(A^+) RNA purified from **aa**, **a**α, and αα cells harvested both during vegetative growth and at 3, 7, 10, and 15 h after transfer to sporulation medium. The autoradiogram in Fig. 2 shows the hybridization pattern of the 38 clones that consistently gave a more intense hybridization signal with the **a**α 7-, 10-, and 15-h sporulation probes (Fig. 2, lanes 8, 11, and 14, respectively) relative to both the αα and **aa** control probe for these time points (Fig. 2, lanes 7, 10, and 13 and lanes 9, 12, and 15, respectively). (Seven of these clones had originally been identified with the 7-h probes; 28 and 3 had been identified with the 10- and 15-h probes, respectively.)

Fifteen of these clones (Fig. 2, clones 84, 97 . . . 94) clearly contain a gene uniquely expressed in **a**α cells during sporulation: the 7-, 10-, and 15-h **a**α sporulation probes show significant hybridization with the plasmid DNAs purified from these clones, whereas the **aa**, **a**α, and αα vegetative probes and the **aa** and αα sporulation medium probes show no (or markedly reduced) hybridization. The hybridization pattern obtained with the remaining 23 plasmid DNAs (Fig. 2, clones 17, 27 . . . 90) is more complex. All of the cDNA probes hybridize to these plasmid DNAs. However, whereas an equivalent hybridization response was obtained with all three cDNA probes (**aa**, **a**α, and αα) prepared with RNA from vegetatively growing cells, the hybridization response with the **a**α sporulation probe was more intense than with the **aa** and αα sporulation medium probes. This hybridization pattern could be a consequence of a plasmid containing two yeast genes, one of which is expressed in all cell types during vegetative growth and a second gene expressed preferentially in **a**α cells during sporulation. Alternatively a single gene (for instance, one whose product is required for DNA

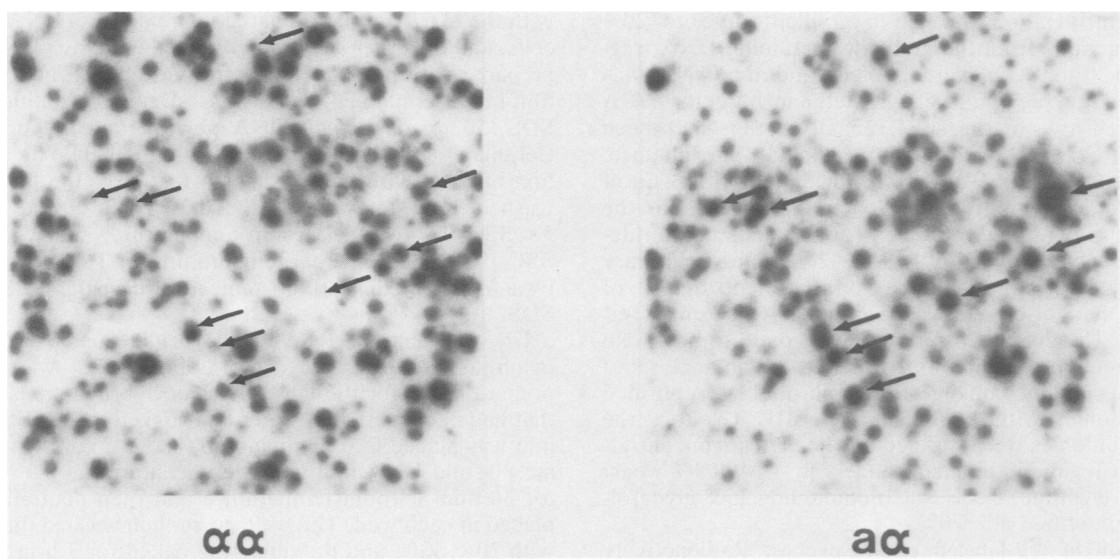

FIG. 1. Differential colony hybridization screen. The autoradiogram (Kodak BB film exposed for 4.5 h at −70°C with a Cronex Lightning Plus intensifying screen) shows 1 pair of the 18 replicate pairs of filters (each containing the DNA print of approximately 600 colonies from the yeast library) hybridized with ^{32}P-labeled cDNA probes prepared with poly(A^+) RNA from αα or **a**α cells at 10 h after transfer to sporulation medium. The arrows indicate colonies hybridizing more intensely with the **a**α probe than with the αα probe.

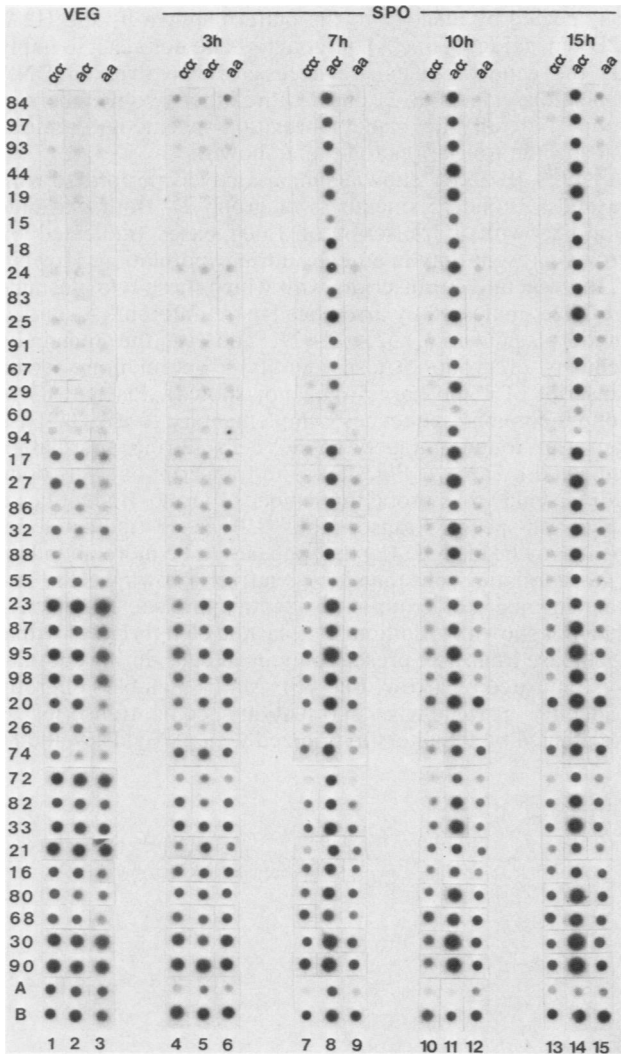

FIG. 2. Dot-blot analysis of the developmental expression of cloned genes. Fifteen nitrocellulose filter sheets were prepared containing 1 μg of each plasmid DNA (as indicated on the left) in a dotted array. A filter was then hybridized with a ^{32}P-labeled cDNA probe prepared either with RNA from αα cells (lanes 1, 4, 7, 10, and 13), aα cells (lanes 2, 5, 8, 11, and 14), or **aa** cells (lanes 3, 6, 9, 12, and 15) growing vegetatively (VEG; lanes 1, 2, and 3) or at 3 h (lanes 4, 5, and 6), 7 h (lanes 7, 8, and 9), 10 h (lanes 10, 11, and 12), or 15 h (lanes 13, 14, and 15) after transfer to sporulation medium (SPO). The film of the autoradiographic representation of the hybridization response (Kodak BB film exposed for 48 h) was then cut into strips to give the arrangement presented in the figure.

replication) may be expressed in all cell types during vegetative growth and only in aα cells during sporulation. These possibilities are addressed further below.

For comparison, the hybridization responses with plasmid DNAs containing the yeast histone H2A and H2B genes (9) and actin gene (21) are shown in Fig. 2, lines A and B, respectively. As expected the histone H2A and H2B genes are expressed in all cell types during vegetative growth and in aα cells in sporulation medium (Fig. 2, line A). The actin gene is expressed in all cell types both during vegetative growth and in sporulation medium (Fig. 2, line B). This latter hybridization pattern validates the relative integrity of the cDNA probes prepared with poly(A$^+$) RNA from αα and **aa** cells in sporulation medium: that is, the enhanced hybridization response with the aα sporulation probe relative to the **aa** and αα control probes for the clones described above is not due to some fortuitous differences in these cDNAs.

Characterization of clones. Considering the size of the screen performed (>5 × 10^4 kb of yeast DNA screened with each set of probes) relative to the size of the yeast genome (1.3 × 10^4 kb) and the possibility that each positive clone could have been identified with the 7-, 10-, and 15-h probes, several of the sporulation-specific genes may in fact be present (at least in part) in several different clones in the collection of 38. To examine this possibility, the clones were first analyzed for distinct yeast sequences by comparing the size of the DNA fragments obtained by digestion of the plasmid DNAs with a restriction endonuclease. Next, the sizes of the transcripts coded for by the cloned genes were compared.

Plasmid DNA purified from each of the clones was restricted with the enzyme *Hin*fI, and the resulting fragments were radioactively labeled and separated by polyacrylamide gel electrophoresis (see above). A comparison of the resultant array of fragments, as visualized in the autoradiograms shown in Fig. 3, allowed the 38 clones to be tentatively divided into 15 groups. (Preliminary comparisons were done with the restriction endonuclease *Sau*3A, but due to the large number [22] of vector fragments generated with this enzyme, we found it simpler to compare the *Hin*fI-generated fragments.)

Group 1 consists of plasmids 21, 23, and 72. A comparison of the array of restriction fragments obtained on digestion of these plasmid DNAs with *Hin*fI indicates that plasmids 21 and 23 are identical and that they differ only slightly from plasmid 72 (Fig. 3A, lanes 2 through 4). Group 2 contains 7 members (plasmids 26, 32, 55, 82, 27, 19, and 44) all of which share five common yeast DNA fragments (indicated by arrows, Fig. 3A, lanes 5 through 11). Within this group there are two sets of apparently identical plasmids: plasmids 26 and 32 (Fig. 3A, lanes 5 and 6) as well as plasmids 19 and 44 (Fig. 3A, lanes 10 and 11). Group 3 contains the two identical plasmids 25 and 83 (Fig. 3A, lanes 12 and 13). Three of the six members of group 4 (plasmids 95, 30 and 33) are very similar (Fig. 3B, lanes 2 through 4). Although digestion of the DNA of the other three members of this group (plasmids 90, 98, and 87) results in a significantly different array of fragments (Fig. 3B, lanes 5 through 7), several fragments (indicated by arrows) are common to all six members of this group.

The patterns of restriction fragments generated by digestion of plasmid DNA from the 8 members of group 5 (plasmids 67, 20, 71, 86, 80, 93, 68, and 17) are quite varied (Fig. 3B, lanes 8 through 15). However these plasmids have preliminarily been grouped together on the basis of sharing *Hin*fI restriction fragments of 420, 350, 120, and 48 base pairs (indicated by arrows). By similar comparisons, plamids 18 and 84 (Fig. 3B, lanes 16 and 17) and plasmids 29 and 88 (Fig. 3B, lanes 18 and 19) have been placed into groups 6 and 7, respectively.

Groups 8 through 15 have only one member each: plasmids 15, 91, 97, 94, 24, 60, 74, and 16, respectively. Digestion of these plasmids with *Hin*fI appeared to generate a unique restriction fragment pattern for each DNA. The autoradiogram shown in Fig. 3C compares the array of fragments obtained on digestion of these latter unique plasmids (lanes 9 through 16) with a representative member of each of the composite groups described above (lanes 2 through 8).

To further characterize these clones, we have identified

the yeast transcripts present in vegetatively growing cells and cells in sporulation medium which are complementary to sequences on the various plasmids. This analysis serves two purposes. First, it confirms the presence of sporulation-specific transcripts complementary to plasmid sequences, in particular for those clones whose hybridization patterns in the DNA dot-blot study (Fig. 2) were difficult to interpret. Second, it serves to verify the assignment of the clones to the groups described above; all plasmids containing the same sporulation-specific gene should identify the same transcript. Poly(A^+) RNA purified from αα, **a**α, and **aa** cells during vegetative growth or at 10 h after transfer to sporulation medium was separated by agarose gel electrophoresis and then transferred to nitrocellulose filter paper. Such a filter was then hybridized with each plasmid DNA which had been radioactively labeled by nick translation.

The hybridization pattern obtained with plasmid 72 (a member of group 1) to probe a filter containing poly(A^+) RNA from the three cell types during vegetative growth or at 10 h after transfer to sporulation medium is shown in Fig. 4A. This clone contains two sporulation-specific genes (or portions thereof); the plasmid DNA hybridizes with two transcripts (indicated by arrows) present only in **a**α cells during sporulation. Additionally, this plasmid contains sequences complementary to two RNAs that are present in all cell types during vegetative growth and only in **a**α cells during sporulation (indicated by open arrowheads). (For simplicity, we refer to this transcriptional pattern as replication specific by analogy to the pattern shown by the H2A, H2B genes [Fig. 2, line A], although we do not mean to imply that the gene products are necessarily involved in DNA replication.) Plasmids 21 and 23, the other two members of group 1, identify the same sporulation-specific and replication-specific transcripts (data not shown).

Figures 4B and C show a similar analysis performed with plasmids 19 and 55 (members of group 2). Both plasmids hybridize with a transcript of 1,400 bases (indicated by arrows) present only in **a**α cells during sporulation. However, the vegetative transcripts with which these two plasmids hybridize (indicated by arrowheads) are different. As anticipated, plasmids 26, 32, 82, 27, and 44 (the remaining members of group 2) also identify a sporulation-specific transcript of 1,400 bases (data not shown). Plasmid 83 of group 3 contains sequences complementary to a 1,000-base transcript found uniquely in **a**α cells during sporulation (indicated by arrow, Fig. 4D). The transcript analysis done with plasmid 95 as probe (a member of group 4) identifies a replication-specific transcript of 950 bases (indicated by open arrowhead, Fig. 4E) that appears to be more abundant in **a**α sporulating cells than in vegetatively growing cells. The other members of group 4 all identify this same transcript (data not shown). Additionally, plasmid 87 hybridizes with a 2,500-base transcript present only in **a**α cells during sporulation (indicated by arrow, Fig. 4F). All the members of group 5 identify a 1,400-base sporulation-specific transcript as exemplified by the filters hybridized with plasmids 67 and 86

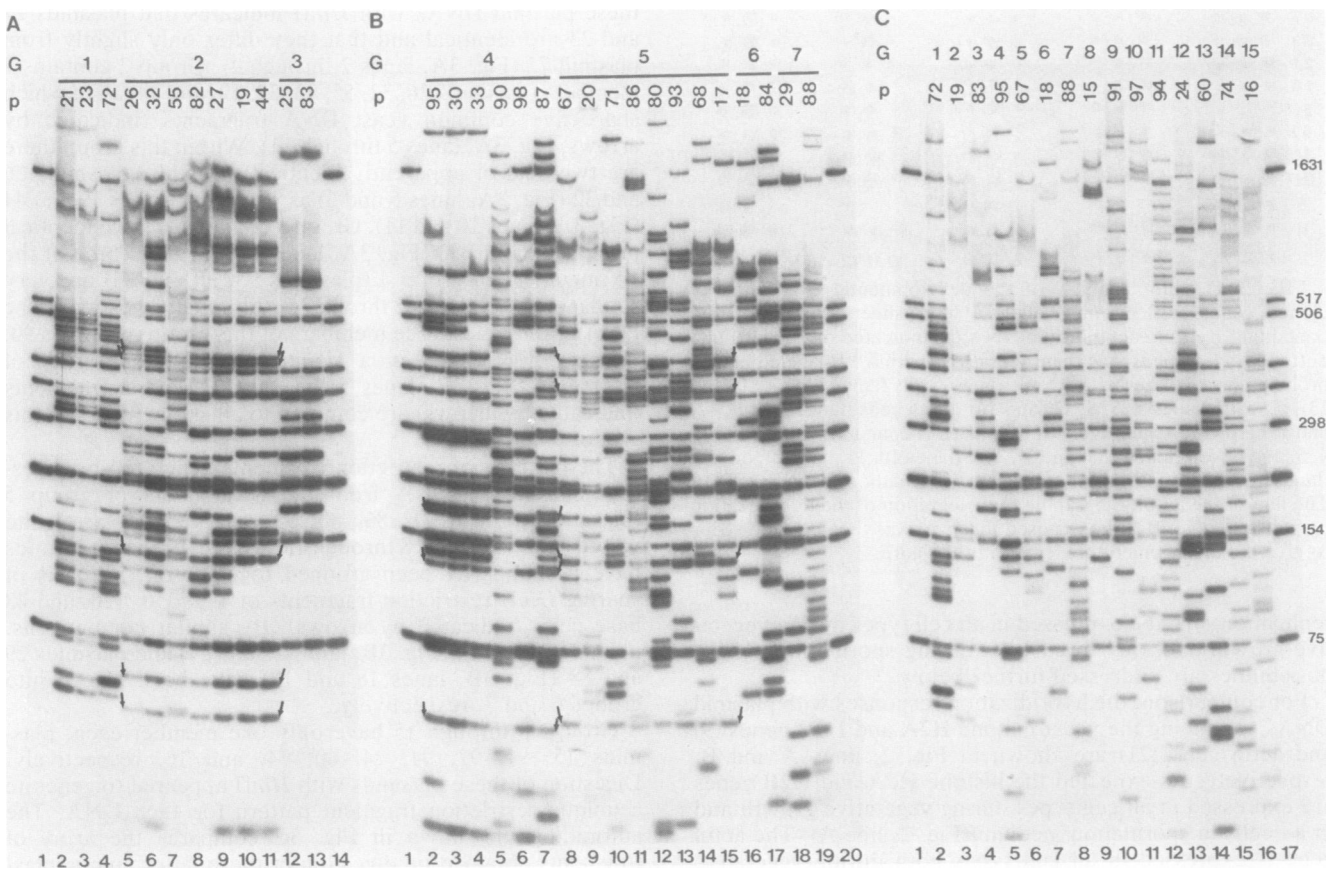

FIG. 3. Comparison of the fragments generated by restriction of plasmid DNAs with *Hin*fI. The various plasmids, as indicated along the top of the radioautograms (G, group number; p, plasmid number), were digested with *Hin*fI, and the resulting fragments were radioactively labeled and separated by electrophoresis through an 8% polyacrylamide gel. The sizes (in base pairs) of the fragments generated by digestion of pBR322 DNA with *Hin*fI (A, lanes 1 and 14; B, lanes 1 and 20; C, lanes 1 and 17) are indicated on the right.

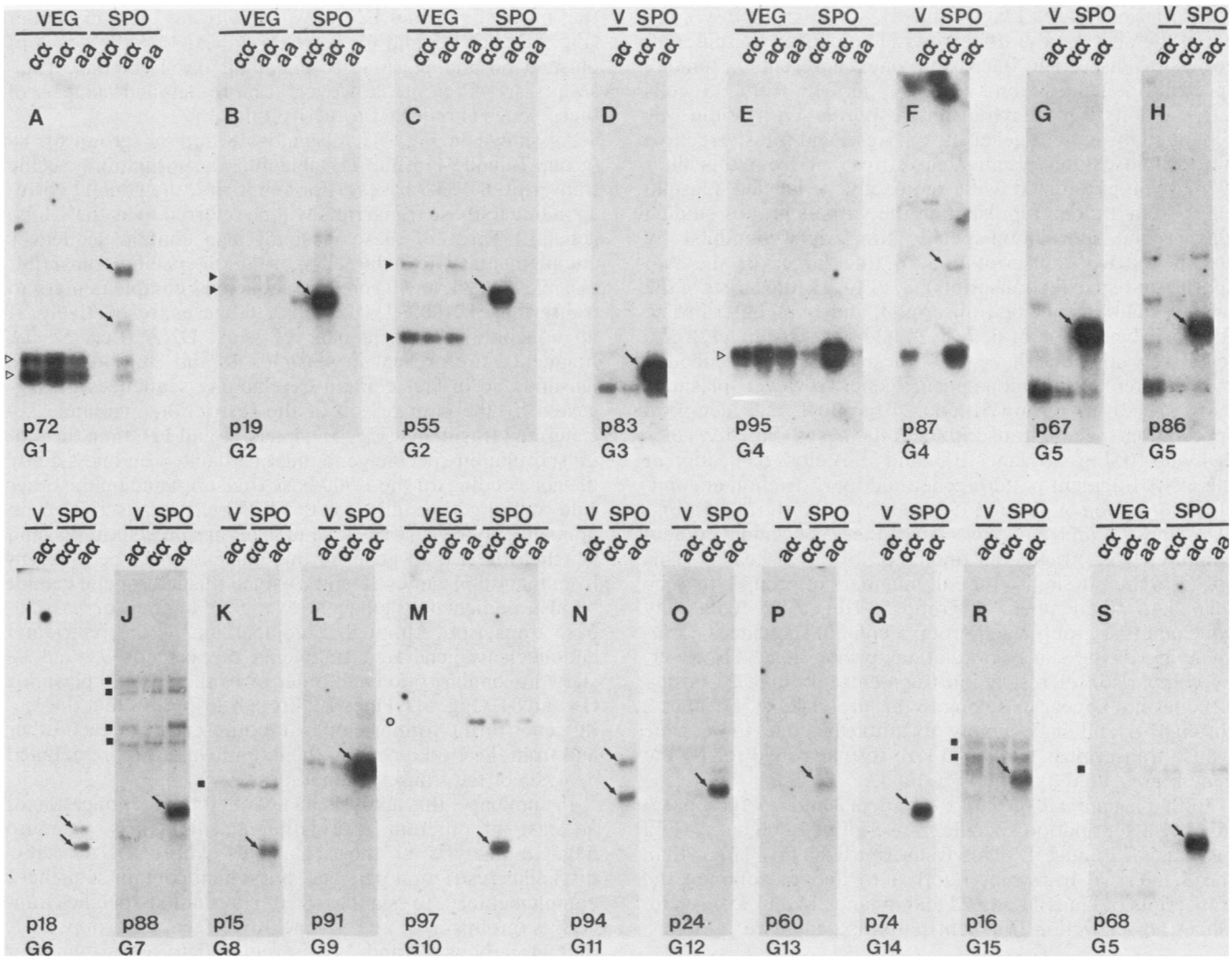

FIG. 4. Examination of the developmentally regulated synthesis of transcripts that hybridize with cloned DNAs. Poly(A^+) RNA purified from either αα, aα, or aa cells (as indicated at the top of each lane) growing vegetatively (VEG, V) or at 10 h after transfer to sporulation medium (SPO) was denatured, separated by electrophoresis through a 1.5% agarose-formaldehyde gel, and then transferred to nitrocellulose paper. The filters were then hybridized with plasmid DNAs radioactively labeled by nick translation. The plasmid DNA (p, plasmid; G, group) used as a probe is denoted by the number in the bottom left-hand corner of each filter. Filters B and C, E and F, and G and H were prepared from the same gels. Sporulation-specific transcripts (—▶), replication-specific transcripts (▷), vegetative transcripts (▶), common transcripts (■), and sporulation-medium specific transcripts (○) are denoted by the indicated symbols. Each lane contained approximately 0.25 μg of poly(A^+) RNA, and the bromophenol blue marker migrated approximately 13 cm. The filters were exposed to Kodak BB film for 5 to 24 h.

(Fig. 4G and H, respectively). Plasmid 18 (Fig. 4I) and plasmid 84 (data not shown), the two members of group 6, both hybridize with two sporulation-specific transcripts of approximately 1,600 and 1,900 bases. Plasmid 88 (Fig. 4J) and plasmid 29 (data not shown), the two members of group 7, hybridize with a sporulation-specific transcript of 1,600 bases.

The RNA filters probed with the plasmids 15, 91, 97, 94, 24, 60, 74, and 16, the sole members of groups 8 through 15, respectively, indicate that these plasmids all contain a gene (or part thereof) preferentially expressed in sporulating cells. (The corresponding sporulation-specific transcripts are indicated by the arrows in Fig. 4K through R.) Plasmid 94 appears to contain two sporulation-specific genes (Fig. 4N). Plasmid 97 also hybridizes with a transcript present in all cell types in sporulation medium (indicated by circle, Fig. 4M). Several plasmids also contain sequences that hybridize equivalently with RNAs from all cell types both during vegetative growth and in sporulation medium (indicated by square symbol, Fig. 4J, K, R, and S). This hybridization pattern establishes the integrity of the RNAs used for the comparisons in these experiments.

This RNA analysis has confirmed that all but five of the potential sporulation-specific clones identified in the DNA dot-blot analysis (Fig. 2) do indeed contain sporulation-specific genes (or portions thereof) (i.e., the plasmids contain sequences complementary to transcripts present only in aα cells during sporulation). Plasmids 95, 30, 33, 90, and 98 of group 4 contain sequences complementary to replication-specific, rather than sporulation-specific, transcripts. Additionally, this analysis has verified the grouping of the various plasmids based on a comparison of the restriction endonuclease-generated fragment patterns: all of the plasmids within a group identify the same sporulation-specific transcript as assessed by size.

Since many of the sporulation-specific transcripts are

1,400 to 1,600 bases long (Table 1), we next directly compared the sizes of the transcripts identified by the different groups to ascertain that each one represents a unique sporulation gene. Poly(A$^+$) RNA purified from **a**α cells harvested at 10 h after transfer to sporulation medium was separated by agarose gel electrophoresis and transferred to a nitrocellulose filter. Equivalent strips cut from this filter were then hybridized with radioactively labeled plasmid DNA from a clone representing the various groups studied above. The sporulation-specific transcripts identified by groups 1 (two transcripts), 3, 6 (the larger of the two sporulation-specific transcripts), 8, 9, 10, 11 (the larger of the two sporulation-specific transcripts), and 14 all differ in size (Fig. 5A, lanes 1, 3, 5, 6, 7, 8, 9, and 13, respectively). We tentatively conclude that each of these transcripts is encoded by a different sporulation-specific gene. However, plasmids 19 (group 2), 67 (group 5), and 24 (group 12) all identify a sporulation-specific transcript of 1,400 bases (Fig. 5A, lanes 2, 4, and 10; Fig. 5B, lanes 1, 2, and 3). A close inspection of the DNA fragment pattern generated by restriction endonuclease digestion of plasmid 19 (group 2) and 67 (group 5) (Fig. 5D, lanes 2 and 3) shows that these plasmids contain common DNA fragments (indicated by arrowheads). This suggests the possibility that all members of group 2 may be related to all members of group 5. Hence we tentatively conclude that group 2 and group 5 contain sequences corresponding to the same sporulation-specific gene. However, by comparison of restriction fragments, plasmid 24 (group 12) does not appear to belong to this group (Fig. 5D, lanes 2 through 4) and may simply fortuitously code for a gene whose transcript is similar in size to that coded for by the gene represented by groups 2 and 5.

Both plasmid 60 (group 13) and plasmid 16 (group 15) identify a sporulation-specific transcript of 1,500 bases (Fig. 5A, lanes 12 and 15). (This transcript is clearly larger than the 1,400-base transcript referred to above; compare the transcripts of lanes 11 and 12 and of lanes 14 and 15.) Again, since the restriction fragment patterns generated by digestion of these plasmid DNAs with *Hin*fI are quite dissimilar (Fig. 5D, lanes 5 and 6), it would appear that the genes of plasmid 60 and plasmid 16 encoding the 1,500-base transcripts are different. However, a more detailed analysis of these genes is required to verify this.

As shown in Fig. 5C, plasmids 18 and 84 (group 6), 88 (group 7), and 94 (group 11) all identify a sporulation-specific transcript of 1,600 bases. (For simplicity, the gene[s] corresponding to these transcripts will be referred to as the 1.6-kb gene[s].) Three of these plasmids also contain sequences complementary to a larger sporulation-specific transcript; plasmids 18, 84, and 94 contain sequences complementary to transcripts of 1,900, 1,750, and 2,300 bases, respectively. If no noncontiguous sequences of yeast DNA (i.e., *Sau*3A fragments) are present in plasmids 18 and 84, and if these plasmids do in fact contain overlapping sequences as suggested by the comparison of the restriction-endonuclease-generated fragments (Fig. 5D, lanes 11 and 12), then the 1.6-kb sporulation-specific gene must be flanked on one side by the gene coding for the 1,900-base transcript and on the other side by the gene coding for the 1,750-base transcript. If no noncontiguous yeast DNA sequences are present in plasmid 94, then the 1.6-kb gene on this plasmid must be different from that on plasmids 18 and 84 since this latter gene cannot be also immediately adjacent to a gene coding for a 2,300-base transcript. Although a comparison of the restriction endonuclease-generated fragments of plasmids 94 and 88 show no similarity to each other or to the group 6 plasmids (18 and 84) (Fig. 5D, lanes 10 through 13), proof that the 1.6-kb genes of the former clones are different from each other and from the 1.6-kb gene of the latter awaits a more detailed analysis of the transcriptional units.

In summary, this analysis has verified the identification of at least 14 different sporulation-specific genes. A more detailed analysis of those plasmids whose DNAs show dissimilar restriction patterns, but which contain sequences complementary to similar-sized sporulation-specific transcripts (groups 2, 5, and 12; the 1.6-kb gene of groups 6, 7, and 11; groups 13 and 15), is required to assess whether these clones contain different sporulation-specific sequences.

TABLE 1. Summary of plasmids containing sequences complementary to sporulation-specific transcripts

Group	Plasmid(s)	Transcript (kb)	
		SPO[a]	REP[b]
1	21/23,[c] 72	2.4, 1.35	0.9, 0.75
2 and 5	26/32, 55, 82, 27, 19/44	1.4	
	67, 20, 71, 86, 80, 93, 68, 17	1.4	
3	25/83	1.0	
4	95, 30, 33, 90, 98		0.95
	87	2.5	0.95
6	18	1.9, 1.6	
	84	1.75, 1.6	
7	29, 88	1.6	
8	15	0.86	
9	91	2.8	
10	97	0.96	
11	94	2.3, 1.6	
12	24	1.4	
13	60	1.5	
14	74	1.3	
15	16	1.5	

[a] Sporulation-specific transcript: present only in **a**α cells during sporulation.

[b] Replication-specific transcript: present in **aa**, **a**α, and αα cells during vegetative growth and in **a**α cells (but not **aa** or αα cells) in sporulation medium (see text).

[c] The shill indicates that the two plasmids are identical.

DISCUSSION

A differential hybridization screen of a genomic yeast library has led to the identification of at least 14 genes expressed preferentially during sporulation in *S. cerevisiae*. Of the several hundred colonies that initially appeared positive in the colony hybridization screen, 38 were consistently found to give an enhanced hybridization response with the sporulation probe relative to the control probe when analyzed in a DNA dot-blot study (Fig. 2). Despite the numerous false-positives, this simple approach has in fact proved surprisingly successful in identifying developmental specific genes. On the basis of a comparison of the DNA fragments generated by restriction endonuclease digestion of the various plasmid DNAs (Fig. 3) and a detailed analysis identifying the transcripts present in αα, **a**α, and **aa** cells during vegetative growth and after transfer to sporulation medium that are complementary to sequences on the plasmids (Fig. 4 and 5), the 38 clones have been divided into 14 groups (Table 1). All members of a group share common restriction fragments and contain sequences complementary to the same-sized transcript. In all but one case (group 4), this analysis demonstrated that the plasmids contain sequences complementary to transcripts found uniquely in **a**α cells during sporulation.

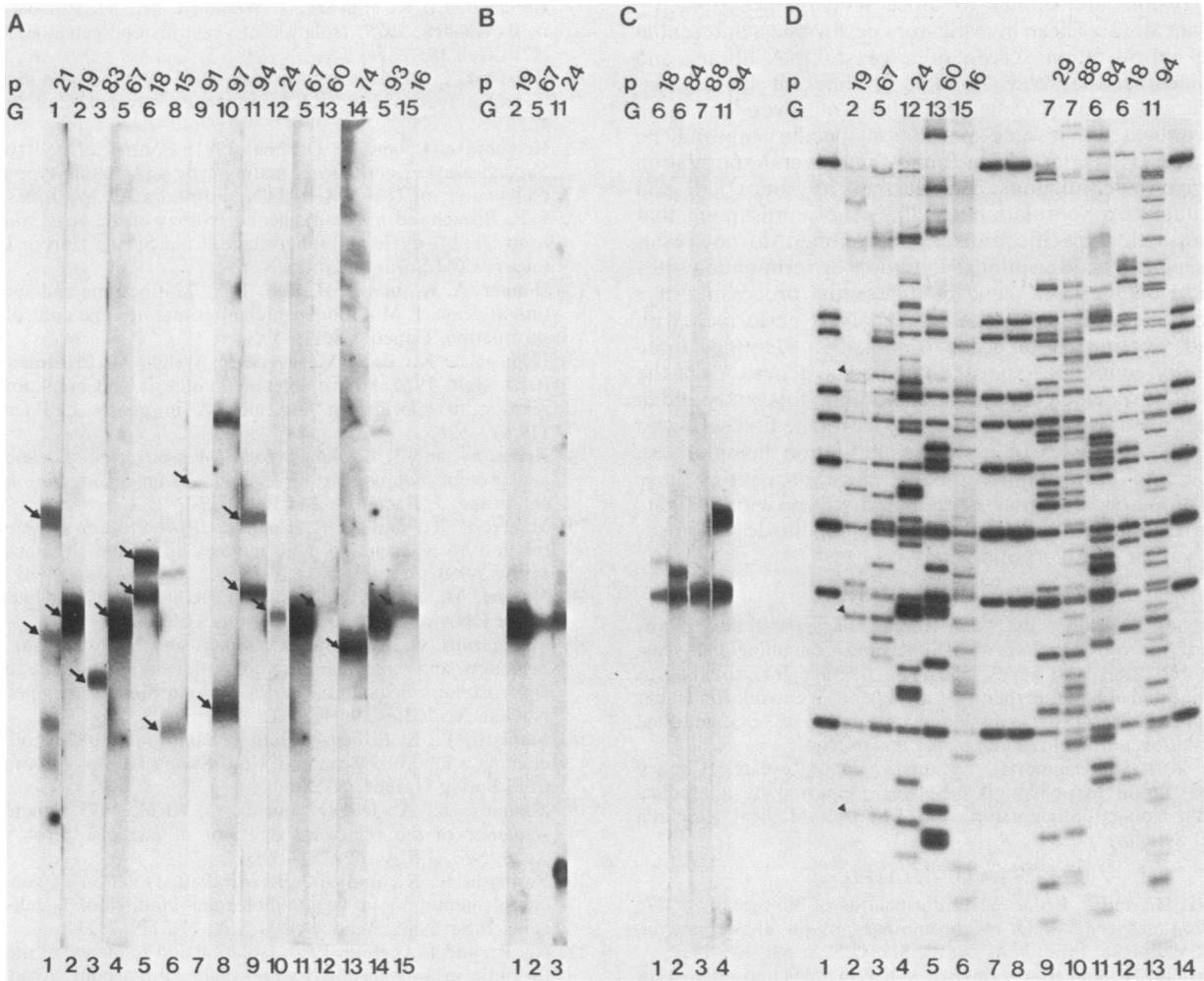

FIG. 5. Comparison of sporulation-specific transcripts. (A, B, C) Poly(A)$^+$) RNA purified from **a**α cells at 10 h after transfer to sporulation medium was separated by electrophoresis through a 1.5% agarose-formaldehyde gel until the bromophenol blue marker had migrated 20 cm, and then the RNA was transferred to nitrocellulose paper. Strips from this filter were then hybridized with plasmid DNAs (denoted by the number at the top of the lane; p, plasmid; G, group) that had been radioactively labeled by nick translation. (The filters in A, B, and C are from different gels.) The arrows denote sporulation-specific transcripts. The filters were exposed to Kodak BB film for (A) 12 h, (B) and (C, lanes 1, 2, and 4) 12 h with a Cronex Lightning-Plus intensifying screen and (C, lane 3) 48 h with an intensifying screen. (D) A comparison of the fragments generated by restriction of plasmid DNAs with *Hin*fI was performed as described in the legend to Fig. 3. Lanes 1, 7, 8, and 14 contain pBR322 DNA.

Given the quasi-random nature of the library and the small insert size, we reasoned that by performing a large screen (so that each yeast sequence would be present on several plasmids of independent origin representing overlapping rather than identical yeast sequences) we would increase the probability that the enhanced hybridization response due to a sporulation-specific cDNA would not be obscured by the hybridization of both probes to any adjacent gene expressed in all cell types in sporulation medium. Since several genes have been cloned more than once (Table 1), this would suggest that most of the sporulation-specific genes that can be identified at the level of sensitivity provided by this screen have indeed been identified. These genes appear to be expressed at about the same level as the actin and histone genes (Fig. 2), suggesting that they code for transcripts of moderate abundancy. Hence, it is possible that a more sensitive screen would be required to identify any sporulation-specific genes whose transcripts are present in very low abundance.

As expected from the nature of the construction of the library, not all of the plasmids within a group are identical. They may contain overlapping yeast sequences (as delimited by partial *Sau*3A fragments) or even disparate sequences that were ligated together and cloned as a contiguous sequence (but all sharing a *Sau*3A fragment[s] from the same sporulation gene). This would explain why some members of a group (e.g., group 5) share so few common restriction fragments. Alternatively, we may have identified a gene(s) present in highly conserved replicate copies in the genome. This latter possibility has not been investigated further, but may apply to the composite group containing groups 2 and 5, which appears to have 15 members. Also, since the analysis we have done does not distinguish between sporulation-specific transcripts of similar size but differing sequence, some plasmids may have been grouped together because they fortuitously contain *Hin*fI restriction fragments of similar size and contain sporulation-specific genes (or a part thereof) coding for similar sized RNAs.

Our results are similar to those recently described by Clancy et al. (3). These investigators performed a differential plaque hybridization screen of a yeast DNA library and estimated that they had identified 15 different sporulation-specific genes (3). A detailed analysis of several of these clones indicated that some sporulation-specific genes may be clustered (3). We have also found that several sporulation genes may be contiguous. The plasmids in groups 1, 6, and 11 contain two sporulation-specific genes, presuming that the sporulation-specific transcripts identified do not result from separate transcriptional initiation or termination sites (or both) on the same gene or differential processing of a unique transcript. The RNA blot analysis performed with plasmids 18 and 84 of group 6 (Fig. 5C) identifies three apparently contiguous sporulation-specific genes. All of the sporulation-specific genes identified both in this study and in that described by Clancy et al. (3) appear to be first activated at 7 h after transfer of cells to sporulation medium. An investigation of the manner in which the expression of these sporulation-specific genes is regulated will provide insights into the mechanisms involved in controlling the developmental activation of eucaryotic genes.

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