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Genetics

Identification, molecular cloning, and mutagenesis of *Saccharomyces cerevisiae* RNA polymerase genes

(plaque hybridization/gene family/sequence conservation/gene disruption/*Drosophila melanogaster*)

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ABSTRACT **Three different regions of *Saccharomyces cerevisiae* DNA were identified by using as hybridization probe a fragment of *Drosophila melanogaster* DNA that encodes an RNA polymerase II (EC 2.7.7.6) polypeptide. Two of these regions have been molecularly cloned. Each contains a sequence related not only to the *D. melanogaster* DNA fragment that was used as a probe in its isolation but also to the immediately adjacent DNA fragment of the *D. melanogaster* RNA polymerase II gene. The two cloned *S. cerevisiae* DNA sequences are each the template for single transcripts *in vivo*, one of 5.9 kilobases and the other of 4.6 kilobases. *In vitro* translation of hybrid-selected cellular RNA indicated that the former locus encodes a protein of M_r 220,000, equal in size to the largest polypeptide subunit of *S. cerevisiae* RNA polymerase II. Disruption of either gene by targeted integration of *URA3*$^+$ DNA demonstrated that each is single-copy and essential in a haploid genome. We suggest that these *S. cerevisiae* loci are members of a family of related genes encoding the largest subunit polypeptides of RNA polymerases I, II, and III.**

In *Drosophila melanogaster* a series of α-amanitin-resistant, lethal, and temperature-sensitive mutations affecting RNA polymerase II activity (EC 2.7.7.6) have all been mapped to a single genetic locus, *RpII* (1, 2). By using the DNA of the transposable element *P* as probe, DNA sequences from a mutant strain of *D. melanogaster* bearing a lethal *P*-element insertion in this *RpII* locus were cloned (3). Of the four different *in vivo* transcripts that originate from this *RpII* region of *D. melanogaster* DNA, only one has a homolog in mammalian DNA (4). In interspecies DNA-mediated gene transfer experiments this conserved sequence was shown to encode the gene conferring sensitivity to inhibition of RNA polymerase II activity by α-amanitin (4). These latter studies (4) clearly identified the DNA of a conserved RNA polymerase II structural gene. This gene in *D. melanogaster* encodes the largest (M_r = 215,000) subunit of RNA polymerase II (5).

The conservation of eukaryotic RNA polymerase II subunit structure and antigenicity extends to fungal species such as *Saccharomyces cerevisiae* (6–11). We have therefore asked if yeast RNA polymerase II DNA could be detected by using the DNA of the *D. melanogaster RpII* region as a probe. Unexpectedly, not one but three different loci were detected in the *S. cerevisiae* genome when the *D. melanogaster* RNA polymerase II DNA was used as a probe. These yeast DNA sequences thus appear to be three members of a family of related genes. We suggest that they encode subunit polypeptides of RNA polymerases I, II, and III.

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MATERIALS AND METHODS

Escherichia coli K-12 strains JF1754 (*hsdR*, *lac*, *gal*, *metB*, *leuB*, *hisB*) and HB101 (*hsdS20*, *recA13*, *ara-14*, *proA2*, *lacY1*, *galK2*, *rpsL20*, *xyl-5*, *mtl-1*, *supE44*) were used as hosts for plasmid propagation. Yeast DNA for Southern blots was purified as described (12). *S. cerevisiae* diploid strain JH101 (*MATa/MATα*, *ade1/ADE1*$^+$, *leu2-3,2-112/LEU2*$^+$, *ura3-52/ura3-52*, *HIS4*$^+$*/his4-912*) was constructed for this study. pBR325 (13) was used as a cloning vehicle. pJH104 is a derivative of pBR325 with a 1.1-kilobase (kb) *Hin*dIII fragment insert carrying the *S. cerevisiae URA3*$^+$ gene. Bacteriophage λ libraries of *S. cerevisiae* DNA were constructed by us, using λgt*WES* (14), or by M. Olson, using λMG14 [a derivative of λ1059 (ref. 15) and λ Charon 30 (ref. 16)]. Transformation of *E. coli* and *S. cerevisiae*, preparation of plasmid and bacteriophage DNA, DNA blotting, RNA blotting, nick-translation, nucleic acid hybridization (4), and *in vitro* translation of hybrid-selected RNA (17) have been described previously and were carried out according to conventional methods (see, for example, refs. 18 and 19).

RESULTS

Cloning Yeast DNA Homologous to a *D. melanogaster* RNA Polymerase II Gene. Two subclones of *D. melanogaster* DNA, p4.1 and p4.2, together contain most of the structural gene information for the largest subunit of *D. melanogaster* RNA polymerase II (4, 5). We determined whether related sequences could be detected by hybridization to *S. cerevisiae* genomic DNA by using either of these *D. melanogaster* DNA species as probe. Yeast DNA (20 μg) was digested with the restriction endonuclease *Eco*RI and fractionated by electrophoresis in agarose gels; a nitrocellulose blot of this was probed under various degrees of stringency with nick-translated p4.2, a plasmid in which the *D. melanogaster* DNA is carried on pBR325. A number of hybridizing fragments were seen (Fig. 1*A*, lanes a and b) when the hybridization solutions contained either 30% or 50% (vol/vol) formamide. Most of these appeared to be vector-DNA related since they were also detected when the same DNA was probed with the labeled vector pBR325 DNA (Fig. 1*A*, lanes c and d). In 30% formamide, however, two additional yeast *Eco*RI fragments, 7.5 and 2.6 kb in size, were detected by the p4.2 probe, in addition to those detected by the vector DNA. These are the same conditions that allowed cross-hybridization of p4.2 with mammalian RNA polymerase II DNA (4). Probing the *Eco*RI-digested yeast DNA at this reduced stringency but with the gel-purified insert of p4.2 revealed, after a longer exposure, the 7.5- and 2.6-kb bands, as

Abbreviations: kb, kilobase(s); Ura$^+$ and Ura$^-$, uracil-independent and dependent.

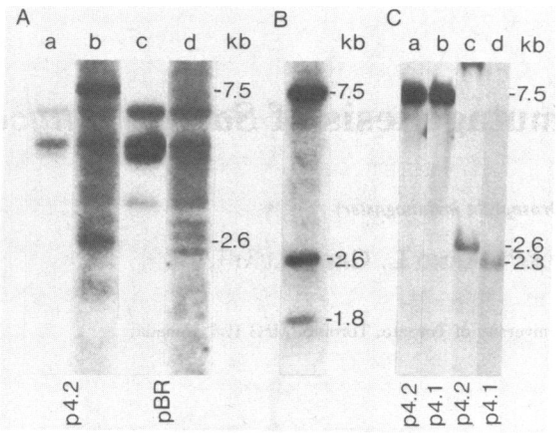

FIG. 1. *S. cerevisiae* DNA sequences homologous to *D. melanogaster* RNA polymerase II DNA. (*A*) Twenty micrograms of genomic DNA from *S. cerevisiae* was digested with *Eco*RI, electrophoretically fractionated on 1% agarose gels, transferred to nitrocellulose, and hybridized with nick-translated ^{32}P-labeled p4.2 DNA (lanes a and b) or vector pBR325 DNA (lanes c and d). p4.2 is a plasmid that consists of the 2.6-kb *D. melanogaster Eco*RI DNA fragment inserted in pBR325 (3). Hybridization solutions (4) contained 50% (vol/vol) formamide (lanes a and c) or 30% formamide (lanes b and d). Sizes of hybridizing DNA bands were determined by comparison to *Hin*dIII-digested λ DNA standards. (*B*) A genomic blot of *Eco*RI-digested *S. cerevisiae* DNA was hybridized (30% formamide) with a ^{32}P-labeled probe prepared from the purified 2.6-kb *D. melanogaster* DNA insert of p4.2. (*C*) One microgram of *Eco*RI-digested DNA from two different recombinant λ phage which were isolated from the genomic library of *S. cerevisiae* DNA in λgt*WES* was hybridized (30% formamide) with ^{32}P-labeled *D. melanogaster* RNA polymerase II DNA, either p4.2 (lanes a and c) or p4.1 (lanes b and d). Lanes a and b, DNA from the bacteriophage carrying the 7.5-kb insert; lanes c and d, DNA from the bacteriophage carrying the 2.6-kb fragment.

well as a third hybridizing band, 1.8 kb (Fig. 1*B*), which did not correspond to any band hybridizing to pBR325 (Fig. 1*A*). On the basis of these results we screened a genomic library of *EcoRI*-digested *S. cerevisiae* DNA carried in the bacteriophage vector λgt*WES*, using the purified insert of p4.2 as labeled probe, and hybridizing in 30% formamide. Thirty-six positive clones were recovered from a total of 16,000 plaques screened.

Characterization of the Yeast RNA Polymerase-Related DNA Clones. The DNA of 30 of the initial 36 positive λ phage clones was examined by restriction endonuclease digestion. Seven of these contained a 7.5-kb *Eco*RI DNA fragment that hybridized to the *D. melanogaster* p4.2 DNA (Fig. 1*C*, lane a). The remaining 23 contained a 2.6-kb *Eco*RI DNA fragment that hybridized with this same probe (Fig. 1*C*, lane c). None of the isolates contained both a 7.5-kb and a 2.6-kb *Eco*RI fragment.

Since the *S. cerevisiae* inserts in this λgt*WES* library were both relatively small and had been generated from genomic DNA by *Eco*RI digestion, we used nick-translated *Eco*RI fragments from two of these initial λ isolates to screen a second genomic library of *S. cerevisiae* DNA. This second library, constructed by M. Olson in the vector λMG14 (a λ1059–λ Charon 30 hybrid bacteriophage), contains yeast DNA inserts generated by partial *Sau*3A digestion in the size range 15–20 kb. Over 100 positive plaques were detected upon screening 17,000 bacteriophage. The DNA from a number of these bacteriophage was in turn characterized by restriction enzyme mapping and hybridization experiments (data not shown, but see Fig. 2). The bacteriophage that we isolated from this second library also clearly represented two distinctly different loci. One set contained the 7.5-kb *Eco*RI fragment of *S. cerevisiae* DNA that was detected by p4.2 DNA in the whole genome Southern blots of *Eco*RI-digested yeast DNA. The other set of bacteriophage contained the 2.6-kb *Eco*RI fragment of *S. cerevisiae* that was detected by this same *D. melanogaster* probe. We have not yet succeeded in isolating a bacteriophage carrying the locus that includes the more weakly hybridizing 1.8-kb *Eco*RI fragment.

The likelihood that both of these cloned sequences represented *bona fide* RNA polymerase-related yeast genes was strengthened by the following observation. Both loci contained sequences that hybridized not only with the *D. melanogaster* RNA polymerase II DNA p4.2, the fragment that was originally used to detect these isolates, but also with a second *D. melanogaster* RNA polymerase II probe, p4.1. Probe p4.1 contains the DNA that lies immediately adjacent to the p4.2 DNA on the *D. melanogaster* chromosome (3, 4); together these two *D. melanogaster* DNA fragments and their flanking sequences make up the gene encoding the M_r 215,000 subunit of RNA polymerase II (5). Both p4.1 and p4.2 DNA hybridized to the 7.5-kb *Eco*RI yeast DNA fragment (Fig. 1*C*, lanes a and b). p4.1 DNA did not hybridize to the 2.6-kb fragment detected by p4.2, but it did hybridize with the adjacent 2.3-kb *Eco*RI fragment (Fig. 1*C*, lanes c and d) in the same recombinant phage. More detailed mapping and cross-hybridization studies of the DNA of a number of the recombinant bacteriophage with *D. melanogaster* p4.1 and p4.2 DNA are summarized in Fig. 2, in which homology

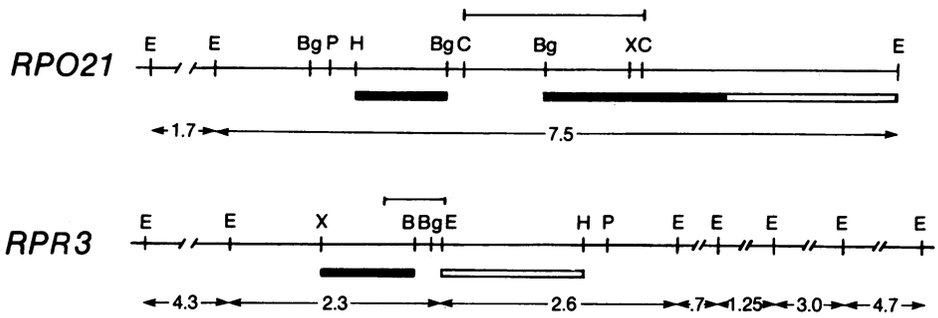

FIG. 2. Chromosomal organization of the DNA of two *S. cerevisiae* RNA polymerase-related loci, *RPO21* and *RPR3*. The cleavage sites for the restriction endonucleases *Eco*RI (E), *Hin*dIII (H), *Bgl* II (Bg), *Bam*HI (B), *Cla* I (C), *Xba* I (X), and *Pst* I (P) in two regions of yeast genomic DNA are shown. The maps are a composite derived for each locus from the analysis of overlapping recombinant λ phage that were isolated from two different λ phage genomic libraries of *S. cerevisiae* DNA (see test). Sites for restriction enzymes other than *Eco*RI are not shown for the DNA that flanks the 7.5-, 2.6-, and 2.3-kb *Eco*RI fragments. Restriction fragments that hybridized to the *D. melanogaster* RNA polymerase II DNA in the p4.1 probe are indicated by filled rectangles and to the p4.2 probe by open rectangles. Note that this does not imply that the *D. melanogaster* probes hybridize to the entire *S. cerevisiae* DNA fragments; more likely they hybridize only to portions of them. The bars above the diagrams indicate the regions of each locus that were subcloned in the nonreplicating *URA3*$^+$ plasmid for use in the gene disruption experiments described in the text. A justification for the nomenclature of these loci is given in the text (see *Discussion*).

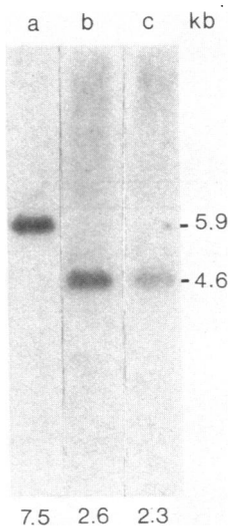

FIG. 3. Analysis of transcripts originating from the cloned *S. cerevisiae* RNA polymerase-related gene loci. Total cellular RNA, 20 μg per lane, was fractionated by electrophoresis in 1% agarose gels containing formaldehyde, transferred to nitrocellulose, and hybridized in 50% formamide with ^{32}P-labeled probes. The DNA probes, each subcloned in pBR325, were as follows: the 7.5-kb *Eco*RI fragment (lane a) from one locus; the 2.6-kb *Eco*RI fragment (lane b) or the 2.3-kb *Eco*RI fragment (lane c) from the second locus. RNA sizes were estimated by comparison to denatured DNA standards (not shown).

between the *D. melanogaster* RNA polymerase II gene fragments and portions of the two RNA polymerase-related *S. cerevisiae* DNA loci are indicated.

Hybridization Analysis of Yeast RNA. Experiments were undertaken to determine the size and number of *in vivo* transcript(s) from each locus. Total RNA was isolated from cells in the logarithmic phase of growth, fractionated by electrophoresis in agarose under denaturing conditions, transferred to nitrocellulose, and probed with DNAs of each of the cloned *S. cerevisiae* RNA polymerase-related loci. When the 7.5-kb *Eco*RI fragment was used as a probe, a single large transcript, approximately 5.9 kb in length, was detected. When either the 2.6-kb or the 2.3-kb *Eco*RI fragment, which together make up the second RNA polymerase-related locus, was used, a single RNA species of approximately 4.6 kb was detected (Fig. 3). Thus each of these yeast RNA polymerase-related loci is expressed as a single RNA species, and the sizes of the two transcripts are different. It is significant that these two transcripts are sufficiently large to encode polypeptides of about M_r 220,000 and M_r 160,000, respectively (see *Discussion*).

Identification of a Polypeptide Encoded by One of the *S. cerevisiae* Genes. We have employed a direct biochemical approach to characterize the polypeptide encoded by one of these genes. DNA from the 7.5-kb *Eco*RI locus, subcloned in pBR325, was used for hybrid-selection of a specific messenger RNA species from total yeast RNA. The selected filter-bound RNA species were eluted and translated *in vitro* in a rabbit reticulocyte system. Fig. 4 shows the fluorograph of the [^{35}S]methionine-labeled proteins synthesized *in vitro*, and separated by sodium dodecyl sulfate gel electrophoresis. A single protein of about M_r 220,000, was the only protein band unique to the 7.5-kb *Eco*RI-selected RNA (Fig. 4, lane b). Control samples, representing the translation products of pBR325-selected RNA (Fig. 4, lane a) or H_2O only (not shown), showed only the presence of reticulocyte-derived lower molecular weight proteins, which were also present in the translation products of the 7.5-kb DNA-selected RNA. This M_r 220,000 polypeptide is the same size as the largest

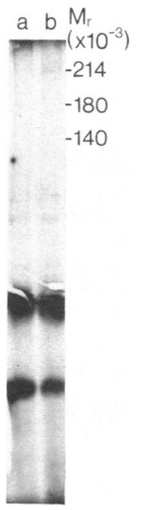

FIG. 4. Cell-free translation of hybrid-selected *S. cerevisiae* RNA. Total cellular RNA (500 μg) was hybridized to linearized plasmid DNA immobilized on nitrocellulose. The RNA was eluted from both control (pBR325) filters (lane a) and filters containing the 7.5-kb *Eco*RI fragment subcloned in pBR325 (lane b) and was translated in a nuclease-treated rabbit reticulocyte lysate (Bethesda Research Laboratories). The [^{35}S]methionine-labeled products were subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis and visualized by fluorography. The molecular weight of the translated polypeptide was estimated to be ≈220,000 by comparison to the indicated mobilities of marker Calf thymus RNA polymerase II polypeptides run on a parallel track of the same gel.

subunit polypeptide of the yeast RNA polymerase II enzyme (10, 20).

Using both total yeast RNA and oligo(dT)-cellulose-selected poly(A)$^+$ RNA, we have as yet been unsuccessful in identifying a polypeptide encoded by RNA selected with DNA of the second (2.6-kb) yeast RNA polymerase-related locus.

Mutagenesis of the Yeast RNA Polymerase-Related Loci by Homologous Recombination. We have used insertional inactivation (21–23) to determine if each locus encodes a unique function essential for yeast growth. To carry out this experiment with the locus containing the 7.5-kb *Eco*RI fragment we used an internal region of the locus, itself flanked on both sides by sequences that hybridize to p4.2 or p4.1 *D. melanogaster* DNA (see Fig. 2). A 2.0-kb *Cla* I fragment was subcloned on a nonreplicating plasmid that also carries the *S. cerevisiae URA3*$^+$ gene (pJH104). A diploid *ura3*$^-$/*ura3*$^-$ strain was transformed with this plasmid DNA, which had been digested with *Bgl* II in order to target integration into the polymerase-related site (21). Uracil-independent (Ura$^+$) recombinants were selected on agar lacking uracil (Fig. 5*A* summarizes this procedure). As indicated by the DNA blots shown in Fig. 5*B*, one of two 7.5-kb *Eco*RI segments in the DNA of diploid recombinants was disrupted by the recombination event leading to Ura$^+$ transformation. Two new *Eco*RI fragments of the expected sizes 10.6 and 6.0 kb were revealed in addition to the original 7.5-kb fragment in DNA blots of *Eco*RI-digested genomic DNA of *ura3*$^-$/*URA3*$^+$ diploid transformants. About one-third of these Ura$^+$ recombinants contained an additional 9.1-kb *Eco*RI fragment (Fig. 5*B*, lane e), the result of tandem integration (24) of the 9.1-kb *URA3*$^+$ plasmid at this same chromosomal site. The *ura3*$^-$/*URA3*$^+$ transformants shown in lanes b and c were then sporulated; dissected tetrads were scored both for viability and for uracil auxotrophy. Of 20 tetrads that were analyzed, 17 contained two viable Ura$^-$ spores and two nonviable spores. The remaining three contained one viable Ura$^-$ spore and three nonviable spores, possibly the result of a gene conversion event or incomplete tetrads. All Ura$^-$ haploids contained only an intact 7.5-kb *Eco*RI segment (two examples are shown in Fig. 5*B*). The integration of the *URA3*$^+$ gene, pBR325, and 2.0 kb of yeast RNA polymerase-related DNA in this *S. cerevisiae* locus was clearly a lethal event. This locus must therefore encode a single-copy essential gene.

A similar strategy was used to examine the second yeast RNA polymerase-related locus. A 640-base-pair *Eco*RI/*Hae* III fragment, also determined on the basis of the hybridization studies with *D. melanogaster* DNA to be internal to this gene (see Fig. 2), was cloned in the same nonreplicating

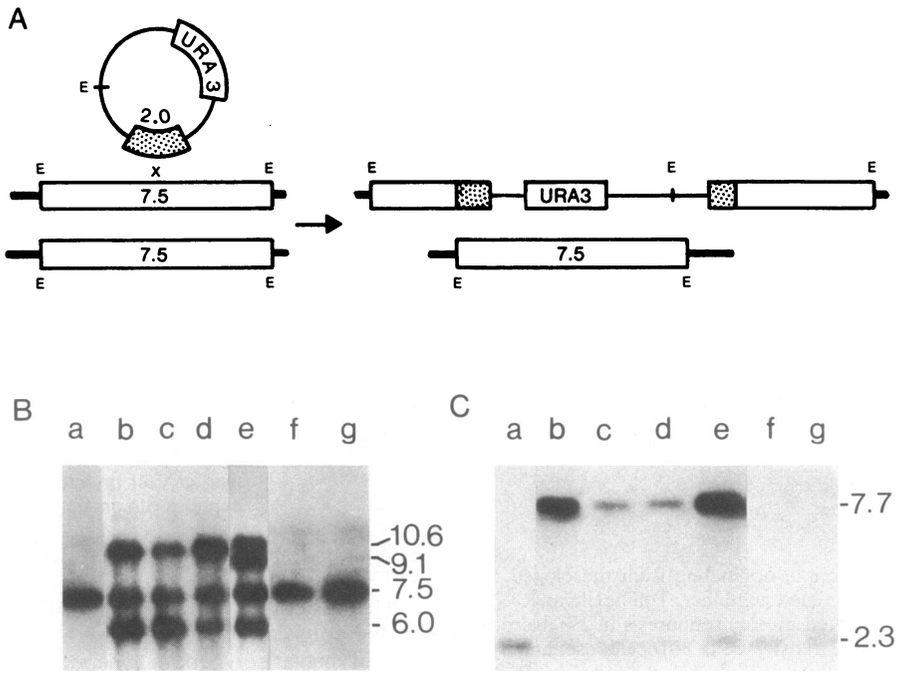

FIG. 5. Gene disruption by plasmid integration of the loci containing RNA polymerase-related functions. (*A*) Diagram of the procedure. Two 7.5-kb *Eco*RI segments are present in a *ura3*$^-$/*ura3*$^-$ diploid strain. Integration by homologous recombination of a *URA3*$^+$ plasmid containing a 2.0-kb *Cla* I fragment, which is internal to the RNA polymerase gene, into one of these two 7.5-kb *Eco*RI segments results in Ura$^+$ transformants that contain the intact locus with the 7.5-kb *Eco*RI segment and the mutated locus with the inserted plasmid sequence. Sites for the restriction enzyme *Eco*RI are indicated (E). (*B*) Genomic blots verifying the disruption of the locus containing the 7.5-kb *Eco*RI segment (RPO21). Lane a, *Eco*RI-digested DNA of parental *ura3*$^-$/*ura3*$^-$ diploid cells. Lanes b–e, *Eco*RI-digested DNA of *ura3*$^-$/*URA3*$^+$ diploids obtained by transformation with the integrating *URA3*$^+$ plasmid that carries the 2.0-kb *Cla* I insert derived from the 7.5-kb *Eco*RI fragment. Lanes f and g, *Eco*RI-digested DNA of surviving Ura$^-$ haploid strains obtained after sporulation and tetrad dissection of the *ura3*$^-$/*URA3*$^+$ diploids. The ^{32}P-labeled probe was the 7.5-kb *Eco*RI fragment in pBR325. (*C*) Genomic blots verifying the disruption of the locus containing the 2.6- and 2.3-kb *Eco*RI segments (*RPR3*). Lane a, *Eco*RI-digested DNA of parental *ura3*$^-$/*ura3*$^-$ diploid cells. Lanes b–e, *Eco*RI-digested DNA of *ura3*$^-$/*URA3*$^+$ diploids obtained after transformation with the integrating *URA3*$^+$ plasmid carrying a 640-base-pair *Hin*dIII/*Eco*RI insert from the *RPR3* locus. Lanes f and g, *Eco*RI-digested DNA of surviving Ura$^-$ haploid cells obtained after sporulation. The ^{32}P-labeled probe was the 2.3-kb *Eco*RI fragment in pBR325. All hybridizations were in 50% formamide.

URA3$^+$ plasmid (pJH104). Integration of this plasmid in the homologous chromosomal locus of *ura3*$^-$/*ura3*$^-$ diploids was targeted with a *Bgl* II cut. Recombination of the plasmid into the homologous site was confirmed by analysis of DNA blots (Fig. 5*C*). Digestion of the DNA of diploid Ura$^+$ recombinants with *Eco*RI showed the presence of a normal 2.3-kb fragment hybridizing with the 2.3-kb *Eco*RI probe and a new fragment of the expected size, 7.7 kb. The copy number of this latter fragment varied, again due to tandem insertions (24) of the *URA3*$^+$ plasmid. Sporulation and analysis of the diploid shown in lane b of Fig. 5*C* indicated, as for the 7.5-kb locus, that this second locus was also single-copy and essential. Eighteen tetrads contained two viable and two nonviable spores; the remaining two contained three viable spores. These viable Ura$^-$ haploids contained only the 2.3-kb *Eco*RI fragment (Fig. 5*C*, lanes f and g).

These loci, which both contain DNA homologous to a single *D. melanogaster* RNA polymerase II structural gene, must represent different structural genes. Both appear to encode essential functions required for the growth of yeast strains. This evidence is consistent with the proposition that each of them encodes a different RNA polymerase polypeptide.

DISCUSSION

Success in using the *D. melanogaster* RNA polymerase II gene as a probe to identify the analogous gene in mammalian cells (4) prompted us to use a parallel approach to identify related genes in yeast. We have discovered and isolated two such genes from the *S. cerevisiae* genome; a possible third gene has been identified but not yet isolated. Analysis of cellular RNA indicated that the two cloned genes are templates for the transcription of RNA species that correspond in size, 5.9 and 4.6 kb, to messenger RNA species that could encode proteins of molecular weight approximately 220,000 and 160,000, respectively. *In vitro* translation of the RNA isolated by hybrid-selection with the DNA of one of these genes yielded a protein of M_r 220,000. Both cloned loci were shown by insertional inactivation to encode single-copy essential genes.

The *in vitro* translation experiment strongly suggests that the gene corresponding to the 7.5-kb *Eco*RI fragment encodes the largest subunit of RNA polymerase II. No other subunit of any of the three yeast RNA polymerases is as large as M_r 220,000 (8, 10, 20). The cloned *D. melanogaster RpII* DNA used as probe to isolate these *S. cerevisiae* genes has been shown to be homologous to the DNA that encodes the subunit of RNA polymerase II determining sensitivity to inhibition by α-amanitin in higher eukaryotes (4). In *D. melanogaster* this DNA is also the site of mutations to α-amanitin resistance (3) and it encodes the M_r 215,000 subunit of the RNA polymerase II enzyme (5). This is the gene we expected to isolate by using cross-species hybridization. We propose to call this *S. cerevisiae* gene *RPO21*.

Further evidence that the locus containing this 7.5-kb *Eco*RI fragment encodes the largest subunit of one of the *S. cerevisiae* RNA polymerases has been provided by R. A. Young (Stanford University). Using the entirely different approach of immunological screening (25) with an antibody raised against RNA polymerase II large subunits, DNA with identical restriction sites for *Eco*RI, *Hin*dIII, *Bgl* II, *Pst* I,

and *Kpn* I has been isolated from a library of *S. cerevisiae* genomic DNA in the bacterial expression vector λgt11 (R. A. Young, personal communication). Because of the known antibody crossreactivity among the large subunits of the three RNA polymerases of *S. cerevisiae* (11), immunological identification alone does not allow an unequivocal determination of which RNA polymerase large subunit gene has been isolated. However, the two kinds of data, ours which show that the product of the gene is a protein of M_r 220,000 and the immunological identification, taken together prove that the 7.5-kb *Eco*RI fragment encodes the largest subunit of RNA polymerase II.

The identification of the second cloned gene (corresponding to the 2.6-kb *Eco*RI fragment) is not as clear. From the size of the transcript (4.6 kb) one can infer a maximum polypeptide product of about M_r 165,000. This corresponds most closely to the largest subunit of RNA polymerase III [M_r, 160,000 (ref. 20)]. Alternatively, it could encode the second-largest subunit of RNA polymerase II [M_r, 150,000 (ref. 20)], the second-largest subunit of RNA polymerase I [M_r, 135,000 (ref. 20)] or some other as yet undescribed polymerase-related protein. We believe, however, that this second locus most likely is the gene for the polymerase III M_r 160,000 polypeptide because not only do immunological studies indicate that there is a conservation of antigenic determinants among the three largest subunits of the different RNA polymerases in *S. cerevisiae* (11) but also the RNA polymerases I and III of different eukaryotic species are to various extents sensitive to inhibition by α-amanitin and may therefore share with RNA polymerase II a closely related α-amanitin-binding domain. Thus it is likely that there are conserved nucleotide sequences among the genes encoding the largest subunits of *S. cerevisiae* RNA polymerase I, II, and III. If this is indeed the RNA polymerase III gene, then we propose naming this second locus *RPO31*, although until further evidence is available, we retain the name *RPR3* (RNA polymerase-related). We are suggesting that *S. cerevisiae* genes encoding subunits unique to RNA polymerase I, II, and III be named *RPO11* through *RPO19*, *RPO21* through *RPO29*, and *RPO31* through *RPO39*, respectively. Subunits in common between RNA polymerases I, II, and III could be designated *RPO1* through *RPO9*. A third, 1.8-kb, fragment was also detected in the whole genome blot by using *D. melanogaster* p4.2 DNA as probe (Fig. 1B), and there are additional p4.1-related DNA sequences in genomic blots of *S. cerevisiae* DNA apart from those of the RPO21 and RPR3 loci (data not shown). However, we have not yet been able to isolate this third gene. The hybridization is relatively weak and it has been difficult to detect positive signals above the background in the plaque assays. On the basis of the foregoing discussion we speculate that the locus represented by the 1.8-kb *Eco*RI band might encode the largest subunit of RNA polymerase I (hence *RPO11*). In this case the large subunits of the three RNA polymerases in *S. cerevisiae* might be members of a gene family that have evolved from a single primordial ancestor (11, 20). Detailed sequence comparison of genes from both yeast and other organisms will be required to establish the validity of this idea.

The demonstration that both of the genes that we have cloned are essential for cell viability was made possible by the fact that they are also single-copy and is, of course, to be expected of RNA polymerase functions. *In vitro* mutagenesis of the cloned genes, followed by gene replacement (24) and screening to obtain other classes of RNA polymerase mutants, not only will verify the nature of the functions that these genes encode but also will be extremely useful in physiological and biochemical studies of transcription. The cloning of the large subunit of the three different RNA polymerases will be of special interest for it has been suggested (20) that the promoter specificities of the class I, II, and III enzymes reside in their larger subunits.

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