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Ribosomal protein genes rp 39(10-78), rp 39(11-40), rp 51, and rp 52 are not contiguous to other ribosomal protein genes in the Saccharomyces cerevisiae genome

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ABSTRACT

A library of recombinant phage containing EcoR1 fragments of Saccharomyces cerevisiae DNA has been constructed. This library was screened with four different recombinant plasmids, each containing a different yeast ribosomal protein gene, in order to isolate chromosomal fragments extending in both directions from these genes. These chromosomal fragments were assayed for the presence of additional ribosomal protein genes by hybridization selection and cell free translation, and none were found. These four regions are not closely linked to each other, since DNA from one domain does not cross hybridize with DNA from any of the other three, except for the sequences within the homologous ribosomal protein 39 gene pair. Northern blots demonstrate that although the concentrations of ribosomal protein mRNAs are diminished significantly in a strain containing the ts mutation rna2, transcripts from genes in these flanking segments are relatively unaffected.

INTRODUCTION

The biosynthesis of ribosomes is a complex process involving the synthesis and assembly of equimolar quantities of 50-70 different proteins and 3-4 different structural RNAs (1). Many of the mechanisms whereby ribosome biogenesis is regulated in the procaryote E. coli have been elucidated in elegant detail in recent years (2). The rate of synthesis of each of the ribosomal proteins of E. coli is identical and varies in proportion to the cellular growth rate (3). This coordinate synthesis in E. coli is mediated by virtue of (1) the clustering of the 52 ribosomal protein genes into at least 15 different transcription units or operons (2), and (2) the ability of one ribosomal protein gene product from each operon to serve as an autogenous feedback inhibitor of translation of the polycistronic ribosomal protein mRNA transcribed from its respective operon (4). Such a detailed understanding of ribosome biogenesis in eucaryotes awaits a similar molecular and genetic analysis. Yeast is a particularly

advantageous eucaryotic organism for such multifaceted studies, in that it is well characterized from a genetic, molecular biological, and (in the case of ribosome biogenesis) physiological point of view. Coordinate regulation of yeast ribosomal protein synthesis is manifested in cells containing ts mutations in each of ten loci, rna2 - rna11. One hour after shifting these mutant cells to the nonpermissive temperature, translatable mRNA for all but six ribosomal proteins is reduced approximately tenfold (5).

Recently we have isolated cloned yeast genomic DNA fragments, each of which contains a single ribosomal protein gene (6). Three of the genes are among those whose mRNAs are decreased in concentration at the nonpermissive temperature in the mutant strain rna2, while two of the clones contain the two different copies of a ribosomal protein gene (repeated twice in the yeast genome) whose expression is not affected in the mutant strains.

In studying the coordinate expression of these genes, we have asked whether these ribosomal protein genes are tightly clustered in the yeast genome as is the case for bacterial ribosomal protein genes. For this purpose, we have isolated fragments of yeast genomic DNA contiguous to those previously purified, and determined whether additional ribosomal protein genes are present in these adjacent chromosomal segments.

MATERIALS AND METHODS

Construction and Screening of Recombinant Phage Library

Separate aliquots of yeast genomic DNA, isolated from spheroplasts of Saccharomyces cerevisiae strain A364A as described by Hereford and Rosbash (7), were digested with Eco R1 restriction endonuclease (New England Biolabs) for varying lengths of time in order to obtain a random collection of various partially digested genomic Eco R1 fragments as well as completely digested fragments. This mixture of fragments was centrifuged on a 10-40% neutral sucrose gradient containing 0.01M Tris HCl, pH 7.5, 0.001M EDTA, 0.1M NaCl, at 27,000 rpm for 21 hours in an SW40 rotor. Twenty drop fractions were collected, and aliquots were analyzed for size fractionation of the DNA by electrophoresis on a 1% agarose gel using Eco R1 digested Charon 4A DNA as a molecular weight standard. Fractions containing $12\text{-}20^{+}$ kb[1] DNA were pooled, phenol extracted twice, ethanol precipitated, and resuspended at 0.4 mg/ml in 0.01M Tris, 0.001M EDTA, pH 7.5 (TE buffer). Purified arms of λ phage Charon 4A DNA (8), generously provided by Dr. Ulrich Schafer, were preannealed for 1 hour at 42°C in 100mM $MgCl_2$, and added to a mixture containing the purified 12-20 kb yeast DNA (66 μg/ml),

66mM Tris HCl, pH 7.6, 10mM $MgCl_2$, 1mM ATP, 15mM DTT, 100 µg/ml BSA, and 1 unit of T4 ligase. This mixture was incubated at 4°C for 20 hours, heated to 65°C for 2 min., and chilled to 0°C. Successful ligation was assayed by agarose gel electrophoresis of the samples. A 2.0 µg aliquot of the ligated DNA was subsequently packaged into phage in vitro, using extracts of lysogenic strains N5428 and N5433, prepared by Drs. Lynn Golden and U. Schafer, as described by Sternberg et al. (9).

The resulting library of phage was amplified by preadsorbing phage to E. coli DP50 supF at 37°C for 15 min., and plating on NZCYM media (10). Top agar containing the amplified phage was scraped from petri dishes into sterile SM buffer (0.01M $MgSO_4$, 0.05M Tris HCl, pH 7.5, 0.1M NaCl, 0.01% gelatin), containing a drop of chloroform for storage at 4°C.

Twelve-thousand phage from the library were screened with each recombinant plasmid probe, at a density of 3,000 plaques per 15 cm petri dish, following the plaque filter hybridization protocol of Benton and Davis (11). Plasmid DNAs were radioactively labelled in vitro to specific activities of $1\text{-}5\cdot10^7$ cpm/µg by nick translation using DNA polymerase I (12). Positive plaques were picked into SM buffer, replated, and rescreened in order to plaque-purify phage containing yeast DNA complementary to the cloned probes. Phage were propagated in liquid cultures of DP50 supF in NZCYM media by a modification of the PDS method of Blattner et al. (8) (J. Lingrel, personal communication), and were purified from lysates by PEG precipitation and subsequent isopycnic banding in CsCl density gradients. DNA was purified from phage by two phenol extractions followed by ethanol precipitation, and was stored in TE buffer at 4°C.

Isolation of Bacterial Plasmid DNA and Yeast mRNA

Recombinant plasmid DNA molecules containing yeast ribosomal protein genes were isolated from a library of yeast genomic DNA cloned in plasmid vector pMB9 (13), as described in Woolford et al. (6). Plasmid DNA was purified from Brij deoxycholate treated bacterial spheroplasts by CsCl ethidium bromide density gradient centrifugation (14), with the modifications of Petes et al. (13). Gradient purified DNA was chromatographed on 1x20 cm agarose A15M columns equilibrated with TE 0.1M NaCl, to resolve plasmid DNA from contaminating RNA.

Poly(A)$^+$ RNA was isolated from exponential phase wild type A364A yeast (15) grown at 23°C, or ts 368 yeast containing the rna2 mutation (16) grown at 23°C and shifted to 36°C one hour prior to extraction, as described in Rosbash et al. (17).

Gel Electrophoresis, Transfer, and Filter Hybridization of DNA and RNA

Restriction endonucleases were purchased from New England BioLabs and were used according to their specifications. Restriction fragments of genomic, phage, or plasmid DNA were electrophoresed on neutral agarose or polyacrylamide gels, as described in Woolford et al. (6). DNA was transferred from gels to nitrocellulose filters (Millipore HAWP 00010 or Schleicher and Schuell BA85R597) by the method of Southern (18). To facilitate transfer of larger fragments, the gels were exposed to ultraviolet light (254 nm) for two minutes prior to denaturation of the DNA. Filter hybridizations were performed as described in Woolford et al. (6).

Total and poly(A)$^+$ yeast RNA was electrophoresed on denaturing CH_3HgOH gels as described in Alwine et al. (19). Transfer of RNA to DBM paper and subsequent hybridization with recombinant plasmid or phage DNA radioactively labelled in vitro by nick translation was performed as described in Alwine et al. (19) and Golden et al. (20).

Hybridization Selection and Cell Free Translation of RNAs Complementary to Cloned Yeast DNA

Proteins encoded by the yeast genomic fragments cloned in the recombinant phage were identified following the protocols of Woolford and Rosbash (21), Ricciardi et al. (22), or Barnett et al. (23). Usually 10 µg of phage DNA were hybridized in solution to 3-5 µg of yeast poly(A)$^+$ RNA, under conditions such that R-loops were formed between the DNA and the complementary mRNA (24). Complementary mRNAs were then purified from unhybridized non-complementary RNAs by gel filtration chromatography in high salt buffer (21). When larger amounts of poly(A)$^+$ mRNA (20-30 µg) were hybridized to the phage DNAs, the protocols of Ricciardi et al. (22) or Barnett et al. (23) were followed using 10-40 µg of denatured phage DNA immobilized on nitrocellulose filters or DBM paper.

RNA samples purified thusly were translated in a wheat germ extract (25) containing L[^{35}S] methionine (Amersham, ∿ 1,100 Ci/mmole) or L-[4,5-^{3}H(N)]-lysine (New England Nuclear, 78.1 Ci/mmole).

Gel Electrophoresis of Proteins

Radioactively labelled proteins synthesized in vitro were analyzed by electrophoresis on SDS slab gels containing a linear 7.5-15% gradient of polyacrylamide (26). Cell-free translation products were identified as yeast ribosomal proteins by two-dimensional gel electrophoresis, as described by Warner and Gorenstein (5). Yeast ribosomes and ribosomal proteins were purified as described in Woolford et al. (6). All gels were subjected

to fluorography at -80°C (27) using Kodak XR5 or AR5 X-ray film.

Physical and Biological Containment

Plasmid bearing strains described herein were grown under P2EK1 containment, and phage were propagated under P2EK2 containment, in compliance with NIH Guidelines for Recombinant DNA Research.

RESULTS

Isolation of Yeast Chromosomal Fragments Contiguous to Cloned Ribosomal Protein Genes

The clones containing yeast ribosomal protein genes that we previously isolated (6) were constructed using randomly sheared fragments of yeast nuclear DNA, which were inserted into the Eco R1 restriction enzyme site of the plasmid vector pMB9 using the homopolymer poly dA : poly dT tailing method described by Wensink *et al.* (28).

That these ribosomal protein gene-containing plasmids could serve as suitable hybridization probes for contiguous yeast chromosomal fragments from a recombinant phage library of partial Eco R1 fragments of yeast DNA requires at least two conditions: (1) the probes contain single copy yeast DNA, and (2) the yeast genomic Eco R1 fragments homologous to the DNA cloned in the plasmids be present in the library of partial and complete Eco R1 fragments; e.g. that the fragments not be so large that they cannot be packaged within a recombinant phage particle. These conditions were tested by mapping the genomic Eco R1 sites within and immediately adjacent to the cloned fragments. This was accomplished by standard restriction enzyme mapping of the plasmid DNAs and by hybridizing each of the plasmids, radioactively labelled *in vitro* with ^{32}P, to yeast genomic Eco R1 fragments separated by agarose gel electrophoresis and transferred to nitrocellulose.

Plasmid pY9-90 hybridizes to 20-30 different Eco R1 genomic fragments of yeast DNA (6), and therefore could not be used as a probe for contiguous sequences unless a fragment of single copy yeast DNA were first subcloned from it. Plasmid pY10-78 includes all of a single Eco R1 fragment 2.9 kb long, plus part of a 4.4 kb Eco R1 fragment including the rp 39 gene, and a small portion of a 2.2 kb Eco R1 fragment (Figure 1). pY11-40 DNA is contained within a single Eco R1 fragment 11.0 kb long which includes a second copy of the rp 39 gene (Figure 1). Since both pY10-78 and pY11-40 contain sequences complementary to the rp 39 gene which is repeated twice in the yeast genome (6), both plasmids hybridize to the two genomic fragments containing the rp 39 sequences. Plasmids pY13-86 and pY11-18 contain only

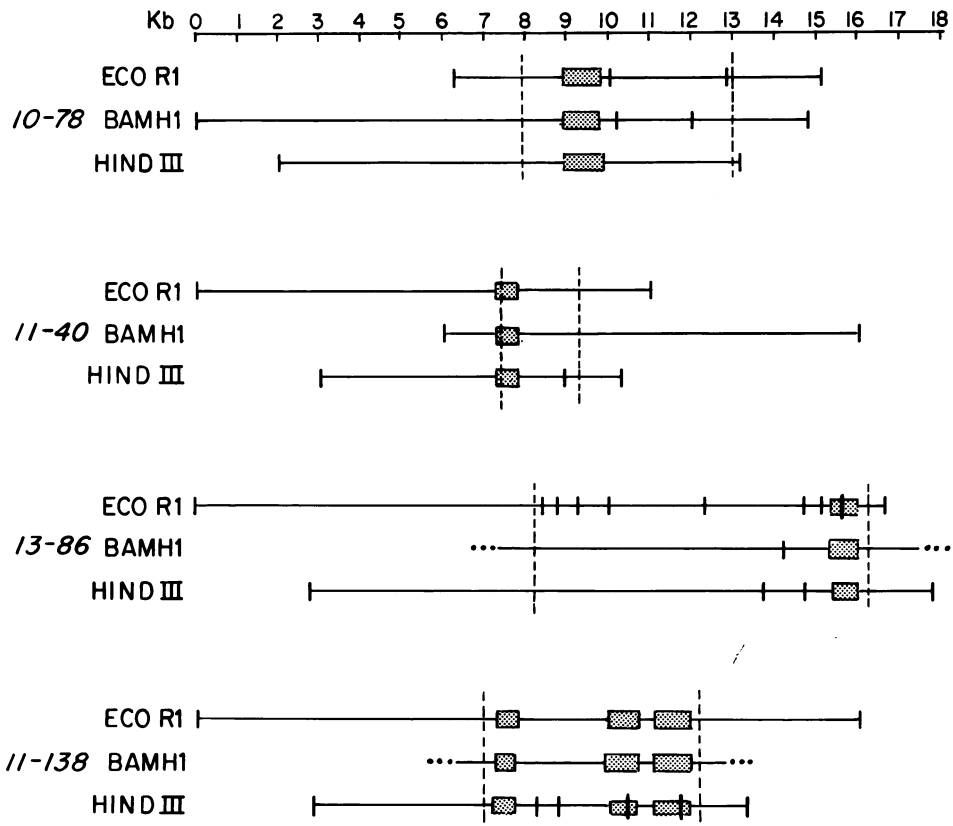

Figure 1. Organization of genomic restriction fragments homologous to yeast ribosomal protein gene containing plasmid DNAs. The dashed vertical lines denote the end points of randomly sheared yeast genomic fragments cloned in plasmids pY10-78, pY11-40, pY11-138, and pY13-86, relative to their respective homologous yeast genomic Eco R1, Bam H1, and Hind III restriction fragments. The solid boxes denote gene sequences, and the solid vertical lines denote restriction enzyme sites. Genes were located relative to restriction enzyme sites by electron microscopic visualization of R-loops (6).

single copy yeast DNA; the DNA cloned in pY13-86 contains seven small Eco R1 fragments plus portions of 0.75 kb and 8.0 kb genomic fragments (Figure 1), and that in pY11-138 is contained within the yeast genome as a single large Eco R1 fragment 13.5 kb long. The latter four plasmids can therefore serve as suitable hybridization probes for the phage library, since the only repeated sequences are the twice repeated rp 39 gene sequences, and none of the overlapping genomic Eco R1 fragments are exceedingly large.

A library of yeast chromosomal DNA clones consisting of partial and

complete Eco R1 fragments was established in the λ cloning vector Charon 4A, as described in Materials and Methods. Twelve-thousand plaques from the library were separately screened with radioactively labelled plasmids pY10-78, pY11-40, pY11-138, and pY13-86. The phage containing yeast fragments homologous to these probes are diagrammed in Figure 2. The order of Eco R1 fragments within each phage was determined by restriction enzyme mapping and by cross-hybridization analysis on Southern gels between restriction fragments of each set of phage or plasmid DNAs.

Ten identical phage containing DNA homologous to pY11-138, designated λ11-138(1)-(10), were identified (Figure 2a). All contained the single 13.5 kb Eco R1 fragment, suggesting that the adjacent Eco R1 fragments were too large to be cloned together with this fragment within a Charon 4A recombinant or that partial Eco R1 fragments containing the 13.5 kb fragment were extremely rare. Four different phage, λ13-86(1), (5), (7), and (10), were isolated that included DNA complementary to a portion of pY13-86 DNA (Figure 2b). These four phage DNAs define a chromosomal region 45 kb long, including 27 kb on one side of the ribosomal protein gene 52 and 17.5 kb on the other. DNA adjacent to the ribosomal protein 39(10-78) gene was isolated in five different recombinant phage, three identified using the pY11-40 probe (λ11-40(2), λ11-40(6), and λ11-40(7)) and two using the pY10-78 probe (λ10-78(1) and λ10-78(14)). These phage together include chromosomal DNA 20 kb long, 8 kb to one side of the ribosomal protein 39(10-78) gene and 12 kb on the other (Figure 2c). Phage from the pY11-40 region of the yeast genome were identified using the pY11-40 probe, and contained 13.2 kb of DNA centered around the ribosomal protein 39(11-40) gene (Figure 2d).

All of the non-vector Eco R1 fragments cloned in these recombinant phage are present in the yeast genome. Hybridization of each radioactively labelled phage DNA to Southern blots of yeast genomic Eco R1 fragments demonstrated that each cloned Eco R1 fragment was present in the yeast genome and that all of these cloned yeast DNA sequences are single copy sequences, except for those sequences within the duplicated rp 39 genes. Since no homology was detected between each of the DNA sequences surrounding these different ribosomal protein genes, it follows that none of the four ribosomal protein genes are linked to each other within the distances of the cloned fragments, and that there is no significantly long stretch of DNA sequence in these clones that is adjacent to more than one of these ribosomal protein genes (or any other since the fragments are single-copy).

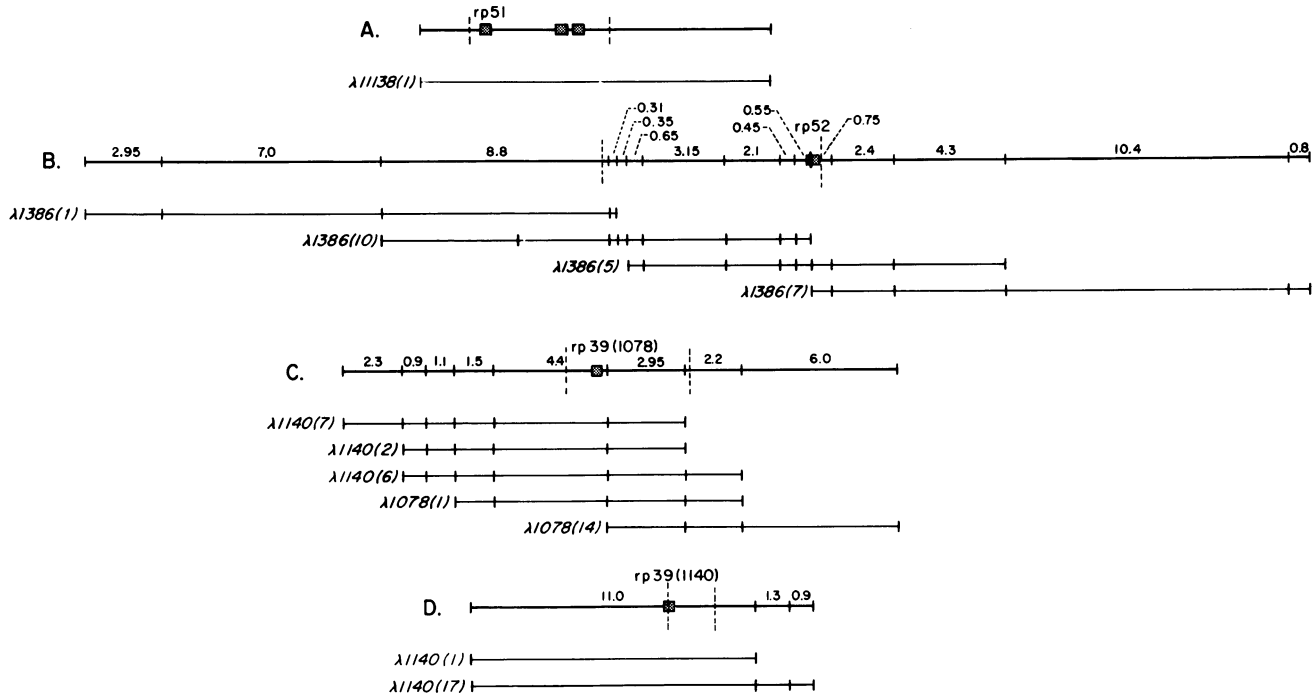

Figure 2. Cloned chromosomal segments overlapping and contiguous to yeast ribosomal protein genes. The Eco R1 restriction fragments, cloned in recombinant phage, which overlap with plasmid DNAs containing yeast ribosomal protein genes 39 (10-78), 39 (11-40), 51, and 52, are indicated. The top line in each series designates the entire cloned region contiguous to each gene, while the lower figures in each series designate the inserts of Eco R1 fragments cloned in each phage. Solid vertical lines delineate Eco R1 sites, and solid boxes designate the location of gene sequences in plasmid-cloned sequences. The dashed vertical lines delineate the randomly sheared fragments cloned in the plasmids. Sizes of restriction fragments are indicated in kilobase pairs.

Screen for Ribosomal Protein Coding Regions in Chromosomal DNA Contiguous to Ribosomal Protein Genes 39(10-78), 39(11-40), 51, and 52

Does the DNA contiguous to ribosomal protein genes 39(10-78), 39(11-40), 51, or 52 contain other ribosomal protein genes? We tested this hypothesis by screening the phage DNAs described above for the ability to hybridize to yeast mRNA that could be translated into a ribosomal protein. Yeast poly(A)$^+$ mRNA complementary to each phage DNA was purified as described in Materials and Methods, and was translated in a wheat germ lysate containing ^{35}S methionine or ^{3}H lysine. Control DNAs, containing a ribosomal protein gene sequence at equivalent or lower concentrations than the sequences being tested, were included in each hybridization. An aliquot of each cell-free translation reaction was electrophoresed on 7.5-15% polyacrylamide slab gels to visualize all of the polypeptides synthesized (data not shown). The remainder of each sample was electrophoresed on the two-dimensional gel system described by Gorenstein and Warner (1976), which resolves most yeast ribosomal proteins. The ribosomal proteins encoded by λ11-138(1) and λ13-86(5) are shown in Figure 3. None of the recombinant DNAs hybridized to any additional ribosomal protein mRNAs. Only phage λ13-86(1) contained any additional (detectable) yeast genes other than those discovered previously in the plasmid clones. Since the control ribosomal protein gene sequences were always detected, it is unlikely that ribosomal protein genes went undetected due to a lack of sensitivity in the assay (see Discussion). Nevertheless, the experiments were repeated using up to five times as much phage DNA, immobilized on nitrocellulose filters or on DBM paper (22, 23), which was hybridized to ten times as much poly(A)$^+$ RNA as is normally necessary to assay for ribosomal protein genes. The results were identical to those in the original experiments.

RNA Transcripts from Contiguous Chromosomal Segments

Perhaps a more sensitive assay for the transcribed regions within DNA is provided by hybridization of the radioactively labelled DNA to Northern blots of poly(A)$^+$ RNA resolved by denaturing gel electrophoresis, and covalently attached to DBM paper. For this reason the recombinant phage DNAs overlapping with pY13-86 plasmid DNAs were nick translated and hybridized to blots of yeast poly(A)$^+$ RNA. Transcripts identical to those detected with the plasmid, as well as additional transcripts, were identified with these probes (Figure 4). The major poly(A)$^+$ RNA, approximately 600 bases in length, is probably the mRNA for rp 52 because: (1) this transcript hybridizes with pY13-86, λ13-86(5) and λ13-86(7), all of which include the rp 52

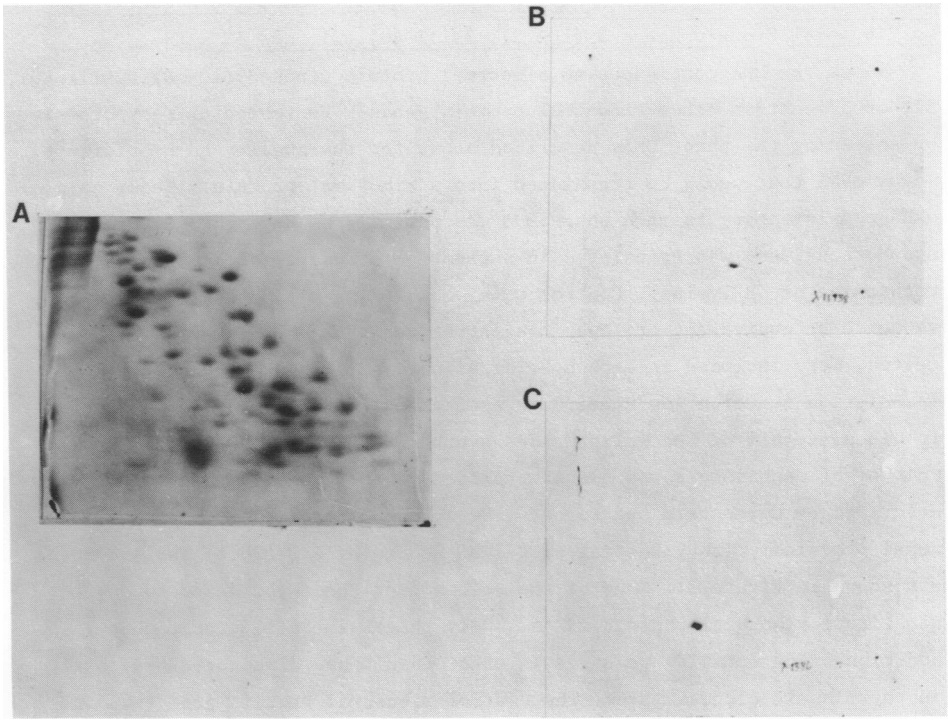

Figure 3. Two-dimensional gel electrophoresis of translation products of poly(A)$^+$ RNA hybridized to recombinant phage DNAs. (A) Coomassie blue-stained gel of total yeast protein, displaying yeast ribosomal proteins. The only non-ribosomal proteins are those in the upper left-hand corner of the gel. (B) Fluorogram of ^{35}S methionine labelled translation products of poly(A)$^+$ mRNA complementary to (B) λ11-138(1) and (C) λ13-86(5) phage DNAs.

gene, and does not hybridize with λ13-86(1), which does not include the rp 52 gene; (2) this transcript is complementary to both the λ13-86(7) and pY13-86 probes, whose overlap consists mainly of a portion of the rp 52 gene (Figure 2); and (3) this is the only transcript which decreases in concentration in rna2 upon incubation at the non-permissive temperature (compare odd lanes with even lanes). This latter observation suggests that the effect of rna2 is specific for individual rp genes and does not affect adjacent (or nearby) genes. A similar observation has been made for the rp 51 gene (17). A single large transcript hybridized to λ13-86(1) (Figure 4, lanes 1 and 2), consistent with the fact that a single large polypeptide (∿ 95,000 daltons) was detected by cell-free translation of

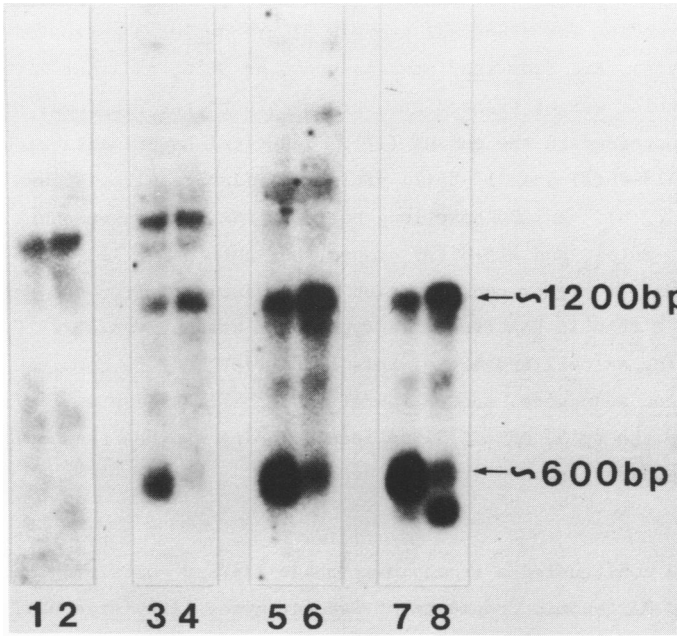

Figure 4. Yeast transcripts complementary to cloned DNA segments surrounding ribosomal protein 52 gene. Yeast poly(A)$^+$ RNA was prepared from log phase wild-type A364A yeast grown at 23°C (lanes 1, 3, 5, 7) or from the temperature-sensitive strain ts368 containing the rna2 mutation, grown at 23°C and shifted to 36°C one hour prior to extraction (lanes 2, 4, 6, 8). One µg of poly(A)$^+$ RNA was electrophoresed in each lane of a CH_3HgOH agarose gel, and was transferred to DBM paper. Phage or plasmid DNAs were radioactively labelled in vitro and approximately $5x10^6$ cpm were hybridized to the blotted RNAs from each pair of lanes.

Lanes 1 and 2: hybridization with λ13-86(1)
Lanes 3 and 4: hybridization with λ13-86(5)
Lanes 5 and 6: hybridization with λ13-86(7)
Lanes 7 and 8: hybridization with pY13-86

poly(A)$^+$ RNAs complementary to λ13-86(1) (data not shown). The level of this large transcript is not altered in RNA isolated from the rna2 strain grown at the nonpermissive temperature (Figure 4, lane 2). Interestingly, there is one small mRNA that is increased in concentration in rna2. This transcript, smaller than rp 52 mRNA, is detected by hybridization with pY13-86 DNA (Figure 4, lanes 7 and 8) and on longer exposure with λ13-86(1) DNA but not λ13-86(5) DNA (data not shown), and must therefore be complementary to the left end of pY13-86.

Northern blotting experiments demonstrate that the levels of the mature mRNA coding for ribosomal protein 51 are depleted approximately ten-fold in poly(A)$^+$ RNA from rna2 strains grown at 36°C, and that levels of higher molecular weight transcripts, precursors to the ribosomal protein 51 mRNA, are increased in the mutant (17). Identical experiments with pY13-86, λ13-86(1), λ13-86(5) and λ13-86(7) yielded similar results (Figure 4, lanes 2, 4, 6, 8). A 1200 base long poly(A)$^+$ RNA is complementary to the pY13-86, λ13-86(5), and λ13-86(7) probes, but not to the λ13-86(1) probe, and is present in higher concentration in RNA isolated from the rna2 strain grown at 36°C than in RNA from wild type A364A yeast grown at 23°C. By the same reasoning as outlined above, this transcript must be complementary to the rp 52 gene sequences, and is therefore probably a high molecular weight precursor to the rp 52 mRNA. S1 nuclease mapping and DNA sequencing experiments are underway to confirm this hypothesis.

DISCUSSION

We have constructed a recombinant phage library containing partial and complete Eco R1 genomic fragments of Saccharomyces cerevisiae DNA, in order to isolate additional fragments of chromosomal DNA adjacent to previously cloned yeast ribosomal protein genes (6). The average size of yeast DNA inserts randomly chosen from this library has been found to be approximately 15 kb. In screening 12,000 phage from this library, there should have been a greater than 99.9% probability of finding any one sequence. Consistent with this prediction, ten to twenty recombinant phage containing DNA complementary to each of the single probes were identified. Furthermore, more than 500 of 12,000 plaques screened hybridized to pY9-90, which contains, in addition to ribosomal protein gene 63, sequences repeated at least 30 times in the yeast genome. No evidence was obtained for the presence of fragments within a single phage that are not contiguous within the yeast genome; multiple isolates of each identified phage were found.

The phage isolated from the library contain 45 kb of DNA surrounding the rp 52 gene, 20 kb surrounding the rp 39 (10-78) gene, 13.2 kb surrounding the rp 39 (11-40) gene, and 13.5 kb surrounding the rp 51 gene. The failure to isolate longer stretches of DNA adjacent to the rp 39 (11-40) gene and to the rp 51 gene probably is due to the presence in the yeast genome of relatively large Eco R1 fragments adjacent to the 11.0 kb and 13.5 fragments containing these genes, resulting in multiple contiguous fragments exceeding the 22 kb cloning capacity of the 4A phage vector. Alternatively, such partial fragments might not have been created in the

Eco R1 digestions of DNA for construction of the library.

None of the cloned DNA surrounding these genes cross-hybridizes with DNA adjacent to one of the other three ribosomal protein genes. Thus, the minimal distance between any pair of these genes is at least 12 kb (Figure 3). These data are consistent with the observation that all four of these plasmids map genetically to unlinked regions of the yeast genome (L. Hyman, unpublished observations).

Only one more structural gene was discovered by hybridization translation assays of the additional 71 kb of DNA cloned adjacent to the ribosomal protein genes, i.e. a total of seven genes have been identified in these 92 kb of yeast DNA (6 were identified in Woolford et al. (6)). Hybridization of excess yeast mRNA to cDNA and to single copy genomic DNA indicates that approximately 3,000 to 4,000 genes are transcribed from 30-40% of the yeast genome (7). Electron microscopic visualization of R-loops formed between high molecular weight yeast chromosomal DNA (100-300 kb) and poly(A)$^+$ RNA substantiates that transcribed regions occur very frequently in the yeast genome, about one 1 kb gene per 3.3 kb DNA (30). The apparent low frequency of genes identified by hybridization translation in DNA contiguous to the four ribosomal protein genes may indicate that these particular regions of the yeast genome are not densely transcribed. However, a more likely explanation is that only genes coding for abundant or moderately abundant mRNAs ($\gtrsim$ 0.1% of total mRNA) are detected by the hybridization-translation assays. Ninety percent of the yeast genes code for low abundance mRNAs (each $\lesssim$ 0.1% of total mRNA), whose detection is probably below the sensitivity of the assay. Hybridization of radioactively labelled DNA probes to Northern blots of electrophoretically separated transcripts is almost certainly a more sensitive assay for genes, since transcripts comprising as low as 0.05% to 0.01% of total poly(A)$^+$ RNA are readily detectable (20). By this means, many transcripts other than those identified by hybridization translation were discovered in DNA overlapping the rp 51 and rp 52 genes (Figure 4, and (17)).

Since the one additional structural gene was not a ribosomal protein gene, it follows that no additional ribosomal protein genes were detected in any of the yeast DNA cloned in these recombinant phage. While a small number of ribosomal protein genes are not visible on the two-dimensional gel assay (e.g. the few acidic ribosomal proteins), it is unlikely that most yeast ribosomal protein genes would escape detection. Although the absolute level of each individual yeast ribosomal protein mRNA is unknown, two dif-

ferent experiments suggest that most of the ribosomal protein mRNAs are present in approximately equal concentrations as moderately abundant yeast mRNAs (each 0.1-0.2% of total poly(A)$^+$ RNA) and are equally detectable. (1) Hybridization kinetic analysis indicates that those mRNAs which are decreased in strains bearing the rna2 mutation are abundant or moderately abundant mRNAs (31). (2) Most ribosomal proteins are readily detectable by cell-free translation of total yeast RNA and subsequent two-dimensional gel electrophoresis ((5); Woolford, unpublished observations). Therefore, all additional ribosomal proteins should have been at least detectable on the standard one-dimensional gel assay, and most of them should have been detectable on the two-dimensional ribosomal protein gel. These negative results are reinforced by the fact that no additional transcripts complementary to 13-86 or 11-138 phage DNA were decreased in concentration at the non-permissive temperature in a rna2 background (Figure 4 and (17)). They are also consistent with data from Fried and Warner (32) on the rp 3 gene and from Fried et al. (submitted for publication) on a number of other yeast ribosomal protein genes.

The lack of clustering of these yeast ribosomal protein genes with each other or with other ribosomal protein genes, at least in the four chromosomal regions studied, is not surprising, in spite of the precedent in E. coli. Several different yeast genes conferring resistance to antibiotic inhibitors of translation, at least one of which has been proven to be a ribosomal protein gene (32), are not linked to each other (32-35). In fact, genetic mapping and recombinant DNA studies have revealed only three clusters of related yeast genes coding for separate polypeptides: the gal 1, 7, 10 cluster (36), the histone genes H2A and H2B (37), and the dal 1, 4, 2 cluster (38).

The fact that yeast ribosomal protein genes are unlinked to each other suggests that coordinate regulation of these genes is based on sequence information within or immediately adjacent to individual genes. The data in Figure 4, and similar data previously published (17), are also consistent with this hypothesis. These latter experiments support the notion that the phenotype of at least some of the rna mutants is due to a block in rp mRNA processing. Further experiments will be required to determine the mechanism(s) which control ribosomal protein synthesis in other genetic and physiological conditions. Presumably these mechanism(s) will also be dependent upon DNA or RNA sequence information and will act independent of the chromosomal location of individual ribosomal protein genes.

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[1]Abbreviations Used

kb	(kilobases), 1,000 bases or base pairs in single or double-stranded nucleic acids, respectively.
rp	ribosomal protein
poly(A)	polyadenylic acid
SDS	sodium dodecyl sulfate
BSA	bovine serum albumin
ts	temperature sensitive

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