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Structural and Functional Analyses of *Saccharomyces cerevisiae* Wild-Type and Mutant *RNA1* Genes

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The yeast gene *RNA1* has been defined by the thermosensitive *rna1-1* lesion. This lesion interferes with the processing and production of all major classes of RNA. Each class of RNA is affected at a distinct and presumably unrelated step. Furthermore, RNA does not appear to exit the nucleus. To investigate how the *RNA1* gene product can pleiotropically affect disparate processes, we undertook a structural analysis of wild-type and mutant *RNA1* genes. The wild-type gene was found to contain a 407-amino-acid open reading frame that encodes a hydrophilic protein. No clue regarding the function of the *RNA1* protein was obtained by searching banks for similarity to other known gene products. Surprisingly, the *rna1-1* lesion was found to code for two amino acid differences from wild type. We found that neither single-amino-acid change alone resulted in temperature sensitivity. The carboxy-terminal region of the *RNA1* open reading frame contains a highly acidic domain extending from amino acids 334 to 400. We generated genomic deletions that removed C-terminal regions of this protein. Deletion of amino acids 397 to 407 did not appear to affect cell growth. Removal of amino acids 359 to 397, a region containing 24 acidic residues, caused temperature-sensitive growth. This allele, *rna1-Δ359-397*, defines a second conditional lesion of the *RNA1* locus. We found that strains possessing the *rna1-Δ359-397* allele did not show thermosensitive defects in pre-rRNA or pre-tRNA processing. Removal of amino acids 330 to 407 resulted in loss of viability.

In all organisms, primary transcripts are processed to generate mature molecules. The biochemical reactions not only require given catalytic activities but, in many cases, also require the participation of ribonucleoprotein particles. Furthermore, in eucaryotes most of the reactions occur in particular subcellular compartments (the nucleus [mRNA and tRNA], the nucleolus [rRNA], and the mitochondria [mitochondrial mRNA, tRNA, and rRNA]) and therefore there is also a requirement for shuttling the catalytic and ribonucleoprotein components to the appropriate locations. Thus, the production of mature RNAs is complex and requires the interaction of a large number of protein and RNA components, yet relatively few of these components have been characterized biochemically. One approach to study such components is to identify mutants that interfere with the production of mature RNAs and then to characterize the wild-type product of the gene in question.

The *Saccharomyces cerevisiae RNA1* gene is 1 of 10 loci identified by Hartwell (20) that affect production of mature RNA. The original *RNA2-RNA10/11* (*PRP2-PRP10/11*) loci (21) and the newly characterized *PRP17-PRP27* loci (U. Vijayraghaven, M. Company, and J. Abelson, submitted for publication) affect the removal of intervening sequences from precursor mRNAs (18, 37, 50; Vijayraghaven et al., submitted). (After this paper was submitted for publication, it was agreed to adopt new nomenclature for the genes affecting precursor RNA processing [*PRP*]. Both nomenclatures are maintained in this report.) Extracts from most of the *rna2-rna10/11* (*prp2-prp10/11*) mutants demonstrate heat lability for the in vitro pre-mRNA splicing reactions (39), and recent experiments have shown that at least the *RNA11* (*PRP11*) and *RNA8* (*PRP8*) gene products are components of pre-mRNA spliceosomes (9, 38). Therefore, it is likely that many, if not all, of the *RNA2-RNA10/11* (*PRP2-PRP10/11*) and *PRP17-PRP27* genes will be found to encode components of the yeast mRNA spliceosome.

Although mutations of the *RNA1-RNA10/11* loci can be suppressed by the same second-site suppressor (*SRN1*) (46; S. L. Nolan, N. S. Atkinson, R. W. Dunst, and A. K. Hopper, manuscript in preparation), the *RNA1* gene appears to define a category distinct from the *RNA2-RNA10/11* (*PRP2-PRP10/11*) genes. The mutant allele, *rna1-1*, causes conditional lethality, and strains bearing this mutation do not grow at temperatures exceeding 30°C. Unlike cells with the *rna2-rna10/rna11* (*prp2-prp10/prp11*) mutations, at the nonpermissive temperature *rna1-1* cells demonstrate pleiotropic defects in RNA production. The best-characterized defect is that involving pre-tRNA processing. Cells possessing the *rna1-1* allele accumulate a category of pre-tRNAs that are highly modified and have mature 5′ and 3′ termini but contain intervening sequences (24, 31, 45). Since the removal of intervening sequences from pre-tRNAs appears to be a late step in processing (41), *rna1-1* cells can be considered to be blocked late in pre-tRNA processing.

The defect in the production of mRNA by *rna1-1* cells is poorly understood. It is known that genes encoding mRNAs are transcribed (27). Analysis of mRNA has shown that for some (57) but not all (59) transcripts, *rna1-1* cells generate novel 3′ termini. It is not known if the novel termini are the products of faulty termination of transcription or faulty 3′ processing of precursor species.

The defect in pre-rRNA production is also not well characterized, but at the nonpermissive temperature *rna1-1* cells transiently accumulate the 35S pre-rRNA (24, 62). Thus, *rna1-1* cells show a defect in the earliest of steps for pre-rRNA processing. It is possible that this defect in pre-rRNA processing is a secondary consequence of the defect in production of mRNA for ribosomal proteins, since

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it has been shown that the ribosomal proteins are necessary for rRNA production (18, 50). In addition, there appears to be a defect in the transport of RNAs from the nucleus to the cytosol. Newly labeled RNA does not appear in the cytosol, and newly synthesized poly(A) RNA does not enter polysomes (27, 55). Therefore, the *rna1-1* lesion either directly or indirectly interferes with the production of all major classes of RNA and in each case does so at a distinct step. Our approach to understand how the *rna1-1* lesion can affect distinct and presumably unrelated steps in RNA production has been to characterize the gene product, localize it to a particular subcellular compartment (A. K. Hopper, H. M. Traglia, and R. W. Dunst, manuscript in preparation), and obtain second-site suppressors of the *rna1-1* lesion (Nolan et al., in preparation). Previously we described the cloning of the *RNA1* and *rna1-1* genes and the generation of a disruption of the *RNA1* locus (1). This paper describes the DNA sequence of the *RNA1* and *rna1-1* genes and the generation of several new point mutations and deletions of the *RNA1* locus.

MATERIALS AND METHODS

Strains, media, and genetic methods. Three *S. cerevisiae* strains were used. EE1b, a haploid strain derived from ts136 (27) is *MAT***a** *rna1-1 ura3-52 ade2-1 tyr1* Gal$^-$ His$^-$. SJ17 (obtained from J. E. Hopper) is a haploid *RNA1* strain with the genotype *MAT*α *ura3-52 leu2-3,112 gal4*. 2b × 3b, a diploid strain also derived from ts136, is *MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 rna1-1/rna1-1 ade2-1/ade2-1 tyr1/TYR1 his7/HIS7* Gal$^-$. These strains were grown in either YEPD or complete medium minus uracil, formulated as previously described (26).

Escherichia coli JM109 [*recA1 endA1 gyrA96 thi hsdR17 SupE44 relA1* λ$^-$ Δ(*lac-proAB*) (*F' traD36 proAB lacI ZΔM15*)] (63) was used for all manipulations involving M13 bacteriophage. Strain MC1066 [F$^-$ *leuB6 pyrF74*::Tn*5* Δ (*lacIPOZYA*) X74 *galU galK* StrA^r *hsdR trpC9830 rpsL*] (8) was used for selecting yeast *URA3*-containing plasmid. Strain RR1 (F$^-$ *pro leu thi lacY* Str^r r_k^- m_k^- *hsdR hsdM endoI*) was used for all other manipulations. Media used to grow these strains have been described previously (40, 43). Yeast cells and bacteria were transformed as described previously by Ito et al. (28) and Maniatis et al. (40), respectively.

Plasmid DNAs. The M13mp8, M13mp9, M13mp10, and M13mp11 vectors (42) were used for DNA sequencing, and the pUC18 and pUC19 vectors were used for plasmid constructions.

Plasmid DNA from *E. coli* transformants was extracted by a modification of the alkaline lysis procedure of Birnboim and Doly (6) as described by Hurt et al. (26). In addition, the final DNA precipitation was performed with isopropanol. Cesium chloride density gradients were used to purify larger quantities of DNA (40).

DNA manipulations. Restriction endonucleases were purchased from Bethesda Research Laboratories, Inc., New England BioLabs, Inc., or Boehringer Mannheim Biochemicals. All digestions were performed according to the instructions of the manufacturers. T4 DNA ligase was purchased from Bethesda Research Laboratories, and reactions were carried out in the buffer supplied at 23°C for 4 h. Klenow fragment of DNA polymerase I was purchased from Pharmacia, Inc., or Boehringer Mannheim. Exonuclease III was purchased from Bethesda Research Laboratories, and S1 nuclease was purchased from Boehringer Mannheim. Reactions to fill in recessed ends of DNA with Klenow fragment, remove 5′ extensions of DNA by using S1 nuclease, or produce random removal of DNA from 3′ termini with exonuclease III were carried out as described by Henikoff (22). Oligonucleotides were end labeled for use as probes, using 5 U of T4 DNA kinase (Bethesda Research Laboratories) in 50 mM glycine (pH 9.5)–10 mM $MgCl_2$–5 mM dithiothreitol–0.2 mM spermidine hydrochloride–25% glycerol, for 1 h at 37°C.

Southern analysis. High-molecular-weight genomic DNA was isolated from yeast cells as described by Sherman et al. (54). Total cellular DNA was digested with the appropriate restriction enzymes for 4 to 5 h and subjected to electrophoresis through 1% agarose gel in 100 mM Tris base–99 mM boric acid–2.5 mM EDTA (pH 8.0). The DNA was transferred to GeneScreen Plus (Dupont, NEN Research Products). The blot was prehybridized and hybridized according to the instructions of the manufacturer.

DNA sequence analysis. Restriction fragments were purified from YEpRNA1(7.7) and cloned into an appropriate M13 vector (42). Single-stranded phage DNA was sequenced by the method of Sanger et al. (52). In some experiments, Sequenase (U.S. Biochemical Corp.) was substituted for the Klenow fragment of DNA polymerase I. The strategies to sequence the wild-type and mutant *RNA1* alleles are diagrammed in Fig. 1. The DNA fragment from *RNA1* sequences bound by *Hin*dIII and *Bam*HI was cloned into M13mp8 and M13mp9. Single-stranded DNA derived from these constructs was sequenced to completion by using forward and reverse sequencing primers (New England BioLabs). The 2.36-kilobase-pair (kbp) *Sst*I restriction fragment known to contain the entire *RNA1* coding region (1) was purified and digested with *Bam*HI. The resulting 1.1- and 1.2-kbp fragments were ligated into homologous restriction sites of M13mp10 and M13mp11. DNA sequencing proceeded in both directions from the *Bam*HI site of the M13mp10 clones and *Sst*I site of the M13mp11 clones. Exonuclease III deletions (22) of random length were generated from the M13mp10 clones to yield a complete overlapping sequence. A continuous open reading frame (ORF) was established. The M13mp11 clones were subjected to exonuclease III deletions from convenient restriction sites to verify sequence from the complementary DNA strand.

Sequence analysis of the *rna1-1* allele (Fig. 1B) proceeded by cloning the two *Sst*I-*Bam*HI fragments from YEprna1-1 (1) into M13mp10 and M13mp11. Exonuclease III deletions, using available restriction sites, were performed on the M13mp10 clones. This generated a series of overlapping fragments that allowed complete sequencing of the ORF. Both point mutations were confirmed by analysis of the complementary DNA from the M13mp11 clone.

To determine the DNA sequence of the *RNA1* region encoded by YEprna1(2.3), a 950-bp *Bam*HI-*Hin*dIII fragment from YEprna1(2.3) extending from the *Bam*HI site internal to *RNA1* to the *Hin*dIII site of YEp24 just outside the tetracycline resistance gene was ligated into the *Bam*HI-*Hin*dIII sites of M13mp8. This generated a plasmid that contained ~350 bp of vector sequences. Segments (156 bp) of these vector sequences were removed by a *Hin*dIII-*Eco*RV double digestion and ligation. This procedure yielded a plasmid that allowed the necessary *RNA1* ORF sequences to be analyzed from a single sequencing reaction, using the forward M13 sequencing primer (Fig. 1C).

Construction of plasmids. pR35, pR6, pR3, and pR8 contain *RNA1*, *rna1-1*, and hybrid *rna1*/*RNA1* (allele *rna1-17*) and *RNA1*/*rna1* (allele *rna1-194*) sequences, respectively. To

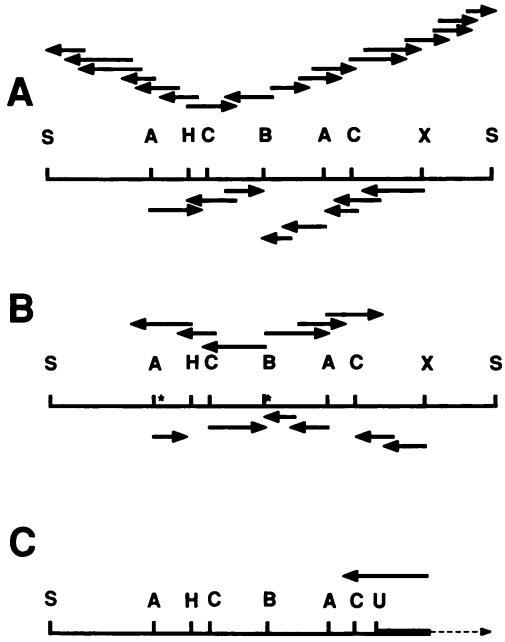

FIG. 1. Strategy for sequencing alleles of the *RNA1* gene. Shown are restriction maps of the *RNA1* alleles. Abbreviations: S, *Sst*I; A, *Asu*II; H, *Hin*dIII; C, *Hin*cII; B, *Bam*HI; X, *Xba*I, U, *Sau*3A. (A) Strategy for sequencing *RNA1*. Arrows at the top show the directions and extents of DNA sequence determination. The region between H and B was determined by sequencing the *Hin*dIII-*Bam*HI fragment. Sequences marked by arrows facing left were determined by using exonuclease III (22) to generate deletions of the *Sst*I-*Hin*dIII fragment; those marked by arrows facing right were determined for exonuclease III deletions of the *Bam*HI-*Sst*I fragment. The strategy for determining the sequence of the second DNA strand is depicted by the arrows below the restriction map. Where convenient, deletions were generated by using restriction sites. Other deletions were generated by using exonuclease III. (B) Strategy for sequencing *rna1-1*. Arrows at the top show the DNA sequence of *rna1-1* in M13mp10 that was determined by generating deletions, using convenient restriction sites or exonuclease III digestions. Arrows at the bottom depict sequences in M13mp11 that were determined by using convenient restriction sites, exonuclease III digests, or oligonucleotides homologous to the *RNA1* sequence. Asterisks mark the positions of amino acid changes in *rna1-1*. (C) Strategy for sequencing *rna1-Δ397-407*. The thick line segment represents pBR322 sequences.

construct these plasmids, the ~1.1-kbp *Sst*I-*Bam*HI fragments containing the 5′ ends of the *RNA1* and *rna1-1* genes were ligated into the respective restriction sites of pUC18. These plasmids, pUCR and pUCr, contain a reconstructed *Bam*HI site and an *Xba*I site in the polylinker region of the vector. *Bam*HI-*Xba*I fragments that contained the 3′ sequences from both wild-type and *rna1-1* genes were ligated into pUCR and pUCr.

To construct the yeast centromere-containing plasmids YCpRNA1, YCprna1-1, YCprna1-17, and YCprna1-194 plasmids pR35, pR6, pR3, and pR8, respectively, were digested with *Eco*RI and *Sal*I. This released ~2-kbp fragments that contained the entire *RNA1* or mutant *rna1* sequences. Each fragment was ligated into the centromere-containing vector YCp50 (35), which had been digested with *Eco*RI and *Sal*I.

To construct pRU35, pRU6, pRU3, and pRU8, the *rnh1*::*URA3* sequence was added to pR35, pR6, pR3, and pR8 by purifying the 1.4-kbp fragment generated by digesting pMY148 (gift of R. Crouch) with *Xba*I and *Sph*I. This fragment, containing the *rnh1*::*URA3* (M. Itaya and R. Crouch, manuscript in preparation) sequence, was ligated into plasmids pR35, pR6, pR3, and pR8, which had been digested with *Xba*I and *Sph*I. This yielded plasmids pRU35, pRU6, pRU3, and pRU8, respectively. The constructs were confirmed to contain the appropriate *RNA1* alleles by double-stranded DNA sequence analysis (23), using *RNA1*-specific oligonucleotide primers.

Plasmid pRU Δ397-407 contains the *rna1-Δ397-407* allele. The DNA fragment from YEprna1(2.3) bound by *Sst*I and *Sma*I sites was ligated into the *Sst*I-*Sma*I sites of pUC19. The DNA fragment from pMY148 encoding the *rnh1*::*URA3* sequence bound by *Xba*I and *Sph*I was added to this construct by using the *Xba*I and *Sph*I sites of the polylinker region of pUC19.

Plasmids pRUΔ359-397 and pRUΔ330-407 were constructed by using site-directed oligonucleotide mutagenesis (34, 65), followed by deletions of DNA bound by newly generated restriction sites. M13 DNA containing the 3′ *Bam*HI-*Sst*I fragment of the *RNA1* gene was subjected to two rounds of oligonucleotide mutagenesis. Three oligonucleotides were used; one would generate a *Bgl*II site, the second would generate a *Sal*I site, and the third would generate a *Kpn*I site. These were used in combinations to generate a *Bgl*II site at codon 395 and a *Sal*I site at codon 359 or a *Bgl*II site at codon 395 and a *Kpn*I site at codon 331. The fragment of DNA bound by *Bam*HI and *Xba*I of the mutant plasmids was used to replace the *Bam*HI-*Xba*I fragment of pRU35. This generated plasmids pRU359,395 and pRU331,395. The plasmids were digested with *Sal*I and *Bgl*II and with *Kpn*I and *Bgl*II, respectively. The overhanging nucleotides were removed by digestion with S1 nuclease, and the resulting linear fragments were blunt-end ligated. This generated plasmids pRUΔ359-397 and pRUΔ330-407, which were used for one-step gene replacements of *rna1-1* in yeast cells.

RESULTS

The *RNA1* gene encodes a 407-amino-acid protein with an acidic domain. Previously, we reported the isolation of genomic and cDNA sequences that encoded the *S. cerevisiae RNA1* locus (1). The 1.4-kbp region coding for the *RNA1* product was mapped by transposon and deletion mutagenesis and by comparing the restriction map of the functional cDNA with the restriction maps of genomic clones. It was concluded that the *RNA1* gene was contained within a 2.36-kbp genomic *Sst*I fragment. That fragment contains ~500 bp 5′ and ~500 bp 3′ to the *RNA1* coding region.

The DNA sequence of the entire 2.36-kbp *Sst*I fragment was determined by using the strategy outlined in Fig. 1A. The DNA sequence of the *Sst*I fragment is shown in Fig. 2. The sequence contains an uninterrupted 1,221-bp ORF that would encode a 407-amino-acid protein of 45,739 daltons. Most yeast genes are preceded by a consensus sequence, TATAAA, at −40 to ~−120 bp from the 5′ end of mRNAs; this sequence is necessary but not sufficient for transcription initiation (for a review, see reference 58). Although the *RNA1* ORF does not contain a perfect yeast consensus sequence, there are TATA-like sequences, TAATA, TATA, and TTTTAA, at positions −67, −45, and −36 (with respect to the first ATG). The first AUG in the vast majority of mammalian and viral mRNAs is preceded by a purine at −3 or followed by a purine at +4 or both (32, 33). Yeast initiator consensus regions have A at −1 and −3 and U at +4 (11). It

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GAGCTCCTTAGGGTTGCGCGTAGGCTTAAATTGGTATTGTGTTCTCGCTGCAATGGCAAGCCGTATGTCTT

CTACTCCTAATCCACTGCCGGCAGAATTTCCACTGCCAGCGTAGTCGTTGTAAACCAACGCGTCCTTAAG

GACTCCTTGTGTGTACCTATGTGCAAAATCCATCAATTGTAGCGTTACTTGGTCCTCATATTGATGAATC

GACTGTGATGCGAGGAGCAGATGCAAAAGACGGACATCTCGTGGCGTTTCCTCTTGTGTTGAGTCCGGAC

CAACTTCTGATACTGAGCCCACTGAGTTTTTATTCAAAACATTCTTGCCTCCGCCGTTCATAATGGTCTG

TGAAGTCCTCTAGTCTTTTCTTGTTGTCGTGTTGTTTCATAATGTCAAATTCTACTTTTGATTTAAAGCT

CTTAGTTTGGTCATGGAATGCATGTCACTATGCACGTTGTGGGACAATTAATGGAAAAAAATTTATTAGG

AAGATATTGATCAAAGGCATAATATCACGGTAAAAGGTGAATATAGGCCATTTTAACAGGCTGTCTTTCG

                  MET ALA THR LEU HIS PHE VAL PRO GLN HIS GLU GLU GLU GLN
AAACAGATTTTTAGCTATG GCT ACC TTG CAC TTC GTT CCT CAG CAC GAG GAA GAA CAA
        PHE
VAL TYR SER ILE SER GLY LYS ALA LEU LYS LEU THR THR SER ASP ASP ILE LYS
GTT TAC TCC ATC TCT GGG AAG GCA CTC AAG TTA ACA ACC AGT GAC GAT ATC AAA
        TTC
PRO TYR LEU GLU GLU LEU ALA ALA LEU LYS THR CYS THR LYS LEU ASP LEU SER
CCA TAC CTG GAA GAA TTG GCA GCT TTG AAA ACC TGT ACC AAA TTA GAC CTT TCA

GLY ASN THR ILE GLY THR GLU ALA SER GLU ALA LEU ALA LYS CYS ILE ALA GLU
GGG AAT ACA ATC GGT ACT GAA GCT TCG GAA GCA TTA GCT AAA TGC ATC GCT GAA

ASN THR GLN VAL ARG GLU SER LEU VAL GLU VAL ASN PHE ALA ASP LEU TYR THR
AAT ACA CAG GTC AGG GAA TCT TTG GTT GAA GTA AAT TTT GCT GAC TTA TAC ACT

SER ARG LEU VAL ASP GLU VAL VAL ASP SER LEU LYS PHE LEU LEU PRO VAL LEU
TCG AGG TTG GTT GAC GAA GTC GTT GAT TCG TTG AAG TTT TTA TTG CCT GTT CTG

LEU LYS CYS PRO HIS LEU GLU ILE VAL ASN LEU SER ASP ASN ALA PHE GLY LEU
TTG AAA TGT CCT CAC TTG GAG ATT GTG AAC CTT TCT GAT AAT GCG TTT GGG CTA

ARG THR ILE GLU LEU LEU GLU ASP TYR ILE ALA HIS ALA VAL ASN ILE LYS HIS
AGA ACA ATC GAG TTA CTA GAA GAT TAC ATT GCA CAT GCC GTG AAT ATC AAA CAT

LEU ILE LEU SER ASN ASN GLY MET GLY PRO PHE ALA GLY GLU ARG ILE GLY LYS
TTG ATC TTA AGT AAC AAT GGT ATG GGC CCT TTT GCT GGT GAA AGG ATT GGT AAG

ALA LEU PHE HIS LEU ALA GLN ASN LYS LYS ALA ALA SER LYS PRO PHE LEU GLU
GCC CTA TTT CAT CTC GCT CAA AAT AAG AAA GCT GCT TCC AAA CCA TTT TTG GAA
                                                              VAL
THR PHE ILE CYS GLY ARG ASN ARG LEU GLU ASN GLY SER ALA VAL TYR LEU ALA
ACT TTT ATC TGT GGT AGA AAT AGA TTA GAG AAT GGA TCC GCA GTC TAC TTA GCT
                                                              GTT
LEU GLY LEU LYS SER HIS SER GLU GLY LEU LYS VAL VAL LYS LEU TYR GLN ASN
CTG GGT TTG AAA AGC CAC TCC GAA GGT TTG AAA GTC GTA AAG CTG TAC CAA AAT
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FIG. 2. DNA and protein sequences of *RNA1* alleles. The DNA sequence of the *Sst*I fragment is shown. Regions sequenced in both directions are located between the arrows. Mutant sequences are boxed; amino acid replacements and DNA changes are shown above and below, respectively, the wild-type sequence.

appears that the consensus nucleotide can affect the efficiency of translation of some yeast genes (2) but not of others (12, 61). The *RNA1* mRNA has G at −3 and +4 and U at −1 and therefore resembles the mammalian consensus more than the yeast consensus. Six and 18 nucleotides beyond the UAG of the ORF there is the sequence AATAAA. This sequence is identical to the poly(A) recognition sequence found 18 to 32 nucleotides upstream of the site of poly(A) addition in mammalian mRNAs (17) and has been found in many but not all yeast genes (4). Another consensus sequence of the structure ... stop ... 1 to 140 bp ... TAG .../// ... TATGT or TAGT .../// ... A+T rich .../// ... TTT has been found 3′ to many yeast ORFs. Deletions of this sequence causes either faulty transcription termination (64) or faulty 3′ processing of pre-mRNA (7). A perfect match to this consensus is found for *RNA1* at +73 bp beyond the TAG of the ORF (TAG ... 39 ... TATGT ... 30 ... TTT). Thus, the *RNA1* gene has regulatory consensus sequences typical of those of yeast or other eucaryotic genes.

Highly expressed yeast genes utilize a biased codon preference, and the most frequently used codons are complementary to the most abundant isoaccepting species of tRNAs (5). The codon choice of genes that are not highly expressed is less biased (5, 53). The codon bias index for *RNA1* is 0.25, which places it among the yeast genes that are not highly expressed. This is consistent with the low mRNA abundance (1).

The predicted RNA1 protein contains a high proportion (32%) of the charged amino acids Asp, Glu, Lys, and Arg. The ratio of Asp + Glu/Lys + Arg is about 2, indicating that the protein is acidic. There is a region particularly rich in acidic residues at the C terminus. From amino acids 347 to 375, 22 of the 28 residues are either Glu or Asp, and no basic residues are within this region. The regions just preceding and just following this domain are also quite acidic (Fig. 3). Hydropathicity analysis (36) of the hypothetical RNA1 protein suggests that the protein lacks any region expected to span a membrane.

The PIR-NBRF, SWISS-Prot, and GenBank libraries were searched to find sequences similar to that of *RNA1*. The Bionet IFIND program was used to search both the DNA and protein sequence banks, and the FASTA-MAIL

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GLY ILE ARG PRO LYS GLY VAL ALA THR LEU ILE HIS TYR GLY LEU GLN TYR LEU
GGT ATT AGG CCT AAA GGT GTC GCC ACG CTA ATT CAT TAC GGT TTA CAG TAC TTG

LYS ASN LEU GLU ILE LEU ASP LEU GLN ASP ASN THR PHE THR LYS HIS ALA SER
AAA AAC TTG GAA ATC TTG GAT CTT CAA GAC AAT ACT TTC ACG AAA CAT GCT TCT

LEU ILE LEU ALA LYS ALA LEU PRO THR TRP LYS ASP SER LEU PHE GLU LEU ASN
TTG ATC CTT GCT AAG GCC TTG CCT ACA TGG AAG GAT AGT TTA TTT GAA TTG AAT

LEU ASN ASP CYS LEU LEU LYS THR ALA GLY SER ASP GLU VAL PHE LYS VAL PHE
TTG AAC GAC TGT CTT TTG AAA ACT GCT GGT TCA GAT GAA GTC TTT AAA GTA TTC

THR GLU VAL LYS PHE PRO ASN LEU HIS VAL LEU LYS PHE GLU TYR ASN GLU MET
ACC GAA GTT AAA TTC CCC AAT TTG CAT GTC TTG AAA TTC GAA TAT AAT GAA ATG

ALA GLN GLU THR ILE GLU VAL SER PHE LEU PRO ALA MET GLU LYS GLY ASN LEU
GCT CAA GAA ACC ATT GAA GTA TCC TTC TTA CCG GCT ATG GAA AAG GGA AAT TTA
                                        THR
PRO GLU LEU GLU LYS LEU GLU ILE ASN GLY ASN ARG LEU ASP GLU ASP SER ASP
CCT GAA TTG GAA AAG CTA GAA ATA AAT GGT AAC AGA TTA GAT GAA GAT TCT GAT
                                        ACC
ALA LEU ASP LEU LEU GLN SER LYS PHE ASP ASP LEU GLU VAL ASP ASP PHE GLU
GCT TTA GAT TTG CTC CAA AGC AAA TTT GAT GAT TTA GAG GTT GAC GAT TTT GAA
          ASP
GLU VAL ASP SER GLU ASP GLU GLU GLY GLU ASP GLU GLU ASP GLU ASP GLU ASP
GAG GTC GAT AGT GAA GAT GAG GAA GGC GAG GAC GAG GAA GAC GAG GAC GAG GAT
          GAC
GLU LYS LEU GLU GLU ILE GLU THR GLU ARG LEU GLU LYS GLU LEU LEU GLU VAL
GAA AAA CTC GAA GAA ATT GAA ACG GAA AGG CTT GAA AAG GAA CTG CTA GAA GTA
          GLU     PRO GLN ASP GLY CYS GLY ARG HIS GLU ARG VAL VAL ASP SER
GLN VAL ASP ASP LEU ALA GLU ARG LEU ALA GLU THR GLU ILE LYS ***
CAA GTA GAT GAT CTT GCT GAA CGT TTA GCT GAA ACT GAA ATC AAA TAGCTATGAAT
          GAA     CCA CAG GAC GGG TGT GGT CGC CAT GAT CGC GTA GTC GAT AGT
GLY SER LYS ***
AAAAGTAAAAATAAACACAATCAACTGTGGACGAGGTTCAGGCAAATATCATGGTGACACGCTTATAGATA
GGC TCC AAG TAG

GTTATCGGGTAAGTTAAAAAACCAAATAGCTGATGTATGTATATGTATGCAAAAGATATAAAATATATATT

TCTATTACAGGTACAACAGGTCCAGTAAGAAGCCAAGCAAAAAACAGCATTATCGTCTAGATGCTCCTTTC

TTCGCCAGAAAATCTGCCATTTCATTTCCTGGATCACCATCGTGTCCTTTTACCCATTCAATCTGAAATTT

ACCATTATTTTTGAAGCACTCTTTATTCAACTCATAGTACTTTTTAACTTTAACGAACCTCTGCACCAAAG

GAACAATTAAATCAGAATTAGGTAGCCCTTCAAGTTTTTTATTATCATACGTCATATACCTGTCATTTAAT

AATTTGGTTACGTACTCTGAATCAGTTTTGATTTGATAATTTACTTTTTCCTTTTCATTAGTTAACTTTTC

CCAGATTTTCTTCAAAGCTTCTGATACTGCTTCGATTTCAGCTCTATTATTAGTTTGAGCTC
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Fig. 2—*Continued.*

program (47) was used to search protein libraries. In addition, by using the Genepro 4.10 program (Riverside Scientific Enterprises, Seattle, Wash.), the entire GenBank DNA sequence library was translated in all six frames, and the deduced ORFs were searched for similarity to *RNA1*. These searches identified many genes with similarities to *RNA1*, but in most cases because of a similarity of the acidic C-terminal domain of *RNA1* to acidic regions of the identi-

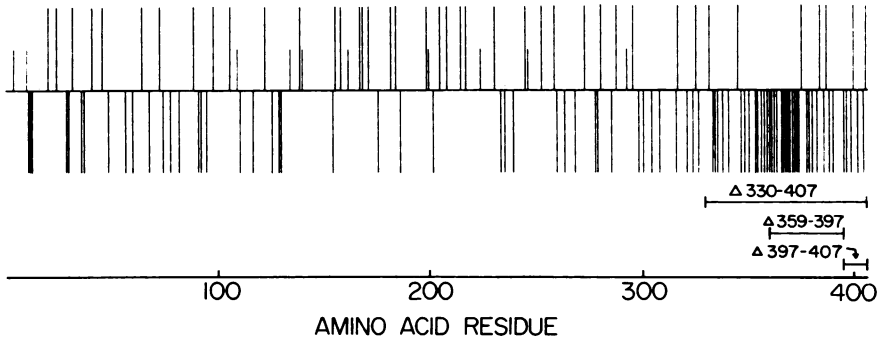

FIG. 3. Charge profile of the RNA1 ORF. Vertical lines below the horizontal line represent the acidic residues Asp and Glu. Long vertical lines above the horizontal line represent the basic residues Arg and Lys; shorter vertical lines represent His. Numbers along the horizontal line indicate amino acid sequence positions. The bracketed regions indicate the amino acids that have been deleted in *rna1-Δ397-407*, *rna1-Δ359-397*, and *rna1Δ-330-407*.

fied proteins. A few weak (<20%) similarities to DNA-binding and regulatory proteins, such as DNA-directed RNA polymerase II, yeast RAD3, GAL4, or adenylate cyclase were found, but in no case was the similarity to a region of known function. The RNA1 protein sequence was also searched for consensus regions of functional significance. The sequence lacks consensus sequences that are found in RNA-binding proteins (for a review, see reference 10), EF hand positions of Ca^{2+}-binding proteins (3; see Discussion), protein kinases (for a review, see reference 25), or zinc fingers (for a review, see reference 30). There is no potential acetyltransferase recognition signal (48). There are two potential sites (Asn X Ser) for N-linked glycosylation and two potential sites for phosphorylation by caseinlike kinases (reviewed in reference 16), but it is not known whether these sequences are used for such purposes. Thus, we have failed to gain information regarding the function of the RNA1 protein by searching for similarities to genes of known function.

The mutant *rna1-1* sequence differs at two amino acids from the wild-type sequence, and both amino acid substitutions are necessary for the temperature-sensitive growth phenotype. DNA sequence analysis revealed that the *rna1-1* allele contains two single-base-pair substitutions, resulting in two predicted amino acid changes from the wild-type sequence at amino acids 17 (Ser→Phe) and 194 (Ala→Val).

We determined the phenotypes of the two missense mutations of *rna1-1* individually by generating hybrid constructs of *RNA1* and *rna1-1* sequences. One nucleotide substitution was 513 bp upstream from the unique *Bam*HI site, and the other mutation was 14 bp downstream from this site. Cleavage at this *Bam*HI site allowed for the convenient splicing of mutant and wild-type sequences. By using pUC vectors as intermediates, plasmids possessing all possible combinations were generated from the wild-type and mutant sequences [pR35(++), pR6(−−), pR3(−+), and pR8(+−); see Materials and Methods and Fig. 4]. The DNA sequences of all four constructs were determined to confirm that each contained the appropriate *RNA1* allele.

Initially, each of the four constructs were placed on a yeast centromere vector, YCp50, to test for the ability to complement the thermosensitive growth defect of *rna1-1* cells. YCpRNA1 harbors the wild-type gene, and YCprna1-1 harbors the *rna1-1* allele. YCprna1-17 contains the allele with the Ser→Phe substitution at position 17, and YCprna1-194 contains the allele with the Ala→Val substitution at position 194. Each of these plasmids was transformed into the haploid yeast strain EE1b (relevant genotype, *rna1-1 ura3-52*). Uracil prototrophs were selected and tested for the ability to grow at 34 or 37°C. The results of this experiment indicated that on a low-copy plasmid, alleles that contained only one of the point mutations (i.e., YCprna1-17 and YCprna1-194) were capable of complementing the *rna1-1* thermosensitive growth defect.

We found it unusual that neither of the single mutations alone caused a temperature-sensitive phenotype. Since the above data were derived for plasmid-encoded mutant sequences, it was possible that these sequences were regulated inappropriately and that complementation of the *rna1-1* phenotype was due to overexpression of the single mutant alleles. This was considered to be feasible, since previously we had shown that when the *rna1-1* allele was carried on the multicopy vector YEp24, recipient *rna1-1* cells were able to grow at elevated temperatures (1). Therefore, the endogenous *rna1-1* allele was substituted with the single mutations of this locus by using one-step gene replacement (51).

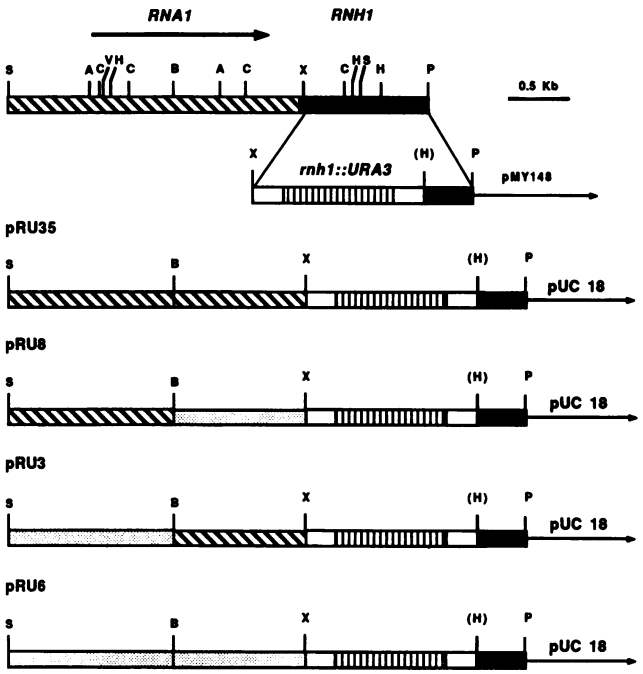

FIG. 4. Strategy for constructing pRU35, pRU6, pRU3, and pRU8. The constructions used plasmids pU35, pU6, pU3, and pU8, containing, respectively, the *Sst*I-*Xba*I fragment encoding *RNA1* (▨) or *rna1-1* (▥) or hybrid sequences consisting of the 5′ *Sst*I-*Bam*HI *rna1-1* fragment and the 3′ *Bam*HI-*Xba*I *RNA1* fragment (▥▨) or vice versa (▨▥) in pUC18. An *Xba*I-*Sph*I fragment containing the *rnh1*::*URA3* allele from pMY148 was added to each of the plasmids at the yeast *Xba*I and pUC18 *Sph*I site of the polylinker. This generated pRU35, pRU6, pRU3, and pRU8, respectively. ■, *RNH1* sequences; ▥, *URA3* sequences; □, yeast vector sequences.

Fortuitously, an unessential gene, *RNH1*, is located close to *RNA1*. The nonlethal disruption of this gene, *rnh1*::*URA3* (Itaya and Crouch, in preparation; gift of R. Crouch) was used as a tag to enable us to detect gene replacements at the *RNA1* locus. The sequence encoding *rnh1*::*URA3* was added to the four pUC constructs containing the different *RNA1* alleles, generating pRU3 (containing allele *rna1-17*), pRU8 (allele *rna1-194*), pRU6 (allele *rna1-1*), and pRU35 (allele *RNA1*) (see Materials and Methods and Fig. 4). Each of these plasmids has homologies to yeast DNA flanking the *RNA1* locus at regions upstream of the *RNA1* ORF and downstream of the *URA3* insert. All four plasmid DNAs were digested to completion with *Sst*I, a restriction site that borders the yeast-bacterial sequences of these plasmids. The digestions resulted in a linear DNA end that was used to target the new alleles to the *RNA1* locus of the yeast genome by homologous recombination (51). Yeast strains 2b × 3b (relevant genotype, *rna1-1*/*rna1-1 ura3-52*/*ura3-52*), EE1b (relevant genotype, *rna1 ura3-52*), and SJ17 (relevant genotype, *RNA1 ura3-52*) were transformed, and uracil-prototrophic transformants were selected. Several transformants for each replacement were tested for growth at nonpermissive temperatures. Consistent with the results obtained for the centromere-containing plasmids described above, EE1b and 2b × 3b transformants containing all chromosomal alleles except *rna1-1*, harboring two amino acid substitutions, were able to grow at nonpermissive temperatures (Table 1). The same results were obtained for

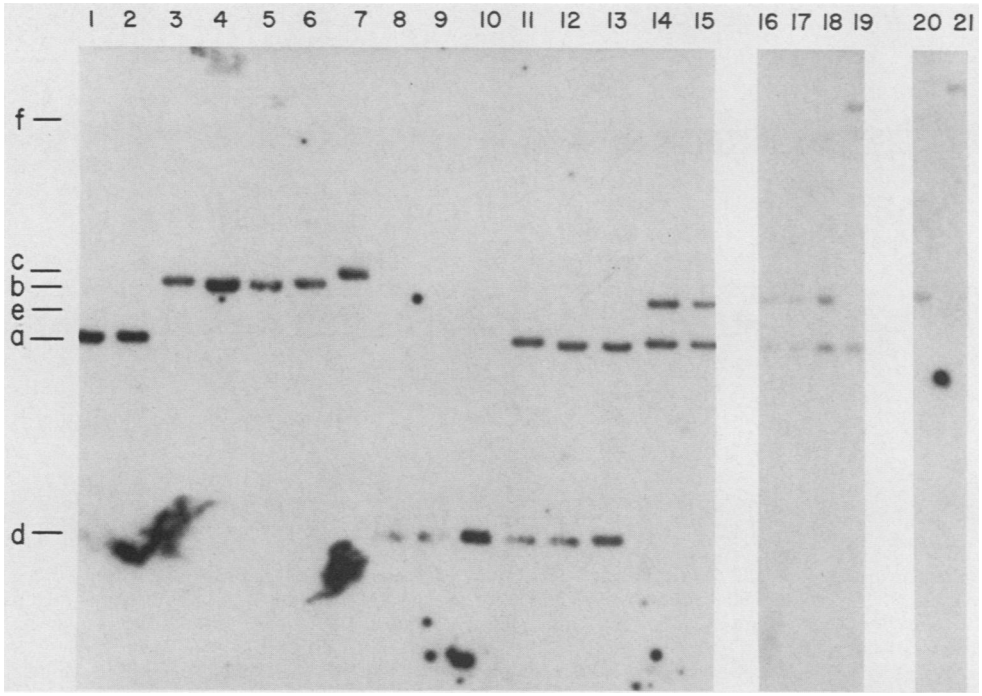

FIG. 5. Southern analysis of yeast constructs. Genomic DNA was extracted from yeast cells, digested with *Bgl*II, resolved on 1% agarose gels, and transferred to GeneScreen Plus membranes. A 5′ ^{32}P-end-labeled 18-mer oligonucleotide homologous to *RNA1* sequences was used as a probe for *RNA1* sequences. Lanes contained yeast strains and *RNA1* alleles as follows: 1, EE1b; 2, 2b × 3b; lane 3, EE1b replaced with *rna1-1*; 4, EE1b replaced with *rnal-194*; 5, EE1b replaced with *rna1-17*; 6, EE1b replaced with *RNA1*; 7, EE1b replaced with *rna1-Δ397-407*; 8 and 9, EE1b replaced with *rna1-331,395*; 10, EE1b replaced with *rna1-359,395*; 11 and 12, 2b × 3b replaced with *rna1-331,395*; 13, 2b × 3b replaced with *rna1-359,395*; 14 and 15, 2b × 3b replaced with *rna1-Δ330-407*; 16 to 18, 2b × 3b replaced with *rna1-Δ359-397*; 19, 2b × 3b temperature-resistant transformant carrying amplified *rna1-Δ359-397* sequences; 20, EE1b replaced with *rna1-Δ359-397*; 21, EE1b carrying amplified *rna1*-Δ359-397. Correct constructions were assessed by calculating the expected effects of the nucleotide additions or deletions on the size of the *Bgl*II fragment containing the *rna1-1* allele (line a). The fragment containing replacements in lanes 3 to 6 (line b) and 7 (line c) are larger than the fragment designated by line a because exogenous *URA3* (line b) or *URA3* and tetracycline resistance gene (line c) sequences were introduced into the *RNA1* locus. The fragments homologous to *RNA1* in lanes 8 to 13 (line d) are smaller than those for lanes 3 to 6 because the introduced sequences contain a *Bgl*II restriction site. The fragments indicated by line e are larger than that indicated by line d and smaller than that indicated by line b because deletions have removed sequences including the *Bgl*II site. Line f indicates fragments that apparently contain multiple *rna1* alleles.

strain SJ17; that is, transformants carrying *RNA1*, *rna1-17*, and *rna1-194* alleles were able to grow at 34 and 37°C, whereas *rna1-1* transformants lost the ability to grow at these temperatures. Therefore, neither single-amino-acid change alone resulted in temperature-sensitive growth.

TABLE 1. Phenotypes of *RNA1* alleles[a]

RNA1 allele	Predicted amino acid alterations	Ability to grow on YEPD at:		
		23°C	34°C	37°C
RNA1	Wild type	+	+	+
rna1-1	aa 17, Ser→Phe; aa 194, Ala→Val	+	–	–
rna1-17	aa 17, Ser→Phe	+	+	+
rna1-194	aa 194, Ala→Val	+	+	+
rna1-Δ397-407	Deletion of aa 397–407	+	+	+
rna1-359,395	Synonymous codon change at 359; aa 395, Asp→Glu	+	+	+
rna1-331,395	aa 331, Asn→Thr; aa 395, Asp→Glu	+	+	+
rna1-Δ359-397	Deletion of aa 359–397	+	–	–
rna1-Δ330-407	Deletion of aa 330–407	NV		

[a] The yeast strains were generated by replacing endogenous *rna1-1* sequences in strain EE1b as described in Materials and Methods. aa, Amino acid; NV, haploids bearing this deletion are not viable.

A Southern analysis (56) was performed to verify that the *RNA1* locus carried the new *rna1* alleles. Genomic DNA from each of the candidates was digested with *Bgl*II. The DNA was analyzed by using a 5′-end-labeled oligonucleotide probe homologous to *RNA1*. The results of the analysis confirmed that all four correct gene replacements had been obtained (Fig. 5, lanes 3 to 6).

An 11-amino-acid deletion-substitution of the C terminus of the RNA1 protein has no apparent growth defect. YEprnal(2.3) contains ~1.8 kbp of yeast sequences and is the smallest subclone of the original 7.7-kbp clone able to complement the temperature-sensitive phenotype of *rna1-1*. This subclone was generated by ligating partial *Sau*3A digests of YEpRNA1(7.7) into the *Bam*HI site of Yep24 (1). Although this subclone generates an mRNA slightly larger than the wild-type *RNA1*, it lacks ~200 bp at the 3′ end that is present in a functional cDNA encoding *RNA1* mRNA (1). Inspection of the DNA sequence of the wild-type *RNA1* gene indicated that there are *Sau*3A sites within the coding region of the *RNA1* sequence (ORF) that could potentially yield 3′ deletions when subjected to partial digestions.

The strategy for sequencing the *RNA1* allele of YEprna1(2.3) is shown in Fig. 1C. This allele was shown to lack 11 3′-terminal codons of the *RNA1* ORF and to contain 16 substituted codons from the antisense direction of the tetra-

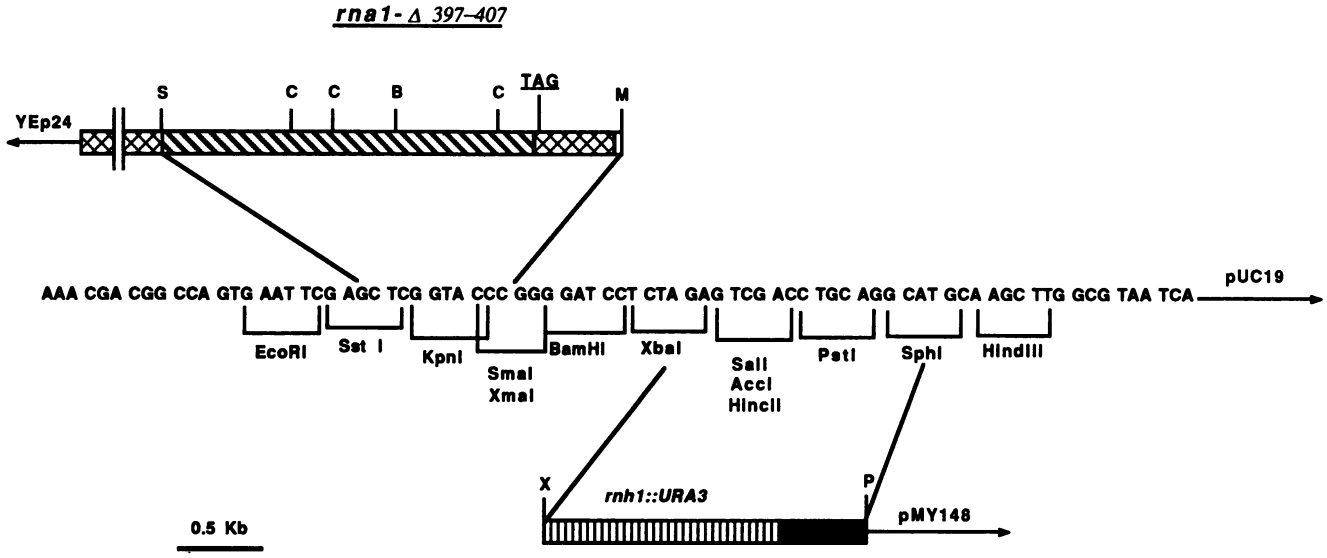

FIG. 6. Strategy for constructing pRUΔ397-407. The *Sst*I-*Sma*I fragment from YEprna1(2.3) was inserted into the *Sst*I-*Sma*I sites of the polylinker region of pUC19. The resulting plasmid was digested with *Xba*I and *Sph*I, and the *Xba*I-*Sph*I fragment from pMY148 containing the *rnh1*::*URA3* sequence was ligated into the respective sites. Symbols and abbreviations: ▨, *RNA1* sequences; ▩, tetracycline resistance gene sequences from YEp24; □, YEp24 vector sequences; ▥, *URA3* sequences; ■, *RNH1* sequences; S, *Sst*I; C, *Hin*cII; B, *Bam*HI; M, *Sma*I; X, *Xba*I; P, *Sph*I. The DNA sequence of the polylinker region of pUC19 is shown in the middle of the figure.

cycline resistance gene of pBR322 (Fig. 2). These results suggested that, at least in high copy, the carboxyl-terminal 11 amino acids are unessential for function. The mRNA encoded by *rna1-Δ397-407* is larger than the mRNA for the wild-type allele (unpublished data). This can be explained by assuming that a new fortuitous termination site within the reverse orientation of the tetracycline resistance gene has replaced the deleted *RNA1* transcription termination or 3′-end processing sites (or both).

To test whether this deletion-substitution functions as a wild-type allele in single copy, a gene replacement was done. Plasmid pRUΔ397-407, containing the allele *rna1-Δ397-407* and the *rnh1*::*URA3* gene, was generated by using a strategy similar to that used to generate the hybrid *RNA1*/*rna1-1* plasmids (Materials and Methods and Fig. 6). The construct was digested with *Sst*I and subsequently transformed into yeast strain EE1b. Uracil prototrophs were assessed for growth at 34 and 37°C. All transformants were able to grow at these nonpermissive temperatures, indicating that in single copy, *rna1-Δ397-407* is indistinguishable from the wild-type *RNA1* allele for growth (Table 1). Southern analysis of genomic DNA extracted from candidates verified that transformants had the *rna1-1* allele replaced with the *rna1-Δ397-407* allele (Fig. 5, lane 7).

Deletion of the C-terminal acidic region causes growth defects. The presence of an acidic domain on the *RNA1* ORF led us to question whether it might perform a functional role. There are other yeast genes possessing such C-terminal acidic regions (13–15, 19, 49). Our strategy to probe the role of the acidic domain in the RNA1 protein was to generate *RNA1* alleles possessing in-frame deletions removing parts of this domain. Since there are no restriction sites in the wild-type *RNA1* gene that would allow for construction of such deletions, we used oligonucleotide-directed mutagenesis (65) to generate appropriate restriction sites. One oligonucleotide generated a *Kpn*I site that was expected to result in a substitution at position 331 of Thr for Asn. The second oligonucleotide generated a *Sal*I site that would change a codon at position 359 without generating an amino acid alteration. The third oligonucleotide generated a *Bgl*II site, resulting in a nucleotide substitution at codon 395 that would generate an Asp→Glu substitution at this position. The mutagenesis was performed such that one construct contained the *Bgl*II and *Sal*I sites and the other contained the *Bgl*II and *Kpn*I sites (see Materials and Methods). These two constructs, pR359,395 and pR331,395, were used to substitute for wild-type sequences in *rnh1*::*URA3*-containing plasmid pRU35 to generate plasmids pRU359,395 and pRU331,395. The plasmids were linearized with *Sst*I, and the linear DNAs were used for one-step gene replacements by transformation into haploid (strain EE1b) and diploid (strain 2b × 3b) *rna1-1* strains. In both cases, the altered alleles rescued the thermosensitive phenotype of *rna1-1* and generated temperature-resistant haploid and diploid transformations (Table 1). The gene replacements were confirmed by Southern analysis (Fig. 5, lanes 8 to 13).

Plasmids pRU359,395 and pRU331,395 were used to generate deletions (see Materials and Methods) that were predicted to contain the in-frame removal of amino acids 359 to 397 and 331 to 397, respectively. DNA sequence analysis confirmed the appropriate deletion for pRUΔ359-397 (data not shown). However, one additional base was removed by S1 nuclease from the *Kpn*I site for the putative pRUΔ331-397 construct. This generated a frameshift at position 330 causing four amino acid substitutions followed by a stop codon (data not shown); hence, we have named this construct pRUΔ330-407. The deletion constructs were used for one-step gene replacements by linearizing the DNAs with *Sst*I and transforming haploid and diploid *rna1-1* strains EE1b and 2b × 3b with the linear DNA.

Both haploid and diploid transformants were obtained for cells that received the *rna1-Δ359-397* construct, and most of the transformants were unable to grow at 34 or 37°C (Table 1). Southern analysis (Fig. 5, lanes 16 to 21) showed that the appropriate gene replacements occurred only in those transformants that were temperature sensitive. The exceptional temperature-resistant transformants showed unexpected DNA restriction patterns (lanes 19 and 21). These patterns

were consistent with the hypothesis that multiple insertions of *rna1-Δ359-397* can generate temperature resistance (data not shown). Diploid transformants with the correct replacements were analyzed by tetrad analysis. Two large Ura^- and two small Ura^+ segregants were obtained for all asci with four viable spores (nearly 100% viability), and all segregants were unable to grow at 34 or 37°C. Thus, it appears that amino acids 359 to 397 of *RNA1* are not required for cell viability but that deletion of these amino acids results in slow growth at 23°C and an inability to grow at elevated temperatures. This conclusion was confirmed when the *rna1-Δ359-397* allele was used to replace the wild-type *RNA1* allele; temperature-sensitive derivatives were obtained. RNA from strains containing the *RNA1*, *rna1-1*, or *rna1-Δ359-397* allele was radioactively labeled with [^{3}H]uracil at 23 and 35.5°C, and the effects of these alleles on rRNA synthesis and pre-tRNA splicing were determined (24). As expected, the *rna1-1* strain at 35.5°C showed marked defects in 25S and 18S rRNA production and accumulated pre-tRNAs. Surprisingly, neither of these defects was detected for the *rna1-Δ359-397* strains (data not shown). These studies, as well as studies to determine the effects of all new *rna1* alleles on rRNA, tRNA, and mRNA production, will be described elsewhere.

Only one haploid and several diploid transformants were obtained for pRUΔ330-407 transformation. None of the transformants were able to grow at elevated temperatures. The Ura^+ temperature-sensitive haploid colony did not have a gene replacement of the *RNA1* locus (not shown). Three diploid candidates that possessed the correct replacement as demonstrated by Southern analysis (two candidates are shown in Fig. 5, lanes 14 and 15) were sporulated, and the haploid spores were analyzed by tetrad analysis. The diploids segregated two viable to two nonviable progeny, and no Uracil-prototrophic progeny were obtained. Therefore, deletion of amino acids 330 to 407 of the *RNA1* gene results in lethality (Table 1).

DISCUSSION

The *RNA1* gene is essential for production of all classes of RNA and apparently for transport of RNA from the nucleus to the cytosol. There are a number of models that might account for the biochemical role of the *RNA1* gene product in yeast cells. These include (i) a protein or modification activity common to disparate RNA processing systems, (ii) a product or signal involved in chaperoning RNAs to appropriate subnuclear locations where precursor processing occurs, (iii) a regulatory product affecting several RNA processing activities, and (iv) a structural component of the nuclear membrane or a protein affecting function of the nuclear membrane. For the last model, one must also propose that the nuclear membrane plays a secondary role in the processing steps that may not occur at the nuclear membrane.

We have attempted to distinguish among such models by scanning the sequence of RNA1 for similarities to other known proteins. The DNA sequence of *RNA1* provided data that render some of the models less likely but failed to provide information necessary to assign a definitive biochemical role to this gene product. If model i or ii were correct, one would expect that the RNA1 protein would be localized to the nucleus and perhaps possess a consensus sequence common to RNA-binding proteins (reviewed in references 10 and 30). Neither of these predictions has been met (Hopper et al., in preparation; this report). If model iii were correct, one might expect to find consensus sequences typical of other regulatory proteins such as protein kinases (reviewed in reference 25) or Ca^{2+}-binding proteins (3). However, no such sequences were identified. We can eliminate the possibility that *RNA1* codes for typical protein kinases and Ca^{2+}-binding proteins, but we cannot eliminate the possibility that *RNA1* plays a regulatory role in RNA processing. The RNA1 protein lacks a region expected to span a membrane. Therefore, if RNA1 plays a role in nuclear integrity or nuclear membrane function (model iv), it either is a peripheral nuclear protein or modifies a component of the nuclear membrane.

We have assessed the effects of nucleotide substitutions of *RNA1* on viability. The original *rna1-1* allele contains two replacements, Ser→Phe at position 17 and Ala→Val at position 194. By in vitro manipulation, we have obtained alleles bearing the single Ser→Phe (allele *rna1-17*) and Ala→Val (allele *rna1-194*) amino acid replacements, and by in vitro mutagenesis we have created the alleles *rna1-359,395*, which contains a Glu→Asp change at position 395, and *rna1-331,395*, which bears two replacements, Asn→Thr at residue 331 and Glu→Asp at residue 395. None of these newly created alleles results in conditional or nonconditional lethality or markedly affects growth rate. The wild-type growth phenotype is somewhat surprising, since at least the replacement at position 17 is nonconservative. What is even more surprising is the finding that neither *rna1-17* nor *rna1-194* cells are thermosensitive, but *rna1-1* cells containing both replacements are unable to grow at temperatures above 30°C. Since single amino acid changes at a variety of residues do not affect viability, this result may help account for the fact that only a single conditional lethal *rna1* strain was found in conventional mutant hunts.

The most unusual feature of the *RNA1* ORF is the carboxy-terminal acidic domain. Our immunological studies have shown that the RNA1 protein is not proteolytically cleaved at the C terminus (Hopper et al., in preparation) and that therefore the acidic domain is part of the mature *RNA1* protein project. There are several reports of other yeast proteins that contain C-terminal acidic domains. The C-terminal amino acids of the RAD6 ORF contain 13 consecutive Asp residues (49), the 355-amino-acid ARGRIII ORF contains 17 consecutive Asp residues beginning at position 285 (15), and the CDC34 ORF contains an acidic carboxy-terminal region also composed primarily of Asp (19). There is also an acidic domain spanning amino acids 501 to 611 of the 729-amino-acid KEX1 ORF, and this domain consists primarily of long runs of Asp and a short run of Glu (13). The RNA1 ORF differs from the C-terminal acidic domains of these gene products in that it contains a mixture of both Asp and Glu. There are reports of two other acidic domains that also consist of a mixture of Asp and Glu. The product of *FUN80* (*MCM1*), an essential gene affecting minichromosome maintenance, contains a stretch of 19 Asp and Glu residues beginning at position 118 of the 157-amino-acid ORF (14). The product of *FUN81*, an essential gene of unknown function, contains a Glu and Asp acidic region also starting near the last one-third of the ORF (14). Most curious, *RNA1*, *FUN80* (*MCM1*), and *FUN81* are all essential genes, their products all contain a Glu and Asp acidic C-terminal domain, and they are all located on chromosome XIII.

We have attempted to determine whether the acidic domain is essential for *RNA1* function. Our strategy was to determine the consequences of genomic deletions of 3′ regions of the *RNA1* gene. The *rna1-Δ397-407* allele contains

a deletion of the C-terminal 11 amino acids that contain three acidic and two basic residues. This allele has a substituted 17-amino-acid region from pBR322 sequences that contains three acidic and four basic residues (Fig. 2). In addition, *rna1-Δ397-407* does not contain the yeast sequences that are normally used to generate the 3′ mRNA terminus. Nevertheless, cells bearing a genomic copy of *rna1-Δ397-407* have a phenotype that is indistinguishable from that of wild-type cells.

Increasing the size of the deletion to include amino acid residues 330 to 407 results in lethality. The region between 330 and 397 contains 36 acidic and 5 basic residues (Fig. 3). However, this region also contains hydrophobic amino acids (Fig. 2). It is not known whether lethality results from the removal of the entire acidic domain or from perturbing the *RNA1* product in a different manner. Cells bearing allele *rna1-Δ359-397* have an in-frame deletion of 39 amino acids. Twenty-four of these amino acids are Glu or Asp, and only three basic residues are in this region. Removal of this part of the acidic domain impairs growth at 23°C and causes an inability to grow at elevated temperatures. Thus, it would appear that this part of the acidic domain is necessary for appropriate regulation, product stability, or protein activity. We will attempt to distinguish among these possibilities. In any case, it is interesting that one can mimic the temperature-sensitive phenotype of point mutations with deletions within an essential gene.

rna1-Δ359-397 now defines a second temperature-sensitive mutation of the *RNA1* gene. Preliminary studies of this lesion indicate that it is possible to uncouple defects in rRNA synthesis and pre-tRNA splicing from thermosensitive growth. Our results are similar to those obtained for *RAD6*. The *RAD6* gene product is required for repair of UV-damaged DNA and has been shown to be a ubiquitin-conjugating (E2) enzyme (29). Removal of a 23-amino-acid highly acidic carboxy-terminal domain of *RAD6* affects ubiquitin-conjugating activity and sporulation but does not affect DNA repair (60). Sung et al. (60) have suggested that the deletion could allow for residual activity necessary for ubiquitination of UV repair products but not be sufficient for modification of products involved in sporulation. Similarly, one could hypothesize that *RNA1* codes for a regulatory or modification activity that is required for RNA processing as well as some other essential product(s) and that the *rna1-Δ359-397* lesion affects modification of the essential product(s) without affecting rRNA synthesis or pre-tRNA splicing. Such a model accommodates the pleiotropy of *rna1-1* alleles and the cytoplasmic location of the RNA1 protein (Hopper et al., in preparation).

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