Replacement of the *Saccharomyces cerevisiae* RPR1 gene with heterologous RNase P RNA genes

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Received October 6, 1993; Revised and Accepted December 8, 1993

**ABSTRACT**

Phylogenetic studies of yeast nuclear RNase P RNA genes have shown a striking conservation of secondary structure for the *Saccharomyces* and *Schizosaccharomyces* RNase P RNAs, yet much of the primary sequence and many substructures vary among the RNAs examined. To investigate which sequences and structural features can be varied and still allow function in a heterologous organism, RNase P genes from several yeast species were tested for the ability to substitute for the *Saccharomyces cerevisiae* RNA. The RNase P genes from *Saccharomyces carlsbergensis* and *Schizosaccharomyces kluveri* could act as the sole source of RNase P RNA within *S. cerevisiae* cells, whereas the genes from *Saccharomyces globosus* and *Schizosaccharomyces pombe* could not. Although heterologous RNase P RNAs were synthesized by the cells in all cases, the RNAs that complemented tended to be processed from longer precursor transcripts into mature-sized RNase P RNA, while the RNAs that did not complement tended to accumulate as the longer precursor form. The results identified sequences and structures in the RNA that are not essential for interaction with species-specific proteins, processing or localization, and suggested other positions that may be candidates for such processes.

**INTRODUCTION**

RNase P is a ribonucleoprotein enzyme that cleaves precursor tRNA molecules to generate mature 5' termini (reviewed in ref. 1). RNase P activity has been identified from numerous prokaryotic and eukaryotic sources including eubacteria, archaeabacteria, fungi, vertebrates and mitochondria (2–11). In eubacteria, both the protein and RNA components are required for function in vivo, but the RNA moiety has been shown to retain catalytic activity *in vitro* in the absence of the protein subunit (12). Conversely, the RNAs from other sources have not been shown to exhibit catalytic function by themselves. The relative roles of the eukaryotic RNA and protein components in substrate binding and catalysis remain unclear.

The nature of the interaction between RNase P RNA and its tRNA substrates has been studied in the eubacterial enzyme (reviewed in ref. 13). The combination of tRNAs' divergent primary sequence, conserved tertiary structure, and lack of sequence complementarity with RNase P RNAs suggests that substrate binding occurs through recognition of higher order pre-tRNA structure by a complex RNase P RNA structure. Phylogenetic comparative analysis has been used to investigate RNase P secondary structures from various sources. Such analyses performed in eubacteria resulted in the discovery of a conserved common RNA core structure (14,15).

Recently, a phylogenetic analysis of yeast nuclear RNase P RNA genes resulted in an eukaryotic secondary structure model (16). We had previously identified the RNA component of *S. cerevisiae* nuclear RNase P (7) and characterized its gene, RPR1 (17). RPR1 was shown to be an essential single-copy gene and to be transcribed by RNA polymerase III *in vivo* (18). Comparative sequence analysis of nuclear RNase P RNAs from six *Saccharomyces* species revealed a consensus RNA secondary structure which also fit the RNAs from more distant *Schizosaccharomyces* species (16,19). Comparison of this consensus core to the conserved eubacterial structure and to vertebrate RNase P RNAs from HeLa cells (8) and Xenopus *laevis* (9) revealed a number of secondary structural similarities, suggesting that eukaryotic RNase P RNAs from evolutionarily divergent sources share a core of conserved structural features, many of which have counterparts in eubacterial RNase P RNA (16).

The RNase P RNA from *S. cerevisiae*, as from other non-eubacterial sources, has not yet been shown to be catalytic in the absence of protein, and our attempts to reconstitute the RNA and protein component(s) *in vitro* have also been unsuccessful. Sedimentation studies suggest that protein makes up a significant proportion of the eukaryotic enzymes (20), in agreement with the recent finding that the protein subunit from the yeast mitochondrial enzyme is significantly larger than that found in eubacterial RNase P (21). The protein complement of the eukaryotic nuclear enzymes has not yet been determined, although RNA footprinting studies suggest it covers a much larger proportion of the RNA subunit than is the case in the bacterial holoenzymes (A. Tranguch, submitted). To augment the phylogenetic analysis with information regarding which altered sequences and structures in heterologous RNAs do or do not allow function in *S. cerevisiae*, we tested the ability of RNase P RNA

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from both closely and distantly related yeast species to substitute for the *S. cerevisiae* RNA *in vivo*. Results obtained from these complementation experiments reveal possible positions in the RNA that are not essential for protein interaction, processing or localization, as well as positions that might be essential for these functions.

**MATERIALS AND METHODS**

**Strains and genetic methods**

Plasmids were constructed and maintained in *Escherichia coli* strain DH5αF' using standard techniques (22). *S. cerevisiae* strain W3031A (MATa ade2-1 his3-11,15 leu2-3,112 trpl-1 1 ura3-1 canl-100, gift of R. Rothstein), was used to construct the strain JLY1 which contains an *rpr1::HIS3* disruption (17). Since the *RPR1* gene has been shown to be essential (17), the haploid strain must be kept viable by a plasmid-borne *RPR1* gene.

Isolation of plasmid DNA from DH5αF' strain was performed using standard techniques (22). Bacterial and yeast transformations were performed by electroporation as described (23,24), using a Bio Rad Gene Pulser and Pulse Controller.

Yeast cells were grown in two different selection media: SD-ura-his-trp (2% dextrose, 0.67% yeast nitrogen bases, amino acids supplements lacking uracil, histidine and tryptophan) for selection of plasmids with *URA3* and *TRP1* markers; or SD-his-trp+5-FOA (same as above but containing uracil and 1g/liter 5-fluoorotic acid) to promote the loss of the *URA3*-marked YCp50-*RPR1* plasmid ('curing') and retention of the *TRP1*-marked plasmid. All media lacked histidine to prevent the loss of the *HIS3* gene that disrupts the chromosomal *RPR1* gene. Cells were incubated at 30°C unless otherwise specified.

**Recombinant plasmids**

YCp50-*RPR1*, a single copy plasmid containing a wild-type copy of the *RPR1* gene was constructed from a 1.3 kilobase (kb) *BglII*—*HindIII* fragment of *RPR1* cloned in the *BamHI*—*HindIII* polynucleotides sites of pUC19 as described previously (17).

The high copy (2μ) plasmid pRS424-5'-3' *RPR* was used as an expression vector for heterologous RNase P RNA cDNAs (Fig. 1A) and was constructed as follows. 408-base pairs (bp) of *RPR1* 5' flanking sequences and 550-bp of *RPR1* 3' flanking sequences were amplified by polymerase chain reaction (PCR) using Taq DNA polymerase (25) and inserted into the *BamHI* and *HindIII* sites of the centromeric plasmid pBM950 (gift of M. Johnston). The resulting construct (5'3'RPRlpBM, unpublished data), contained an *EcoRI* site (created by addition of *EcoRI* linkers) at the junction of the two flanking regions. Plasmid 5'-3'RPRlpBM was restricted with *BamHI* and *SalI* and the resulting fragment containing the *RPR1* flanking sequences was ligated into the *BamHI*—*SalI* sites of the *TRP1*-marked plasmid pRS424 (gift of P. Hieter; 26) to create pRS424-5'-3' *RPR* (Fig. 1A). In the constructs used for complementation experiments, cDNAs representing the mature domain of the different yeast species' RNase P RNAs ('test genes') were inserted in the *EcoRI* site of the pRS424-5'-3' *RPR* plasmid (Fig. 1A) between the *RPR1* promoter/leader sequences (84-bp contained within the 5' flank sequences) and the terminator region (37-bp contained within the 3' flank). Correct orientation of the test genes in pRS424-5'-3' *RPR* was determined by PCR.

The *S. cerevisiae* *RPR1* cDNA used as a positive control in the complementation experiments was obtained as a 374-bp *EcoRI* fragment containing the *RPR1* mature domain coding region cloned into pGEM-3Z plasmid (Promega) as previously described (18). The heterologous cDNAs were synthesized by PCR amplification of the mature region of the RNase P genes. The
templates used for PCR amplification were genomic DNA clones of *S. carlsbergensis*, *S. kluyveri* and *S. globosus* (16), and a genomic DNA clone of *S. pombe* (6). Primers with sequences specific for the 5' or 3' ends of the mature domain of each RNase P RNA gene were used for the PCR amplification. The primers also introduced EcoRI sites at each end of the cDNA to allow subcloning in the EcoRI site of pRS424-5'S3'RPR plasmid. The hybridization positions of the primers were the following: Eco5' carlsbergensis (+1 to +18) and Eco3' carlsbergensis (+339 to +359), Eco5' kluyveri (+1 to +17) and Eco3' kluyveri (+316 to +336), Eco5' globosus (+1 to +18) and Eco3' globosus (+319 to +339), Eco5' pombe (+1 to +25) and Eco3' pombe (+256 to +286). The numbers of RNase P RNA positions are relative to the mature RNA 5' end as described earlier (16). PCR conditions were 25 rounds of: 94°C for 1 min, 42°C for 30 s and 72°C for 1 min.

**RNA isolation and analysis**

RNA was isolated from exponentially growing yeast cultures (27). Approximately 10μg per lane were used for Northern blot analysis. RNA was normalized by measuring absorbance at 260 nm and by ethidium bromide staining to visualize ribosomal RNA bands after electrophoresis in agarose gels (Figures 3 and 4), and by quantitating U6 RNA levels as an internal control (Figure 4). The RNA used for the Northern blots was isolated from yeast cells containing both the single-copy plasmid carrying the *RPRI* gene and the high-copy plasmid containing the heterologous test RNase P RNA genes.

Northern blot analyses were performed essentially as described previously (16). [32P]-radiolabeled antisense oligodeoxynucleotides were used as probes to detect specific RNAs on the Northern blots. The probes' signal was roughly the same in all cases. For the Northern blot analysis of the *S. cerevisiae* RPR1 RNA (Fig. 4A), a 16-nucleotide probe that hybridizes between nucleotides +33 and +48 of RPR1 RNA was used. U6 RNA (28) was detected by a 17-nucleotide probe that hybridizes between nucleotides +62 and +88. For Northern blot analyses of RNase P RNA from the other four yeast species (Fig. 3), probes that hybridize specifically to the 3' end of each heterologous RNase P RNA were used (described under Recombinant plasmids). These probes did not show cross-hybridization to the RPR1 RNA under the conditions used for the experiments. Radiolabeled RNA size markers used to determine the sizes of RNA products were prepared as described previously (16). Quantitation of RNA from *S. cerevisiae* Northern blot (Fig. 4) was performed by exposing the blot to a Molecular Dynamics PhosphorImager and by performing volume integration with the ImageQuant program (Molecular Dynamics Corp.). The values obtained after volume integration are expressed in arbitrary units. For comparison of the levels of RPR1 RNA, the numbers obtained
from the volume integration of RNAs from individual clones were averaged for each isolate.

RESULTS

Complementation of the RPR1 disruption by RNase P genes from evolutionarily divergent yeast species

The ability of RNase P RNAs from both closely and distantly related yeasts to complement the disrupted S. cerevisiae RPR1 gene was tested for several reasons. First there is precedent for mixed RNase P protein and RNA components from distantly related bacteria being able to reconstitute functional holoenzyme in vitro (12). Additionally, in vivo complementation of an RNase P RNA gene deletion in E. coli by genes from distantly related eu-bacteria has been demonstrated (29). Second, results from phylogenetic studies of RNase P RNAs from several yeast species showed a striking evolutionary conservation of RNA secondary structure. The purpose of the present study is to identify sequences and structures in the yeast RNA that are essential for function within an individual organism, even though they are variable between species. The yeast species used in these experiments and the percent sequence identity of their RNase P RNA with S. cerevisiae RPR1 RNA were: Saccharomyces carlsbergensis (86%), Saccharomyces Kluyveri (70%), Saccharomyces globosus (65%) and Schizosaccharomyces pombe (51%) (16).

The plasmid constructs and the experimental strategy for the complementation experiments are outlined in Figure 1. The cDNAs of the RNase P RNA coding sequences from the four yeast species were cloned in a high copy plasmid between the leader/promoter and terminator of the S. cerevisiae RPR1 gene. The RNA is expected to be synthesized as a precursor containing the RPR1 leader and terminator sequences, with subsequent removal of those sequences, as was shown to be the case when the S. cerevisiae mature region cDNA is cloned into this high copy vector (17). By using this expression system, we were able to test whether heterologous RNase P RNAs' mature coding regions were functional without concern over whether the heterologous transcriptional promoters were functional in S. cerevisiae. The plasmids carrying the RNase P cDNA inserts will be referred to as 'test RNase P genes' (Fig. 1). Four to six

![Figure 3](image-url)

**Figure 3.** Expression of heterologous RNase P RNAs in S. cerevisiae. Total RNA isolated from cells containing both the RPR1 gene and a test gene was subjected to Northern blot analysis using antisense probes specific to each species' RNA (see Materials and Methods). Two individual positive clones of each RNase P were inspected (a and b). Estimated sizes of hybridizing RNAs (+/-10 nt) relative to markers are indicated at the left of each blot.

![Figure 4](image-url)

**Figure 4.** Expression of RPR1 RNA in S. cerevisiae cells that also contain the heterologous RNase P RNA genes. (A) Total RNA isolated from cells containing both the RPR1 gene and one of the test genes was subjected to Northern blot analysis using an antisense probe specific for the RPR1 RNA. The first lane (JLY1) shows RNA obtained from the strain containing only a single copy of RPR1. The other experimental lanes show RNAs from cells containing a single copy of RPR1 and also high copy of the indicated test gene. Two clones from each species were inspected (a and b). Both the precursor (upper) and mature (lower) forms of RPR1 RNA are indicated. A U6 RNA probe was used as an internal control for RNA levels. (B) The relative levels of the precursor and mature RPR1 RNAs shown in Figure 4A were quantitated using a PhosphorImager (Molecular Dynamics) and expressed as arbitrary units using the ImageQuant program. The levels of RNA were normalized to U6 RNA levels. The amounts of S. cerevisiae precursor and mature RNA are given when each test gene is also present in high copy. RPR1 RNA from JLY1 cells shows the levels with no test gene.
independent clones of each cDNA in the correct orientation were chosen and transformed into the RPR1-disrupted yeast strain JLY1. Multiple isolates were tested in all cases to ensure that phenotypes in yeast were reproducible and not due to any unexpected mutations acquired in the cloning process. To test the ability of the heterologous RNase P genes to complement the RPR1 disruption, the YCp50-RPR1 plasmid containing the wild type RPR1 gene was eliminated from the transformed yeast cells by plating on 5-FOA medium lacking histidine and tryptophan. Growth on this medium induces the loss of the URA3-marked plasmid, YCp50-RPR1, and viability of the cells becomes dependent on the ability of the test RNase P gene to function as the source of RNase P RNA.

The results of the complementation experiments are shown in Figure 2. Eight single colonies derived from transformation with each of the RNase P test gene plasmids were plated on 5-FOA medium to test for complementation. In Figure 2, two independent plasmids (a and b) and two independent yeast transformants with each plasmid clone are shown (a, a'; b, b'). Cells were streaked on medium retaining the YCp50-RPR1 plasmid as control for growth and for the number of cells plated (Fig. 2, panel A). Growth of colonies on 5-FOA medium (Fig. 2, panel B) indicates complementation. The genes from S.cerevisiae (positive control), S.carlsbergensis and S.kluyveri were able to complement the RPR1 disruption, but the S.globosus and S.pombe genes were not. Non-complementing transformants were also found to be non-viable at 25°C (data not shown).

Expression of heterologous RNase P RNAs in S.cerevisiae

We investigated whether the lack of complementation of the RPR1 gene deletion in S.cerevisiae by RNase P genes from S.globosus and S.pombe might be due to lack of expression or to instability of the foreign RNA in S.cerevisiae. Northern blot analyses were performed on total RNA extracted from JLY1 cells containing both the plasmid-borne wild type RPR1 gene and plasmids carrying the RNase P cDNAs from the other yeast species (Figs. 3 and 4). Figure 3 shows the results where species-specific probes were used to detect the test RNase P RNAs. The RNase P RNAs from all the species, including those that did not complement the RPR1 disruption, are expressed in S.cerevisiae. This is in contrast to reports from in vivo complementation experiments performed in bacteria which showed that in most cases, the inability to complement an RNase P RNA gene deletion correlated with the absence of stable heterologous RNase P RNA (29).

The pattern of RPR1 RNase P RNA on Northern blots, described previously (18), consists of a 369-nucleotide RNA (mature RPR1 RNA), and a longer less abundant precursor form of RNA containing an 84-nt 5' leader and 27–37-nt of extra 3' sequences. This pattern can be observed in Figure 4 (panel A, first lane) where RPR1 RNA was detected using an RPR1-specific probe in all the transformants which also contained the foreign

![Figure 5. Sequence and structural comparison of S.cerevisiae's RNase P RNA with the heterologous RNase P RNAs that are able to complement the RPR1 disruption. The sequences and phylogenetically-derived secondary structures (16) of the RNase P RNAs from S.carlsbergensis and S.kluyveri were compared to that from S.cerevisiae RNA. On the S.carlsbergensis or S.kluyveri RNA nucleotides that differ from the S.cerevisiae RNA are denoted by filled circles. These positions are combined on the S.cerevisiae RNA.](image-url)
RNase P gene. The estimated sizes of the RNA products in Figure 3 match (within error for estimating the sizes) the predicted RNase P RNA products. The expected RNA sizes for the precursor and mature form of RNase P RNAs from the other yeast species in Figure 3 are respectively: 490-nt and 359-nt for S.carlsbergensis, 462-nt and 336-nt for S.kluyveri, 467-nt and 339-nt for S.globosus and 417-nt and 286-nt for S.pombe (16). The RNA pattern of the species that complemented the RPR1 disruption, S.carlsbergensis and S.kluyveri, showed a distribution similar to that of RPR1 RNA where the smallest RNA is the size of the expected mature form and the largest RNA is the size expected to contain the extra 5' and 3' sequences. These two heterologous RNAs also showed a third intermediate-sized form that is less prevalent for S.kluyveri RNA and slightly more prevalent than the mature form for S.carlsbergensis RNA. A phenomenon similar to this one has been previously observed for RPR1 RNA, where multiple RNAs larger than the fully processed one are detected on Northern blots (17). It is possible that in S.carlsbergensis and S.kluyveri the intermediate-sized RNA could also be an intermediate with 3' or 5' unprocessed ends.

For S.globosus and S.pombe, the RNase P RNAs that were not able to complement the RPR1 disruption, there is accumulation of the precursor RNA form. The intermediate-sized and the fully processed RNAs are either very faint or absent. These results suggest that these two RNase P RNAs might not be recognized by the assembly and/or processing machinery. This interpretation is consistent with the effect of the different heterologous RNAs from high copy genes on the stability of RPR1 from a single copy gene in the same cell (see below).

**Effects of heterologous RNase P RNAs on endogenous RPR1 RNA expression**

The levels of expression of the RPR1 RNA were also inspected by Northern blot analysis in cells containing both the wild type RPR1 gene in single copy and a test RNase P gene in high copy (Fig. 4). Figure 4B shows the quantitation of the RPR1 RNAs from the Northern blot in Figure 4A. The levels of both the precursor RNA and mature RNA forms were normalized to U6 RNA levels, used as an internal control. Cells transformed with the native RPR1 cDNA in multiple copies accumulate considerably more of the larger precursor RNA and only somewhat more of the smaller mature 369-nt RNA, indicating a possible overloading of the assembly and processing machinery (17, Fig. 4). In particular, it is possible that the RNase P protein subunit(s) become limiting for assembly, and that without assembly into a nucleoprotein particle processing of the RNase P RNA does not proceed. The hypothesis that full assembly of the holoenzyme occurs before terminal RNA processing is supported by co-purification of the larger and smaller RNA forms as nucleoproteins on multiple ion exchange resins (7).

Accumulation of precursor RNA is observed in Figure 4 (S.cerevisiae a and b lanes) showing RNA from JLY1 cells.

Figure 6. Sequence and structural comparison of S.cerevisiae's RNase P RNA with the heterologous RNase P RNAs that are not able to complement the RPR1 disruption. The sequences and phylogenetically-derived secondary structures (16) of the RNase P RNAs from S.globosus and S.pombe were compared to S.cerevisiae's RNase P RNA. Nucleotides that differ from the S.cerevisiae RNA are highlighted by filled circles and combined on the S.cerevisiae RNA.
transformed with high copy RPR1 cDNA. In these S.cerevisiae lanes, two RNA products are actually present in the precursor region (not obvious on this exposure). The largest, most intense RNA corresponds to the RPR1 cDNA expression construct precursor containing extra nucleotides from the EcoR1 linkers, whereas the less abundant RNA corresponds to the wild type gene's primary transcript. Steady state levels of both precursor and mature RPR1 RNAs are severely reduced in cells that also contain RNase P RNAs that complement the RPR1 disruption (S.carlsbergensis and S.kluyveri). The levels of RPR1 RNA in the cells containing the non-complementing RNase P RNA genes from S.globosus or S.pombe were similar to those produced in cells containing only the single copy wild type RPR1 gene (first lane Fig. 4). The reduction in the levels of RPR1 RNA in cells containing the S.carlsbergensis and S.kluyveri were 3 to 4-fold for the precursor RNA and 6 to 8-fold for the mature RNA compared to the expression of wild type RPR1. In cells containing the S.globosus gene there was a small reduction (2-fold) in the level of the mature RNA whereas the level of the precursor was close to the control. The RNA levels in the cells containing S.pombe gene did not show any reduction. Taken together with the data in Figure 3, these results suggest that the heterologous RNase P RNAs that fail to complement neither process to mature form nor compete for limiting determinants of maturation. It is not clear whether these limiting determinants are the protein subunits, but the failure to assemble a mature holoenzyme is a possible cause of the inability of the RNAs to functionally complement.

DISCUSSION

Since the eukaryotic nuclear RNase P RNAs have not yet been shown to have catalytic activity when purified from holoenzyme preparations or when synthesized in vitro, it is necessary to characterize the effect of any changes made to the RNA component in the context of the holoenzyme. The availability of the S.cerevisiae strain with an RPR1 disruption has made it possible to perform studies of RNase P structure and function in vivo by substituting heterologous or mutated RNase P RNA genes. This approach has been used to study RNase P in eubacteria (29, 30), but its use has been limited in eukaryotic systems.

In this work, RNase P RNA genes from evolutionarily divergent yeast species were introduced into the RPR1-disrupted yeast strain in an attempt to identify sequences and structural domains in the S.cerevisiae RNA that might be important for enzyme function in vivo. In order to function in S.cerevisiae, the heterologous RNase P RNAs need to not only be synthesized, but also to fold properly, bind the protein components, be processed, and localize correctly within the cells. The RNAs from S.globosus and S.pombe are clearly not intrinsically defective for substrate binding and catalysis, since they function in their normal cellular environment. Their inability to complement correlates with their failure to be processed from precursor to smaller RNAs, but it is not clear that the inability to be processed per se causes loss of function. Suppression of wild type RPR1 levels by overexpression of the complementing RNAs S.carlsbergensis and S.kluyveri suggests that these compete for some component that stabilizes RNase P RNA, perhaps limiting amounts of RNase P protein. The lack of such suppression by the non-complementing S.globosus and S.pombe RNAs might conversely suggest that these RNAs are unable to compete successfully for the S.cerevisiae protein components. This lack of protein binding could then lead to both lack of RNA maturation and lack of function.

The sequence and proposed secondary structure for S.cerevisiae RNase P RNA (16) were compared with the structures of the RNAs that complemented the RPR1 disruption (Fig. 5) and with those that did not complement (Fig. 6). In each case, nucleotides that are not conserved between S.cerevisiae and the heterologous RNAs are represented as filled circles surrounding white letters. For purposes of the discussion, the regions of the RNA will be referred to by nucleotide position numbers in the S.cerevisiae model. When comparing the sequences of the S.cerevisiae RNA with the sequences of the S.carlsbergensis and S.kluyveri counterparts, it is clear that there are some structural domains in which sequences are not conserved (Fig. 5). The fact that these RNAs are able to complement the RPR1 disruption suggest that there might not be sequence recognition between these regions of the RNA and protein component(s). The regions that showed less conservation of sequence are the hairpin stems Sce 47–54/59–68, Sce 123–134/139–150, Sce 216–226/231–241 and part of Sce 269–282/289–303, four extra nucleotides in the region of Sce 23–31, and the 6-nt bulge Sce 167–172 (absent in S.kluyveri) (Fig. 5). There are also several sequence patches not part of obvious secondary structures that are not conserved, e.g., nucleotides in the regions of Sce 69–80, Sce 113–119 and Sce 190–197. For entire stems, nucleotide sequence may not be important, but the presence of the structure may be. However, initial mutational studies guided by this work confirm that independent deletions of stems Sce 123–134/139–150 and Sce 117–226/231–241 leave functional RNase P RNAs (manuscript in preparation).
When the *S.cerevisiae* RNA is compared to the RNA species that did not complement the *RPR* disruption, numerous sequence and structural differences are observed (Fig. 6). Some of the most obvious differences are the presence of the stem 212–218/223–229 in *S.globosa* RNA, the absence of stem Sco 269–282/289–303 in *S.pombe* and absence of stem Sco 123–134/139–150 in both species. Some of the differences in Figure 6 overlap the set of non-essential sequences shown in Figure 5.

Positions that are conserved in complementing RNAs, but not conserved in non-complementing RNAs are shown as open circles in Figure 7. In addition, dark circles with white letters in Figure 7 show positions that are invariant among yeast (16) and as such also suspected of playing essential functional roles. Many of the sequences denoted by open circles in the *S.cerevisiae* structure cluster near nucleotides that are invariant among yeast (dark circles). Most of the open circles correspond to nucleotides that are conserved within the Saccharomyces RNAs but changed in Schizosaccharomyces. These highlighted positions in Figure 7 are part of the highly conserved regions that have been identified as highly conserved according to phylogenetic studies (16). Some of these highly conserved regions are the terminal stem Sco 2–14/355–367, stem Sco 17–22/318–323, Sco 31–36, Sco 38–44, Sco 81–98, Sco 100–111/262–272, Sco 204–216/241–249, Sco 299–317 and Sco 337–354 (Fig. 7).

Interestingly, there is a group of nucleotides that were conserved only between *S.cerevisiae* RNA and the RNAs that complemented, *S.carlsbergensis* and *S.kluyveri*, complemented, and structural differences are observed (Fig. 6). Some of the open circles correspond to nucleotides that are not essential for function (16,31). This study serves a slightly different purpose than previous studies (8,46,53,62,63) and for gifts of plasmids, cloned DNA and yeast strains. We acknowledge support services provided by the DNA Synthesis Facility/University of Michigan Biomedical Research Core. This work was supported by National Institutes of Health Grant NIH GM 34869 (to D.R.E) and Grant MO1 RR00042. Oligonucleotide synthesis was partially subsidized by National Institutes of Health Grant P30CA46592 to the University of Michigan Cancer Center. EPR was supported by the Merck Minority Graduate Student Fellowship and by the Rackham Merit Fellowship, University of Michigan. AJT is a fellow in the Medical Scientist Training Program at the University of Michigan and was supported by a Young Scientist M.D./Ph.D. Scholarship provided by the Life and Health Insurance Medical Research Fund. DWK was supported by the NIH National Research Service Award #5-T32-GM07544-15 from the National Institute of General Medical Sciences.

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