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Yeast RNase P: Catalytic activity and substrate binding are separate functions

(*Saccharomyces cerevisiae*/tRNA binding assay/acceptor-stem mutants)

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ABSTRACT **During tRNA biosynthesis the 5′-leader sequences in precursor tRNAs are removed by the ribonucleoprotein RNase P, an enzyme whose RNA moiety is required for activity. To clarify some aspects of the enzyme mechanism, we examined substrate binding and product formation with mutant precursor tRNAs. Mutations G-1→A or U-2→C in the *Schizosaccharomyces pombe* sup3-e tRNA^{Ser}, which cause mispairing at or near the top of the acceptor stem, prevent the removal of the 5′-leader sequences by *Saccharomyces cerevisiae* RNase P. Equilibrium binding studies involving specific gel retardation of RNase P–precursor tRNA complexes showed that complexes with wild-type and A-1 and C-2 mutant precursor tRNAs had very similar dissociation constants (average K_d for sup3 = 1.5 ± 0.2 nM). Thus, the 5′-terminal nucleotides of mature tRNA, on the 3′ proximal side of the RNase P cleavage site, affect the enzyme's catalytic function but not substrate binding. The catalytic integrity of the RNA component of RNase P is not essential for binding of tRNA precursors, as demonstrated by gel retardation of micrococcal nuclease-inactivated enzyme. This suggests a possible role for the protein component of the enzyme in substrate binding. Upon restoration of base pairing to the acceptor stem in the A-1 or C-2 mutants, we found that, in addition to a requirement for pairing at these positions, conservation of the wild-type first and second nucleotides of the tRNA was necessary to obtain maximal cleavage by RNase P. This indicates a distinct sequence preference of this enzyme.**

The transcription products of eukaryotic tRNA genes interact with many enzymes as they undergo 5′- and 3′-end maturation, splicing in some cases, transport to the cytoplasm, nucleotide modification, aminoacylation, and finally, interaction with translation factors and ribosomal components during protein synthesis. Some of these enzymes act upon various tRNAs and have presumably evolved to recognize general features of all tRNAs, rather than specific features common to only a few. During tRNA biosynthesis one such enzyme, the ribonucleoprotein RNase P, is responsible for removing the extra 5′ nucleotides found in precursor tRNAs. In *Escherichia coli* and *Bacillus subtilis*, RNase P has been shown to belong to a class of enzymes in which the RNA moiety itself, under certain conditions, is catalytic (1, 2). Several groups have studied, to various degrees, eukaryotic RNase P enzymes from *Saccharomyces cerevisiae* (3), *Schizosaccharomyces pombe* (4), *Bombyx mori* (5), *Xenopus laevis* (G. Tocchini-Valentini, personal communication), and HeLa cells (6). So far, all of these have been found to be RNA-containing enzymes, but as yet, "ribozyme" activity has not been demonstrated for any of them. In addition to the RNA, these enzymes contain various amounts of protein; the *Sc. pombe* enzyme was shown to contain 270 kDa of protein and 180 kDa of RNA (4). An equivalent activity, designated 5′ pre-tRNAase, in which no RNA was detected, has been isolated from *X. laevis* oocytes (7).

We have characterized numerous suppressor-inactivating mutations in the *sup3-e* and *sup9-e* tRNA^{Ser} genes of *Sc. pombe* (8, 9). *In vitro* and *in vivo* analysis of these mutants in *Sa. cerevisiae* has shown that most of the mutations in the tRNA^{Ser} coding region affect RNase P cleavage, to some degree. In general, those mutations that disturb tRNA secondary or tertiary structure are inhibitory to RNase P cleavage. There are, however, domains within the tRNA that vary in their importance for RNase P catalysis. The observation that alterations in acceptor stem structure can compensate for mutations elsewhere in the tRNA that decrease RNase P processing has shown this region to be of primary importance (10). Furthermore, single mutations that form A·C mismatches at five of the seven base pairs in the acceptor stem are deleterious to RNase P cleavage (8, 9). In particular, the A-1 and C-2 mutations affect RNase P cleavage drastically. These mutations cause mispairing in the tRNA acceptor stem on the 3′-proximal side of the site of RNase P cleavage; the resultant mutant precursor tRNAs are not detectably cleaved by RNase P *in vivo* or *in vitro* (8, 9). Similar mutants exist in *E. coli*; temperature-sensitive mutants of *su3*$^+$ tRNA^{Tyr} genes show recovery of suppression if complementary base pairing is restored to the acceptor stem (11).

We have further mutated the A-1 and C-2 mutant *sup3-e* tRNA^{Ser} genes *in vitro* in an attempt to convert their corresponding precursors into efficient substrates for RNase P and to restore suppressor activity. We show that the first and second nucleotides of the mature tRNA, as well as the base-pairing potential of the acceptor stem, are important for RNase P catalytic rate but do not affect substrate binding. The results of RNase P–tRNA precursor binding studies of micrococcal nuclease "inactivated" enzyme imply that the protein component of *Sa. cerevisiae* RNase P may play a more important role in 5′-flank removal than the corresponding proteins of the *E. coli* and *B. subtilis* enzymes.

MATERIALS AND METHODS

Primer-Directed Mutagenesis. The conditions were as in Stewart *et al.* (12). The oligodeoxyribonucleotides [for mutation G-71, d(GCTGGTGGCGGTATT); for mutation T-72, d(CTGGTGATGGTATTT)] were kindly provided by DuPont. A 1.0-kilobase *Hin*dIII–*Bam*HI DNA fragment containing the *Sc. pombe sup3-e* gene (13) was cloned into M13mp8 and used as the template to make the mutations G-71 and T-72. The *sup3-e* template containing the A-1 or C-2 mutation was used to make the A-1,U-72 or C-2,G-71 double mutations, respectively. Each of the mutant genes, as

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Abbreviations: IVS, intervening sequence; nt, nucleotide(s).

well as the parental *sup3-e* gene, was recloned as a 1.0-kilobase *Hin*dIII–*Bam*HI fragment into the *Sa. cerevisiae* centromeric vector YCp50 (gift of K. Struhl and R. Davis, Stanford), containing the *URA3*$^+$ gene. They were subsequently used for the *in vitro* and *in vivo* assays described below.

Transcription and Processing Assays in an *Sa. cerevisiae* Extract. These reactions have been described in detail (8, 9).

Suppression Assay. *In vivo* suppressor activity was assayed in *Sa. cerevisiae* strain 3A84 (*ura3-52 ade1-1*$_{op}$ *leu2-2*$_{op}$ *his4-260*$_{op}$). Parental and mutant *sup3-e* genes, cloned on the single-copy YCp50 vector, were transformed into strain 3A84, and opal suppressor activity was determined by monitoring the red color that forms in the yeast, on limiting adenine plates (3 mg/liter), if the *ade1-1* mutation is not suppressed. Suppression was quantitated by spotting dilutions of saturated cultures on minimal plates and selecting for suppression of all three genes with opal mutations. A 1% suppressor efficiency indicates that 100 times as many cells were needed to give an equivalent number of colonies when selecting for suppression. Both assays gave similar, relative measures of suppression.

RNA Gel Blot Hybridization Analysis. Reclones in YCp50 of the various *sup3-e* alleles were transformed into *Sa. cerevisiae* strain 3A84. RNA gel blot analysis was performed after electroblotting RNA to Zeta-probe membranes (8). The hybridization probe consisted of the 1.0-kilobase *Hin*dIII–*Bam*HI, *sup3-e* gene fragment.

RNase P Gel Retardation Assays. Substrates for gel retardation assays were generated by SP6 RNA polymerase from clones of the various tRNA genes in pSP64 (14). The 793-base-pair *Alu* I–*Bam*HI fragment of *sup3-e* (13) or its mutants were cloned into pSP64 that had been digested with *Hin*dIII, treated with mung bean nuclease, and then cleaved with *Bam*HI. Synthesis of a 143-nucleotide (nt) precursor consisting of a 12-nt 5′-leader sequence, the intron-containing *sup3-e* serine tRNA, and a 34-nt 3′ trailer (which includes the intragenic spacer and the first 27 nt of the adjacent tRNA^{Met}) was carried out after digestion of the templates with *Ban* II and mung bean nuclease. A pSP64 clone containing the *Sc. pombe supS1* gene and the precursor produced after digestion with *Taq* I have been described (4). Transcription reactions (20 μl) were performed as described by Melton *et al.* (14) and contained 12 μM [α-^{32}P]GTP (200 Ci/mmol; 1 Ci = 37 GBq). To generate a non-tRNA substrate, the 596-base-pair *Hin*dIII–*Xho* I fragment of plasmid pGC106 (15) was cloned into pGEM-3. A 357-nt transcript was synthesized by SP6 RNA polymerase after digestion with *Sca* I. Transcripts were purified by electrophoresis on 6% polyacrylamide/8.3 M urea gels (16).

Binding reactions were carried out by using RNase P fractionated from *Sa. cerevisiae* as described by Engelke *et al.* (3). Each reaction mixture (20 μl) contained 10% (vol/vol) glycerol, 20 mM Hepes·KOH (pH 7.9), 100 mM KCl, 10 mM $MgCl_2$, 2.5 mM dithiothreitol, and 0.46–9.2 × 10^5 dpm of precursor tRNA. All components were kept at 0°C prior to and following (for 10 min) enzyme addition. With the running voltage applied (250 V, 25 mA), reaction mixtures were loaded onto 4% polyacrylamide [acrylamide/*N*′,N′-methylenebisacrylamide, 39:1 (wt/wt)] gels (20 × 20 × 0.15 cm) containing 50 mM Tris borate (pH 8.3), 1 mM EDTA, and 5% (vol/vol) glycerol. Electrophoresis was at 4°C. Gels were dried and autoradiographed, and the amount of complexed precursor tRNA was determined by densitometry or Cerenkov counting. Substrate dissociation constants were calculated from Scatchard plots by a linear regression analysis of the data.

Preparative complex formation assays were carried out as described above, with 2.3 × 10^6 dpm of tRNA precursor and sufficient enzyme to maximize the amount of complex. After electrophoresis and autoradiography (4°C for 30 min), RNA was eluted from the complex by an overnight incubation at 30°C in RNase P reaction buffer. The eluate was phenol extracted, ethanol precipitated, and analyzed on a 6% polyacrylamide/8.3 M urea gel. Assays for RNase P activity were identical to binding reactions but were incubated at 30°C for 10 min. Products were purified and analyzed as described above.

Micrococcal nuclease digestions of *Sa. cerevisiae* RNase P were performed as described by Krupp *et al.* (4) in a volume of 15 μl and over a range of nuclease concentrations from 10 to 2000 units/ml. Digests were terminated after incubation at 37°C for 30 min by adding 10 μl of 10 mM EGTA (pH 8.5). The amount of this pretreated enzyme that was used subsequently for assaying tRNA precursor binding and RNase P activity was equivalent to the amount used (per reaction mixture) for the experiment shown in Fig. 5.

RESULTS

Mutants Altered in the First Two Base Pairs of the tRNA Acceptor Stem Are Differentially Sensitive to RNase P Cleavage. To generate improved substrates for RNase P and thereby recover suppressor activity, the A-1 and C-2 mutant *sup3-e* tRNA genes were mutagenized by using oligonucleotides to restore Watson–Crick base pairing to the tRNA acceptor stem. In addition, we generated the mutations T-72 and G-71 alone in the parental *sup3-e* gene to test the effect of G·U pairing at these positions on RNase P processing and opal suppression. Acceptor-stem secondary structure of these mutant tRNAs is shown in Fig. 1.

Initial analysis of the mutants was carried out by using *Sa. cerevisiae* cell extracts active in class III gene transcription and processing of tRNA precursors. Processing of the initial dimeric tRNA precursors derived from *sup3-e* and the A-1 and C-2 mutant genes has been described in detail (9). The latter are severely defective in RNase P cleavage and yield little, if any, 5′-end-matured precursors [shown in Fig. 2 by the absence of the third dimeric tRNA precursor and the tRNA^{Ser} plus the intervening sequence (IVS) species]. The abundance of these products provides a relative measure of the efficiency of RNase P cleavage. Accordingly, the Watson–Crick base-repair mutants A-1,U-72 and C-2,G-71 show a substantial improvement in RNase P cleavage over the A-1 and C-2 mutants (Fig. 2). The most efficient RNase P substrates among the mutants examined were those with the mutations G-71 or U-72. These were cleaved at rates comparable to the parental sup3-e precursor (Fig. 2). Thus, while base pairing in the acceptor stem, proximal to the site of RNase P cleavage, is essential for catalysis, various base-pair combinations at these positions result in substrates that are differentially sensitive to cleavage. Interestingly, the rate of cleavage by RNase P does not vary with the thermodynamic stability of the base pairs at these positions (17) but rather appears to be correlated with the nature of the base occupying positions 1 and 2 of the mature sup3-e tRNA.

RNase P Cleavage and Suppression *in Vivo*. To determine whether the *in vivo* activities correlated with the *in vitro* results, the mutant genes were transformed into *Sa. cerevisiae* strain 3A84, and suppressor activity was examined. As in *Sc. pombe*, the A-1 and C-2 mutants were inactive (9). Mutations involving the second base pair of the acceptor stem were fully active in suppression at 30°C if either Watson–Crick or U·G pairing was possible. Both C-2,G-71 and G-71 gave 100% suppression relative to sup3-e. Mutations in the first base pair allowed very weak suppression, with G-1,U-72 (5%) retaining more activity than A-1,U-72 (1%). These results were surprising in that the U-72 mutant gene produced significantly more mature-sized serine tRNA

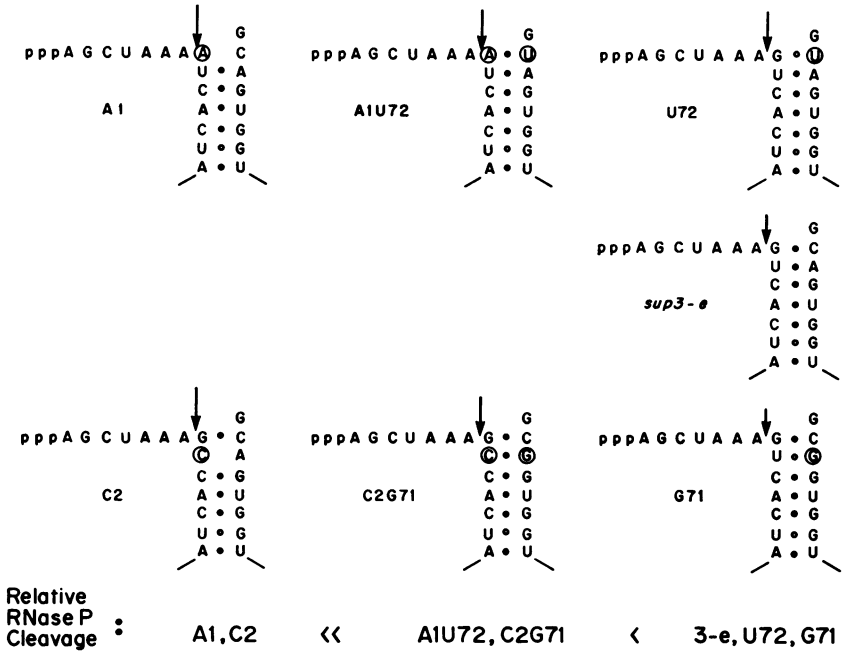

FIG. 1. Acceptor-stem secondary structures of the mutant and parental sup3-e precursor $tRNA^{Ser}$ molecules. The normal site of RNase P cleavage is shown by an arrow. The mutated nucleotides are circled. The relative ability of these precursors to be cleaved by RNase P (based on the data in Figs. 2 and 3) is also indicated.

than the C-2,G-71 double mutant in the *in vitro* extract (Fig. 2), yet was a much weaker suppressor than C-2,G-71 *in vivo* (5% vs. 100%). Other work with the *in vitro* extracts has shown a good correlation between the amount of mature $tRNA^{Ser}$ produced in the extract and the level of suppression in *Sa. cerevisiae*. We, therefore, examined the *in vivo* processing characteristics of the mutants by RNA gel blot analysis. The results (Fig. 3) show that the steady-state levels of 5′-flanked and end-matured $tRNA^{Ser}$ plus IVS precursors reflected the same relative extents of RNase P cleavage as were seen *in vitro* (Fig. 2). Thus, conservation of the wild-type first and second nucleotides of the tRNA again correlates with better 5′-end maturation by RNase P. Further, the RNA gel blot analysis reveals that despite a 20-times lower efficiency of suppression, the U-72 mutant yields more $tRNA^{Ser}$-specific products than the C-2,G-71 mutant (Fig. 3). These data and the *in vitro* results (Fig. 2) suggest that a function subsequent to tRNA maturation may be defective for U-72.

RNase P Equilibrium Binding Studies. The various abilities of the mutant precursors to be cleaved by RNase P may reflect differences in RNase P binding, catalytic rate, or both of these processes. To investigate these possibilities, we developed an RNase P–tRNA precursor binding assay. RNase P was purified ≈50-fold from *Sa. cerevisiae*. Based on *in vitro* assays with a variety of tRNA precursors (data not shown) and known elution characteristics of various tRNA processing enzymes (18, 19), this fraction was devoid of detectable 3′-processing nucleases, splicing endonuclease, and tRNA ligase. Incubation of this RNase P fraction with various monomeric tRNA precursors under binding-reaction conditions resulted in the formation of a single, high molecular weight complex that could be resolved from unbound precursor tRNA by electrophoresis (Figs. 4*A* and 5*A*). These retarded complexes were electrophoretically indistinguishable from complexes formed with an RNase P preparation purified >1000-fold by a different protocol (J. Lee and D. Engelke, personal communication; data not shown). The RNA contained in these complexes could be eluted under conditions that permit RNase P catalysis to

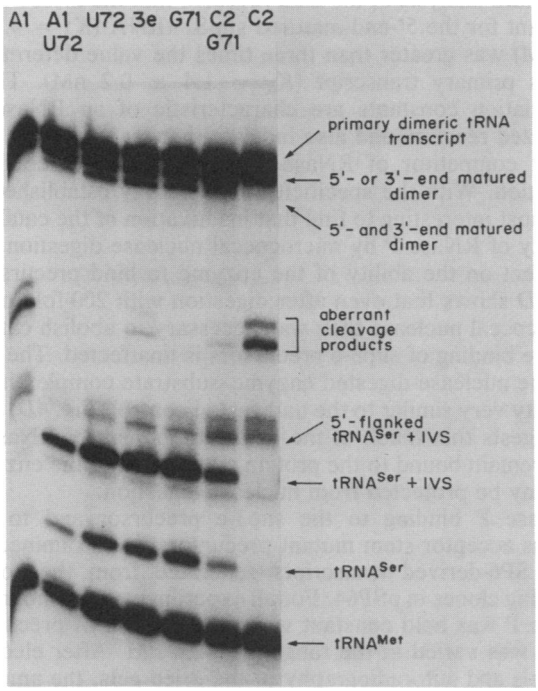

FIG. 2. Transcription and processing of the *Sc. pombe* sup3-e acceptor-stem mutants in an *Sa. cerevisiae* extract. The product assignments have been described (16).

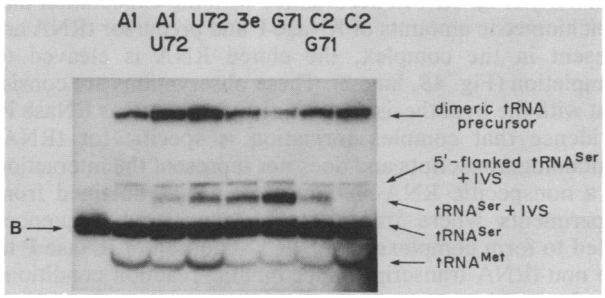

FIG. 3. RNA gel blot analysis of RNA isolated from *Sa. cerevisiae* 3A84 transformed with wild-type and mutant *sup3-e* genes in YCp50. Each lane contains 50 μg of RNA. The far-left lane contains RNA isolated from a control YCp50 transformant and shows the extent of cross-hybridization (denoted B) between *Sa. cerevisiae* RNAs and the *Sc. pombe* sup3-e probe.

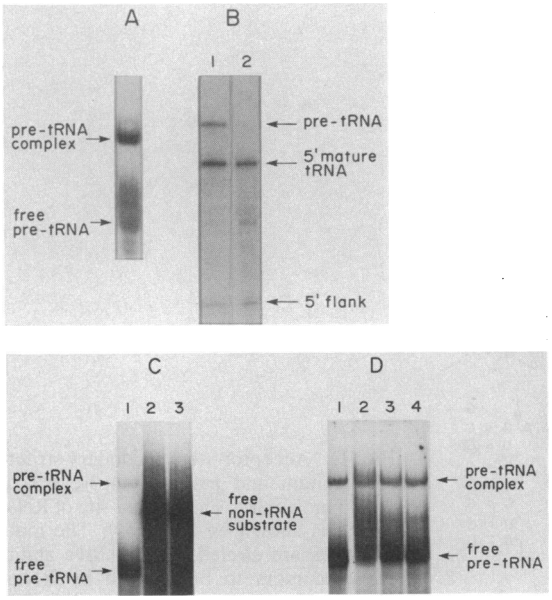

FIG. 4. RNase P–precursor tRNA binding experiments. (*A*) Products of a binding reaction between *Sa. cerevisiae* RNase P and *Sc. pombe* supS1 $tRNA^{Ser}$ precursor were resolved on a native polyacrylamide gel. (*B*) Products obtained after digestion of the supS1 $tRNA^{Ser}$ precursor with RNase P (lane 1) and the RNAs eluted with RNase P reaction buffer from the supS1 $tRNA^{Ser}$ complex in *A* (lane 2) are shown after electrophoresis on a denaturing polyacrylamide gel. (*C*) Products obtained by incubation of equimolar amounts of sup3-e tRNA precursor or a non-tRNA transcript with an equal amount of RNase P (under binding-reaction conditions) are shown in lanes 1 and 3, respectively, after electrophoresis on a native polyacrylamide gel. Lane 2 is a control lane showing the non-tRNA transcript without added RNase P. (*D*) Binding studies with micrococcal nuclease-inactivated RNase P. The concentrations of RNase P and sup3-e tRNA precursor are identical and correspond to the amounts used for the reaction mixtures in lanes 5 and 14 of Fig. 5. At these concentrations approximately two in every five RNase P molecules are complexed with tRNA precursor (assuming a stoichiometry of 1:1). Lanes: 1, RNase P preincubated with micrococcal nuclease (10 units/ml) in the presence of EGTA; 2–4, digestions of RNase P with micrococcal nuclease at 2000, 500, and 10 units/ml, respectively, were carried out prior to the addition of EGTA. Assays for RNase P catalytic activity showed that the lowest concentration of nuclease tested (10 units/ml) abolished all enzymatic activity (data not shown).

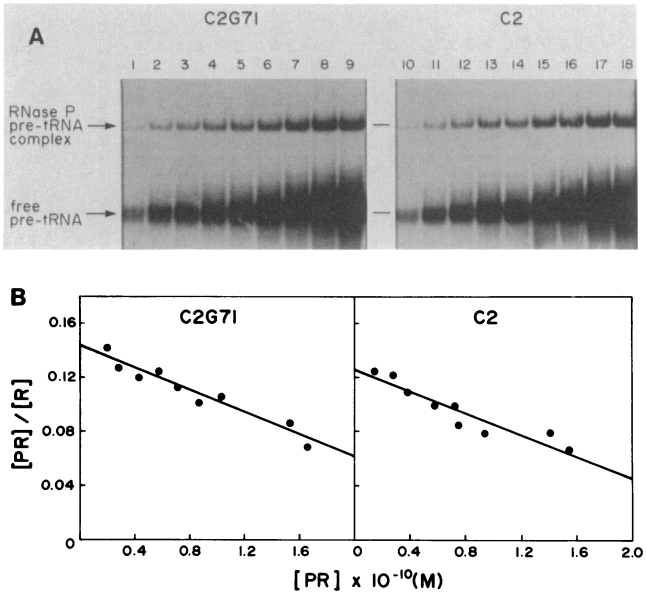

FIG. 5. RNase P binding to precursor tRNA transcripts of mutants C-2,G-71 and C-2. (*A*) Increase in complex formation with increasing amounts of tRNA precursor (0.46–9.20 × 10^5 dpm) and fixed amounts of RNase P. The concentrations of tRNA precursor in lanes 1–9 are equivalent to lanes 10–18, respectively. (*B*) Scatchard plots of the data obtained from *A* for both mutants. [PR] represents the concentration of RNase P–tRNA precursor complex, and [R] is the concentration of unbound precursor tRNA.

yield 5′-end-matured tRNA and the 5′-leader sequence. For the supS1 $tRNA^{Ser}$ precursor, these data are shown in Fig. 4. Excision of the complex (shown in Fig. 4*A*) followed by elution of the RNA with RNase P reaction buffer yields the same products that are obtained in a typical assay for RNase P activity (Fig. 4*B*). In accordance with the expectation that stoichiometric amounts of RNase P and precursor tRNA are present in the complex, the eluted RNA is cleaved to completion (Fig. 4*B*, lane 2). These observations are consistent with the hypothesis that the complex contains RNase P. Evidence that complex formation is specific for tRNA-containing transcripts and does not represent the interaction of a nonspecific RNA-binding protein was obtained from experiments where transcripts lacking tRNA sequences failed to form complexes (Fig. 4*C*). Addition of RNase P to the non-tRNA transcript under binding-reaction conditions failed to produce a retarded complex in contrast to a control reaction containing an equimolar amount of sup3-e tRNA precursor. In other experiments, carried out at saturating substrate concentrations, we observed that a 50-fold molar excess of a nonspecific competitor RNA (*Sc. pombe* 5S RNA) did not affect the amount of complexed precursor tRNA (data not shown). Finally, kinetic evidence has shown that the observed complexes represent the specific interaction of RNase P and precursor tRNA. The 5′ flank of the supS1 precursor was removed in a preparative-scale RNase P digestion and the 5′-end-matured tRNA was purified by gel electrophoresis. Binding assays were then carried out to compare the preprocessed supS1 product of RNase P cleavage to the precursor from which it was derived (i.e., both RNAs were from the same run-off transcription reaction). Scatchard analysis of the data revealed that the dissociation constant for the 5′-end-matured supS1 tRNA (K_d = 4.3 ± 0.5 nM) was greater than three times the value determined for its primary transcript (K_d = 1.4 ± 0.2 nM). These dissociation constants are characteristic of an RNase P-catalyzed reaction and also indicate that mature tRNA is a potent competitor of RNase P–precursor tRNA complex formation. With the specificity of the assay established, it was most interesting to find that inactivation of the catalytic activity of RNase P by micrococcal nuclease digestion had no effect on the ability of the enzyme to bind precursors. Fig. 4*D* shows that even after digestion with 200-fold more micrococcal nuclease than was necessary to abolish catalysis, the binding of sup3-e precursors is unaffected. The fact that the nuclease-digested enzyme–substrate complex has a mobility very similar to the undigested control (Fig. 4*D*, lane 1) suggests that much of the RNA component of RNase P must remain bound to the protein component of the enzyme and may be protected from nuclease digestion.

RNase P binding to the sup3-e precursor and to the various acceptor stem mutant precursors was examined by using SP6-derived transcripts generated from the corresponding clones in pSP64. For all experiments the amount of RNase P was held constant while the amount of precursor tRNA was varied in the range 0.13–2.6 nM. After electrophoresis and autoradiography of the dried gels, the amount of radioactivity in the retarded complex was determined by densitometry (for mutations at positions 1 and 72) or by Cerenkov counting (for mutations at positions 2 and 71). Both methods were used on different sets of data obtained

for sup3-e, and the values agreed within 20%. The binding of each mutant precursor was analyzed at least once, certain precursors were examined twice, and three experiments were performed with the parental sup3-e precursor. A comparison of the complexes formed for the mutant precursors C-2 and C-2,G-71 is shown in Fig. 5*A*, and Scatchard plots of these data are presented in Fig. 5*B*. Despite the fact that the C-2 mutation prevents RNase P catalysis, the binding of this precursor by RNase P is no different than for C-2,G-71. In the experiment shown in Fig. 5, identical substrate dissociation constants were determined for the two precursors (for C-2, K_d = 2.4 ± 0.4 nM; for C-2,G-71, K_d = 2.4 ± 0.3 nM). In a second, independent experiment, a value of 1.7 ± 0.4 nM was calculated for the C-2 mutant. Similar results were obtained for precursors with different potential structures in the terminal base pair of the acceptor stem. The K_d values determined from one experiment for the catalytically inert substrate A-1 and the A-1,U-72 precursor were 1.2 ± 0.1 nM and 1.3 ± 0.2 nM, respectively. Overall, the dissociation constants of each of the mutant precursors were as similar to one another as they were to the parental sup3-e precursor. In 11 determinations, K_d values varied in the range of 0.6 ± 0.1 nM for U-72 to 2.4 ± 0.4 nM for C-2 with an average of 1.5 ± 0.2 nM. We conclude that the various abilities of the mutant precursors to be cleaved by RNase P do not result from alterations in the affinity of RNase P for these substrates but, rather, represent differences in the rate of catalysis by RNase P.

DISCUSSION

Nucleotides Proximal to the Cleavage Site of RNase P Affect Its Catalytic Rate. We have shown (8, 9) that maintenance of the characteristic "L" conformation of tRNA through the conservation of secondary and tertiary interactions is essential if RNase P is to carry out efficiently its catalytic function. In the work described here, we have examined the effect on RNase P cleavage of mutations that alter the structure of the two base pairs in the tRNA acceptor stem proximal to the site of RNase P cleavage. We found that while base pairing at these positions (positions 1 and 72 and positions 2 and 71) is essential for catalysis, simple Watson–Crick interactions may not be sufficient to permit maximal rates of cleavage by RNase P. Both *in vitro* and *in vivo* methods of analysis showed that the base-paired double mutants U-1,A-72 and C-2,G-71 produced substrates that were less efficiently end-matured by RNase P than those derived from sup3-e or from the single-point mutants G-71 and U-72. Since the thermodynamic stabilities of the various base-pair combinations do not provide an explanation for these data, we propose that RNase P may simply prefer sup3-e substrates that conserve the wild-type first and second nucleotides of the mature tRNA. That is, as long as some type of base pairing is possible, the nucleotides on the 3′-proximal side of the RNase P cleavage site determine the reaction rate. Clearly, this implies that RNase P can, in some way, recognize the base at positions 1 and 2 in the mature tRNA. To determine at what stage of the reaction these effects are being exerted, we examined the binding of mutant and wild-type tRNA precursors to RNase P with a mobility shift assay. Since all of the acceptor-stem mutants exhibited essentially wild-type binding affinity to RNase P, we conclude that it is the catalytic step in the reaction that is influenced by the tRNA sequence on the 3′-proximal side of the RNase P cleavage site. How may this effect be brought about? The mechanism of RNase P catalysis may involve a direct interaction of the enzyme with nucleotides of the tRNA adjacent to the cleavage site. If this is the case, the quality of these interactions and hence the efficiency of the reaction may be dependent upon the available hydrogen donor and acceptor sites. In this context it is not difficult to imagine that various bases may function more or less well. Alternatively, recognition of the base at these positions may be indirectly mediated by subtle differences in the base-paired structures that are formed.

A Role for the Protein Component of *Sa. cerevisiae* RNase P. Since the demonstration that the RNA component of prokaryotic RNase P enzymes possesses all the properties necessary to function as an enzyme (1), attention has been focused on the role of the protein component in the holoenzyme. Because gel-shift analysis of RNase P complexes with mutant and wild-type precursor tRNAs had permitted a separation of the catalytic and the substrate-binding function of the yeast enzyme, we were able to test the contribution of the intact RNA component to both functions. The results showed the inactive enzyme (due to micrococcal nuclease inactivation of the RNA component) retained the full capacity of binding precursor tRNA substrates. To the best of our knowledge, there are no reports that the RNA components alone of eukaryotic RNase P enzymes display catalytic activity. This may be due to lack of substrate binding potential on their part, a function that may be assumed by the protein component of the enzyme. Thus, it appears that the protein component of the *Sa. cerevisiae* RNase P may play a more prominent role in the enzymatic mechanism than the proteins of the corresponding *E. coli* and *B. subtilis* enzymes. This could be reflected by the fact that eukaryotic RNase P enzymes have a much higher protein/RNA weight ratio than do the prokaryotic enzymes studied to date.

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