184–186 Nucleic Acids Symposium Series No. 41

Characterization of chimeric Archaeal RNase P RNAs

Daniel Williams* and James W. Brown
Department of Microbiology, North Carolina State Universtiy, Raleigh, NC 27695, USA
*Presenting and Corresponding author

ABSTRACT

Archaeal RNaseP RNAs were previously thought to be unable to carry out catalysis *in vitro* (1). However, the RNA alone is active, but very high mono- and divalent salt concentrations are required for minimal catalytic activity (2). Unlike in Archaea, their bacterial counterparts are very catalytically active *in vitro* and do not need extremely high salts to carry out catalysis. The core structure of RNase P RNA between Bacteria and Archaea are very similar, however, there are differences in the peripheral structure of these molecules in respect to variation in size, helical placement and length (3). The question we propose is; What are the differences and/or similarities in structure that contribute to RNase P RNA from the archaeon *Methanobacterium thermoautotrophicum* being less catalytically proficient than Bacteria? Homologous element (s) from the bacterial RNA (i.e. *E. coli*) were inserted into the archaeal RNA in hopes of making the archaea RNase P RNA as catalytically proficient. The results showed that insertion of helical element P18 and base pair changes in P8 did not increase the catalytic proficiency of the molecule.

INTRODUCTION

RNase P is a ribonuclease that cleaves the 5' leader sequence of precursor tRNAs (pre-tRNA) by phosphodiester hydrolysis to produce a mature tRNA molecule. This endonucleolytic event is site specific and results in a mature tRNA retaining a 5' phosphate and a leader fragment with a 3' hydroxyl. RNase P is ubiquitious in all organisms and subcellular organelles involved in tRNA biogenesis. RNase P RNAs of Bacteria are ribozymes- the catalytic subunit of the enzyme is RNA. Bacterial RNase P RNAs are catalytically proficient *in vitro*, unlike the archaeal counterparts. The bacterial RNase P RNAs (primarily from *Escherichia coli* and *Bacillus subtilis*) are the only RNase P RNAs that have been extensively studied (4). The RNase P holoenzyme of Bacteria are composed of the large RNA (ca. 400 nucleotides) and small protein (ca. 120 amino acids). Archaeal and Eucaryal RNase P holoenzymes contain more protein relative to their bacterial counterpart. The most useful way to understanding the structure and function of RNase P has been through comparative analysis (5), which has enabled the identification of a minimum consensus bacterial (6) and archaeal (7) RNase P RNA structure. The bacterial RNase P RNA has been well characterized *in vitro*; this has proved very useful in determining what structural elements contribute to the catalytic proficiency of the molecule. There are core helices (P1-P11 and P15) that are present in all bacterial RNase P RNAs that may be essential for function given the conservative nature of the helices. Most of the non-conserved helices are subject to variation in length, which suggest that they are located in the periphery of the RNaseP RNA catalytic core (8). Experiments have been done where helices varying in length have been deleted. This results in a destabilized structure, but the molecule still retains most of its enzymatic activity (9, 10). A few of the dispensable elements are P16/P17/P6, P12, P13/P14, and P18. There is no dramatic reduction in catalytic activity after individual removal of these elements. However, the enzyme does seem to be less stable (temperature sensitive, higher ionic requirement) than the native RNA. It has recently been shown that some archaeal RNase P RNAs are active under extreme ionic conditions (requiring 3M NH_4OAc and 300 mM $MgCl_2$) but are less catalytically proficient compared to bacterial RNase P RNA (2). This fact raises the question of what are the differences in structure that make one molecule more active than the other. We plan to determine what changes in structure may reduce the high salt requirement for minimal catalytic activity in the archaeal RNA. We know that in Bacteria, helix P18 and P14 form a tertiary interaction with helix P8 in *E. coli* (11). It is believed that these interactions help stabilize the global structure and contribute to correct folding of the molecule *in vitro*.

In this paper we report the catalytic properties of two chimeric RNase P RNAs based on *M. thermoautotrophicum* RNA containing the *E.coli* P18.

METHODS

Preparation of RNAs

The RNAs were synthesized as run off transcripts using the T7 promoter. Transcription products were purified by electrophoresis on 5% denaturing polyacrylamide/8M urea gels. The RNAs were excised and eluted overnight. Samples were ethanol precipitated and resuspended into 75 µl elution buffer.

Generation of the Chimeric RNAs

Two archaeal chimeras (one chimera with only P18 inserted and the second chimera with P18 and base pair changes in P8) were made by insertion of P18 (based on that of *E. coli*) into the RNA from *M. thermoautotrophicum* by *in vitro* site-directed mutagenesis and polymerase chain reaction (PCR). In the second chimera, the appropriate base pair changes were made in helix P8 to ensure that the tertiary interaction was established between the two helices. The primers used are as follows: Mth P18 (5'-CGGCAGCATTCATCTAGGCCAGCAATTGCTCACTGGCTCGGGCGGACTACCTC-3') and Mth P8: (5'-CTCAGCTGATGGCTCACGCCACCACGG-3').

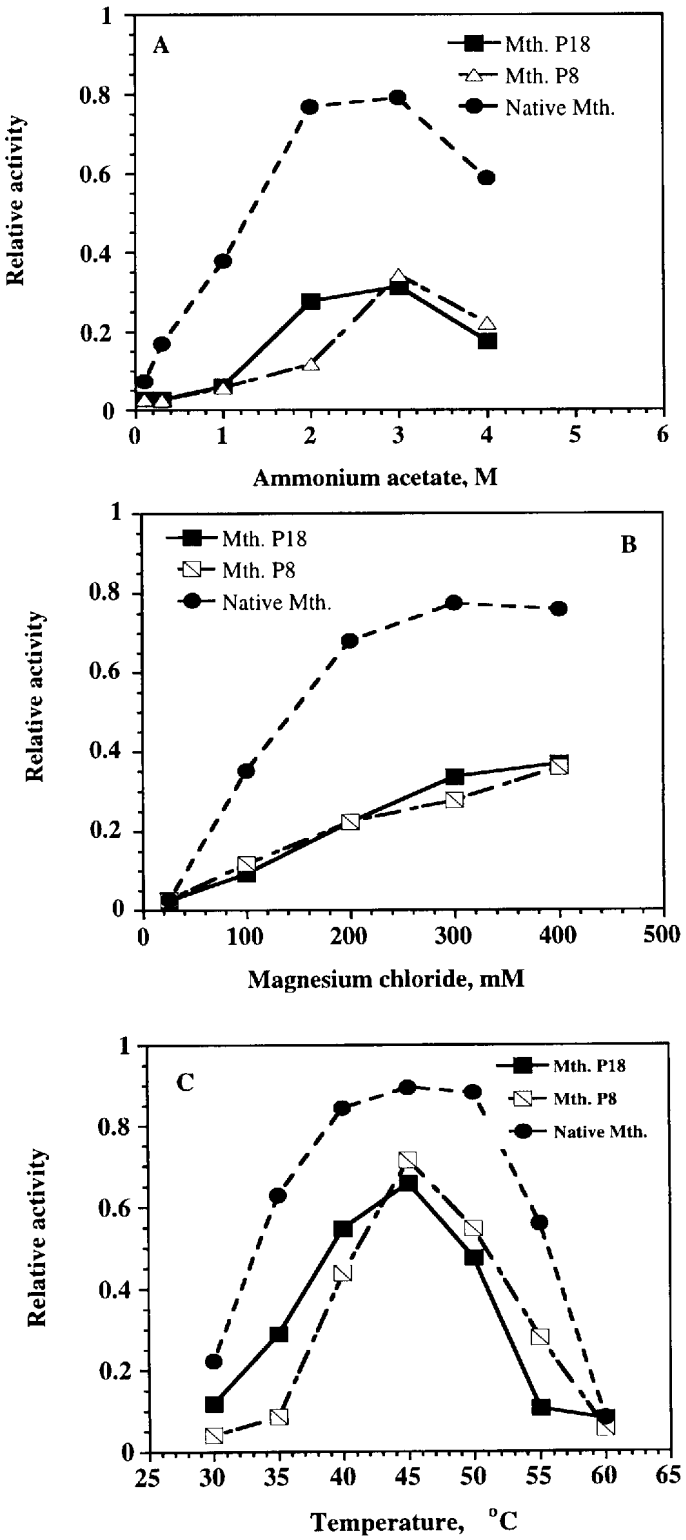

Figure 1. Reaction condition for native and chimeric RNAs. **A:** Influence of ammonium acetate on cleavage activity. Reactions contained 10 nM RNA, 300 mM $MgCl_2$, 50mM Tris-pH 8, 2 nM [^{32}P] pre-tRNA^{Asp}, and varying concentrations of NH_4OAc (.1M-4M). **B:** Influence of magnesium chloride on cleavage activity. Reaction conditions are the same except to an adjustment in the magnesium concentration (25 mM-400 mM) and a 3M NH_4OAc. **C:** Influence of the temperature on cleavage activity. These reactions were performed at various temperatures under standard reaction conditions (300 mM $MgCl_2$, 50mM Tris-pH 8, 3M NH_4OAc).

RNase P Assays

The assays were done in RNase P RNA buffer (50mM Tris-pH 8, $MgCl_2$, NH_4OAc) under varying concentrations of NH_4OAc (.1 mM-4M), $MgCl_2$ (25 mM-400mM) and temperature (30-60° C). Each RNA (10 nM) was assayed in the presence *B. subtilis* ^{32}P-labeled pre-tRNA^{Asp} (2 nM) at 46°C for 45 minutes and then stopped by adding an equal amount of RNA loading dye. Samples were run out on 12% denaturing polyacrylamide/ 8M urea gels at 300 volts to visualize cleavage activity. Cleavage products were quantified using Phosphorimagery.

RESULTS

Helix P18 was inserted into the *M. thermoautotrophicum* RNase P RNA based on the homologous sequence of helix P18 in *E. coli*. The two chimeric RNAs produced are as follows: *M. thermoautotrophicum* P18 (designated Mth P18, contains only the addition of helix P18) and *M. thermoutotrophicum* P8 (designated Mth P8, contains helix P18 plus appropiate changes in helix P8 to allow docking with P18).

Native *M. thermoutotrophicum* RNase P RNA (native Mth) requires a high concentration of monovalent (3M NH_4OAc) salt for optimal activity as shown in Figure 1 A. This result is consistent with earlier experiments of RNase P RNAs from other Archaea (2). The monovalent salt requirement for minimal catalytic activity of Mth P18 and Mth P8 was the same as the native RNA (Figure 1A). There is ca. 50% difference in relative activity between the native RNA and the two chimeric RNAs at 3M ammonium acetate. The previous salt titration showed that the optimal activity for the RNAs is at 3M ammonium acetate, so all of the $MgCl_2$ reactions were done at 3M ammonium acetate. Figure 1 B shows that native Mth has the greatest optimal activity at 300 mM magnesium chloride ($MgCl_2$) in comparison to the lesser active chimeric RNAs, which also have an optimum at the same $MgCl_2$ concentration. Figure 1 C shows that the temperature optimum for native Mth, Mth P18, and Mth P8 is 45 °C. Mth P8 is slightly more active than Mth P18 at the temperature optimum and continues to remain more active as the temperature increases. The tertiary interaction in chimera Mth P8 may be stabilizing the molecule better than the P18 alone insertion, which could explain the slight difference in relative activity between the chimeric RNAs. The P18 insertion without an established tertiary interaction with P8 could be further destabilizing the molecule, therefore, reducing its ability to perform catalysis as well.

DISCUSSION

We have constructed and characterized chimeric archaeal RNase P RNAs. P18 was chosen as the first helical element in constructing the archaea chimera based on earlier experiments with *Chlorobium* spp. (green-sulfur bacteria) that naturally lack P18 but were still catalytically active *in vitro* (10). This finding was surprising since P18 is universally present and conserved in other bacterial RNase P RNAs. The *Chlorobium* spp. and *E. coli* RNase P RNA with P18 removed are comparable to the native *E. coli* RNase P RNA in catalytic activity; the mutant requires an increase in monovalent salt

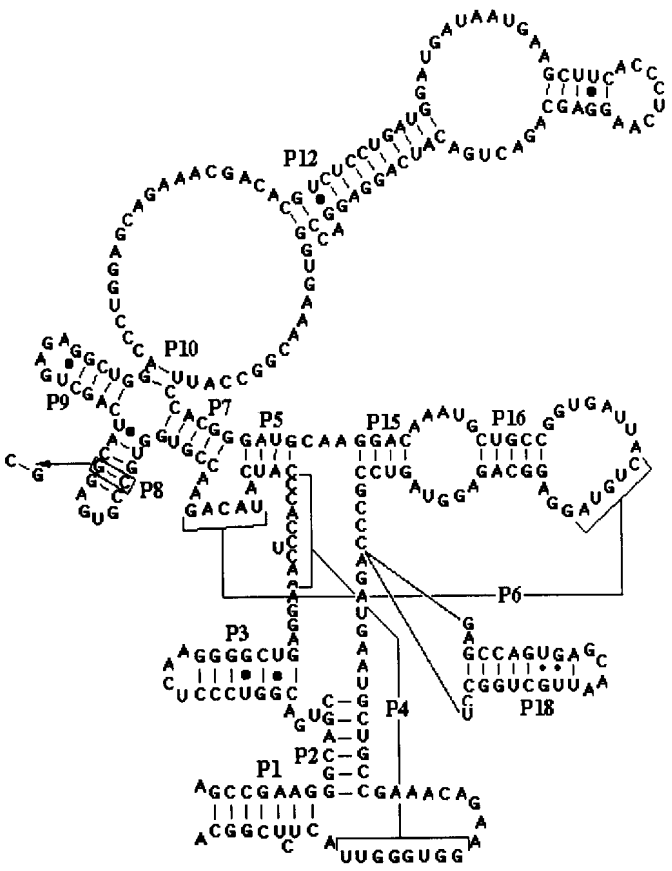

Figure 2. Secondary structure of *M. thermoautotrophicum*. The changes in P8 and the site of insertion of P18 in the chimeras are indicated.

(NH_4OAc) concentration from 1M to 3M. The increase in salt suggests that P18 assists in stabilizing the molecule but is not directly involved in catalytic activity. Helix P18 from *E. coli* was inserted into RNase P RNAs from the archaeon *Methanobacterium thermoautotrophicum*, but did not reduce the high mono- and divalent salt requirements of the molecule for catalysis; these chimeric RNAs were less catalytically active than the native RNA. However, an interesting finding was that it is necessary to establish the P8 and P18 tertiary interaction in order to maintain catalytic activity comparable to the native RNA. We propose that the addition of helix P18 into the molecule further destabilizes the RNA unless the helix is anchored into position by interaction with P8. The next step will be to insert helix P14, establish its tertiary interaction with P8, completing the tertiary structure of the region. Another approach will be to use *in vitro* selection techniques to generate mutant RNase P RNAs that function in moderate ionic conditions. *In vitro* selection has proven very efficient in assaying large sets of mutations introduced into RNase P RNAs of Bacteria (12, 13). This will involve the use of a self-cleaving conjugate containing the RNA from the archaeon *Methanobacterium formicicum* RNase P RNA, and its substrate, precursor tRNA (cpPT RNA).

ACKNOWLEDGEMENTS

I would like to thank Dr. James Brown for his support and guidance as my mentor. In addition, I thank Dr. Elizabeth Haas, Tom Hall and Andy Andrews for their constant technical advice. Lastly, it is important that I acknowledge Kenya Stokes and Leslie Hadley for their continous words of encouragement in all of my scientific endeavors. This work is supported by NIH grant GM52894.

REFERENCES

1. LaGrandeur, T.E., Darr, S.C, Haas, E.S., and Pace, N.R. (1993) *J. Bacteriol.* **175**: 5043-5048.
2. Pannuci, J.A., Haas, E.S., Hall, T.A., Harris, J.K. and Brown, J.W. (*in press*) *Proc. Natl. Acad. Sci.USA.*
3. Nolan, J.M. and Pace, N.R. (1996) *Nucleic Acids & Mol. Biol.* **10:** 109-128.
4. Haas, E.S., Banta, A.B, Harris, J.K., Pace, N.R. and Brown, J.W. (1996) *Nucleic Acids Res.* **24:** 4775-4782.
5. Gutell, R.R., Power, A., Hertz, G.Z., Putz, E.J. and Stormo, G.D. (1992) *Nucleic Acids Res.* **20:** 5785-5795.
6. Siegel, R.W., Banta, A.B., Haas, E.S., Brown, J.W. and Pace, N.R. (1996) *RNA.* **2:** 452-462.
7. Haas, E.S., Armbruster, D.W., Vucson, B.M., Daniels, C.J. and Brown, J.W. (1996) *Nucleic Acids Res.* **24:** 1252-1259.
8. Chen, J-.L. and Pace, N.R. (1997) *RNA.* **3:** 557-560.
9. Darr, S.C., Zito, K., Smith, D. and Pace, N.R. (1992) *Biochemistry.* **31:** 328-333.
10. Haas, E.S., Brown, J.W., Pitulle, C. and Pace, N.R. (1994) *Proc. Natl. Acad. Sci. USA.* **91:** 2527-2531.
11. Brown, J.W., Nolan, J.M., Haas, E.S., Rubio, M.T., Major, F. and Pace, N.R. (1996) *Proc. Natl. Acad. Sci. USA.* **93:** 3001-3006.
12. Frank, D. N., Ellington, A.E. and Pace, N.R. (1996) *RNA.* **2:** 1179-1188.
13. Frank, D.N. and Pace, N.R. (1997) *Proc. Natl. Acad. Sci.USA.* **94:** 14355-14360.