Supplemental Data

Structure and Function of Eukaryotic Ribonuclease P RNA

Steven M. Marquez, Julian L. Chen, Donald Evans and Norman R. Pace

Supplemental Figure

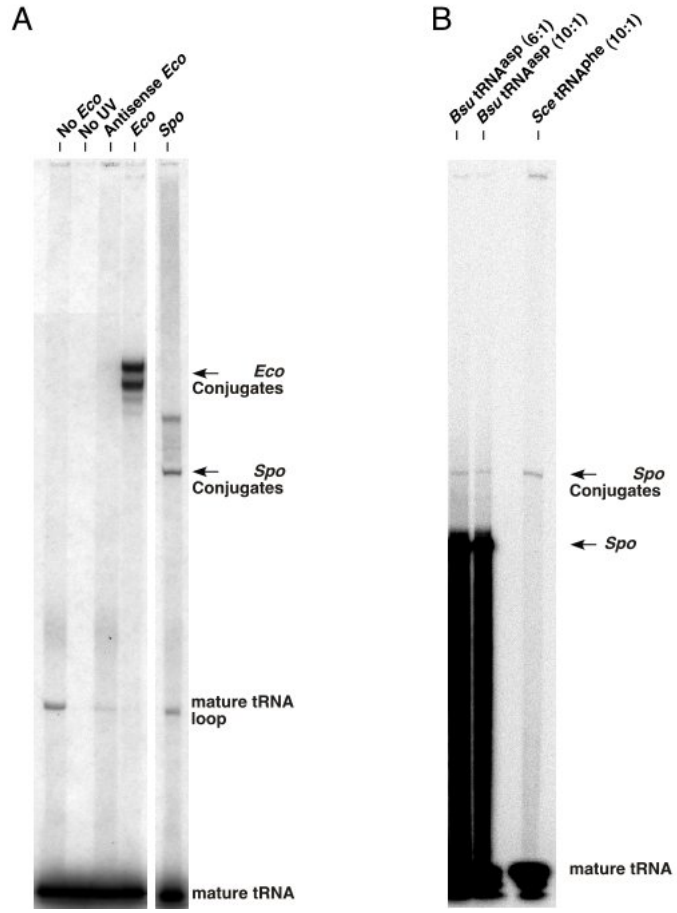

Figure 1S. *S. pombe* RNase P RNA crosslinks to precursor tRNA and *S. cerevisiae* tRNA. (A) 5'-arylazido-*B. subtilis* precursor-tRNA^{asp} (containing a 33 nt leader sequence) crosslinks to *S. pombe* RNase P RNA. All reactions are identical except: (1) no *E. coli* RNase P RNA, (2) the reaction contained *E. coli* RNase P RNA but was not exposed to UV, (3) RNA complementary to *E. coli* RNase P RNA was included instead of *E. coli* RNase P RNA, (4) *E. coli* RNase P RNA and (5) *S. pombe* RNase P RNA was included. (B) Photoactive-5'-6-thioguanosine bacterial and eukaryal tRNAs crosslink to

S. pombe RNase P RNA. All the reactions were identical except: (1) The reaction contained uniformly labeled *S. pombe* RNase P RNA and unlabeled *B. subtilis* mature $tRNA^{asp}$ (from a transcription reaction with a ratio of s6G to GTP of 6:1), (2) same as 1 but the ratio of s6G to GTP was 10:1, (3) *S. cerevisiae* $tRNA^{phe}$ was uniformly labeled in a transcription reaction with a ratio of s6G to GTP of 10:1.

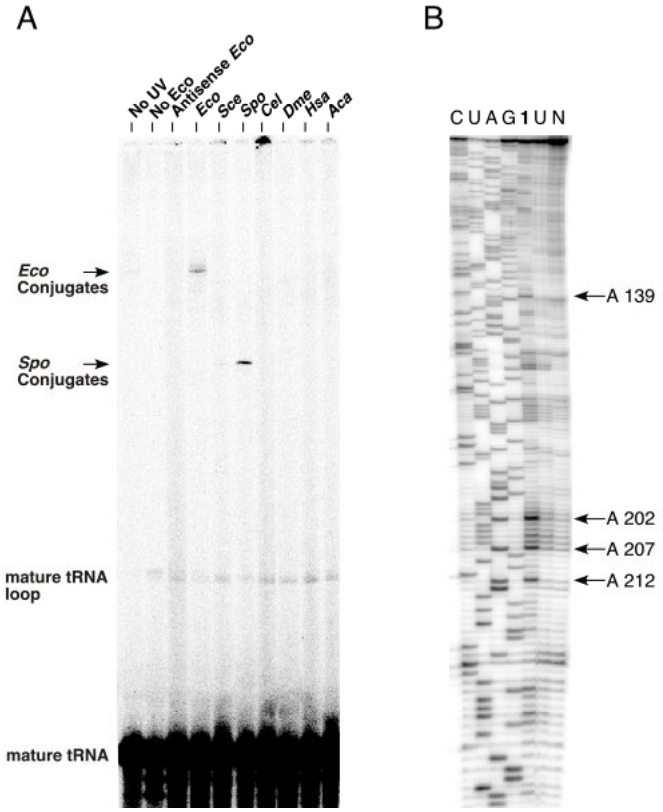

Figure 2S. Photoactive-5'-6-thioguanosine *Bsu* $tRNA^{asp}$ crosslinks to *S. pombe* RNase P RNA. (A) All the reactions were identical except: (1) The reaction contained *E. coli* RNase P but not exposed to UV, (2) no *E. coli* RNase P RNA, (3) RNA complementary to *E. coli* RNase P RNA was included instead of RNase P RNA, (4) *E. coli* RNase P RNA, (5) *S. cerevisiae* RNase P RNA, (6) *S. pombe* RNase P RNA, (7) *C. elegans* RNase P RNA, (8) *D. melanogaster* RNase P RNA, (9) *H. sapiens* RNase P RNA and (10) *A. castellanii* RNase P RNA was included. (B) The *S. pombe* RNase P RNA-5'- s^6G-tRNA conjugates were analyzed by primer extension. Unlabeled *S. pombe* RNase P RNA- s^6G-

tRNA conjugates were prepared, purified and quantified as described in Experimental Procedures. Lane 1 contains primer extension products using oligonucleotide 250R. Lanes C, U, A, and G correspond to sequencing reactions with non-crosslinked RNA template, lane U contains primer extension products from unconjugated *S. pombe* RNase P RNA isolated from the preparative gel and lane N is a control primer extension without dideoxynucleotides of unmodified *S. pombe* RNase P RNA. The termination sites of primer extension are indicated to the right of each gel. Equal amounts of *S. pombe* RNase P RNA were added to each primer extension reaction in lanes 1, U and N.

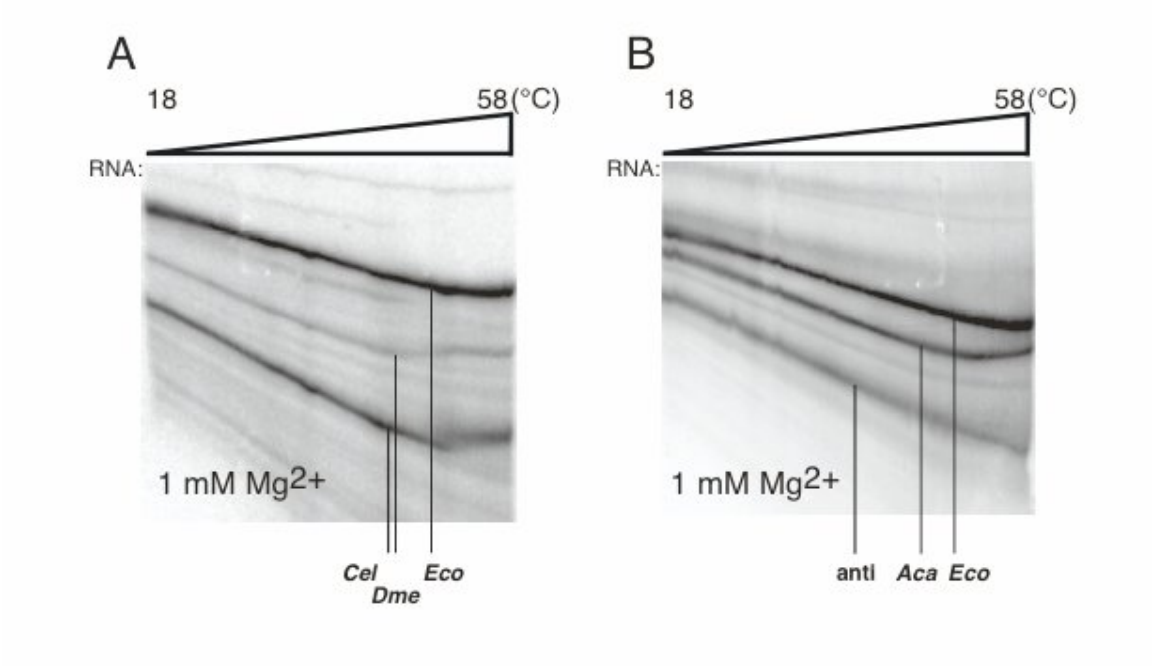

Figure 3S. Eukaryotic RNase P RNAs are less stable than Bacterial RNase P RNAs (A) TGGE (1X THE pH 7.4, 4.5% acrylamide, 100 mM NH_4OAc, 1 mM MgOAc) with *Eco*, *Escherichia coli*; *Dme*, *Drosophila melanogaster* and *Cel*, *Caenorhabditis elegans* RNAs. The temperatures at which the RNAs unfold are indicated. (B) TGGE with *Eco*; *Aca*, *Acanthamoeba castellanii* and antisense *E. coli.* Antisense *E. coli* is RNA complimentary to *E. coli* RNase P RNA and presumably represents a non-functional, unfolded RNA - consistent with the observation that throughout the temperature range no deflection is seen.

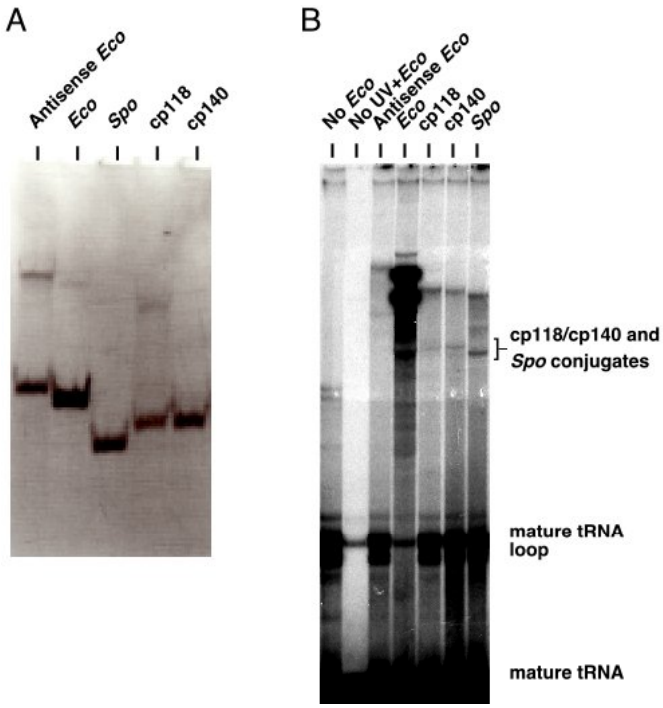

Figure 4S Circularly permuted *S. pombe* RNase P RNAs are globally folded and bind tRNA. (A) cp118 and cp140 comigrate on native gels. The native gel mobility of cp118 and cp140 were compared to each other. RNAs were folded and analyzed on 4.5% acrylamide 1X THE native gels containing 100 mM NH_4OAc and 1 mM Mg^{2+}. The gel was stained with ethidium bromide and the RNA was visualized with ultraviolet light. The fact that cp118 and cp140 comigrate as a single species suggests that cp140 crosslinks, not consistent with the bacterial structure, are not the result of a globally misfolded P RNA. (B) 5'-arylazido-*B. subtilis* $tRNA^{asp}$ cross-links to cp 118 and cp140 RNase P RNAs. All the reactions were identical except: (1) The reaction did not contain *E. coli* RNase P, (2) contained *E. coli* RNase P RNA but was not exposed to UV, (3) RNA complementary to *E. coli* RNase P RNA was included instead of RNase P RNA, (4) *E. coli* RNase P RNA, (5) cp118 RNase P RNA, (6) cp140 RNase P RNA, (7) *S. pombe* RNase P RNA. Based on the extent of crosslinking, it is approximated that cp118 and cp140 bind tRNA at 25% and 50% the level of native *S. pombe* RNase P RNA, respectively.