

***In vitro* selection identifies a large RNA with an incredible propensity to crystallize**

Kara Juneau, Elaine Podell, Daniel J. Harrington, and Thomas R. Cech
Howard Hughes Medical Institute, University of Colorado

An *in vitro* selection system was developed based on RNA tertiary structural stability, independent of RNA activity. The P4-P6 domain from the *Tetrahymena thermophila* group I intron, whose 2.8Å crystal structure was solved by Cate et al.¹, was partially randomized. Molecules were selected for their ability to fold into compact structures using native gel electrophoresis in the presence of decreasing quantities of MgCl₂ (10 mM - 1.1 mM). The selection identified a single deletion of C209 within the P4 helix, which significantly stabilized tertiary folding of P4-P6². Considering the proposed correlation between stability and crystallizability, we set up a sparse matrix with the ΔC209 mutant. Indeed, it crystallized much more rapidly and reproducibly than the wild-type P4-P6, and in a much wider variety of precipitants (MPD; hexanediol; PEG 400-8000; Li₂SO₄; and ethanol), pH's (6.0-8.0), polyamines (spermine, spermidine, and putrecine) and temperatures (4, 21, 25, 30, and 37°C). Crystals of ΔC209 diffracted to 2.25Å and the structure was solved. These results, coupled with analysis of another more-stable mutant (ΔA210), suggest that stabilizing the secondary structure of RNA can help stabilize tertiary structure; additionally, *in vitro* selection may provide a general approach for obtaining crystallizable RNAs. Detailed analysis of the ΔC209 structure has revealed an intricate solvent scaffolding that functions to stabilize the overall RNA fold (see Figure 1). The solvent-RNA interactions in P4-P6 may be generalizable to other nucleic acids, thus improving our total understanding of nucleic acid structure and folding.

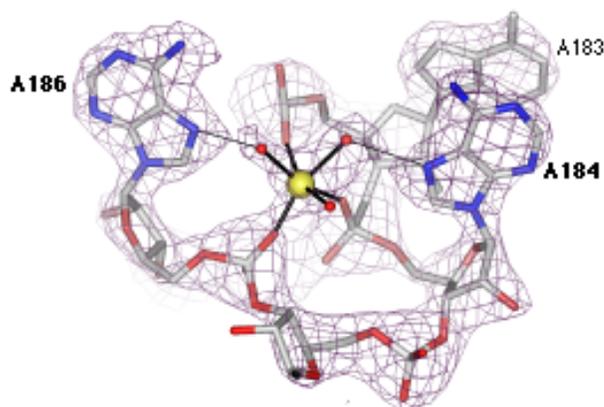


Figure 1: Inner- and outer-sphere coordination by the same magnesium ion. This magnesium ion located in the A-rich bulge of the AC209 RNA bends the RNA around itself using both inner-sphere coordination to three phosphate oxygens (nucleotides A183, A184, and A186) and a water-mediated, outer-sphere coordination to the N7 positions of A184 and A186. At 2.25 Å resolution, the hydration shell of the magnesium is distinct and the geometry of the coordination is well defined.

REFERENCES

1. J.H. Cate, A.R. Gooding, E. Podell, K. Zhou, B.L. Golden, C.E. Kundrot, T.R. Cech, and J.A. Doudna, *Science* 273, 1678 (1996).
2. K. Juneau and T.R. Cech, *RNA* 5, 1119 (1999).

This research was supported by a grant from Howard Hughes Medical Institute to T.R.C., and grant GM28039 from the National Institute of Health to K.J.

Principal Investigator: Thomas R. Cech, Howard Hughes Medical Institute, University of Colorado. Email: thomas.cech@colorado.edu. Telephone: 303-492-8606.