July 16-21, 2017 at UC San Diego, CA, USA

Identification of the NTP binding site in the polymerase ribozyme

Arvin Akoopie[1] and Ulrich F. Müller[1],
[1]Department of Chemistry % Biochemistry, University of California, San Diego
* e-mail of Correspondence Author: ufmuller@ucsd.edu

The RNA world hypothesis describes an early stage in the evolution of life in which RNA would have served as genome and the only genome-encoded catalyst. RNA world organisms would have required catalysts for the template-dependent polymerization of RNA polymer for the replication of RNA polymers. A ribozyme (catalytic RNA) for the template-dependent polymerization of RNA was developed by the Bartel lab [1].

The structure of this polymerase ribozyme has been partially elucidated. Crystal structures of the catalytic core show the positions of critical residues and functional groups contributing to catalysis [2]. The structure of the ribozyme's accessory domain, which is important for binding of nucleoside triphosphates (NTPs), has not been determined. Results from the Unrau lab suggest that NTPs are bound by a purine-rich loop in the accessory domain [3].

To test the location of NTP binding we performed an in vitro evolution experiment of the polymerase ribozyme in the presence of a modified NTP, 6-thio GTP (6sGTP). After ten rounds of evolution, 27 clones were analyzed for their sequence, and their ability to utilize 6sGTP. All clones showed a specific mutation in the purine-rich loop. The most efficient clone was ~200-fold more efficient in utilizing 6sGTP, and contained six mutations. Reverting the mutation in the purine-rich loop reduced activity to within ~2-fold of the starting construct, and introducing this mutation alone into the starting construct raised activity to ~3-fold within the activity of the most active clone. SHAPE analysis suggested a change in flexibility of the purine-rich loop by the mutation. Together, these results confirm that the purine-rich loop of the polymerase ribozyme is the central part of the accessory domain responsible for binding NTPs.

References

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