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IN VITRO SELECTION OF NOVEL LIGASE RIBOZYMES

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Dinucleoside 5'-5'-pyrophosphates may have played an important role in prebiotic and RNA world chemistry (Orgel, 1986; Orgel, 1991). The utilization of AMP-activated oligonucleotide substrates by modern enzymatic ligases (Lehman, 1974) may be indicative of this early importance of pyrophosphate structures. In order to investigate the ability of RNA to catalyze a ligation reaction analogous to that of modern ligase enzymes, an *in vitro* selection from a pool of 10^{15} different RNA molecules was begun. The RNA pool sequences consisted of a 30 nucleotide mutagenized ATP aptamer domain (Sassanfar, 1993) incorporated within the context of 210 random nucleotides. After four iterative rounds of selection, a variety of ribozymes capable of ligating RNA to RNA "capped" with an adenosine 5'-5'-pyrophosphate structure have been isolated. Characterization and optimization of the ribozymes is currently in progress.

Lehman, I.R.: 1974, Science 186, 790.
Orgel, L.E.: 1986, Journal of Theoretical Biology 123, 127.
Rodriguez, L. and Orgel, L.E.: 1991, Journal of Molecular Evolution 32, 101.
Sassanfar, M. and Szostak, J.W.: 1993, Nature 364, 550.