HAMMERHEAD RIBOZYME LIGATION OF RNA IN FROZEN SOLUTION. L. Lie[1] and R. M. Wartell[1]
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Abstract: The RNA World Hypothesis proposes that RNA played a central role in the origin of life, serving as both catalysts and self-replicating information storage. Although considerable evidence supports RNA as the key precursor of current genetic systems, its inherent fragility in aqueous conditions poses a difficulty for the emergence of self-replicating RNA. Previous work has shown that ice provides an environment that minimizes RNA degradation yet supports condensation of activated nucleotides into RNA oligomers[1], RNA ligation by the hairpin ribozyme[2], and RNA synthesis by a RNA polymerase ribozyme[3].

The current study examined the activity of a 49 nt hammerhead ribozyme in frozen solution. The *Schistosoma mansonii* ribozyme (Fig 1), which predominantly cleaves RNA, can ligate its cleaved products (P1 and P2) with yields up to ~23% in single turnover experiments at 25 ^{o}C. $Mg^{2+}$is required for cleavage and ligation. Our studies show that in frozen solutions lacking divalent metal ions the hammerhead ribozyme ligates RNA oligomers. Anions with carboxylate groups and properties associated with strong ion-water interaction and high solubility enhanced ligation.

Yields up to 43% were observed in one freeze-thaw cycle and a maximum of 60% was obtained after several freeze-thaw cycles. P2 substrates with a mixture of bases at 5 of its 16 nucleotides, as well as truncated and mutated P1 substrates were ligated by the ribozyme in frozen solutions. 297 of 1024 possible P2 sequences were represented in ligated products. Our results indicate that frozen solutions provide conditions where the hammerhead ribozyme can generate an ensemble of diverse RNA sequences from shorter RNA oligomers.

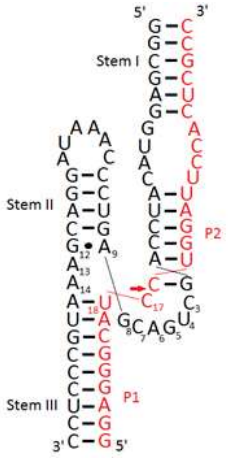

Figure 1

References::

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