Diversifying the Portfolio for RNA Catalysis: Modification of Nucleobase by an RNA Enzyme . R. R. Poudyal[1], P. D. M Nguyen[1], M. K. Callaway[2], D. H. Burke[131]Department of Biochemistry (rrpfz5@mail.missouri.edu) , [2]Department of Engineering, [3]Life Sciences Center (burkedh@mail.missouri.edu)., University of Missouri, Columbia USA

The RNA world hypothesis posits that Ribonucleic Acids (RNA) served as the repository for genetic material and as catalysts during the early evolution of life and metabolism. Phosphoryl transfer is one of the key reactions in metabolic pathways, signal transduction and gene regulation in all domains of contemporary life. Ribozyme 1.140 is an artificial ribozyme that came out of an in vitro selection for self-thiophosphorylation. The ribose 2'OH is also important in many functional RNA molecules for hydrogen bonding, metal ion coordination, self-cleavage and other functions. To understand the role of 2'OH in ribozyme 1.140, we uniformly substituted all A, C, G or U nucleotides with 2'F nucleotide analogs, yielding four 2'F-substituted ribozyme constructs. Catalytic property of the ribozyme was unaffected in the 2'F version of the molecule. Previous work showed that activity of the all-ribose ribozyme was stimulated by higher pH. The pH dependence of the 2'F- substituted ribozymes was indistinguishable from that of the all-ribose ribozyme.

Separating the catalytic and substrate functions into individual polynucleotide chains provided opportunities for studying the reaction in more detail through substitution of substrate analogs. Surprisingly, both RNA and DNA substrate strands were modified by the ribozyme with similar observed rate constants, even though DNA lacks the 2'OH that serves as phosphoryl acceptor in all other known kinase ribozymes. These results, along with primer-extension mapping, established the nucleobase of the G2 as the site of modification. Substrate analogs with 7-deaza-dG or 2-Aminopurine at this position showed evidence of modification catalyzed by the ribozyme, while Inosine abolished modification, thus establishing that N2 exocyclic amine is the likely site of adduct formation. This is the first observation of ribozyme-catalyzed covalent modification of the nucleobase. In modern metabolism, nucleobase phosphorylation is catalyzed by protein kinases during nucleotide biosynthesis; study of this ribozyme could help us understand mechanisms utilized by kinase ribozymes to catalyze early biosynthetic reactions.