In vitro Evolution of Regio-specific Nucleoside Kinase Ribozymes that target a quasi-diffusible substrate.

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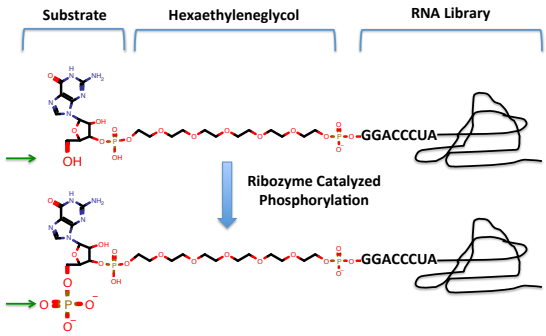
The RNA World hypothesis posits that RNA played an important role during the origin and evolution of life by acting as both genetic information carrier and catalyst. The existence of catalytic RNAs and regulatory RNAs strengthens this hypothesis and provides RNA tools for synthetic biology. As phosphoryl transfer is one of the most fundamental chemical reactions in modern biology, the study of Kinase RNA enzymes (Ribozymes) is of great interest.

In vitro evolution of RNA molecules has yielded many kinase ribozymes, however previously used strategies have had no constraints on phosphoryl acceptor sites. Ligation based strategy have been used previously to select for DNAzymes that self-phosphorylate the 5'OH of the polynucleotide chain[1]. Our lab has developed a similar ligation based strategy for the selection of nucleoside kinase ribozymes that specifically requires the evolving ribozymes to phosphorylate the 5'OH of a tethered mononucleoside. In addition, the rationally designed libraries positions ATP and GTP aptamers within a structural scaffold that is organized by a tetraloop-tetraloop receptor element to nucleate folding and to increase the frequency of active ribozymes. After 8 rounds of selection, we generated ribozymes that use ATP as the donor and phosphorylate the 5'OH of a Guanosine that is linked to the ribozymes via hexaethylene glycol (HEG). Finally, the conformational and diffusional entropy of the HEG-linked substrate was intended to mimic some of the challenges of constraining a freely diffusible substrate within the active site. Current efforts include investigating phosphorylation of free guanosine by our newly selected kinase ribozymes. Our study will enable us to better understand the capabilities and limitations to catalyze chemical reactions in a multiple turnover fashion.



References:

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