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

# Advancing Polymerase Ribozymes Towards Self-Replication

K. F. Tjhung[1,2] and G. F. Joyce*[1]
[1]Jack H. Skirball Center for Chemical Biology and Proteomics, The Salk Institute, La Jolla, CA
[2]Department of Chemistry, The Scripps Research Institute, La Jolla, CA
* gjoyce@salk.edu

RNA-catalyzed replication has long been hypothesized as the basis of RNA life.[1] RNA bridges the gap between genotype (DNA) and phenotype (protein), as RNA acts as both the information carrier and the enzyme. We aim to achieve sustained autocatalytic replication and evolution *in vitro* through two avenues: (i) a general cross-chiral RNA polymerase ($Pol_L$) that can catalyze template-directed polymerization of activated mononucleotides (NTPs) of the opposite handedness, enabling PCR-like amplification of nuclease-resistant, L-RNA in the short-term and enabling the long-term goal of cross-chiral replication; (ii) non-covalent assembly of component fragments of an existing homochiral RNA polymerase ribozyme.

(i) Our laboratory developed the first example of an enzyme with cross-chiral activity that has achieved template-directed assembly of its own enantiomer from oligonucleotide building blocks, but has limited ability to polymerize mononucleotides.[2] Beginning with a library (~$10^{15}$ diversity), we selected for a population of $Pol_L$ composed of D-RNA that can achieve template-directed polymerization of multiple monomers of L-RNA. After 26 rounds of selection, $Pol_L$ is capable of polymerization using all 4 L-NTPs, with particular efficiency in adding L-GTP.

(ii) The 24-3 D-RNA polymerase ribozyme is the most robust RNA polymerase ribozyme published to date.[3] However, it is unable to synthesize itself in its entirety. The ribozyme has been split into four fragments that assemble non-covalently to form a functional RNA polymerase ribozyme that is capable of synthesizing short RNA products.

[1] Crick FH (1968) *Journal of Molecular Biology* 38:367–379. [2] Sczepanski JT and Joyce GF (2014) *Nature* 515:440-442. [3] Horning DP and Joyce GF (2016) *Proceedings of the National Academy of Sciences USA* 9786-9791.

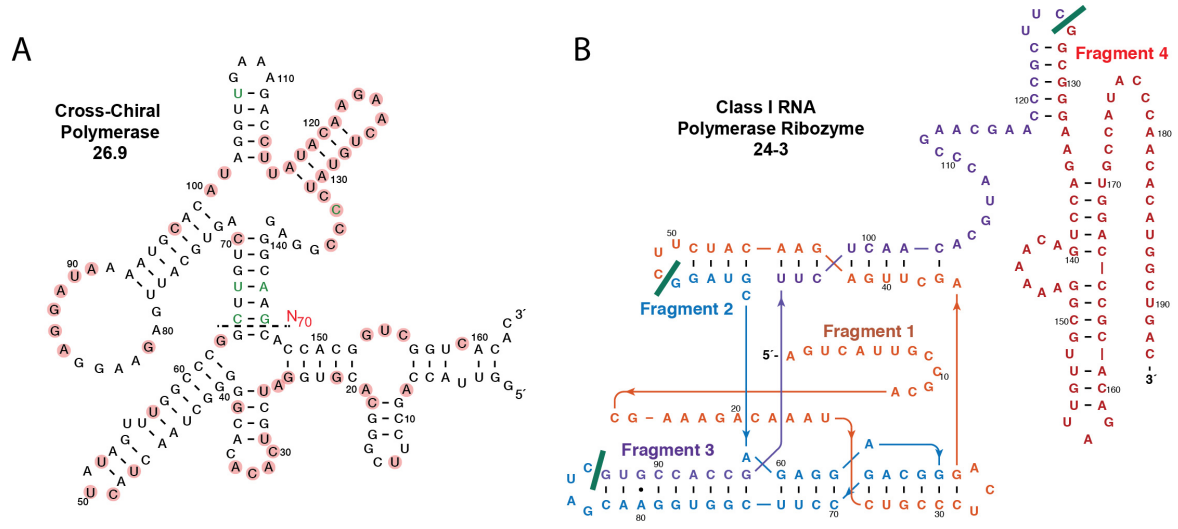


**Figure 1** – RNA polymerase ribozymes evolved *in vitro*. (A) A cross-chiral RNA polymerase ribozyme composed of D-RNA, capable of template-directed polymerization of enantiomeric L-RNA. (B) An RNA polymerase ribozyme capable of template-directed synthesis of such structured, functional RNA as aptamers and tRNA.