# *In vitro* Evolution and Engineering for Improved Ribozyme polymerase Production

*Israr Khan[1], Muhammad Waheed Akhtar[2] and Ayaz Ali Khan[3]

[1,2] University of the Punjab, School of Biological Sciences, 54590, Lahore, Pakistan.

[3] University of Malakand, Department of Biotechnology, Khyberpakhtunkhwa, Pakistan.

*Corresponding author E mail: khanisrar07@gmail.com



The biggest challenge for origin-of-life scientists is to account for the first self-replicating molecule. Genes are needed to make proteins and proteins are needed to make genes, so which came first? The answer is in ribozymes probably, RNA molecules capable of both coding and catalysis. An earlier, simpler biology might have relied on RNA for both heredity and metabolism. Evidence for such an **"RNA world"**, preceding modern life includes the central catalytic and informational roles of RNA in splicing, gene expression, and translation, as well as the versatility of RNA in forming specific receptors and catalysts. Forming the glycosidic link between the nucleoside bases and ribose is thought to be the most difficult step in prebiotic nucleotide synthesis in the presumed scarcity of RNA nucleotides on the prebiotic earth. Organisms of the putative RNA world would have required an RNA polymerase ribozyme for both RNA-based heredity and the expression of "RNA genes". **In vitro evolution** of nucleic acids involve typically randomized synthetic DNA libraries that are either selected directly for desired binding or catalytic activities, or are transcribed into RNA prior to selection. The DNA –encoding molecules with desired activities are amplified (and in some cases intentionally diversified) either by PCR or by reverse transcription followed by PCR. The

resulting enriched DNA pool then re-enters the in vitro evolution cycle. (Wochner *et al*.,2011)

**Table 1: Characteristics of some engineered ribozyme polymerases**

| RIBOZYME | R18 | C19 | tC19 | Z | tC19Z |
|---|---|---|---|---|---|
| **Size (bases)** | 190 bases | 269 | 199 | 221 | 198-bases |
| **Origin** | class I ligase ribozyme | R18 | C19 | R18 | tC19/Z |
| **Activity** | sequence-dependent | template-dependent | | | general |
| **Polymerization(nt)** | 14 nucleotides | 19-95 nt | 21 nt | 40 nt | 70-280 nt |
| Structure | | | | | C60U, G93A, G95A, A159C and 5′-extension GUCAUUGAAAA) to R18 |
| **fidelity** | $4.3 \times 10^{-2}$ | About1.4 fold | $2.7 \times 0^{-2}$ 1.6fold | About 3 fold | $8.8 \times 0^{-3}$ 5-20 fold |

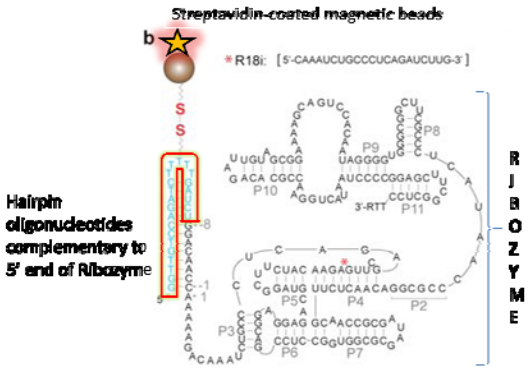


Figure 2. Secondary structure of the ribozyme polymerase R18i

As far as the origin of these polymerases is concerned, Class I Ligase was obtained from the parental group I introns engineering. R18 was isolated from a random sequence pool by in vitro evolution and stepwise engineering of the initial class I ligase ribozyme. Although R18 is a general RNA polymerase, its activity is both sequence-dependent and limited to transcribing stretches of RNA up to 14 nucleotides (nt) long on a favorable RNA template . R18 is further engineered to R18i,C19, tC19, Z and finally the hybrid tC19Z that can efficiently polymerize upto 95 nucleotides. The tC19Z phenotype arises from the contributions of four mutations (C60U, G93A, G95A, and A159C), as well as a short 5′-extension (5′-GUCAUUGAAAA) to the parental R18 ribozyme. There is a lot of hope that having a ribozyme that can polymerize its own full sequence will give rise to an RNA/Ribozyme self Replication Model system. Such a system would mimic/proxy the earliest life self replicating molecule independent of any other polymers present in the ambient soup. For such a molecule to survive, replicate and exist in the ambient natural selection like conditions of directed evolution the system needed to be compartmentalized. Moreover such a closed system would retain any beneficial mutations arising without loss into the environment. One such system is the oil water emulsion system, having the hydrophobic ends inside and hydrophilic ends

outside forming closed circular compartments that can retain the ribozyme/RNA self **polymerization reaction** in a manner similar to the reaction catalyzed by proteins except the speed/velocity of the reaction. **Directed evolution** thus harness the power of natural selection to evolve proteins or RNA with desirable properties not found in nature. (Muller.,2006)

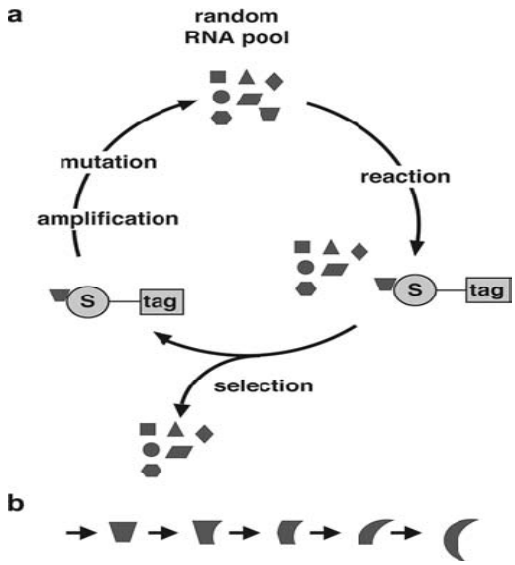


Figure 2: *In vitro* evolution of Ribozymes. (*a*) A pool of random RNAs is reacted with a substrate that is covalently linked to a tag. Those RNAs that reacted with the substrate/RNA in this case are selected via the tag and amplified by RT-PCR and transcription. Mutagenic PCR generates sequence variants that facilitate the improvement of selected ribozymes. (*b*) Repetitions of this cycle lead to the optimization of initially selected ribozymes.

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**References:**

Muller., U.F.(2006). Recreating an RNA World. *Cel Mol. Life Sci*., 63 1278-1293.

Wochner A, A. J., Coulson A, Holliger P. (2011). Ribozyme-Catalyzed Transcription of an Active Ribozyme. *Sci, 332*(6026), 209-212