Effect of co-solutes on template-directed enzyme-free copying of Ribonucleic acid

Niraja Bapat[1], Sudha Rajamani[1*]
[1]Indian Institute of Science Education and Research (IISER), Dr. Homi Bhabha Road, Pashan, Pune 411 008, Maharashtra, India, [*]E-mail of Corresponding Author: srajamani@iiserpune.ac.in

Introduction: The transition from chemistry to biology that resulted in the origin of life still remains a daunting mystery. A widely accepted hypothesis of the 'RNA world' [1] presumes that RNA played a key role during the emergence and evolution of early life on Earth by acting both as a genetic material, and a replicase that could self-propagate this genetic information. However, faithful replication of the encoded information would have been a crucial step for RNA molecule to have acted as an efficeint enzyme. It has been previously shown that the addition of non-cognate nucleotides during nonenzymatic replication stalls this process [2]. Furthermore, this initial misincorporation also leads to a cascade of mismatches [3], giving selective advantage to the accurately replicating nucleic acids. However, these studies were carried out in a chemically simple environment without accounting for the presence of any 'background molecules' in the reaction mixture. This scenario is not prebiotically realistic as the prebiotic soup would have been a heterogenous solution containing a mixture of many different molecules. Presence of molecular crowding agents is known to affect the kinetics of many contemporary biochemical reaction [4,5]. Hence, it becomes important to analyze the effect of presence of co-solutes on prebiotically relevant nonenzymatic reactions.

Results: We report here the effect of presence of Poly Ethylene Glycol (PEG) and double chain surfactant lipid as co-solutes on enzyme-free template-directed RNA primer extension reactions using 5'-imidazolides as monomers.

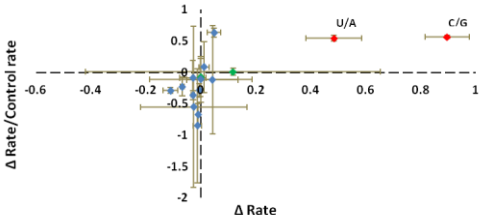

Fig. 1: The 'Δ Rate' (rate of control reaction minus the rate of reaction in presence of both the co-solutes) is significantly different from zero for addition of 'G across C' (point C/G) and addition of 'A across U' (point U/A)

It was observed that the rate of primer extension was slowed down in reactions which involved a 'matched' addition of a purine across the cognate template base (Fig. 1). Importantly, combined use of PEG and lipid in these reactions led to a greater decrease in the aforementioned extension rates in comparison to when each moeity was used alone. We envisage that the diffusion of the potentially stacked purine monomers is possibly reduced in the presence of co-solutes, thus resulting in a slower extension of the primer. Efforts are ongoing to dissect the underlying cause of this phenomenon using pertinent biophysical techniques.

Furthermore, we also observed that reactions involving the addition of a mismatched monomer across the non-cognate template base, were not notably affected (Fig. 1). This resulted in elevated frequency of misincorporations, specifically against 'C' and 'U' template bases (Fig. 2). The mutation rate in the presence of co-solutes was found to be higher than what is observed under control reaction conditions. It, therefore, is critical to consider the heterogeneity of the prebiotic soup while studying pertinent enzyme-free reactions. Our results suggest direct implications for efficient replication of functional nucleic acid sequences in a complex prebiotic milieu.

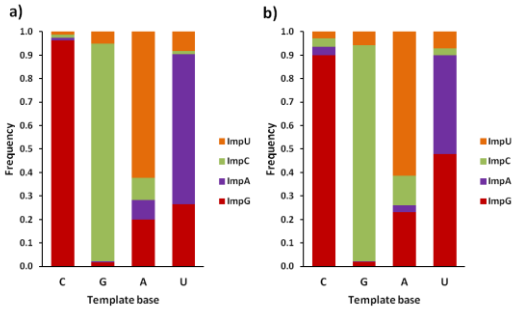

Fig. 2 : Incorporation frequencies for addition of cognate and non-cognate bases. a) In the absence of any co-solutes. b) In the presence of lipid and PEG as co-solutes

References:

[1] Gilbert W. (1986) *Nature, 319,* 618. [2] Rajamani S. et al. (2010) *JACS, 6,* 1008-1011. [3] Leu K. et. al. (2013) *JACS, 135,* 354-366. [4] Ellis R. J. (2001) *Trends Biochem. Sci. 26,* 597-604. [5] Minton A. P., (2001) *J. Biol. Chem., 276,* 10577-10580. [6] Bapat N. V. and Rajamani S. (2015) *J. Mol. Evol., 81,* 72-80.