RIBOZYME EVOLUTION IN AN RNA-MINERAL WORLD J. D. Stephenson[1], M. Popović[1,2], T. F. Bristow[1], and M. A. Ditzler[1] [1]Exobiology Branch, NASA Ames Research Center, Mail Stop 239-4, Moffett Field, CA 94035; mark.a.ditzler@nasa.gov, [2]Blue Marble Space Institute of Science, Seattle, WA 98145; milena.popovic@nasa.gov.

Abstract: Mineral surfaces can support several processes that likely play a role in the origin of life including the concentration, selective sorption[1], protection[2], organization[3-5], and polymerization[6] of organic molecules. Clay minerals are predicted to be present in prebiotic environments and provide a large surface area for reactions with organic molecules. Interactions between early biomolecules and clay minerals are likely and may play an important role in the emergence of life.

The many roles of RNA in contemporary biology, and evidence of even larger roles for RNA in the earliest forms of life[7,8] have motivated multiple investigations into the interactions between clay minerals and nucleic acids. Montmorillonite clay minerals have been shown to bind RNA[1], polymerize activated monomers[6,9], and drive the formation of RNA encapsulating vesicles[3,10]. Additionally, molecular dynamics simulations indicate that RNA folding is altered through its interaction with mineral surfaces[11], and experimental activity assays show the activity of certain ribozymes is altered by the presence of montmorillonite [12]. This suggests that clay minerals have the potential to both interfere with the folding of some functional structures and stabilize other structures that cannot properly fold without the mineral. The impact of clay minerals on RNA folding could facilitate the emergence of RNA based life by supporting a wider variety of functional structures. Alternatively, their impact on folding could present significant challenges to emerging life. For example, if evolution in the presence of a clay mineral leads to populations in which most RNAs cannot properly fold in the absence of that mineral, transitioning away from that mineral would be difficult.

To examine the impact of clay minerals on the evolution of functional RNAs we evolved multiple RNA populations *in vitro* in the presence or absence of montmorillonite[13]. The RNAs were evolved to catalyze self-cleavage. The RNA populations were evolved in parallel, along separate evolutionary trajectories, starting from a shared, multi-copy population of random sequences. The populations evolved in the presence of montmorillonite are strikingly similar to populations evolved in the absence of montmorillonite. The populations have similar levels of activity after the same number of rounds of in vitro evolution. They are largely composed of the same sequences and those sequences exhibit similar levels of catalysis in both the presence and absence of montmorillonite. These results indicate that montmorillonite has a minimal impact on RNA folding. This suggests that folding in the presence of montmorillonite does not improve RNA's ability to evolve functional structures; however, it also suggests that RNAs that evolve in contact with montmorillonite can adopt the same structures in mineral-free environments. This could allow for a smooth evolutionary transition away from mineral surfaces to environments more like contemporary cellular environments.

While the presence of montmorillonite had surprisingly little impact on the evolution of ribozymes that catalyze self-cleavage, our preliminary experiments with ligase ribozymes suggest that intermolecular functions may be more strongly impacted by the presence of montmorillonite than the intramolecular self-cleavage reaction. Additionally, other mineral surfaces vary in their characteristics [9,14] and may have different impacts on RNA folding and evolution. Future experiments with both additional minerals and functions are likely to further improve our understanding of the potential role of RNA-mineral interactions in the origin of life.

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