The influence of environment in shaping RNA fitness landscapes

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The fitness landscape, first proposed by Sewall Wright [1], is a classic concept in evolutionary biology. Fitness refers to the ability of organisms to survive and reproduce in a given environment. In the space of all possible sequences, the mapping of genotypes to their fitness values results in a fitness landscape. This idea can be applied to understand the evolution of nucleic acids. In a typical experiment, one would construct variants of the biopolymer with all possible mutations along its full length or around the active site, and then experimentally determine which mutations preserve, enhance or diminish the catalytic activity. The result is a fitness landscape for the biopolymer in a given environment.

Life is believed to have originated using RNA-based genetic and metabolic systems [2-4]. RNA can store and pass on genetic information, as seen in RNA viruses. Beginning in the 1990s, RNAs have also been discovered to catalyze a variety of fundamental biochemical reactions. Therefore, it is crucial to understand RNA fitness landscapes. Experimental efforts to determine comprehensive fitness landscapes for long RNAs are severely limited due to the astronomical size of sequence space. Therefore, experiments along this line have focused on smaller, functional RNAs. While much work has already been devoted to understanding fitness landscapes under a given condition [5], little is known about how these fitness landscapes respond to changes in their environment. What little we do know suggests that seemingly innocuous changes in chemical environment may give rise to tectonic shifts in the fitness landscape of a given functional RNA.

Typically, *in vitro* selection (or directed molecular evolution) experiments to identify ribozymes are performed in physiological or saturating concentrations of Mg^{2+}. This is because Mg^{2+} plays crucial roles in extant biology. RNA requires Mg^{2+} for effective folding and function. When large RNAs fold into compact structures, negatively charged phosphate groups achieve close proximity. Folded RNAs are stabilized, in part, by inorganic cations that accumulate in and around the RNA envelope. “Diffuse” cations remain hydrated and make primary contributions to global stability by mitigating electrostatic repulsion of the negatively charged backbone. Chelated ions are less frequent, but in some instances are essential for achieving specific local conformation of the RNA. It is now known that Mg^{2+} plays important roles in folding of essentially all large RNAs [6,7]. In addition, Mg^{2+} ions assist directly in stabilizing transition states of some ribozymes [8,9]. Thus, Mg^{2+} is critical for functional RNAs we know of today. But is it the “optimal” divalent cation? Let us consider some alternatives.

The RNA world is believed to have flourished during the early Archean eon, prior to the Great Oxidation Event. This period in history was characterized by an anoxic environment in which iron was much more soluble and abundant than in our current oxidative environment. Life evolved in these conditions for a billion years before the rise of photosynthesis. The concentration of dissolved iron ($[Fe^{2+}]_{marine}$)in the ancient oceans is open to debate. Based on different geological models, the $[Fe^{2+}]_{marine}$ could be as high as 100-1000 μM [10,11], compared to 0.3–0.8 nM in the modern ocean [12]. It seems very likely that the early Archean earth provided a variety of Fe^{2+}-rich microenvironments. Fe^{2+}, when oxidized, generates insoluble Fe^{3+} and hydroxyl radicals that are detrimental to RNA stability. However, in the anoxic early earth ocean, Fe^{2+} would have been available, soluble and non-toxic.

How similar is Fe^{2+} to Mg^{2+} in its ability to support RNA structure and function? There is evidence that, under anaerobic conditions, Fe^{2+} can in fact substitute for Mg^{2+} in RNA folding and catalysis [13]. Quantum mechanical calculations indicate that RNA that forms multiple first-shell interactions with Mg^{2+} will not change conformation when Mg^{2+} is replaced by Fe^{2+}. Experimental evidence suggests that Fe^{2+} can replace Mg^{2+} in the structural and catalytic core of RNA (Figure 1).

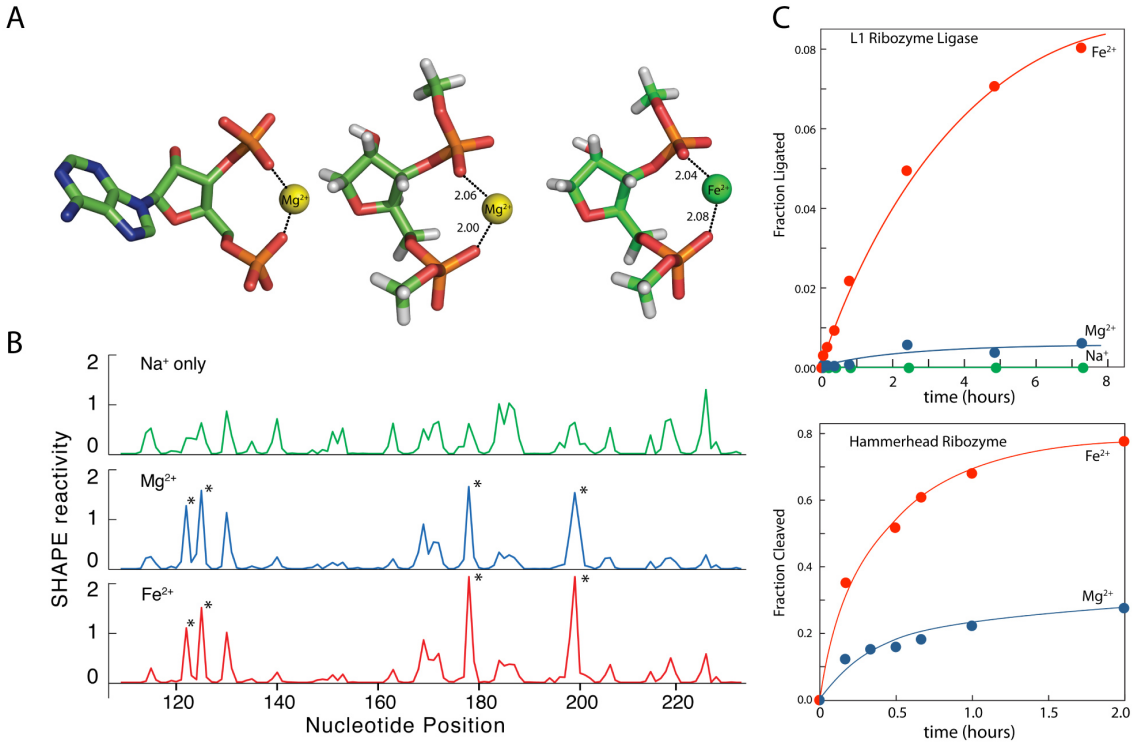

Figure 1. (adapted from [13]). Fe^{2+} can substitute for Mg^{2+} in RNA folding and catalysis. (A) Quantum mechanical calculations show that conformations of RNA-Mg^{2+} and RNA-Fe^{2+} clamps are identical. (B) RNA SHAPE probing of P4-P6 domain of the *T. thermophila* Group 1 intron in presence of Mg^{2+} and Fe^{2+} results in identical profiles. (C) Catalytic activity of L1

ligase and hammerhead ribozyme is enhanced when Mg^{2+} in the catalytic core is replaced by Fe^{2+}.

Fe^{2+}-mediated RNA folding and catalysis, in combination with paleogeological information, suggest that Fe^{2+}, either instead of or in combination with Mg^{2+}, seems to be a possible partner of RNA in the biology of the pre-photosynthesis anoxic earth. The RNA-Fe^{2+} complexes recently observed in extant biology [14] could be molecular fossils from the RNA world. The injection of Fe^{2+} into RNA World models opens broad new possibilities for ancient biochemistry. RNA and Fe^{2+} could potentially support an array of RNA structures and catalytic functions far more diverse than RNA with Mg^{2+} alone. Recent evidence demonstrates that RNA-Fe^{2+} complexes are capable of catalyzing electron-transfer reactions, a catalytic activity not possible in RNA-Mg^{2+} complexes [15].

In light of these observations, especially given that Fe^{2+} can uncover "latent" catalytic activity in RNA, it is interesting to ask how different RNA fitness landscapes would look if selection were done in the presence of Fe^{2+}. However, using *in vitro* selection, Zivarts et al. isolated five classes of allosteric hammerhead ribozymes [16]. Each of these ribozyme classes was activated by Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}. Interestingly, their allosteric binding sites rejected Mg^{2+}, Ca^{2+} and Sr^{2+}. Although the authors did not do the selection experiments in the direct presence of Fe^{2+}, apparently the selection relied on the 5-ppm Fe^{2+} contamination found in commercially available $MgCl_2$, effectively selecting in the presence of Fe^{2+}. Landweber and Pokrovskaya, using *in vitro* selection in the presence of high (60 mM) $MgCl_2$, isolated a ribozyme that catalyzes a template-directed RNA ligation reaction [17]. Surprisingly, they observed that substitution of Mg^{2+} with Mn^{2+} triggered the switch from ligase activity to self-cleaving activity (for which they had not been selected). This switch further supports the idea that different cationic conditions could lead to unexpectedly large functional changes.

Similarly, large effects have been triggered by cation substitution in DNA enzymes, in particular, the RNA-cleaving 8-17 DNA enzyme (DNAzyme). The 8-17 DNAzyme motif has been identified using *in vitro* selection in presence of 10 mM Mg^{2+} (called 8-17) [18], 0.5 mM Mg^{2+}/50 mM histidine (called Mg5) [19], or 100 μM Zn^{2+} (called 17E) [20]. The DNAzymes were found to be of different lengths and base compositions. Interestingly, it was found that all the variants were most active with a cation not present in the original selections, Pb^{2+} (8-17: k_{obs} ~0.5 min^{-1}; Mg5: k_{obs} ~2 min^{-1}; 17E: k_{obs} ~1 min^{-1} with 200 μM Pb^{2+} at pH 5.0) [21]. Peracchi introduced single- and double-site mutations at the core positions of the 8-17 DNAzyme, and examined the effects of those mutations on activity in the presence of Mg^{2+}, $Mn^{2+}$and Ca^{2+} [22]. All the variants in this case showed 20-fold higher reaction rates in 3mM Ca^{2+} and 50- to 150-fold higher rates in 3mM Mn^{2+}, compared to 3mM Mg^{2+} [22]. Vanella and Adriaens reported RNA-cleaving DNAzymes that were *in vitro* selected in the presence of Hg^{2+} and As^{5+} [23]. They found that the Hg^{2+}- and As^{5+}-dependent DNAzymes had catalytic rates similar to most Mg^{2+}-dependent nucleic acid enzymes under comparable conditions.

In conclusion, several studies suggest that the cationic environment of directed molecular evolution experiments affects the outcome, possibly dramatically. It will be interesting to study evolution of functional RNA under representative early earth conditions, i.e., in the presence of abundant Fe^{2+}. Once such ribozymes are characterized, they can be further subjected to selection in the presence of Mg^{2+}. The resulting changes in sequence and structure will shed new light on how metal substitutions affected fitness landscapes in the RNA world. Yet divalent cations represent only one dimension of the environment. Using allosteric selection, Koizumi et al. engineered RNA containing the hammerhead ribozyme capable of self-cleaving in the presence of 3',5'-cyclic monophosphates (cGMP and cAMP) [24]. Other dimensions, such as pH, water activity, fluctuations, or crowding, represent additional fertile ground for further exploration.

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