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Sequence Duplication as an Evolutionary Mechanism in Functional RNAs

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Introduction: RNA plays a central role in contemporary biology, and is thought to have been even more important during the origin and early evolution of life. The "RNA World" hypothesis posits that RNAs preceded protein enzymes and DNA as catalysts and carriers of genetic information, respectively [1]. Understanding the adaptive mechanisms available to RNA is therefore useful when attempting to reconstruct the early evolutionary history of life. Sequence duplication is a primary driver of molecular evolution in proteins, with many modern proteins exhibiting repetitive architectures [2], but the role of duplication events in RNA evolution is less well understood. Our study uses *in vitro* evolution to explore the ways in which duplication of a functional RNA sequence affects the range of secondary structures and associated functions available to the RNA through evolution.

Methods: We designed two experimental RNA constructs, the first containing a single sequence for an ATP-binding aptamer [3] and the second containing two tandem copies of the aptamer sequence. We then generated mutagenized populations from each via error-prone PCR of the DNA templates followed by transcription. Using affinity chromatography, we first selected for RNA molecules in each population capable of binding to ATP, and subsequently selected for molecules capable of binding both ATP and GTP simultaneously. After several rounds of selection, RNA populations exhibiting the desired binding affinities were reverse-transcribed and sequenced using high-throughput sequencing, and their sequences analyzed using RNA secondary structure-prediction software.

Results and Conclusions: Preliminary secondary structure predictions generated for the total range of possible point mutants suggest that aptamer formation will be disrupted in 76.4% (91 of 119) of point mutants for the single-aptamer construct, but only 48.5% (116 of 239) of double-aptamer point mutants will exhibit disruption of both aptamers. Results from the ATP-binding selection appear consistent with these predictions, as a larger proportion of the mutagenized double-aptamer population retains ATP-binding activity than is observed for the mutagenized single-aptamer population. Additionally, both single and double-aptamer mutagenized populations were observed to increase in their ability to bind both ATP and GTP columns over the course of the selection process, but it appears that dual-affinity binding is more pronounced in the mutagenized double-aptamer population. These findings suggest that duplication of a functional sequence can facilitate the evolution of novel functions in RNA while retaining historical functions.

References:

[1] Robertsen M.P. and Joyce G.F. (2012) *Cold Spring Harbor Perspectives in Biology* 4(5):a003608.

[2] Goldman A.D. et al. (2016) *Journal of Molecular Evolution* 82:17-26.

[3] Sassanfar M. and Szostak J.W. (1993) *Nature* 364:550-553.