# *In Vitro* Selection and Characterization of Transition Metal-Dependent DNAzymes and RNAzymes

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Since the discoveries that RNA[1] and DNA[2] could catalyze chemical reactions, much research has been devoted to understanding how these systems operate. Almost all of these "DNAzymes" and "RNAzymes" require divalent metal ions for structure and catalysis.[3,4] Understanding the roles that metal ions play in these systems has been a subject of intense research. However, the majority of the systems currently known are dependent on metal ions such as $Mg^{2+}$, $Ca^{2+}$, $Zn^{2+}$, or $Pb^{2+}$ that lack useful spectroscopic properties. This has made detailed investigations of metal binding sites in these systems difficult.

To help overcome this limitation in many current DNAzyme and RNAzyme systems, a combinatorial process known as *in vitro* selection was employed to obtain RNA-cleaving DNAzyme systems that are dependent on transition metal ions, more specifically $Co^{2+}$, for activity. Transition metal ions were used due to the fact that they can improve the catalytic efficiency of RNA-cleaving DNAzymes as well as broaden the overall diversity of reactions catalyzed by DNAzymes.[4] $Co^{2+}$ was chosen due to its spectroscopic properties, which could be exploited to obtain a better understanding of metal binding sites in DNAzymes.

A negative selection strategy was employed to increase the metal specificity of the *cis*-cleaving DNAzymes,[5,6] as some previously selected systems showed high cleavage activity with metal ions other than the one used during the selection process.[7,8] The selection process was performed twice, resulting in two active sequences, both of which demonstrated rapid cleavage rates. One sequence also possessed remarkable specificity for $Co^{2+}$.[5]

The active sequences that resulted from the two selections were truncated into *trans*-cleaving systems as shown in Figure 1. Unique structures resulted from each selection,

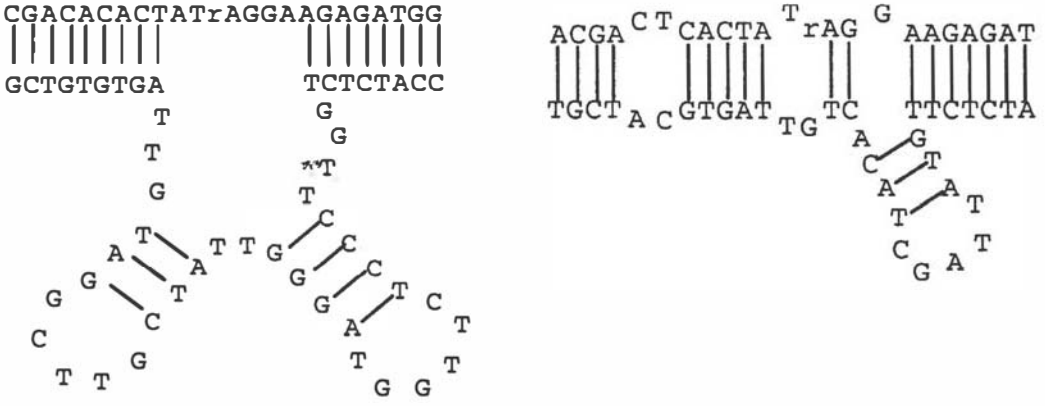


**Figure 1:** Proposed secondary structures of truncated clones 69 (left) and 11 (right).

despite the fact that both *in vitro* selection processes began from the same randomized DNA pools. These truncated systems, called 69E-Full based on clone 69 and 11B based on clone 11, both demonstrated similar reaction characteristics to known DNAzyme and RNAzyme systems; these characteristics included pH profiles, activity dependence on NaCl, and cleavage of an all RNA substrate *versus* a chimeric DNA/RNA substrate. The systems were able to cleave RNA substrates in a site-specific manner, suggesting further utility as sequence-specific nucleases.

The 11B system demonstrated inhibition of cleavage activity in the presence of $Co(NH_3)_6^{3+}$, similar to the results observed with the hammerhead RNAzyme.[9] However, the 69E-Full system was unique in its initial increase in activity in the presence of $Co(NH_3)_6^{3+}$ followed by an inhibition in activity. These data suggest that the 69E-Full system might possess reaction or structural characteristics different from those observed in other DNAzyme and RNAzyme systems.

Both truncated systems were also examined regarding their metal dependence. For both 69E-Full and 11B, the tightest binding affinity for $Co^{2+}$ *versus* $Pb^{2+}$ and $Zn^{2+}$, despite slower observed cleavage rates with $Co^{2+}$ relative to $Pb^{2+}$ and $Zn^{2+}$. The fact that both the truncated systems demonstrated tight cobalt binding was used to characterize the metal binding sites present in the systems by UV-Visible spectroscopic titrations with $Co^{2+}$. The results of multiple titrations show that the $Co^{2+}$ is remaining in an octahedral environment in all the systems, with one or more of the oxygen ligands likely being replaced by nitrogenous ligands, as evidenced by an increased molar absorptivity and a blue shift in the observed $\lambda_{max}$ values relative to $Co(H_2O)_6^{2+}$.[10] These results are consistent with the proposed structure of the $Mn^{2+}$ binding site present in the hammerhead RNAzyme.[11]

Based on the observation that the $Co^{2+}$ is in an octahedral environment in all examined systems and the fact that both the 11B and 69E-Full systems shown in Figure 1 demonstrate tight binding of $Co^{2+}$, future characterization of the metal binding sites by nuclear magnetic resonance (NMR) spectroscopy and electron paramagnetic resonance (EPR) spectroscopy should be possible. The systems can also be developed into fluorescent biosensors for $Co^{2+}$ based on techniques developed in our laboratory[8,12] or sequence specific nucleases based on their abilities to perform site-specific cleavage of all-RNA substrates.

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