Optimization and Generality of a Small Deoxyribozyme that Ligates RNA

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Figures in this Supplementary Material are prefixed by the letter X (e.g., Figure X1) to distinguish them from the manuscript Figures. See the manuscript's Materials and Methods section for details.

Determination of the RNA substrate sequence requirements near the ligation junction for 7Q10

As described in the manuscript, the RNA nucleotides surrounding the ligation junction (UAUA↓GGAA) were systematically altered and the effects on ligation activity by 7Q10 were determined. This was initially done without altering the sequence of the deoxyribozyme, including maintaining its substrate binding arms. The results of these initial ligation assays are shown in Figures X1 and X2 and summarized in the manuscript text.

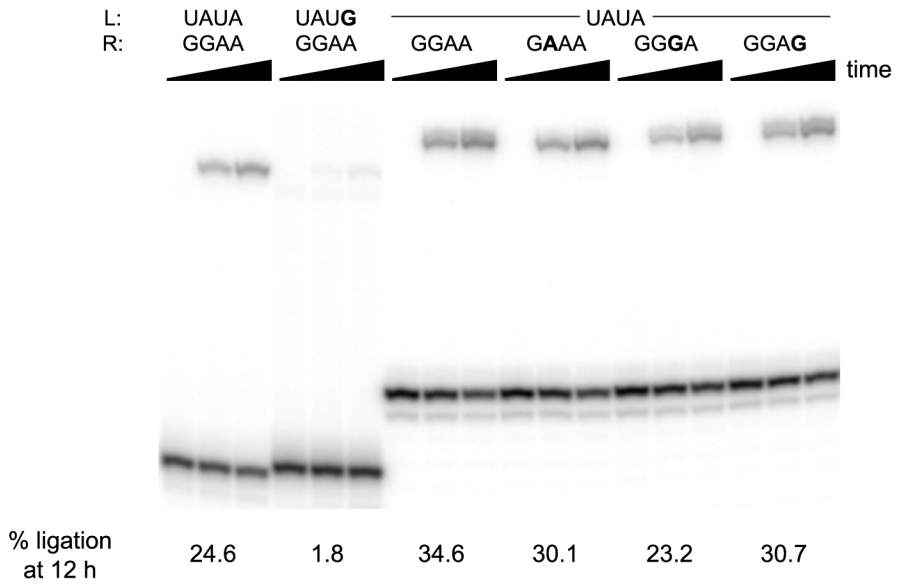

Figure X1. The first group of experiments to analyze changes to RNA substrate nucleotides and their effects on ligation by the 7Q10 deoxyribozyme. Boldface nucleotides denote those changed from the parent sequence UAUA↓GGAA. Experiments were performed in 10-μL assays as described in detail in ref. 28. Timepoints were taken after 0, 4, and 12 h incubation at 37 °C, pH 7.5. For the first two assays, the shorter left-hand substrate was used; for the other assays, the longer substrate was used (see Materials and Methods). In the latter assays, the right-hand substrate was a T7 polymerase transcript, purified as a mixture of *n* and (*n*+1) transcripts, which explains the double bands observed for the ligated products.

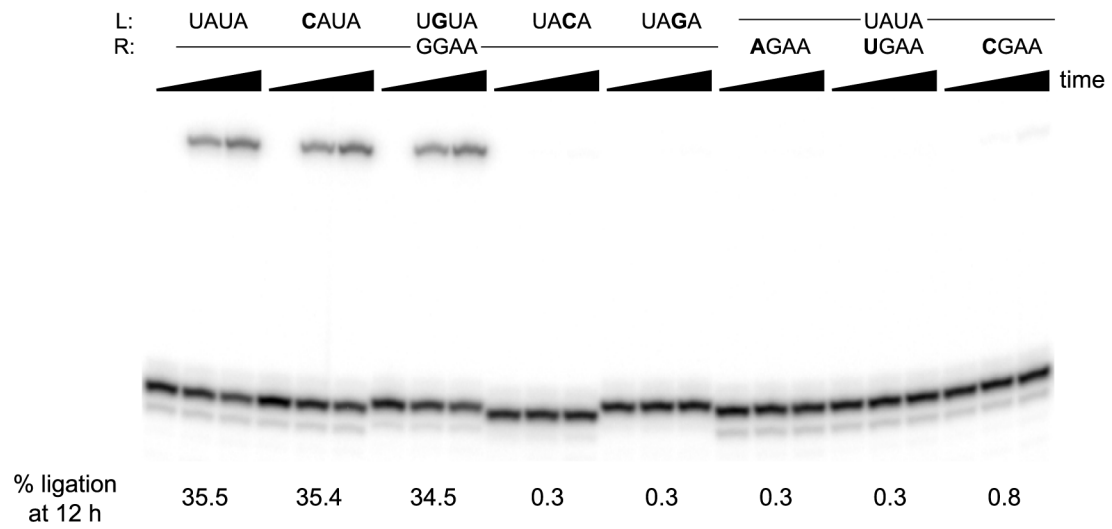

Figure X2. The second group of experiments to analyze changes to RNA substrate nucleotides and their effects on ligation by the 7Q10 deoxyribozyme. Boldface nucleotides denote those changed from the parent sequence UAUA↓GGAA. Experiments were performed in 10-μL assays as described in detail in ref. 28. Timepoints were taken after 0, 4, and 12 h incubation at 37 °C, pH 7.5.