SUPPLEMENTARY MATERIAL, WAN *et al.*

Table S1. Folding of variant ribozymes monitored by catalytic activity [1]

Ribozyme variant [2]	Folding rate constant (min^{-1}) [3]	Relative rate constant [4]	Fraction N [5]	Relative fraction N [4]
WT	1.5 ± 0.3	(1)	0.079 ± 0.018	(1)
L9.1	1.3 ± 0.4	0.85 ± 0.32	0.068 ± 0.012	0.86 ± 0.25
L2.1	0.76	0.50 ± 0.19	0.14	1.77 ± 0.54
L2	2.4 ± 0.2	1.5 ± 0.4	0.030 ± 0.001	0.38 ± 0.09
L5c	>9	>6	0.042	0.53 ± 0.16
L5b	>9	>6	0.057 ± 0.006	0.72 ± 0.18
P5a	>9	>6	0.079 ± 0.004	1.00 ± 0.24
L9	6.6 ± 5.2	4.3 ± 3.5	0.060 ± 0.001	0.76 ± 0.17

[1] Reactions were performed at 25 °C, 50 mM Na-MOPS, pH 7.0, and 50 mM Mg^{2+}. Values reflect averages and standard deviations of three independent determinations except for the L2.1 and L5c variants, for which a single determination was performed.

[2] Each variant ribozyme is denoted by the mutated segment or loop (see Fig. 1).

[3] Values are the rate constant for native ribozyme formation for the fastest observed phase of folding. This rate constant reflects the folding of the fraction of the ribozyme that avoids formation of M. The average values are also shown as bars in Fig. 4. For variant ribozymes that folded too fast to be measured (L5c, L5b, and A-rich bulge variants), only lower limits could be established on the rate constants, which are shown here without standard deviations and with upward arrows in Fig. 4.

[4] Values relative to wild-type ribozyme were determined by dividing each value by the corresponding value for the wild-type ribozyme, and the standard deviations shown reflect propagation of the standard deviations from the three independent determinations for each variant and the wild-type ribozyme. For variants L2.1 and L5c, for which a single determination was performed, the standard deviations were assumed to be similar to the other ribozymes (30% error for rate and 20% for the fraction native).

[5] This value is the amplitude of the first phase of folding, reflecting the fraction of ribozyme that folded directly to the native state without forming the misfolded intermediate M.

FIGURE LEGEND

Fig. S1. Hydroxyl radical and DMS footprinting comparisons of the native ribozyme with conformations formed earlier in folding. **(a)** Comparison of native and unfolded ribozyme. Positions that are more protected in the native species are shown in blue, and those that are more exposed in the native species are shown in yellow. Below the secondary structure is a stereoview of the three-dimensional model with the most prominent protections from hydroxyl radicals in N shown in blue and the most prominent enhancements shown in yellow. Analogous views of the native ribozyme relative to I_{trap} and M are shown in Fig. 2d and 2e, respectively. Regions are highlighted in blue or yellow if at least two consecutive nucleotides had differences of at least 0.2 in their average values or at least five consecutive nucleotides had differences of at least 0.15. This latter criterion was also considered for Fig. 2d and e, but no regions qualified. The colored segments include the 'nucleus' that met one of the above criteria and the extensions of the region to the boundaries (see Methods). **(b)** Comparison of native ribozyme and the I_{trap} intermediate, with results shown on the secondary structure as in panel a. **(c)** Comparison of the native and misfolded ribozyme (M). For each comparison positions that are more protected in the native species are shown in blue, and those that are more exposed in the native species are shown in yellow. In all panels, the nucleotides with the most prominent changes in DMS reactivity are highlighted with asterisks (see Methods).