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**Magnesium and Temperature Dependence of the Folding of the *Tetrahymena* Ribozyme**

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ABSTRACT: Many macromolecular folding reactions, such as the  $Mg^{2+}$ -dependent folding of the *Tetrahymena thermophila* group I intron, occur on timescales ranging from milliseconds to minutes. Kinetic progress curves describing the millisecond folding of P4-P6 and other domains within the L-21 ribozyme have been obtained using a new  $\bullet OH$  "footprinting" technique, stopped-flow synchrotron x-ray "footprinting". The folding of P4-P6 is a highly concerted reaction; the regions of  $\bullet OH$  protection within the interior of the folded domain appear at rates of  $\leq 1.0 \text{ sec}^{-1}$ . The rates of  $\bullet OH$  protection of the "triple helix" junction between P4-P6 and the P3-P7 domain and those protections in P4-P6 ascribed to interaction with the P9 domain at rates of  $\sim 0.3 \text{ sec}^{-1}$ . These results suggest that these tertiary interactions guide the folding of the catalytic core against an extensively folded P4-P6 domain. To further dissect the folding mechanism of the ribozyme, which we have solved in detail for a single set of conditions, the salt and temperature dependence of the folding mechanism will be explored in order to correlate the changes in energetics and structure that occur along the folding pathway. In addition, we are examining the Mg-induced folding of the RNA in the presence of sub-denaturing concentration of urea.