Folding Pathways of the *Tetrahymena* Ribozyme

David Mitchell III and Rick Russell

Department of Molecular Biosciences, *Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA*

Correspondence to Rick Russell: *rick_russell@cm.utexas.edu.*
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Abstract

Like many structured RNAs, the *Tetrahymena* group I intron ribozyme folds through multiple pathways and intermediates. Under standard conditions *in vitro*, a small fraction reaches the native state (N) with $k_{obs} \approx 0.6\ min^{-1}$, while the remainder forms a long-lived misfolded conformation (M) thought to differ in topology. These alternative outcomes reflect a pathway that branches late in folding, after disruption of a trapped intermediate (I_{trap}). Here we use catalytic activity to probe the folding transitions from I_{trap} to the native and misfolded states. We show that mutations predicted to weaken the core helix P3 do not increase the rate of folding from I_{trap} but they increase the fraction that reaches the native state rather than forming the misfolded state. Thus, P3 is disrupted during folding to the native state but not to the misfolded state, and P3 disruption occurs after the rate-limiting step. Interestingly, P3-strengthening mutants also increase native folding. Additional experiments show that these mutants are rapidly committed to folding to the native state, although they reach the native state with approximately the same rate constant as the wild-type ribozyme ($\sim 1\ min^{-1}$). Thus, the P3-strengthening mutants populate a distinct pathway that includes at least one intermediate but avoids the M state, most likely because P3 and the correct topology are formed early. Our results highlight multiple pathways in RNA folding and illustrate how kinetic competitions between rapid events can have long-lasting effects because the "choice" is enforced by energy barriers that grow larger as folding progresses.

Introduction

Structured biopolymers face the difficult task of folding to a functional native conformation from the vast collection of non-functional conformations. For RNA, the energetics of specifying a native state may be simplified, at least conceptually, by the prevalence and independent stability of its local secondary structure [1]. Local helices can form easily and persist even in the absence of tertiary structure, creating a hierarchy in which most of the secondary structure is determined locally and by relatively simple base-pairing rules, while the overall architecture is determined by the orientations of the helical elements and fixed by tertiary contacts between them [2,3]. On the other hand, the stability of local structure may amplify the challenge of finding the native structure rapidly [35,4,5]. Non-native secondary structures can be long-lived, like their native counterparts [6–9], and even native contacts can slow folding if they form prematurely and must be disrupted to allow resolution of non-native structure elsewhere [10–16]. In light of these properties, it is perhaps not surprising that RNA folding is found experimentally to be rife with kinetically trapped folding intermediates [17,18]. Still, relatively little is known about the folding pathways that lead to the formation and resolution of these intermediates or the non-native structural features of the intermediates.

The *Tetrahymena thermophila* group I intron and its ribozyme derivative have provided a powerful system to study a complex folding process (Fig. 1a). Early work using the ribozyme demonstrated multiple folding pathways, with the dominant pathways including two kinetically trapped intermediates

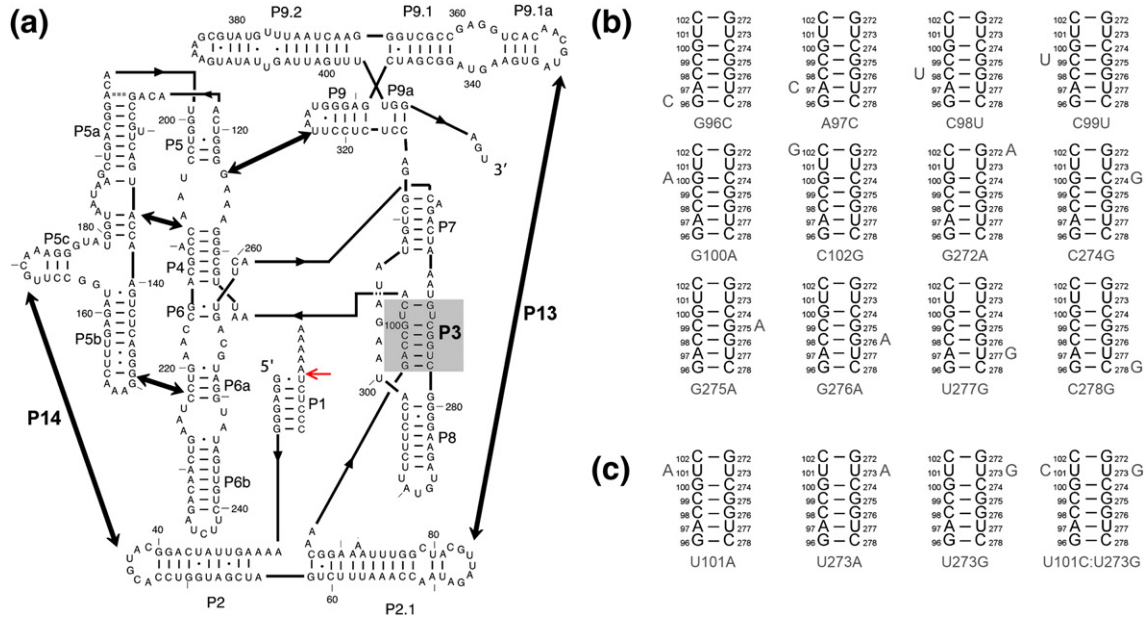
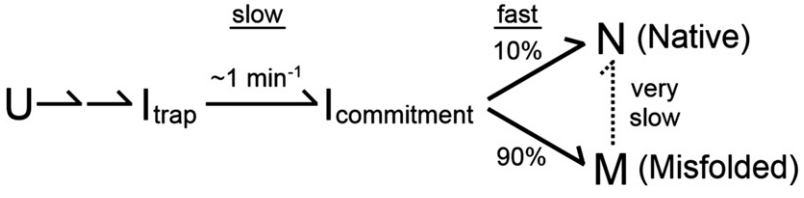


Fig. 1. The *Tetrahymena* ribozyme and mutants used herein. (a) Ribozyme secondary structure. The ribozyme contains five long-range contacts, as indicated by thick arrows. The base paired oligonucleotide substrate is also shown, forming the P1 helix, and the cleavage site is indicated by a red arrow. The P3 helix is shaded in gray. (b and c) Point mutations are designed to weaken the P3 helix by disrupting a single base pair (b) or to strengthen P3 by creating A-U, U-A, or U-G pairs or a C-G pair (c).

(Scheme 1) [19–23]. Under standard conditions *in vitro*, essentially the entire population folds in seconds to the first intermediate, I_{trap} [10,19,24]. From I_{trap}, a small fraction folds along a pathway to the native state, N, on the timescale of 1 min, while the remainder forms a long-lived misfolded intermediate termed M, from which refolding to N occurs on the timescale of hours [13,14,20].

Further work has probed these two intermediates and their folding transitions to the native state. The misfolded state M closely resembles the native state [13,25], yet extensive native structure is disrupted during its refolding to the native state, including all five long-range peripheral tertiary contacts and a native core helix named P3 [12,13]. To explain why the M state must unfold to reach the native state even though it is structurally similar to the native state, we proposed that the M state differs in topology, such that extensive unfolding of the periphery and the P3 helix would be required for exchange to the native topology [12,13]. The I_{trap} intermediate is also compact [25–28] and features extensive native secondary and tertiary structure [14,23,27], including the P3 helix [14]. The folding transition from I_{trap} to the native state depends on unfolding of peripheral structure [10,14], but it is not known whether core unfolding occurs in this transition and specific physical models have not been constructed.

Here we test whether the folding transitions from I_{trap} to the native and misfolded states require the transient disruption of the P3 helix. By using catalytic activity to monitor folding from I_{trap} for a series of P3 mutants, we show that P3 is indeed disrupted during folding to the native state, whereas P3 remains intact during the folding transition from I_{trap} to the M state. This result extends the known similarities of I_{trap} and M, and it suggests that the non-native topology is established early in folding along this pathway and exchanges to the native topology when P3 is disrupted during folding



Scheme 1. Dominant folding pathways for the wild-type ribozyme. The transition from the M state to the N state is shown with a dashed line to indicate that this transition may proceed through the $I_{commitment}$ intermediate or through a different pathway.