Navigating the RNA folding landscape

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Analysis of individual RNA folding reactions reveals that, as in proteins, cooperative interactions selectively drive RNA toward its biologically active, native conformation. This new work establishes a platform for future investigations of the physical principles underlying the assembly of large RNA enzymes.

Presented with myriad possible folding pathways, individual ribozyme molecules have the intrinsic capacity to select that route that culminates in a stably folded, fully active native state (**Fig. 1a**). In order to negotiate this folding landscape within a biologically relevant timescale, the molecule must have an energetic feedback system that expeditiously steers it toward the native state. For decades, scientists have sought to identify the predominant components of this feedback system and expose their underlying physical principles. By investigating single RNA molecules, Sattin *et al.* solve a critical experimental challenge and demonstrate that, like proteins, RNA molecules are directed through the folding landscape by the cooperative coupling of native intramolecular interactions[1].

A powerful aspect of single-molecule observation is the ability to accurately observe transitions between conformational states, irrespective of the population-averaged behavior of the bulk sample. Taking elegant advantage of this, Sattin *et al.* have revolutionized our experimental ability to measure the energetic contribution and mechanistic underpinnings of cooperativity in guiding RNA folding[1]. Cooperativity is generally measured by comparing the thermodynamic stability of a wild-type molecule with that of engineered mutants in which two intramolecular interactions are disrupted, first separately and then together[2]. Thermodynamic stabilities obtained from bulk folding experiments provide population-averaged measurements of the fractions of RNA in the unfolded and native states, from which the equilibrium constant for the folding reaction is calculated. The measurements are taken near the folding transition midpoint, where they are most accurate (**Fig. 1b**). Unfortunately, disruption of RNA tertiary structure typically yields large shifts in the Mg^{2+} dependence of folding[3] and, conse-

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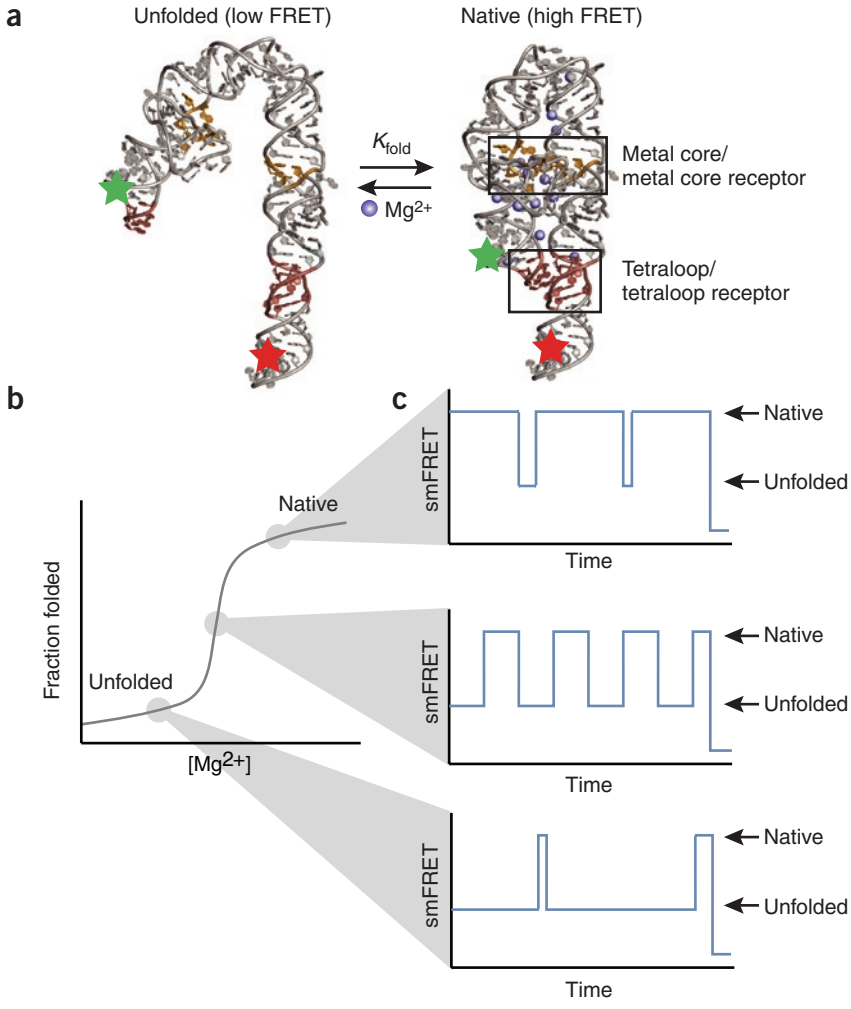

Figure 1 Direct measurement of cooperativity in RNA folding using single-molecule Förster resonance energy transfer. (**a**) The Mg^{2+} (blue spheres)-dependent cooperative formation of the metal core–metal core receptor (MC–MCR, gold nucleotides) and tetraloop–tetraloop receptor (TL–TLR, maroon nucleotides) tertiary interactions in the folding of the P4-P6 domain of a group I intron can be energetically dissected using smFRET to directly measure the thermodynamic stabilities of wild-type P4-P6 and two mutants in which either the MC–MCR or TL–TLR have been disrupted. The general positions of the donor and acceptor fluorophores have been denoted with green and red stars, respectively. (**b**) Thermodynamic stabilities can be obtained from bulk folding experiments in which the population-averaged measurements of the fractions of RNA in the unfolded and native states are used to calculate the equilibrium constant for the folding reaction near the transition midpoint, where the population-averaged measurements are most accurate. (**c**) Thermodynamic stabilities can also be obtained from smFRET studies in which the fractional unfolded and native populations for individual RNA molecules, accurately determined at any point along the folding transition, are used to calculate the equilibrium constant for the folding reaction.

quently, in the folding transition midpoint. The required Hill-type analysis, in which the folding equilibria of the various RNA constructs are extrapolated to a common Mg^{2+} concentration[4,5], presumably introduces large errors into the analysis. By providing an opportunity to accurately measure the fractional unfolded and native population at any point along the folding transition (**Fig. 1c**), the single-molecule approach reported by Sattin *et al.* permits equilibrium measurements under identical Mg^{2+} concentrations for all wild-type and mutant RNA constructs, obviating the need for a Hill-type analysis and directly revealing the extent of cooperativity in RNA tertiary contact formation[1].

Group I introns are large ribozymes that precisely catalyze their own excision from precursor RNAs[6]. In order to observe folding transitions in individual RNA molecules, Sattin *et al.* constructed a fluorescently labeled variant of P4-P6, an independently folding group I intron domain[7], and implemented a single-molecule Förster resonance energy transfer (smFRET) assay in which unfolded and native P4-P6 yielded low and high FRET values, respectively. Analogous constructs were prepared in which two well characterized P4-P6 tertiary contacts, the metal core–metal core receptor (MC–MCR) and the tetraloop–tetraloop receptor (TL–TLR) (**Fig. 1a**)[8], were selectively disrupted by nucleotide mutation. smFRET thermodynamic stability measurements were then performed under identical Mg^{2+} concentrations for all P4-P6 constructs[1]. The result is the direct measurement of the energetic contribution of tertiary contact cooperativity in the folding of P4-P6, revealing that the extent of cooperativity in RNA folding (~3 kcal mol^{-1})[1] is comparable to that observed in protein folding (~2–9 kcal mol^{-1})[9,10]. This result will undoubtedly frame future investigations into the role of cooperativity in directing the assembly of the entire, catalytically active, group I intron. With at least two more domains[11,12] and various interdomain and active site tertiary contacts[12], it is likely that cooperativity will play a pivotal role not only in orchestrating the global architecture of the intron, but also in organizing the critical tertiary interactions at the active site that are responsible for splice site selection and catalysis.

Although cooperativity guides the folding of both proteins and RNA to comparable extents, the physicochemical principles underlying the cooperativity observed in these two classes of biomolecules are surely distinct. Decades of protein folding studies support the view that at the heart of cooperative folding is the formation of the first long-range native interaction, which helps guide organization of proximal native interactions. Although a similar reduction of conformational entropy is certain to play a role in RNA folding, the energetic penalty imposed by the close packing of the negatively charged phosphate backbone is expected to contribute significantly to the barrier that is cooperatively traversed in RNA folding. Nonspecific screening of the phosphate backbone by metal ions, as well as the specific binding of Mg^{2+} ions within the geometrically unique and highly negatively charged pockets forged by RNA tertiary interactions, are therefore likely to take center stage. Interestingly, recent studies on protein-dependent group I introns have demonstrated that accessory protein splicing factors serve to lower the Mg^{2+} dependence of the catalytic reaction in these introns[13,14] and function by serving as structural surrogates for essential RNA elements typically involved in RNA tertiary contacts, including the P4-P6 MC–MCR and TL–TLR interactions typically absent from protein-dependent group I introns[13,14]. Future smFRET studies investigating how *trans*-acting splicing factors replace *cis*-acting RNA elements and Mg^{2+} in cooperatively guiding the folding of protein-dependent group I introns will provide unprecedented insight into the assembly of larger ribonucleoprotein enzymes such as RNase P, the spliceosome and the ribosome.

1. Sattin, B.D. *et al. J. Am. Chem. Soc.* **130**, 6085–6087 (2008).
2. Horovitz, A. *et al. J. Mol. Biol.* **214**, 613–617 (1990).
3. Battle, D.J. *et al. Proc. Natl. Acad. Sci. USA* **99**, 11676–11681 (2002).
4. Silverman, S.K. *et al. RNA* **5**, 1665–1674 (1999).
5. Das, R. *et al. J. Am. Chem. Soc.* **127**, 8272–8273 (2005).
6. Cech, T.R. *Annu. Rev. Biochem.* **59**, 543–568 (1990).
7. Murphy, F.L. *et al. Biochemistry* **32**, 5291–5300 (1993).
8. Cate, J.H. *et al. Science* **273**, 1678–1685 (1996).
9. Kloss, E. *et al. Arch. Biochem. Biophys.* **469**, 83–99 (2008).
10. Batey, S. *et al. J. Mol. Biol.* **349**, 1045–1059 (2005).
11. Golden, B.L. *et al. Science* **282**, 259–264 (1998).
12. Adams, P.L. *et al. Nature* **430**, 45–50 (2004).
13. Weeks, K.M. *et al. Science* **271**, 345–348 (1996).
14. Paukstelis, P.J. *et al. Nature* **451**, 94–97 (2008).

Deciphering a protolanguage for bacteria–host communication

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Many of the phenotypes shown by bacteria at high population densities are only beneficial when they are associated with eukaryotic hosts. A new study confirms that some bacteria may couple quorum sensing to host-derived signals to refine such interactions.

Many bacteria show distinct phenotypes, such as biofilm formation, swarming, luminescence or virulence factor production, as a function of their population density. This phenomenon, known as 'quorum sensing', is driven by the detection of a variety of small molecules, collectively referred to as 'autoinducers'[1]. The best characterized autoinducers are the *N*-acylated L-homoserine lactones (HSLs) used by Gram-negative bacteria (**Fig. 1a**). These simple, low molecular weight ligands differ significantly only in the constitution of their fatty acid–derived acyl chains. Homologs of both the HSL synthase (LuxI-type synthase) and HSL receptor (LuxR-type receptor) are found in many Gram-negative bacteria and regulate quorum sensing in response to structurally distinct HSLs[2]. The potential to manipulate quorum sensing circuits and thereby regulate bacterial group behaviors has garnered considerable attention[3]. In a recent issue of *Nature*, Harwood and co-workers report that the structural diversity of natural autoinducers available

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