Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 270, No. 36, Issue of September 8, pp. 20871–20874, 1995

RNA Chaperones and the RNA Folding Problem*

Daniel Herschlag‡

From the Department of Biochemistry, Stanford University, Stanford, California 94305-5307

Functional and structural inter-relationships of RNA and proteins in the execution and control of biological processes such as RNA processing, RNA splicing, and translation are increasingly apparent. In this minireview, I present an RNA chaperone hypothesis, which fosters the view that constraints imposed by fundamental problems in the folding of RNA have profoundly influenced the nature of RNA/protein interactions in biology. The origin of this view is outlined as follows. RNA has two fundamental folding problems: a tendency to fold into and become *kinetically* trapped in alternative conformations and a difficulty in specifying a single tertiary structure that is *thermodynamically* strongly favored over competing structures. RNA-binding proteins can help solve both RNA folding problems. Nonspecific RNA-binding proteins[1] solve the kinetic folding problem *in vivo* by acting as RNA chaperones that prevent RNA misfolding and resolve misfolded RNAs, thereby ensuring that RNA is accessible for its biological function. In addition, specific RNA-binding proteins can solve the thermodynamic folding problem by stabilizing a specific tertiary structure. The emergence of nonspecific RNA-binding peptides with chaperone-type activities may have been an early step in the transition from the RNA world to the RNA/protein world. Specific RNA-binding proteins may also have RNA chaperone activities that help prevent misfolding of their cognate RNAs. RNA-dependent ATPases may act as RNA chaperones that spatially and temporally control RNA conformational rearrangements.

"RNA chaperone" refers to *proteins* that aid in RNA folding and is not meant to refer to chaperones made of RNA.[2] For clarity, the classical chaperones that aid protein folding are referred to as "protein chaperones." In keeping with the accepted definition of protein chaperones, RNA chaperones are defined as proteins that aid in the process of RNA folding by preventing misfolding or by resolving misfolded species. This is in contrast to proteins that help protein or RNA folding by catalyzing steps along the folding pathway or by stabilizing the final folded protein or RNA structure.[2]

There are no established examples of RNA chaperones that act *in vivo*. This hypothesis is presented because the *in vitro* data reviewed herein provide support for the hypothesis and this view provides a conceptual framework for RNA folding and RNA/protein interactions. The kinetic problem in RNA folding is emphasized, while space constraints have greatly limited discussion of the thermodynamic problem.

* This minireview will be reprinted in the 1995 Minireview Compendium, which will be available in December, 1995. This work was supported by National Institutes of Health Grant GM49243.

‡ Lucille P. Markey Scholar in Biomedical Sciences and a Searle Scholar.

[1] "Nonspecific" is used for simplicity, even though there is presumably no truly nonspecific RNA-binding protein. It refers to RNA-binding proteins with low or wide binding specificities. However, RNA-binding proteins, even those that bind a particular target RNA *in vivo*, bind other RNAs with reasonably high affinity. The difference between specific and nonspecific or widely specific proteins is quantitative rather than qualitative, so that an absolute distinction is not possible.

[2] The term RNA chaperone is already in use by some in the field (1–8). I suggest that only proteins with demonstrated biological roles as chaperones in RNA folding be referred to as RNA chaperones, while the ability to facilitate folding *in vitro* be referred as "RNA chaperone activity." The definition of RNA chaperones is further honed in the text, and some possible ambiguities are addressed. For example, specific RNA-binding proteins can exhibit biological or nonbiological RNA chaperone *activity* in the folding of cognate or noncognate RNAs (see Fig. 2 and text). I suggest that these proteins *not* be referred to as RNA chaperones, in deference to their other functions.

The Two Fundamental Folding Problems of RNA

Many of the examples of RNA misfolding *in vitro* suggest that the inactive or alternative conformer is kinetically trapped such that it does not revert to the active conformation even after long periods of time. Early work showed that several tRNAs were isolated in two conformations, only one of which could be charged by the cognate aminoacyl-tRNA synthetase (11–14). An inactive $tRNA^{Leu}$ was stable on the hour time scale in the presence or absence of Mg^{2+}, but was converted to an active conformation upon heating in the presence of Mg^{2+} (12). These inactive tRNAs apparently adopt stable alternative secondary structures (15–19).

Larger RNAs provide much additional evidence for a kinetic folding problem. For example, *in vitro* self-splicing reactions of group I introns, which are >200 nucleotides, typically do not proceed to completion. This suggests the presence of kinetically trapped, alternatively folded conformers (see also Refs. 20–26).

The RNA folding problems observed *in vitro* could be relevant to the *in vivo* behavior of RNA or could instead arise as an artifact of *in vitro* handling of RNA, as RNA is typically purified under denaturing conditions and then renatured. A comparison of the primary, secondary, and tertiary structure of RNA and proteins, based in part on an insightful analysis of tRNA structure (27), suggests that the kinetic folding problems described above and additional thermodynamic folding problems are intrinsic to RNA (summarized in Fig. 1 and Table I).

Primary Structure—RNA has a paucity of primary structure diversity compared with proteins, with just 4 side chains instead of 20. Furthermore, the 4 RNA side chains are more similar to one another than the protein side chains. The RNA side chains come in only two "sizes," purine and pyrimidine, and each is a planar group decorated with hydrogen bond donors and acceptors, whereas the protein side chains comprise hydrophobic, hydrophilic, and charged groups of varying sizes and shapes. The dearth of primary structure diversity, or low "information content," of an RNA polymer (relative to a protein polymer) would be expected to render it more difficult for an RNA sequence to specify a unique tertiary structure.

Secondary Structure—The high thermodynamic stability of RNA duplexes is expected to result in kinetic folding problems. The most stable protein α-helices dissociate on the sub-microsecond time scale (28). In contrast, an RNA duplex of 10 base pairs has a half-time for dissociation of ~30 min, and G/C-rich duplexes of 10 base pairs have dissociation half-times up to ~100 years at 30 °C (30). Thus, RNA can get stuck in the wrong conformation (Fig. 1). This kinetic problem could prevent a structured RNA from adopting the correct conformation, could prevent access to mRNA, and could even prevent turnover of an RNA subsequent to correct folding.

The potential for alternative folds appears to be a common property of RNAs. Even random RNAs are predicted to have structures with about half of the residues base-paired, consistent with the estimated helical content of randomly associated RNAs (35, 36).

Tertiary Structure—The problem of stable alternative secondary folds is exacerbated by fortuitous tertiary interactions with 2′-hydroxyls, phosphoryl groups, and metal ions and by the formation of nonstandard base/base interactions that can further stabilize incorrect RNA conformers. Even after RNA adopts the correct secondary structure, it is not yet "out of the woods." The low information content of RNA primary structure is further decreased by sequestering the base-pairing faces of residues in the interior of duplexed regions, while the side chains of proteins face outward in α-helices and β-sheets. Each RNA secondary structure element thus has a strong resemblance to others, so that RNA can have a difficult time specifying a unique tertiary structure. For example, a duplex of the *Tetrahymena* group I ribozyme docks into tertiary interactions incorrectly approximately 1/1000 of the time, and mu-

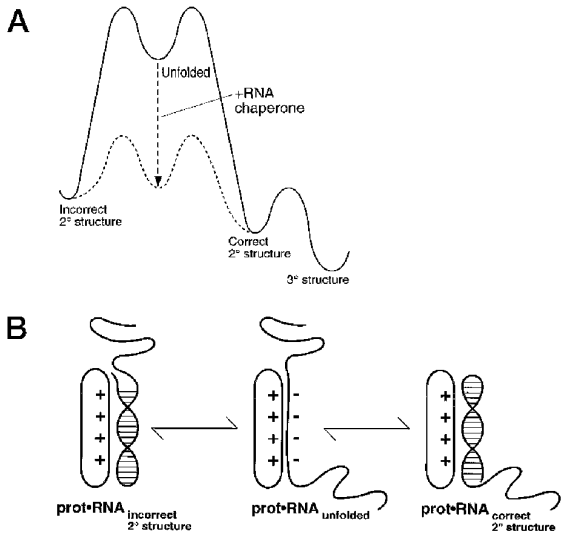

FIG. 1. **RNA folding and the effect of RNA chaperones.** *A*, schematic free energy profiles for folding of RNA in the absence (*solid line*) and presence (*dashed line*) of an RNA chaperone. Species with only secondary structure are shown to emphasize the stability of RNA secondary structure and the tendency to be trapped in incorrect secondary structures, even though such species may not exist as discrete intermediates. Only one alternative RNA secondary structure and only one tertiary structure are shown for simplicity. The barrier for going from unfolded to the correct secondary structure with and without chaperone is shown as the same, although binding of a chaperone could slow correct folding, as observed with protein chaperones. A protein that lowers this barrier to speed the folding process can be referred to as a "guide." Chaperones work by decreasing the barrier for escaping from the incorrectly folded structure. *B*, one of several physical models of how an RNA chaperone could facilitate refolding of misfolded RNAs. Imperfect charge complementarity between the protein surface and RNA secondary structure (*prot·RNA*$_{\text{incorrect 2° structure}}$). The protein speeds unfolding by favoring conformational excursions that increase charge complementarity. The protein binds more strongly to the unfolded RNA (*prot·RNA*$_{\text{unfolded}}$) relative to the folded RNA because the unfolded RNA is free to rearrange to give charge complementarity with the protein. Refolding can occur from this state to either the correct or incorrect secondary structure and is driven by the stabilization from base pair formation, which counters the destabilization from loss of charge complementarity.

tations increase this misdocking to about one-half (37).[3] Thus, although there may be difficulty in ensuring that the correct tertiary structure of an RNA is formed, this problem is not insurmountable; a free energy preference of only 2 kcal/mol is sufficient to ensure >95% correct folding.[4]

Solutions to the RNA Folding Problems: RNA Chaperones and Specific RNA-binding Proteins

RNA Chaperones[2]—The underlying basis for the idea of RNA chaperones is simple. RNA has a fundamental folding problem, a tendency to be kinetically trapped in misfolded forms (Fig. 1). Nonspecific RNA-binding proteins can overcome this problem *in vitro*. It is therefore suggested that RNA-binding proteins act as RNA chaperones to solve this RNA folding problem *in vivo*.

This idea was apparently suggested for RNA over 20 years ago (38, 39). It was shown that the protein UP1, a fragment of hnRNP[5] A1 protein, could renature 5 S and tRNAs that were kinetically trapped in alternative conformations and suggested that such activities would be necessary in biology. DNA annealing experiments appear to have provided the intellectual roots for these ideas (40). Long single strands of RNA or DNA reassociate orders of magnitude slower than short oligonucleotides, in part because the longer nucleic acids form intramolecular structures that limit access by the complementary strand. Catalysis of polynucleotide annealing by single-strand nucleic acid-binding proteins, such as T4 gene 32 protein and *Escherichia coli* SSB, would then arise from a disruption of intramolecular structure that enhances access for intermolecular base-pairing (40, 41). The RNA chaperone proposal brings

[3] G. J. Narlikar and D. Herschlag, unpublished results.
[4] Questions about the thermodynamic folding problem include the following. To what extent does the precision of long range base-pairing interactions compensate for the low information content of secondary structure elements to specify a single strongly favored tertiary structure?
[5] The abbreviations used are: RNP, ribonucleoprotein; NC, nucleocapsid; HIV, human immunodeficiency virus.

TABLE I
Comparison of RNA and protein structural features

	RNA	Protein
Primary structure		
Side chains	4 (similar)	20 (diverse)
Secondary structure		
Side chains face	In	Out
Stability	High	Low
Tertiary structure		
Packing	Loose, multiple possibilities	Tight, precise
Cooperativity	Low	High

molecular chaperones full circle, as early speculation about the involvement of chaperones in *protein* folding, which is now well established, was framed by analogy to the ability of single-strand nucleic acid-binding proteins to catalyze *nucleic acid* duplex formation: both facilitate correct folding by preventing misfolding (42). The energetics of RNA chaperone action are depicted schematically in Fig. 1*A*, and one physical model is portrayed in Fig. 1*B*.

Several recent experiments strongly support such an *in vitro* RNA chaperone activity of RNA-binding proteins. Slow physical steps in the reaction of a hammerhead ribozyme limit turnover and specificity (43) so this system provides an intermolecular model for the kinetic problems in RNA folding, *i.e.* dissociation of intermolecular duplexes is crucial for turnover and for discrimination against incorrect (mispaired) RNA substrates (44). This can be likened to the unraveling of RNAs that have adopted incorrect secondary structures during folding. Proteins such as the NC protein from HIV-1 and the hnRNP A1 protein were shown to facilitate these physical steps and thereby enhance the ribozyme reaction. In addition, the NC protein resolved a kinetically trapped misfolded complex with HH16 (4, 6, 46, 47).

As mentioned above, the self-splicing of group I introns *in vitro* is often slow and inefficient, whereas splicing *in vivo* appears to be fast and efficient (48). In some cases, proteins facilitate splicing *in vivo* by binding specifically to and stabilizing the catalytically active conformation of the intron (49–51). In contrast, the *E. coli* S12 ribosomal protein facilitates proper folding of group I introns by nonspecific binding, suggesting a second mechanism for aiding group I intron splicing *in vivo* (5). Characterization of the S12 protein facilitation further strengthened the analogy between RNA chaperones and protein chaperones. (i) The S12 protein shows no preferential binding to group I introns over exons or other RNAs, suggesting that S12 does not act by specifically stabilize the intron's catalytic conformation. (ii) The S12 protein is also able to facilitate a hammerhead ribozyme reaction, analogous to the NC and hnRNP A1 proteins, further suggesting a nonspecific rather than specific mode of action. (iii) The S12 protein promotes splicing of a population of kinetically trapped, unreactive precursor RNA, suggesting an ability to resolve misfolded RNAs. (iv) Protein chaperones function solely during a folding step and are not present in the final active species. The same stimulatory effect on group I self-splicing was observed whether or not S12 was removed by proteolysis prior to initiation of the self-splicing reaction. Thus, the S12 protein is required solely for folding and can act as a true chaperone.

Why a Specific RNA-binding Protein Would Also Act As an RNA Chaperone: a "Preassociation" Binding Mechanism—Even if a specific RNA-binding protein solves RNA's thermodynamic folding problem by stabilizing the correct RNA conformation, kinetic problems of attaining that conformation remain, as depicted in the bottom pathway of Fig. 2. (i) The RNA can be *kinetically* trapped in misfolded conformations (k_{misfold}), and (ii) the correctly folded RNA may lack the *thermodynamic* stability to exist long enough to be trapped efficiently by its cognate protein (k_{trap} *versus* k_{unfold}). The ability of the S12 ribosomal protein to act as an RNA chaperone in the folding of group I introns (5) raises the intriguing possibility that this chaperone activity also solves these kinetic folding problems. Both problems could be avoided by following a preassociation binding mechanism (Fig. 2, *top pathway*), in which the protein initially uses nonspecific interactions and/or a subset of specific interactions to bind the unfolded RNA and prevent misfolding. The high levels of nonspecific binding exhibited by many specific RNA-binding proteins could allow this chaperone activity. Subsequently, the RNA might undergo conformational rearrangements within the

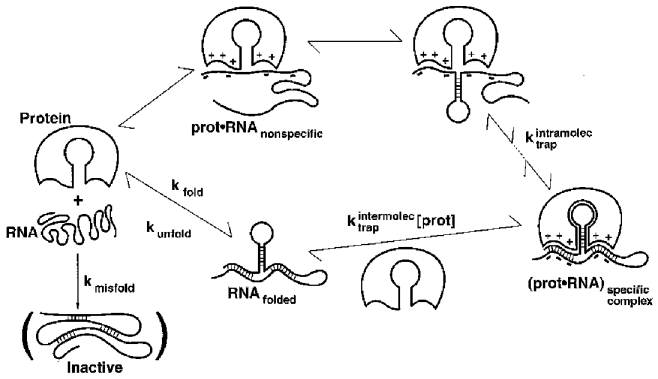

FIG. 2. **Preassociation binding pathway in which a specific RNA-binding protein also has RNA chaperone activity, using nonspecific interactions to facilitate proper folding of its cognate RNA.** The nonspecific and specific interactions are represented schematically by the absence and presence, respectively, of charge and shape complementarity within the complexes.

complex (or via multiple partial or complete dissociations and reassociations, not shown) until the correct conformation is attained and trapped by specific interactions with the protein. Proteins that act via the top pathway of Fig. 2 can be referred to as specific RNA-binding proteins that exhibit RNA chaperone "activity."[2]

The Analogy between Protein and RNA Chaperones—Protein chaperones have suggested that they facilitate the process of protein folding by preventing misfolding (52, 53). As described above, proteins also appear to facilitate RNA folding by preventing misfolding. The following comparisons between RNA and protein chaperones may help elucidate properties that are unique to RNA chaperones as well as concepts that are fundamental to both RNA and protein chaperones.

Protein chaperones appear to be a distinct class of molecules designed to facilitate protein folding. In contrast, the significant extent of nonspecific binding by RNA-binding proteins suggests that many RNA-binding proteins may exhibit RNA chaperone *activity in vitro*.[2] Over 20 different proteins from *E. coli* extracts were able to facilitate group I intron splicing *in vitro* (5), but it is not known which proteins, if any, act as cellular RNA chaperones. The hnRNP proteins, which coat pre-mRNA as it is transcribed, represent the most obvious candidate class for cellular RNA chaperones (see also Ref. 54).

Protein chaperones facilitate folding but do not remain bound to the final folded protein product, whereas RNA chaperones may facilitate the folding process and subsequently remain bound because of high levels of nonspecific binding affinity. This may represent a basic difference in the primary recognition element for the two classes of chaperones. Protein chaperones appear to recognize unfolded proteins because of exposed hydrophobic residues; when the protein folds and these residues are buried, the chaperone no longer binds strongly (52). In contrast, the charged phosphodiester backbone and their bases are likely to be at least partially exposed in unfolded or misfolded RNA, allowing nonspecific binding, especially via electrostatic interactions.

Mechanistic studies of protein chaperones have suggested that they prevent misfolding by sequestering unfolded forms so that they cannot aggregate (52, 55, 56). RNA chaperones may act similarly by binding to regions of an RNA and preventing or slowing formation of certain intramolecular structures. The RNA chaperones have also been shown to resolve RNAs that have already misfolded (see above), whereas the protein chaperones that have been best characterized can bind and sequester unfolded proteins but appear unable to bind efficiently to and resolve protein aggregates. The high nonspecific binding activity of RNA-binding proteins may account for this difference by allowing RNA chaperones to bind and subsequently to resolve misfolded RNA conformers (Fig. 1*B*). However, recent *in vivo* characterization of the Hsp104 protein has suggested that it actively resolubilizes *protein* aggregates (57, 58), although the molecular basis for this is not known.

There are proteins other than the chaperones referred to above that aid proper protein folding such as prolyl isomerases and protein disulfide-isomerases (55). Specific RNA-binding proteins can exhibit RNA chaperone activity by helping to prevent and resolve misfolding of both cognate and noncognate RNA (Fig. 2). Specific RNA-binding proteins could also aid the process of folding for the cognate RNA by acting as "guides" in the folding process, *i.e.* by trapping correctly folded domains or subdomains to help bias the RNA to follow along the folding path toward the final correctly folded structure. In addition, protein/protein interactions can bring together two RNAs (or two regions of one RNA), thereby increasing the probability of duplex formation or other interactions (2, 59, 60). Proteins that do this might be referred to as matchmakers, rather than chaperones (7). Such proteins may be involved in spliceosome assembly. There is evidence that the hnRNP A1 protein can act as both a chaperone and matchmaker (2, 6, 7). RNA chaperones, matchmakers, and guides each can increase the observed rate of RNA/RNA assembly, so that it often may be difficult to distinguish these mechanistically.

RNAs could also act as RNA chaperones to assist in the folding of other RNAs. "Facilitators" are RNAs that base-pair to a ribozyme adjacent to the substrate (61); they presumably prevent the ribozyme from folding up upon itself, thereby increasing access for base-pairing to the substrate. This is analogous to the facilitation of duplex formation by single-strand binding proteins. There are several examples of intramolecular changes that either introduce or resolve problems in folding of an RNA (*e.g.* Refs. 62 and 63). This might be likened to the role of the prosequence in reducing a kinetic barrier in the folding of certain bacterial proteases (32).

Extending the RNA/Protein Folding Analogy to RNA-dependent ATPases—Most or all of the known protein chaperones use ATP (52), in contrast to the RNA chaperones discussed above. The RNA-dependent ATPases (Rd-ATPases), which constitute a large family of proteins (64),[6] may be more akin to the protein chaperones as they use the energy of ATP hydrolysis to facilitate structural transitions. However, despite this gross similarity, there appear to be mechanistic distinctions. The GroEL/GroES chaperonin appears to use ATP to strike a balance between allowing the unfolded protein the opportunity to fold in solution *versus* sequestering it to prevent aggregation with other unfolded proteins (65, 53, 66). In contrast, Rd-ATPases are presumably more akin to helicases, using ATP to disrupt duplex and other structured regions in a stepwise fashion (67). It is not known to what extent ATP-independent and ATP-dependent proteins are employed *in vivo* in the folding and unfolding of RNA.

The use of energy by Rd-ATPases could also allow RNA folding and unfolding steps to be integrated and regulated within complex biological phenomena. For example, an Rd-ATPase may be used to dissociate the U4-U6 snRNP complex at just the right time in spliceosomal assembly, facilitating assembly of a catalytically active spliceosome and/or preventing inappropriate or premature splicing (68). U4 may act as an RNA chaperone made of RNA that prevents misfolding of U6.) Rd-ATPases could also help select between alternative splice sites and prevent inaccurate splicing via a proofreading function that limits the time allotted for individual assembly and catalytic steps (69). Rd-ATPases have also been implicated in ribosomal assembly and translational initiation.

An Evolutionary Perspective

The above ideas can be placed within a unifying but speculative evolutionary context in which an early step in the transition from the RNA world to the RNA/protein world was the emergence of nonspecific RNA-binding peptides with chaperone-type activities. These peptides could have provided a selective advantage in a primitive RNA-dominated world by rescuing RNAs from kinetic traps, aiding in the structural transition of a postreplicative duplex to a folded, functional single-stranded RNA, and helping RNAs more broadly explore structural alternatives. The appearance of a functional nonspecific RNA-binding peptide is expected to be more probable than the appearance of a specific RNA binder because there are more solutions to the problem of nonspecific binding and because a non-

[6] The term RNA-dependent ATPase is used instead of the more common term RNA helicase, as it is likely that some Rd-ATPases facilitate structural transitions other than duplex disruption. The Rd-ATPases have been identified predominantly based on sequence similarities; there is little mechanistic information available concerning helicase or other activities.

specific binder would have many potential functional targets.

Later in evolution, the *problems* in folding RNA could have been parlayed into new *opportunities* for biological systems via cooperation between RNA and proteins, with nonspecific RNA-binding proteins with RNA chaperone functions developing binding preferences and ATP-dependent activities for use in control and regulation. For example, the hnRNP A1 protein has RNA chaperone activity (6, 7, 46) and also appears to be involved in splice site selection (70), and the NC protein from HIV has chaperone activity and appears to bind viral RNA specifically during packaging (4).

Orchestration of RNA Chaperone Activity in Vivo

The nonspecific RNA-binding proteins that enhance RNA function can also shut down RNA function at higher concentrations, so that there is a limited "window of opportunity" for each protein to be functional (1, 6, 7). How then can a cell orchestrate the function of a large number of such proteins amidst a pool of near-random RNA without merely binding to and obscuring the function of a large subset of the RNAs? How are the chaperones removed to allow the RNA to function? How does a specific RNA-binding protein find its cognate RNA?

The answers to these questions are not known. Although the concentrations of the various RNA and protein components and their affinities can be regulated to influence RNA processing and function (*e.g.* Refs. 31, 33, 71, 72), it is not clear that affinities can be tuned and concentrations regulated precisely enough to fully avoid problems of inappropriate RNA/protein pairings and proteins obscuring RNA function. Higher order temporal and spatial cellular organization could be used to avoid these problems and to integrate RNA/protein interactions into other cellular processes. RNA could be "handed off" from one protein to another, with hnRNP proteins binding pre-mRNA as it is transcribed, perhaps being selectively replaced by proteins to set the stage for spliceosomal assembly and splicing (9, 34). After splicing, the mRNA may be escorted to the cytoplasm by a subset of the hnRNPs and by other proteins (10) and then delivered to the ribosome for translation. Evidence for hand offs of proteins from one chaperone to another during folding (29, 56) provides a conceptual precedent for analogous action by RNA chaperones. The replacement of one protein by another could be spatially or temporally regulated by spatial segregation of specific RNAs and specific RNA-binding proteins within the nucleus (as for ribosomal assembly within the nucleolus) or by fast initial binding of more weakly bound proteins followed by slower binding of more strongly bound proteins and complexes.

A general advantage of keeping RNA molecules protein-bound is that proteins can dissociate faster than some RNA self-structures unravel on their own, allowing the RNA to change partners in a timely fashion throughout its processing odyssey. However, in some instances, the kinetic stability of RNA structures has been co-opted for cellular function; the classical example is attenuation of the *trp* and other bacterial operons that are regulated via a choice between alternative RNA secondary structures (45).

It will be fascinating over the coming years to learn how RNA folding is controlled within the organization of RNA processing and RNA function. On the molecular level, it will be fascinating to unravel the mechanisms of RNA folding and RNA chaperone activity.

REFERENCES

1. Munroe, S. H., and Dong, X. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 895–899
2. Pontius, B. W., and Berg, P. (1992) *J. Biol. Chem.* **267,** 13815–13818
3. Sundquist, W. I., and Heaphy, S. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3393–3397
4. Tsuchihashi, Z., Khosla, M., and Herschlag, D. (1993) *Science* **262,** 99–102
5. Coetzee, T., Herschlag, D., and Belfort, M. (1994) *Genes & Dev.* **8,** 1575–1588
6. Herschlag, D., Khosla, M., Tsuchihashi, Z., and Karpel, R. L. (1994) *EMBO J.* **13,** 2913–2924
7. Portman, D. S., and Dreyfuss, G. (1994) *EMBO J.* **13,** 213–221
8. Tsuchihashi, Z., and Brown, P. O. (1994) *J. Virol.* **68,** 5863–5870
9. Staknis, D., and Reed, R. (1994) *Mol. Cell. Biol.* **14,** 7670–7682
10. Dreyfuss, G., Matunis, M. J., Pinol-Roma, S., and Burd, C. G. (1993) *Annu. Rev. Biochem.* **62,** 289–321
11. Gartland, W. J., and Sueoka, N. (1966) *Proc. Natl. Acad. Sci. U. S. A.* **55,** 948–956
12. Lindahl, T., Adams, A., and Fresco, J. R. (1966) *Proc. Natl. Acad. Sci. U. S. A.* **55,** 941–948
13. Adams, A., Lindahl, T., and Fresco, J. R. (1967) *Proc. Natl. Acad. Sci. U. S. A.* **57,** 1684–1691
14. Ishida, T., and Sueoka, N. (1968) *J. Biol. Chem.* **243,** 5329–5336
15. Cole, P. E., and Crothers, D. M. (1972) *Biochemistry* **11,** 4368–4374
16. Cole, P. E., Yang, S. K., and Crothers, D. M. (1972) *Biochemistry* **11,** 4358–4368
17. Yang, S. K., and Crothers, D. M. (1972) *Biochemistry* **11,** 4375–4381
18. Uhlenbeck, O. C., Chirikjian, J. G., and Fresco, J. R. (1974) *J. Mol. Biol.* **89,** 495–504
19. Hawkins, E. R., Chang, S. H., and Mattice, W. L. (1977) *Biopolymers* **16,** 1557–1566
20. Davila-Aponte, J. A., Huss, V. A. R., Sogin, M. L., and Cech, T. R. (1991) *Nucleic Acids Res.* **19,** 4429–4436
21. Riesner, D., Henco, K., Rokohl, U., Klotz, G., Kleinschmidt, A. K., Domdey, H., Jank, P., Gross, H. J., and Saenger, H. L. (1979) *J. Mol. Biol.* **133,** 85–115
22. Tanner, N. K., and Cech, T. R. (1985) *Nucleic Acids Res.* **13,** 7741–7758
23. Walstrum, S. A., and Uhlenbeck, O. C. (1990) *Biochemistry* **29,** 10573–10576
24. Jaeger, L., Westhof, E., and Michel, F. (1991) *J. Mol. Biol.* **221,** 1153–1164
25. Pace, U., and Szostak, J. W. (1991) *FEBS Lett.* **280,** 171–174
26. Michel, F., Jaeger, L., Westhof, E., Kuras, R., Tihy, F., Xu, M.-Q., and Shub, D. A. (1992) *Genes & Dev.* **6,** 1373–1385
27. Sigler, P. B. (1975) *Annu. Rev. Biophys. Bioeng.* **4,** 477–527
28. Gruenewald, B., Nicola, C. U., Lustig, A., Schwarz, G., and Klump, H. (1979) *Biophys. Chem.* **9,** 137–147
29. Hammond, C., and Helenius, A. (1994) *Science* **266,** 456–458
30. Turner, D. H., Sugimoto, N., and Freier, S. M. (1990) *Thermodynamics and Kinetics of Base-pairing and of DNA and RNA Self-assembly and Helix Coil Transition: Nucleic Acids*, pp. 201–227, Springer-Verlag, Berlin
31. Dreyfuss, G., Matunis, M. J., Pinol-Roma, S., and Burd, C. G. (1993) *Annu. Rev. Biochem.* **62,** 289–321
32. Baker, D., and Agard, D. A. (1994) *Biochemistry* **33,** 7505–7509
33. Matunis, E. L., Matunis, M. J., and Dreyfuss, G. (1993) *J. Cell Biol.* **121,** 219–228
34. Wu, J. Y., and Maniatis, T. (1993) *Cell* **75,** 1061–1070
35. Doty, P., Boedtker, H., Fresco, J. R., Haselkorn, R., and Litt, M. (1959) *Proc. Natl. Acad. Sci. U. S. A.* **45,** 482–499
36. Gralla, J., and DeLisi, C. (1974) *Nature* **248,** 330–332
37. Herschlag, D. (1992) *Biochemistry* **31,** 1386–1399
38. Karpel, R. L., Swistel, D. G., Miller, N. S., Geroch, M. E., Lu, C., and Fresco, J. R. (1974) *Brookhaven Symp. Biol.* **26,** 165–174
39. Karpel, R. L., Miller, N. S., and Fresco, J. R. (1982) *Biochemistry* **21,** 2102–2108
40. Alberts, B. M., and Frey, L. (1970) *Nature* **227,** 1313–1318
41. Christiansen, C., and Baldwin, R. L. (1977) *J. Mol. Biol.* **115,** 441–454
42. Rothman, J. E., and Kornberg, R. D. (1986) *Nature* **322,** 209–210
43. Hertel, K. J., Herschlag, D., and Uhlenbeck, O. C. (1994) *Biochemistry* **33,** 3374–3385
44. Herschlag, D. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6921–6925
45. Yanofsky, C. (1981) *Nature* **289,** 751–758
46. Bertrand, E. L., and Rossi, J. J. (1994) *EMBO J.* **13,** 2904–2912
47. Muller, G., Strack, B., Dannull, J., Sproat, B. S., Surovoy, A., Jung, G., and Moelling, K. (1994) *J. Mol. Biol.* **242,** 422–429
48. Brehm, S. L., and Cech, T. R. (1983) *Biochemistry* **22,** 2390–2397
49. Gampel, A., and Cech, T. R. (1991) *Genes & Dev.* **5,** 1870–1880
50. Guo, Q., and Lambowitz, A. M. (1992) *Genes & Dev.* **6,** 1357–1372
51. Mohr, G., Zhang, A., Gianelos, J. A., Belfort, M., and Lambowitz, A. M. (1992) *Cell* **69,** 483–494
52. Ellis, J. R., and van der Vies, S. M. (1991) *Annu. Rev. Biochem.* **60,** 321–347
53. Todd, M. J., Vitanen, P. V., and Lorimer, G. H. (1994) *Science* **265,** 659–666
54. van Dieijen, G., van Knippenberg, P. H., and van Duin, J. (1976) *Eur. J. Biochem.* **64,** 511–518
55. Gething, M.-J., and Sambrook, J. (1992) *Nature* **355,** 33–45
56. Hendrick, J. P., and Hartl, F.-U. (1993) *Annu. Rev. Biochem.* **62,** 349–384
57. Parsell, D. A., Kowal, A. S., Singer, M. A., and Lindquist, S. (1994) *Nature* **372,** 475–478
58. Chernoff, Y. O., Lindquist, S. L., Ono, B., Inge-Vechtomov, S. G., and Liebman, S. W. (1995) *Science* **268,** 880–884
59. Pontius, B. W., and Berg, P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 8237–8241
60. Pontius, B. W. (1993) *Trends Biochem. Sci.* **18,** 181–186
61. Goodchild, J. (1992) *Nucleic Acids Res.* **20,** 4607–4612
62. Dammel, C. S., and Noller, H. F. (1993) *Genes & Dev.* **7,** 660–670
63. Woodson, S. A., and Emerick, V. L. (1993) *Mol. Cell. Biol.* **13,** 1137–1145
64. Linder, P., Lasko, P. F., Leroy, P., Nielsen, P. J., Nishi, K., Schnier, J., and Slonimski, P. P. (1989) *Nature* **337,** 121–122
65. Martin, J., Mayhew, M., Langer, T., and Hartl, F. U. (1993) *Nature* **366,** 228–233
66. Weissman, J. S., Kashi, Y., Fenten, W. A., and Horwich, A. L. (1994) *Cell* **78,** 693–702
67. Lohman, T. M. (1993) *J. Biol. Chem.* **268,** 2269–2272
68. Guthrie, C., and Patterson, B. (1988) *Annu. Rev. Genet.* **22,** 387–419
69. Burgess, S. M., and Guthrie, C. (1993) *Trends Biochem. Sci.* **18,** 381–384
70. Caceres, J. F., Stamm, S., Helfman, D. M., and Krainer, A. R. (1994) *Science* **265,** 1706–1709
71. Amero, S. A., Raychaudhuri, G., Cass, C. L., vanVenrooij, W. J., Habets, W. J., Krainer, A. R., and Beyer, A. L. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 8409–8413
72. Bennett, M., Pinol-Roma, S., Staknis, D., Dreyfuss, G., and Reed, R. (1992) *Mol. Cell. Biol.* **12,** 3165–3175

Minireviews:

RNA Chaperones and the RNA Folding Problem

Daniel Herschlag

J. Biol. Chem. 1995, 270:20871-20874.
doi: 10.1074/jbc.270.36.20871
