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Review

Recent insights on RNA folding mechanisms from catalytic RNA

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Abstract. Methods for probing RNA structure in real time have revealed that initial folding steps are complete in less than a second. Refolding of large catalytic RNAs in vitro often results in long-lived intermediates that reach the native structure very slowly. These kinetically trapped intermediates arise from alternative secondary structures that form early in the folding process. In cells, proteins modulate the outcome of RNA folding reactions by stabilizing specific conformations or by accelerating refolding of misfolded intermediates. At the same time, competition between metastable conformations provides a means for regulating the biological activity of transcripts.

Key words. RNA folding; ribozyme; energy landscape; translation attenuation; RNA structure; group I intron; RNase P; folding kinetics; transcription elongation.

The many biological functions of RNA are reflected in the diversity of three-dimensional structures that RNA sequences can adopt. For example, stable rRNA and snRNAs play integral roles in determining the architecture of the ribosome and spliceosome, and their function in translation and splicing is well established [1, 2]. In addition, secondary and tertiary structure in precursor and mature mRNAs can also determine their post-transcriptional activity, in part by modulating interactions with diverse RNA-binding proteins [e.g., refs 3–5].

The link between structure and function is most apparent in autocatalytic introns and self-cleaving RNAs. For this reason, they have been valuable model systems for understanding how complex RNA structures are assembled [6–9]. Recent high-resolution structures have begun to reveal the folded conformations of several ribozymes [10–12]. Here, I discuss how experiments designed to probe the folding kinetics of catalytic RNAs are beginning to unravel the mechanisms by which large RNA structures are assembled. The conformational dynamics of smaller self-cleaving RNAs, such as the hammerhead and hairpin ribozymes, have been reviewed elsewhere [13] and will not be discussed here.

Early studies on tRNA

Experiments carried out on transfer RNAs in the 1960s and 1970s laid the foundation for more recent work on the folding mechanisms of catalytic RNAs [14]. The discovery that the cloverleaf secondary structure was organized into a compact tertiary fold provided the first hint that RNA structures were topologically complex [15, 16], and the three-dimensional structure of yeast $tRNA^{phe}$ uncovered many of the interactions that stabilize RNA tertiary structures [17, 18]. These include base stacking across helix junctions and in loops, non-Watson-Crick base pairs, and neutralization of negative charge on the phosphates by di- and trivalent cations.

Temperature-jump and nuclear magnetic resonance (NMR) experiments on tRNAs and small RNA hairpins established that local secondary structures form with time constants of 10–100 μs [19, 20]. Although the rate of helix formation was largely independent of sequence and temperature, the rate of helix opening depended on the stability of the base pairs, and varied strongly with temperature [21, 22]. Refolding of stem-loops in *Escherichia coli* initiator $tRNA^{fMet}$ was also found to correlate with the length of the loop, with the acceptor stem forming about 25 times more slowly than the anticodon helix [23].

Tertiary interactions in tRNA were formed more slowly, typically in 10–100 ms [23–25]. For some tRNAs, however, the folding rate was much less (seconds), due to the need to rearrange non-cloverleaf secondary structures which were particularly likely to form at low ionic strength [23]. Indeed, it was apparent very early that RNAs were prone to forming competing structures of similar stabilities, and that the exchange between these conformational states was slow at physiological temperatures [26, 27].

Folding of large catalytic RNAs

The discovery that certain RNAs could function as catalysts stimulated interest in understanding how their tertiary structures are assembled [28, 29]. Of particular interest were large ribozymes that contain two or more domains of tertiary structure. Group I autocatalytic introns range from about 200 to 1000 nt, group II are about 600–2000 nt long, and the RNA subunits of bacterial RNase Ps are typically 300–400 nt [30]. Initial experiments to determine the folding pathway of the group I ribozyme from *Tetrahymena thermophila* rDNA and the ribozyme from *Bacillus subtilis* RNase P revealed that the active conformation of the RNA was formed over a period of minutes or hours, and that partially folded intermediates persisted for long times [31–34]. Thus, the assembly of large multi-domain RNAs is a more demanding process than the folding of tRNA.

Folding pathway of a group I ribozyme

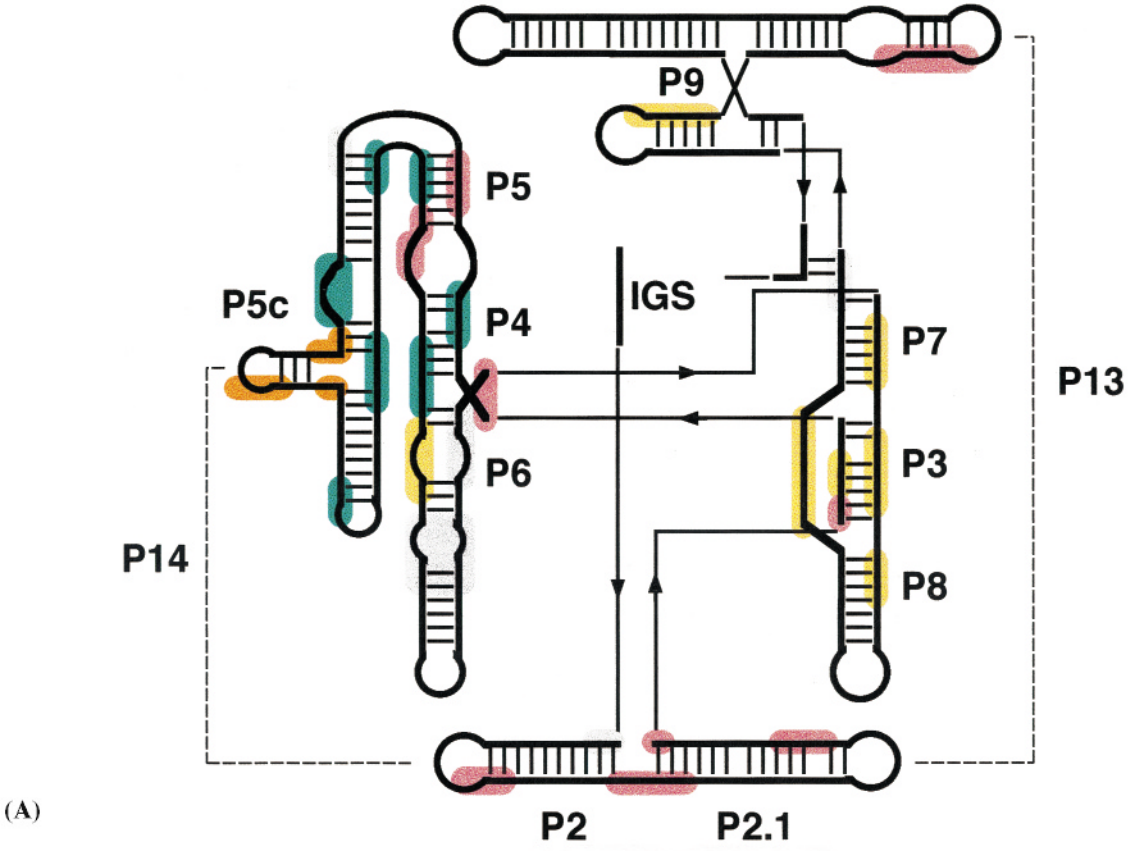
The active site of the *Tetrahymena* group I ribozyme is formed by the close packing of two major helical domains at the center of the structure in the presence of Mg^{2+}, which is required for catalytic activity [29]. One of these, containing paired (P) regions P4–P6, is independently stable [35], and forms a compact structure in which the RNA is bent 180° [36] (fig. 1). A high-resolution crystallographic structure of the 160 nt P4–P6 RNA [37] revealed a network of interactions that stabilize close packing between helices P4, P5, and P6 on the one side, and a subdomain formed by the junction of helices P5a, 5b, and 5c (P5abc) on the other. Paired regions P3, P7, P8, and P9 form a separate helical domain (P3–P9) that wraps around P4–P6, creating a cleft that binds a helix containing the 5′ splice site (P1) [38]. P2.1, P9.1, and P9.2, which are not essential for catalytic activity, are proposed to wrap around the exterior of the structure [39], and play an important role in stabilizing the folded structure of the ribozyme [40–42].

Knowing *how* large RNA structures are formed requires methods capable of rapidly probing the conformation in a sequence-specific manner. Zarrinkar and Williamson [32] developed an approach for measuring RNA folding kinetics based on competitive hybridization of complementary deoxyoligonucleotides. Unpaired regions of the RNA that are available to base pair with the DNA probe are cleaved by RNase H. The folding rate can be obtained from the fraction of cleaved RNA, under conditions in which cleavage is more rapid than dissociation of the probe. Experiments with oligonucleotides complementary to different regions of the *Tetrahymena* ribozyme showed that P4 and P6 were base paired at the earliest time that could be assayed by this method (15 s to 1 min), while the P3 and P7 helices were formed much more slowly ($k_{obs} = 0.7$ min^{-1}). These results, demonstrating that there was at least one intermediate in which only the P4–P6 domain of the ribozyme was folded, were consistent with the ability of the P4–P6 domain to fold at lower Mg^{2+} concentrations than the P3–P9 domain in equilibrium titration experiments [31]. Similar intermediate species were also detected by time-dependent photo-crosslinking and by chemical base modification of the RNA [43, 44].

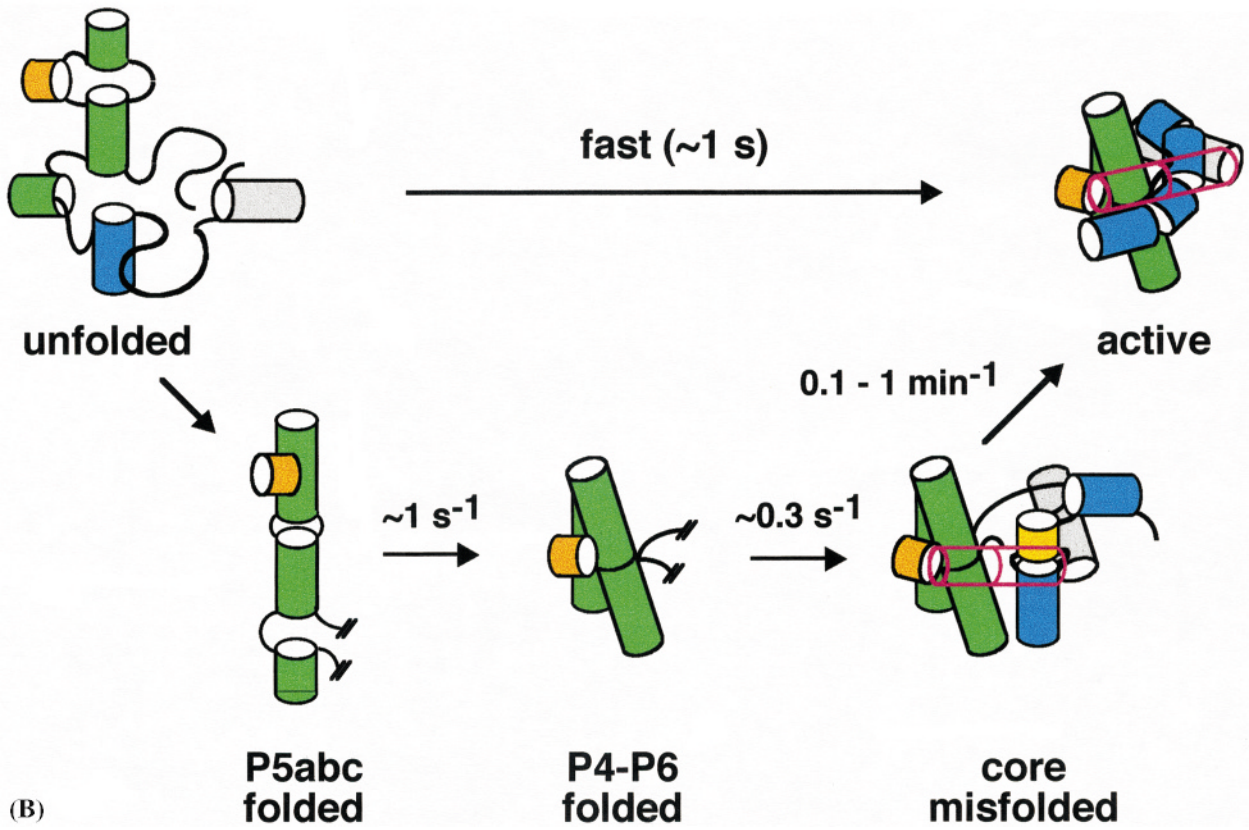
The P4–P6 and P3–P9 domains are oriented with respect to each other by a triple helix at the center of the ribozyme [45]. The triple helix is required for reconstitution of the active ribozyme from the separate P4–P6 and P3–P9 domains [46]. A mutation that disrupts the triple helix decreased the rate at which P3 and P7 became base paired [47]. These results, plus the observation that the P3–P9 domain could not fold in the absence of P4–P6 RNA, initially suggested that the P4–P6 domain acts as a 'scaffold' for folding of P3 and P7 [47, 48]. Recent experiments suggest that P4–P6 stabilizes the folded conformation of the P3–P9 domain, but is not required for its assembly [49, 50].

Spectroscopic probes for RNA folding

One drawback to biochemical probes of RNA structure is that the reactions can only rarely be carried out in


P9
P5
P5c
P4
IGS
P7
P13
P6
P3
P14
P8
P2
P2.1

(A)


fast (~1 s)
unfolded
active
0.1 - 1 min-1
~1 s-1
~0.3 s-1
P5abc folded
P4-P6 folded
core misfolded

(B)

Fig. 1.

less than 1 min, and are usually not suitable for detecting rapid conformational changes. Spectroscopic methods, such as fluorescence emission or circular dichroism, can be used to track conformational changes in milliseconds or even microseconds, as long as one can construct a robust structural framework for interpreting changes in signal intensity. Stopped-flow fluorescence was used to detect base pairing and tertiary interactions involved in binding of substrates to the *Tetrahymena* ribozyme by covalently linking pyrene to the 5′ end of the substrate RNA [51]. A similar approach has also been used to monitor conformational rearrangements associated with substrate binding to the hairpin ribozyme and a group II intron [52, 53], and a conformational switch in a spliced leader RNA [54]. Pyrene has also been used to follow global tertiary transitions in the P4–P6 domain of the *Tetrahymena* ribozyme [55]. If two fluorophores are linked to the RNA, the distance between them during the folding reaction may be monitored using fluorescence resonance energy transfer (FRET) [56, 57].

X-ray footprinting of RNA

More recently, a method for probing RNA tertiary structure by hydroxyl radical cleavage on the millisecond timescale has been developed [58]. Regions of the nucleic acid backbone that are solvent inaccessible are protected from cleavage in the presence of hydroxyl radical [59, 60]. Thus, the relative extent of hydroxyl radical cleavage can be used to monitor tertiary structure in RNA [61]. Irradiation of an aqueous solution with a synchrotron white light X-ray beam generates sufficiently high steady-state concentrations of hydroxyl radical (0.1–1 μM) to carry out the cleavage reaction in 10–50 ms, about three orders of magnitude faster than can be achieved in Fe(II)-EDTA reactions [58]. This advance made it possible to collect 'snapshots' of the *Tetrahymena* ribozyme tertiary structure at 10–20-ms intervals, after initiating folding by rapid mixing with $MgCl_2$.

X-ray footprinting of the *Tetrahymena* ribozyme showed that the tertiary structure of the P4–P6 domain was formed in several seconds ($k_{obs} = 1\ s^{-1}$) [62]. In contrast, nucleotides in the P3–P9 domain became protected from solvent much more slowly ($k_{obs} \approx 1\ min^{-1}$), consistent with earlier results from oligonucleotide hybridization studies and with the onset of catalytic activity [32, 50, 63]. Nucleotides at the interface between P4–P6 and peripheral helices (P2, P2.1, and P9.1a) were protected at an intermediate rate ($k_{obs} \approx 0.3\ s^{-1}$). As a result, the overall folding process is limited by a slow conformational search in the catalytic core of the ribozyme.

Kinetic traps and misfolded intermediates

Slow steps in the folding process indicate the presence of one or more stable intermediates. These intermediates can be resolved by native polyacrylamide gel electrophoresis at low temperatures [64]. After 15–30 s at 30 °C, only a small amount of pre-RNA containing the *Tetrahymena* ribozyme (5–10%) was in the splicing-competent conformation. Most of the pre-RNA migrated as a broad band in the gel, corresponding to many partially folded, inactive conformations [65]. After long incubations with Mg^{2+} or annealing at high temperatures, 70–90% of the RNA was converted to the active form at rates (0.1–0.5 min^{-1}) that were consistent with activity assays [63]. Chemical modification interference and site-directed mutagenesis showed that the predominant intermediate of the pre-RNA is stabilized by incorrect secondary structure in the P3–P9 domain [66]. Subsequent experiments found that the ribozyme also becomes kinetically trapped in stable intermediates [67, 68], and these intermediates arise from misfolding of the P3 helix [66].

In the *Tetrahymena* pre-RNA, the equilibrium between inactive and active conformations also depends on interactions with rRNA exon sequences. Self-splicing is inhibited by a stem-loop in the 5′ exon that competes with formation of the P1 splice site helix [69], and formation of this rRNA stem-loop increases misfolding of P3 in the intron core [66]. In fact, there are many examples known in which adjacent sequences alter the stability and folding kinetics of autocatalytic RNAs. Splicing of a group I intron from phage T4 is inhibited by base pairing between the 5′ exon and nucleotides in the intron near the 3′ splice [70]. Similarly, interactions

Figure 1. Parallel folding pathways of the *Tetrahymena* group I ribozyme. (*A*) Secondary structure [from refs 39, [155]]. Paired (P) regions are numbered 5′ to 3′. The P4–P6 domain (left) contains helices P4, P5, and P6; P7–P9 domain (right) consists of P3, P7, P8, and P9. Dashed lines indicate base pairing between hairpin loops (P13, P14). Colors indicate regions that are protected from hydroxyl radical cleavage [62]: orange, 2 s^{-1}; green, 1 s^{-1}; pink, 0.2–0.4 s^{-1}; yellow, 0.02–0.09 s^{-1}; gray, rate not determined. (*B*) A small fraction of the wild-type ribozyme folds rapidly to the active structure without stable intermediates (top), the remainder of the population becomes trapped in several metastable intermediates that must partially unfold before forming the native structure (bottom) [65]. These intermediates are stabilized by mispairing of the P3 helix (yellow cylinder) and tertiary interactions in the P4–P6 domain (green) [66, 68]. The unfolded state has many conformations; there are at least several populated intermediates. [Model of active ribozyme based on refs 38, 39.]

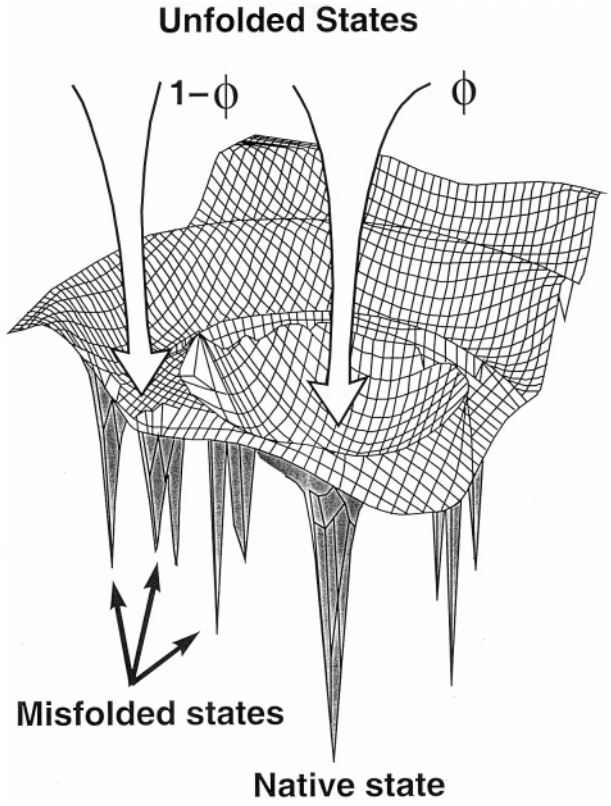


Figure 2. Kinetic partitioning mechanism for folding. Metastable intermediates correspond to local minima in the conformational free energy surface and compete with the native structure (global minimum). The 'ruggedness' of the landscape arises from the tendency of the RNA to form incorrect interactions (e.g., to misfold) [from ref. 85].

with flanking sequences have been found to inhibit splicing of a group II intron in yeast mitochondria [71], splicing of pre-mRNA in yeast [72], and cleavage of the hepatitis delta ribozyme [73, 74] among others. In a few cases, flanking sequences enhance ribozyme activity by stabilizing the active structure [75–78].

Kinetic partitioning mechanism of RNA folding

The results above point to an emerging theme of RNA folding, which is that structures form rapidly, but the degeneracy of local interactions leads to alternative conformations that are resolved slowly at physiological temperatures. As discussed below, these concepts are equally illustrated by parallel experiments on folding of the RNase P ribozyme [79]. This dichotomy between a rapid collapse of secondary and tertiary interactions and slow transitions from intermediates to the native structure late in the folding process can be explained by 'energy landscape' models analogous to those that were initially developed to describe protein folding kinetics [80–83]. In these models, the protein or RNA explores a free energy surface that corresponds to the conformational space available to the molecule (fig. 2). The unfolded state is represented by a large number of conformations of similar free energy, while the native state most often corresponds to the global minimum in the free energy surface [83].

Since each amino acid or nucleotide can make many energetically favorable interactions (besides those that it makes in the correctly folded structure), there are several minima in the free energy surface [84]. The 'rough' features of the energy landscape led us to propose a kinetic partitioning mechanism (KPM) for RNA folding [85]. In brief, this model states that a fraction of the population folds directly and rapidly to the native structure (represented by the global minimum), while the remainder of the population becomes trapped in misfolded intermediates (local minima). Transitions from the intermediates to the native state are slow, because at least some interactions must be broken before the correct structure can be formed (fig. 2).

Since folding is cooperative (that is, each interaction stabilizes the next), the RNA chain will rapidly become more and more ordered until a low free energy state has been reached. If all the contacts are consistent with the native structure and no incorrect interactions that require unfolding are introduced, the native RNA forms rapidly [85]. In this case, folding appears to be a two-state mechanism, because intermediates (if any) are transient and difficult to observe. This direct folding mechanism appears to fit the behavior of some tRNAs, a domain of RNase P RNA (see below) [86], and perhaps the P4–P6 domain of the *Tetrahymena* ribozyme at high ionic strength [S. Silverman, T. R. Cech, M. Deras and S. Woodson, unpublished data].

As we have already seen, most RNAs can adopt many alternative conformations, and the conformational free energy diagram for RNA folding is in reality many times more complex than indicated by figure 2. For long RNA sequences, the number of possible conformations is large, and the fraction of the RNA population that folds rapidly becomes small. Only 5–10% of the *Tetrahymena* precursor RNA and even less of the ribozyme reaches the active conformation within 30 s at physiological temperatures [50, 65].

A prediction of the KPM is that the rates of slow folding processes are determined by the relative stabilities of the trapped intermediates and the native conformation. Consistent with this idea, denaturants such as urea that destabilize metastable intermediates were found to increase folding rates [65, 67, 79]. Conversely, conditions that stabilize RNA structures, such as higher concentrations of Mg^{2+}, resulted in slow folding rates [87, 88].

Optimizing RNA sequences for folding

If RNAs misfold so readily, how can the folding process be improved? Treiber et al. [68] carried out in vitro selection for mutations in the *Tetrahymena* ribozyme that increase the rate at which P3 is base paired, and found that they mapped to P5abc, a particularly stable region of tertiary structure in the P4–P6 domain [68]. The most straightforward interpretation was that native interactions in the P4–P6 domain stabilize one or more folding intermediates, thereby decreasing the overall folding rate [67, 68]. Similarly, long-range base pairing between the terminal loops of P5c and P2 (P14; fig. 1) was found to decrease the rate of folding by stabilizing misfolded intermediates [89]. Tertiary interactions in P5abc and loop-loop interactions such as P14 are required to stabilize the active conformation of the ribozyme under physiological conditions. The fact that they also slow down the folding process suggests that there is a trade-off between the stability of the final structure and the efficiency of assembly.

In contrast, a point mutation that stabilizes the P3 helix enables as much as 80% of the ribozyme to fold within 15 s at 22 °C [89a]. Thus, stabilization of core interactions enables most of the RNA population to avoid becoming trapped in kinetically stable intermediates, resulting in rapid and cooperative formation of active ribozyme. X-ray footprinting of the mutant ribozyme shows that nucleotides in P3 and in the P4–P6 domain are protected from hydroxyl radical cleavage at roughly the same rate, indicating that folding of the mutant is much more cooperative than that of the wild-type RNA [89a].

Folding of a ribozyme from RNase P

The RNA subunit from RNase P is another example of a large catalytic RNA whose folding mechanism has been extensively investigated. Three-dimensional models for the structure of P RNA, which cleaves pre-tRNA substrates to form the 5′ end of the mature tRNA, have been developed from extensive biochemical data and comparative sequence analysis [90, 91]. P RNA from *B. subtilis* can be divided into two domains of tertiary structure that are independently stable and have distinct functions [33, 92]. The larger 'catalytic' domain (~ 250 nt) contains the active site for cleavage [93], while the smaller 'specificity' domain (~ 150 nt) contributes to the specific binding of pre-tRNA substrates [92].

Like the *Tetrahymena* ribozyme, the *B. subtilis* P RNA is susceptible to becoming trapped in misfolded intermediates when refolded in vitro [86]. The overall rates of folding (about 0.1–1 min^{-1}) are limited by the slow transition from the intermediate(s) to the active structure (fig. 3) [34], but can be increased by denaturants such as urea and are highly dependent on temperature and Mg^{2+} concentration [79]. Slow folding is in part due to non-native interactions at the junction of the catalytic and specificity domains.

In the wild-type ribozyme, the catalytic domain is formed from nucleotides at both the 5′ and 3′ ends of the RNA. Permutation of the sequence so that the catalytic domain is placed entirely at the 5′ end of the RNA enabled the catalytic domain to fold rapidly (< 10 s), although the specificity domain still folded slowly under most conditions [86]. These results again demonstrated that single domains of tertiary structure can fold on the 100-ms timescale, if stable misfolded intermediates can be avoided. As the folding kinetics of P RNA were sensitive to the relative order of the catalytic and specificity domains, it also seemed possible that the order in which RNA interactions are formed could determine the folding pathway.

Role of transcription

Various methods used to probe RNA structure during transcription have found that local secondary and tertiary interactions begin to form as soon as the RNA is extruded from the elongation complex, which for *E. coli* RNA polymerase requires about 14–16 nt [94–96]. Since 5′ sequences have an opportunity to interact before the 3′ end is synthesized, the intermediates that are populated during transcription may not be the same as those that prevail during refolding of full-length RNA. Since folding intermediates can be quite stable, they can persist even after transcription is complete.

When Pan and co-workers [97] examined the folding kinetics of RNase P RNA during transcription by either phage T7 or *E. coli* RNA polymerases in vitro, they observed a greater number of metastable intermediates with different stabilities, compared to refolding after transcription (fig. 3). Addition of Nus A elongation factor to reactions of *E. coli* RNA polymerase, however, accelerated folding of the specificity domain at least 10-fold, and increased the overall rate of folding of the permuted RNA about four times. This was most likely due to increased pausing at a specific site, since Nus A had no effect on folding when the RNA was transcribed by a mutant polymerase that displays reduced pausing and is insensitive to Nus A [97].

The distribution of folding intermediates is also expected to depend on the rate of transcription elongation. The elongation rates of phage polymerases are much higher than those of bacterial and eukaryotic polymerases (~ 250 nt s^{-1} vs 5–50 nt s^{-1}) [98, 99], and there have been reports that RNAs expressed from phage T7 promoters are biologically inactive [100, 101]. After slowing down the rate of T7 transcription in vitro,

thermal gradient gel electrophoresis was used to separate metastable structures that appeared during synthesis of potato spindle tuber viroid RNA [102]. Over time, these conformers were observed to refold into thermodynamically more stable structures. As we shall see below, sequential folding of RNA during transcription can also serve as a normal mechanism for regulating gene activity.

Folding of catalytic RNA in vivo

If large ribozymes are susceptible to becoming trapped in long-lived folding intermediates in vitro, how are active RNA structures formed rapidly in the cell? First, binding of specific proteins (or even other RNAs) could promote assembly by selectively stabilizing the native structure [8]. A number of group I and group II introns in fungal mitochondria require specific splicing factors for processing in vivo [103]. Biochemical experiments on the CYT 18 mitochondrial tyrosyl synthetase from *Neurospora*, the CBP2 protein from yeast, and the intron-encoded COB maturase from *Aspergillus nidulans* have shown that these proteins facilitate splicing by binding to the intron RNA and stabilizing its active conformation [104–106]. There is evidence that interactions with the protein also assist folding of the intron RNA [107–109].

Tetrahymena pre-rRNA has a half-life of about 2 s in *Tetrahymena* cells, which is about 20–50 times shorter than that of self-splicing in vitro [110, 111]. The discrepancy between the intracellular activity of the RNA and the very long times required for folding in vitro implies that there are mechanisms for avoiding or overcoming misfolding in vivo. When the intron was inserted into the homologous position of the *E. coli* rRNA, splicing was as rapid in bacteria as it is in *Tetrahymena* cells [112]. Thus, a species-specific splicing

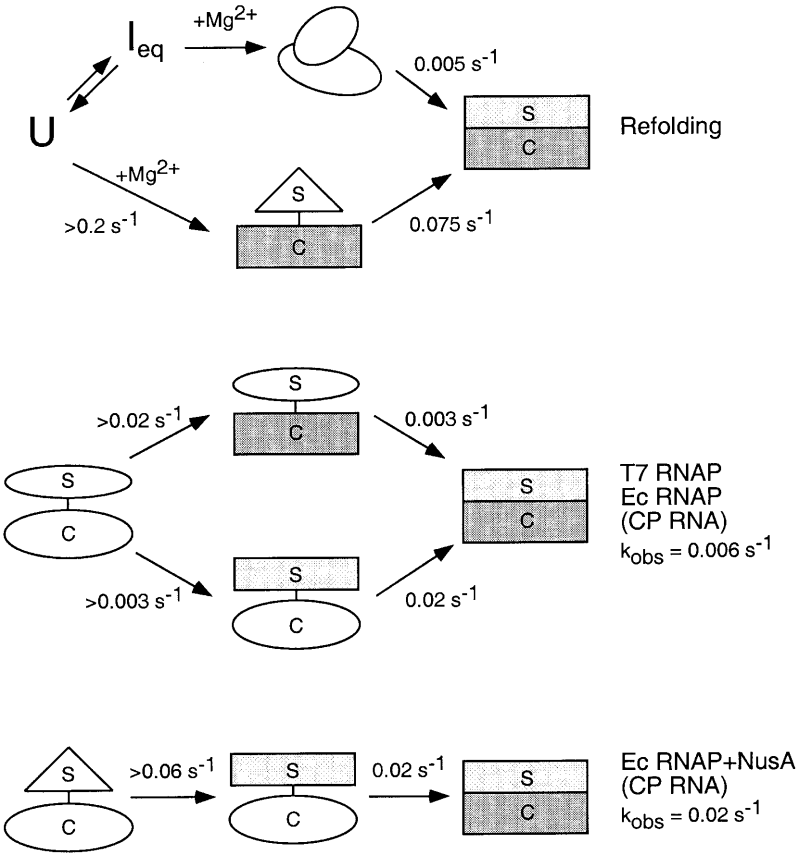


Figure 3. Cotranscriptional folding of *Bacillus subtilis* RNase P ribozyme. The folded specificity (S) and catalytic (C) domains are indicated schematically by gray boxes. The 5′ and 3′ ends of the wild-type ribozyme occur in the middle of the C domain. In the circularly permuted (CP) RNA, the 5′ end begins the C domain and the S domain is at the 3′ end of the transcript. Refolding in vitro (top) involves several metastable intermediates (indicated by open shapes) [86], which are different to those observed during transcription (middle) and in the presence of Nus A (bottom) [from ref. 97].

factor is not required to achieve high intracellular splicing rates of this group I intron. Instead, a more general mechanism must promote folding of the intron in bacteria. Interestingly, mutations in the intron that cause misfolding in vitro also resulted in decreased splicing activity at low temperatures in *E. coli* [113]. These results suggested that the structures of the folding intermediates in bacteria are similar to those identified in vitro.

RNA chaperones

Second, RNA-binding proteins can help unfold non-native intermediates and accelerate their conversion to the active structure [114] in a manner that resembles the action of protein chaperones [115]. A number of proteins that bind preferentially to single-stranded RNA with low sequence specificity have been shown to have RNA chaperone activity in vitro, including the HIV nucleocapsid protein, hnRNP A1 [116], ribosomal protein S12, and a novel protein Stp A from *E. coli* [117, 118]. The *E. coli* cold shock protein Csp A accelerates RNA strand exchange in vitro, suggesting that one of its functions is to help dissociate misfolded RNAs at low temperatures [119]. The yeast La protein, which binds to the oligo-U sequence at the 3′ end of pol III transcripts, is proposed to function as a chaperone for the assembly of newly transcribed pol III RNAs into functional RNPs [120]. A hallmark of these proteins is that they are not required for activity once the RNA is properly folded [117]. Indeed, La is not essential for survival of wild-type yeast, but is required in cells containing mutations that compromise pre-tRNA processing or the assembly of U6 snRNAs [120].

The observation that ribozymes fold slowly in vitro because they form incorrect structures provides a basis for expecting that proteins will be generally required to accelerate conformational changes in RNA. A good illustration of this is the assembly of spliceosomes, which requires auxilliary factors that destabilize RNA helices [121]. These include members of the DEAD/DEAH-box family of RNA-dependent ATPases, many of which have been directly linked to RNA secondary structure rearrangements in the spliceosome [121–123]. Mutations in these proteins often result in cold-sensitive phenotypes, consistent with their role in unwinding RNA duplexes. Similarly, in vitro reconstitution of ribosomal subunits is slow and requires incubation periods above 37 °C [124]. In addition to DEAD box ATPases, ribosome biogenesis depends on transient base pairing interactions between the pre-rRNA and small nucleolar RNAs, raising the interesting possibility that the snoRNPs function as 'chaperones' for ribosome assembly [125, 126].

Refolding of mRNAs during translation: ribosomes as RNA chaperones?

Recent work in bacteria has revealed a role for ribosomes in stimulating the unfolding and refolding of mRNAs. In some examples, refolding of the mRNA is coupled to translational control of downstream genes by disrupting RNA structures that inhibit translational initiation [127, 128]. A particularly clear example of this idea comes from work on the group I intron from the thymidylate synthase (*td*) gene of bacteriophage T4. Analysis of a large number of mutations in the *td* intron showed that splicing activity in *E. coli* correlates closely with the stability of the intron tertiary structure in vitro [129]. Less expected, however, was the observation that splicing activity was almost undetectable when stop codons were introduced upstream of the *td* intron, just before a region of the 5′ exon that is complementary to a sequence at the 3′ end of the intron (fig. 4) [70]. Base pairing between the 5′ exon and the *td* intron blocks self-splicing, because the nucleotides in the intron normally form interactions that stabilize the catalytic center and enable 3′ splice site recognition [40]. Schroeder and co-workers have established that the correlation between translation and splicing activity was due to dissociation of the inhibitory base pairing by ribosomes moving through the upstream portion of the *td* mRNA [70, 130].

Refolding of metastable structures as a mechanism of gene regulation

The idea that ribosomes can alter the equilibrium between alternative mRNA structures dates back to the discovery of anti-termination and translation attenuation of the *trp* operon in *E. coli* [131]. When levels of tryptophan are low, ribosomes stall on adjacent trp codons in the leader sequence of the *trp* operon, permitting formation of an anti-terminator stem-loop and transcription of tryptophan biosynthetic genes. When tryptophan is abundant, ribosomes read through the anti-terminator stem-loop, resulting in formation of the terminator stem-loop instead. In *B. subtilis*, the anti-terminator stem-loop is unfolded by a protein called TRAP that binds the trp leader in the presence of tryptophan [132]. More recently, TRAP has also been shown to effect translational control by triggering refolding of the mRNA, such that the ribosome-binding site of *trp* E, which is 100 nt downstream of the anti-terminator, becomes sequestered in a stable hairpin [133]. The pyrimidine (pur) biosynthetic operon in *B. subtilis* is another well-studied example in which a regulator, Pyr R, effects translational attenuation by modulating the secondary structure of the mRNA [134].

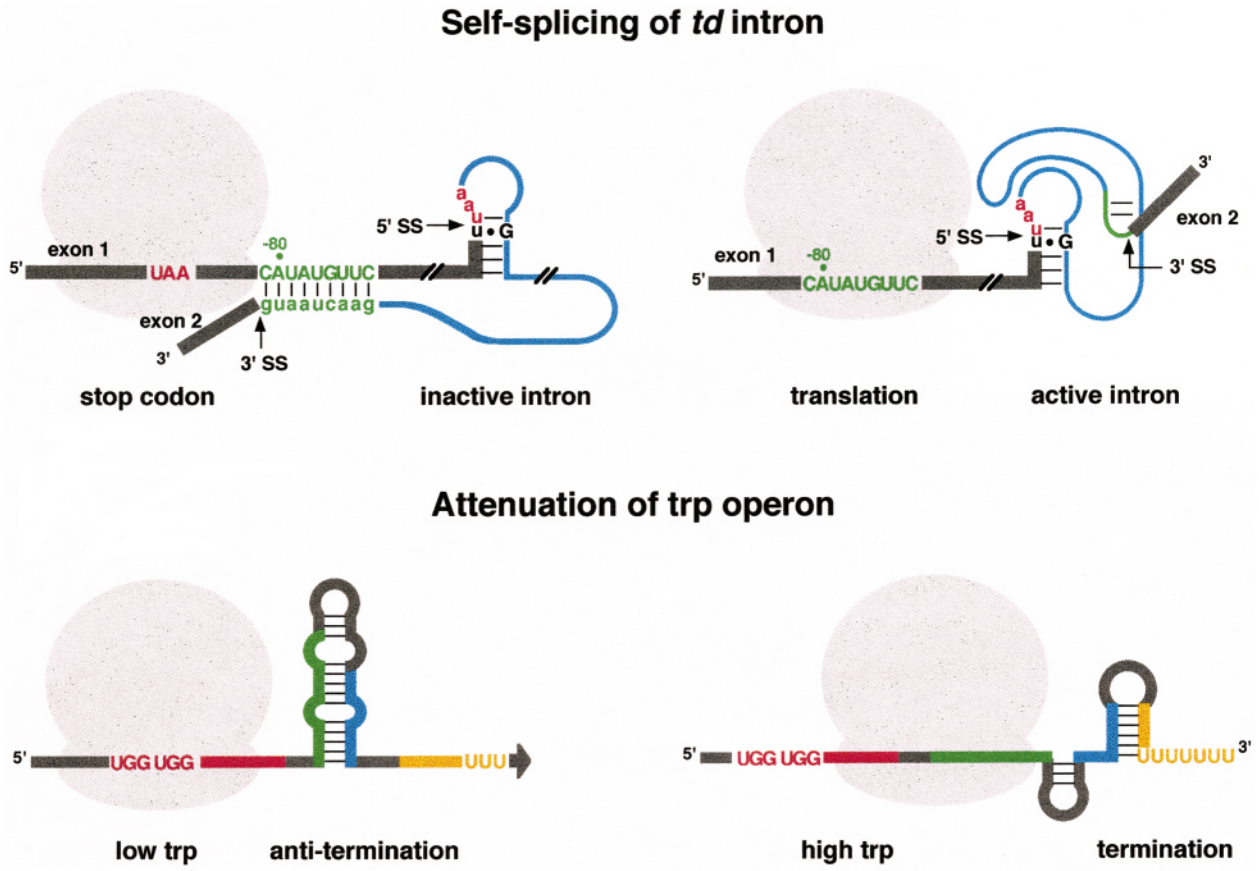


Figure 4. Ribosomes remodel mRNA structure. (*A*) Stop codons upstream of a group I intron in the phage T4 *td* pre-mRNA prevent translocating ribosomes from disrupting a base-pairing interaction (green) between the 5′ exon and nucleotides at the 3′ end of the intron [70]. Self-splicing is inhibited because interactions required for folding and 3′ splice site recognition cannot form. (*B*) Model for translational attenuation and anti-termination in the *trp* operon of *Escherichia coli* [adapted from ref. 131]. In low tryptophan, ribosomes stall on tandem trp codons (red), permitting an anti-terminator stem-loop (green + blue) to form in the mRNA. In high tryptophan, a transcriptional terminator (blue + yellow) is formed.

There are now many examples in bacteria in which the kinetics of RNA folding can be effectively exploited to regulate the timing of translational control [135–137]. Translation of the A protein of phage MS2 is repressed by a cloverleaf structure in the 5′ leader of the mRNA that sequesters the ribosome-binding site in mature mRNAs [138], and only nascent transcripts can be translated. Slow formation of the cloverleaf structure creates a time window in which the ribosome-binding site remains accessible [139]. Slow equilibration of metastable structures has also been proposed to provide a timing mechanism for regulating the expression of plasmid-encoded toxins by anti-sense RNAs [140, 141].

Post-transcriptional control in eukaryotes

Can metastable RNA conformations influence the processing of messages in eukaryotic cells? In eukaryotes, every stage of an mRNA life cycle is orchestrated by an array of RNA-binding proteins, and it is not known to what extent these proteins reorganize or suppress sequence-specific structures in the transcript. All the same, there are hints that RNA structure is important for gene activity. Stable secondary structures involving splice site sequences have been shown to inhibit splice site recognition in yeast and mammals [72, 142–144]. Yeast ribosomal protein L30 and hnRNP A1 down-regulate splicing of their own pre-mRNA by binding and stabilizing secondary structures that sequester the 5′ splice site from components of the spliceosome [145–147]. At the same time, base-pairing interactions that bring together the 5′ and 3′ ends of an intron appear to improve intron/exon recognition [148]. A more subtle effect of pre-mRNA structure could be to promote the synergistic action of splicing enhancer proteins [149]. As the association of splicing factors with pre-mRNA is coupled to transcription by RNA pol II [150], it seems

reasonable to expect that short-term fluctuations in the conformation of the nascent RNA may influence the recognition of RNA sequences by specific proteins [151]. In the cytoplasm, many types of translational control depend on interactions between regulatory proteins and specifically folded structures in the 5′ or 3′ untranslated regions of the mRNA [5]. Developmental timing in *Caenorhabditis elegans* is regulated by lin-4 RNA via an anti-sense mechanism that is not well understood [152]. Masking of maternal *Xenopus* mRNAs depends on binding of members of the Y-box protein family, whose members have many functions in controlling gene expression [153]. The Y-box proteins share structural homology with bacterial cold shock proteins [154], which have also been proposed to modify the stability of RNA duplexes.

Future prospects

New techniques for probing the structures of catalytic RNAs have begun to reveal the mechanisms by which they fold into unique tertiary structures. A more detailed picture of the folding process will continue to emerge as more physical methods are applied to the study of RNA conformation. Perhaps more importantly, RNA folding mechanisms provide a basis for understanding the assembly of RNA-protein complexes and the conformational changes that regulate their function. Genetic and biochemical experiments are still needed to understand the intracellular folding of RNA.

1 Madhani H. D. and Guthrie C. (1994) Dynamic RNA-RNA interactions in the spliceosome. Annu. Rev. Genet. **28:** 1–26
2 Noller H. F. (1998) Ribosomal RNA. In: RNA Structure and Function, pp. Simons R. W. and Grunberg-Manago M. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
3 Balvay L., Libri D. and Fiszman M. Y. (1993) Pre-mRNA secondary structure and the regulation of splicing. Bioessays **15:** 165–169
4 Hertel K. J., Lynch K. W. and Maniatis T. (1997) Common themes in the function of transcription and splicing enhancers. Curr. Opin. Cell Biol. **9:** 350–357
5 McCarthy J. E. G. (1998) Posttranscriptional control of gene expression in yeast. Microbiol. Mol. Biol. Rev. **62:** 1492–1553
6 Brion P. and Westhof E. (1997) Hierarchy and dynamics of RNA folding. Annu. Rev. Biophys. Biomol. Struct. **26:** 113–137
7 Doudna J. A. and Doherty E. A. (1997) Emerging themes in RNA folding. Fold. Des. **2:** R65–R70
8 Weeks K. M. (1997) Protein-facilitated RNA folding. Curr. Opin. Struct. Biol. **7:** 336–342
9 Treiber D. K. and Williamson J. R. (1999) Exposing the kinetic traps in RNA folding. Curr. Opin. Struct. Biol. **9:** 339–345
10 Ramos A., Gubser C. C. and Varani G. (1997) Recent solution structures of RNA and its complexes with drugs, peptides and proteins. Curr. Opin. Struct. Biol. **7:** 317–323
11 Conn G. L. and Draper D. E. (1998) RNA structure. Curr. Opin. Struct. Biol. **8:** 278–285
12 Ferre D. A. A. R. and Doudna J. A. (1999) RNA folds: insights from recent crystal structures. Annu. Rev. Biophys. Biomol. Struct. **28:** 57–73
13 Lilley D. M. (1999) Structure, folding and catalysis of the small nucleolytic ribozymes. Curr. Opin. Struct. Biol. **9:** 330–338
14 Draper D. E. (1996) Parallel worlds. Nat. Struct. Biol. **3:** 397–400
15 Fresco J. R., Adams A., Ascione R., Henley D. and Lindahl T. (1966) Tertiary structure in transfer ribonucleic acids. Cold Spring Harbor Symp. Quant. Biol. **31:** 527–537
16 Kim S. H., Quigley G. J., Suddath F. L., McPherson A., Sneden D., Kim J. J. et al. (1973) Three-dimensional structure of yeast phenylalanine transfer RNA: folding of the polynucleotide chain. Science **179:** 285–288
17 Kim S. H. (1978) Three-dimensional structure of transfer RNA and its functional implications. Adv. Enzymol. Relat. Areas. Mol. Biol. **46:** 279–315
18 Saenger W. (1984) Principles of Nucleic Acid Structure. Springer, New York
19 Coutts S. M. (1971) Thermodynamics and kinetics of G-C base pairing in the isolated extra arm of serine-specific transfer RNA from yeast. Biochim. Biophys. Acta. **232:** 94–106
20 Cole P. E. and Crothers D. M. (1972) Conformational changes of transfer ribonucleic acid: relaxation kinetics of the early melting transition of methionine transfer ribonucleic acid (*Escherichia coli*). Biochemistry **11:** 4368–4374
21 Craig M. E., Crothers D. M. and Doty P. (1971) Relaxation kinetics of dimer formation by self complementary oligonucleotides. J. Mol. Biol. **62:** 383–401
22 Pörschke D. and Eigen M. (1971) Co-operative non-enzymic base recognition. III. Kinetics of the helix-coil transition of the oligoribouridylic · oligoriboadenylic acid system and of oligoriboadenylic acid alone at acidic pH. J. Mol. Biol. **62:** 361–381
23 Crothers D. M., Cole P. E., Hilbers C. W. and Shulman R. G. (1974) The molecular mechanism of thermal unfolding of *Escherichia coli* formylmethionine transfer RNA. J. Mol. Biol. **87:** 63–88
24 Riesner D., Maass G., Thiebe R., Philippsen P. and Zachau H. G. (1973) The conformational transitions in yeast $tRNA^{Phe}$ as studied with $tRNA^{Phe}$ fragments. Eur. J. Biochem. **36:** 76–88
25 Lynch D. C. and Schimmel P. R. (1974) Cooperative binding of magnesium to transfer ribonucleic acid studied by a fluorescent probe. Biochemistry **13:** 1841–1852
26 Adams A., Lindahl T. and Fresco J. R. (1967) Conformational differences between the biologically active and inactive forms of a transfer ribonucleic acid. Proc. Natl. Acad. Sci. USA **57:** 1684–1691
27 Uhlenbeck O. C., Chirikjian J. G. and Fresco J. R. (1974) Oligonucleotide binding to the native and denatured conformers of yeast transfer RNA(Leu)3. J. Mol. Biol. **89:** 495–504
28 Cech T. R. and Herschlag D. (1996) Group I ribozymes: substrate recognition, catalytic strategies, and comparative mechanistic analysis. In: Nucleic Acids and Molecular Biology, vol. 10, pp. 1–17, Eckstein F. and Lilley D. M. J. (eds), Springer, Berlin
29 Cech T. R. and Golden B. L. (1999) Building a catalytic active site using only RNA. In: The RNA World, 2nd edn, pp. 321–349, Gesteland R. F., Cech T. R. and Atkins J. F. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30 Cech T. R. (1993) Structure and mechanism of the large catalytic RNAs: group I and group II introns and ribonuclease P. In: The RNA World pp. 239–269, Gesteland R. F. and Atkins J. F. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31 Celander D. W. and Cech T. R. (1991) Visualizing the higher order folding of a catalytic RNA molecule. Science **251:** 401–407

32 Zarrinkar P. P. and Williamson J. R. (1994) Kinetic intermediates in RNA folding. Science **265:** 918–924
33 Pan T. (1995) Higher order folding and domain analysis of the ribozyme from *Bacillus subtilis* ribonuclease P. Biochemistry **34:** 902–909
34 Zarrinkar P. P., Wang J. and Williamson J. R. (1996) Slow folding kinetics of RNase P RNA. RNA **2:** 564–573
35 Murphy F. L. and Cech T. R. (1993) An independently folding domain of RNA tertiary structure within the *Tetrahymena* ribozyme. Biochemistry **32:** 5291–5300
36 Murphy F. L. and Cech T. R. (1994) GAAA tetraloop and conserved bulge stabilize tertiary structure of a group I intron domain. J. Mol. Biol. **236:** 49–63
37 Cate J. H., Gooding A. R., Podell E., Zhou K., Golden B. L., Kundrot C. E. et al. (1996) Crystal structure of a group I ribozyme domain: principles of RNA packing. Science **273:** 1678–1685
38 Golden B. L., Gooding A. R., Podell E. R. and Cech T. R. (1998) A preorganized active site in the crystal structure of the *Tetrahymena* ribozyme. Science **282:** 259–264
39 Lehnert V., Jaeger L., Michel F. and Westhof E. (1996) New loop-loop tertiary interactions in self-splicing introns of subgroup IC and ID: a complete 3D model of the *Tetrahymena thermophila* ribozyme. Chem. Biol. **3:** 993–1009
40 Jaeger L., Westhof E. and Michel F. (1993) Monitoring of the cooperative unfolding of the sunY group I intron of bacteriophage T4: the active form of the sunY ribozyme is stabilized by multiple interactions with 3′ terminal intron components. J. Mol. Biol. **234:** 331–346
41 Laggerbauer B., Murphy F. L. and Cech T. R. (1994) Two major tertiary folding transitions of the *Tetrahymena* catalytic RNA. EMBO J. **13:** 2669–2676
42 Ikawa Y., Ohta H., Shiraishi H. and Inoue T. (1997) Long-range interaction between the P2.1 and P9.1 peripheral domains of the *Tetrahymena* ribozyme. Nucleic Acids Res. **25:** 1761–1765
43 Banerjee A. R. and Turner D. H. (1995) The time dependence of chemical modification reveals slow steps in the folding of a group I ribozyme. Biochemistry **34:** 6504–6512
44 Downs W. D. and Cech T. R. (1996) Kinetic pathway for folding of the *Tetrahymena* ribozyme revealed by three UV-inducible crosslinks. RNA **2:** 718–732
45 Green R. and Szostak J. W. (1994) In vitro genetic analysis of the hinge region between helical elements P5–P4–P6 and P7–P3–P8 in the sunY group I self-splicing intron. J. Mol. Biol. **235:** 140–155
46 Doudna J. A. and Cech T. R. (1995) Self-assembly of a group I intron active site from its component tertiary structural domains. RNA **1:** 36–45
47 Zarrinkar P. P. and Williamson J. R. (1996) The kinetic folding pathway of the *Tetrahymena* ribozyme reveals possible similarities between RNA and protein folding. Nat. Struct. Biol. **3:** 432–438
48 Doherty E. A. and Doudna J. A. (1997) The P4–P6 domain directs higher order folding of the *Tetrahymena* ribozyme core. Biochemistry **36:** 3159–3169
49 Doherty E. A., Herschlag D. and Doudna J. A. (1999) Assembly of an exceptionally stable RNA tertiary interface in a group I ribozyme. Biochemistry **38:** 2982–2990
50 Russell R. and Herschlag D. (1999) New pathways in folding of the Tetrahymena group I RNA enzyme. J. Mol. Biol. **291:** 1155–1167
51 Bevilacqua P. C., Kierzek R., Johnson K. A. and Turner D. H. (1992) Dynamics of ribozyme binding of substrate revealed by fluorescence-detected stopped-flow methods. Science **258:** 1355–1358
52 Esteban J. A., Banerjee A. R. and Burke J. M. (1997) Kinetic mechanism of the hairpin ribozyme: identification and characterization of two nonexchangeable conformations. J. Biol. Chem. **272:** 13629–13639
53 Qin P. Z. and Pyle A. M. (1997) Stopped-flow fluorescence spectroscopy of a group II intron ribozyme reveals that domain 1 is an independent folding unit with a requirement for specific Mg^{2+} ions in the tertiary structure. Biochemistry **36:** 4718–4730
54 LeCuyer K. A. and Crothers D. M. (1994) Kinetics of an RNA conformational switch. Proc. Natl. Acad. Sci. USA **91:** 3373–3377
55 Silverman S. K. and Cech T. R. (1999) RNA tertiary folding monitored by fluorescence of covalently attached pyrene. Biochemistry **38:** 14224–14237
56 Bassi G. S., Murchie A. I., Walter F., Clegg R. M. and Lilley D. M. (1997) Ion-induced folding of the hammerhead ribozyme: a fluorescence resonance energy transfer study. EMBO J. **16:** 7481–7489
57 Walter N. G., Burke J. M. and Millar D. P. (1999) Stability of hairpin ribozyme tertiary structure is governed by the interdomain junction. Nat. Struct. Biol. **6:** 544–549
58 Sclavi B., Woodson S., Sullivan M., Chance M. and Brenowitz M. (1998) Following the folding of RNA with time-resolved synchrotron X-ray footprinting. Methods Enzymol. **295:** 379–402
59 Tullius T. D. and Dombroski B. A. (1985) Iron(II) EDTA used to measure the helical twist along any DNA molecule. Science **230:** 679–681
60 Dixon W. J., Hayes J. J., Levin J. R., Weidner M. F., Dombroski B. A. and Tullius T. D. (1991) Hydroxyl radical footprinting. Methods Enzymol. **208:** 380–413
61 Latham J. A. and Cech T. R. (1989) Defining the inside and outside of a catalytic RNA molecule. Science **245:** 276–282
62 Sclavi B., Sullivan M., Chance M. R., Brenowitz M. and Woodson S. A. (1998) RNA folding at millisecond intervals by synchrotron hydroxyl radical footprinting. Science **279:** 1940–1943
63 Emerick V. L., Pan J. and Woodson S. A. (1996) Analysis of rate-determining conformational changes during self-splicing of the *Tetrahymena* intron. Biochemistry **35:** 13469–13477
64 Emerick V. L. and Woodson S. A. (1994) Fingerprinting the folding of a group I precursor RNA. Proc. Natl. Acad. Sci. USA **91:** 9675–9679
65 Pan J., Thirumalai D. and Woodson S. A. (1997) Folding of RNA involves parallel pathways. J. Mol. Biol. **273:** 7–13
66 Pan J. and Woodson S. A. (1998) Folding intermediates of a self-splicing RNA: mispairing of the catalytic core. J. Mol. Biol. **280:** 597–609
67 Rook M. S., Treiber D. K. and Williamson J. R. (1998) Fast folding mutants of the *Tetrahymena* group I ribozyme reveal a rugged folding energy landscape. J. Mol. Biol. **281:** 609–620
68 Treiber D. K., Rook M. S., Zarrinkar P. P. and Williamson J. R. (1998) Kinetic intermediates trapped by native interactions in RNA folding. Science **279:** 1943–1946
69 Woodson S. A. and Cech T. R. (1991) Alternative secondary structures in the 5′ exon affect both forward and reverse self-splicing of the *Tetrahymena* intervening sequence RNA. Biochemistry **30:** 2042–2050
70 Semrad K. and Schroeder R. (1998) Ribosomal function is necessary for efficient splicing of the T4 phage thymidylate synthase intron in vivo. Genes Dev. **12:** 1327–1337
71 Seraphin B., Simon M. and Faye G. (1988) MSS18, a yeast nuclear gene involved in the splicing of intron AI5(of the mitochondrial cox-1 transcript. EMBO J. **7:** 1455
72 Goguel V., Wang Y. and Rosbash M. (1993) Short artificial hairpins sequester splicing signals and inhibit yeast pre-messenger RNA splicing. Mol. Cell. Biol. **13:** 6841–6848
73 Lazinski D. W. and Taylor J. M. (1995) Intracellular cleavage and ligation of hepatitis delta virus genomic RNA: regulation of ribozyme activity by cis-acting sequences and host factors. J. Virol. **69:** 1190–1200
74 Perrotta A. T. and Been M. D. (1998) A toggle duplex in hepatitis delta virus self-cleaving RNA that stabilizes an inactive and a salt-dependent pro-active ribozyme conformation. J. Mol. Biol. **279:** 361–373
75 Woodson S. A. and Emerick V. L. (1993) An alternative helix in the 26S rRNA promotes excision and integration of the *Tetrahymena* intervening sequence. Mol. Cell. Biol. **13:** 1137–1145

76 Zaug A. J., McEvoy M. M. and Cech T. R. (1993) Self-splicing of the group I intron from *Anabaena* pre-tRNA: requirement for base-pairing of the exons in the anticodon stem. Biochemistry **32:** 7946–7953

77 Esteban J. A., Walter N. G., Kotzorek G., Heckman J. E. and Burke J. M. (1998) Structural basis for heterogeneous kinetics: reengineering the hairpin ribozyme. Proc. Natl. Acad. Sci. USA **95:** 6091–6096

78 Thomson J. B. and Lilley D. M. (1999) The influence of junction conformation on RNA cleavage by the hairpin ribozyme in its natural junction form. RNA **5:** 180–187

79 Pan T. and Sosnick T. R. (1997) Intermediates and kinetic traps in the folding of a large ribozyme revealed by circular dichroism and UV absorbance spectroscopies and catalytic activity. Nat. Struct. Biol. **4:** 931–938

80 Bryngelson J. D. and Wolynes P. G. (1989) Intermediates and barrier crossing in a random energy-model (with applications to protein folding). J. Phys. Chem. **93:** 6902–6915

81 Bryngelson J. D., Onuchic J. N., Socci N. D. and Wolynes P. G. (1995) Funnels, pathways, and the energy landscape of protein-folding – a synthesis. Proteins **21:** 167

82 Thirumalai D. (1995) From the minimal models to real proteins: time scales for protein folding kinetics. J. Phys. I France **5:** 1457–1467

83 Chan H. S. and Dill K. A. (1998) Protein folding in the landscape perspective: chevron plots and non-Arrhenius kinetics. Proteins **30:** 2–33

84 Wolynes P. G., Onuchic J. N. and Thirumalai D. (1995) Navigating the folding routes. Science **267:** 1619–1620

85 Thirumalai D. and Woodson S. A. (1996) Kinetics of folding of protein and RNA. Acc. Chem. Res. **29:** 433–439

86 Pan T., Fang X. and Sosnick T. (1999) Pathway modulation, circular permutation and rapid RNA folding under kinetic control. J. Mol. Biol. **286:** 721–731

87 Pan J., Thirumalai D. and Woodson S. A. (1999) Magnesium-dependent folding of self-splicing RNA: exploring the link between cooperativity, thermodynamics, and kinetics. Proc. Natl. Acad. Sci. USA **96:** 6149–6154

88 Rook M. S., Treiber D. K. and Williamson J. R. (1999) An optimal $Mg2+$ concentration for kinetic folding of the Tetrahymena ribozyme. Proc. Natl. Acad. Sci. USA **96:** 12471–12476

89 Pan J. and Woodson S. A. (1999) The effect of long-range loop-loop interactions on folding of the Tetrahymena self-splicing RNA. J. Mol. Biol. **294:** 955–965

89a Pan J., Deras M. L. and Woodson S. A. (2000) Fast folding of a ribozyme by stabilizing core interactions: evidence for multiple folding pathways in RNA. J. Mol. Biol. **296:** 133–144

90 Chen J. L. and Pace N. R. (1997) Identification of the universally conserved core of ribonuclease P RNA. RNA **3:** 557–560

91 Massire C., Jaeger L. and Westhof E. (1998) Derivation of the three-dimensional architecture of bacterial ribonuclease P RNAs from comparative sequence analysis. J. Mol. Biol. **279:** 773–793

92 Loria A. and Pan T. (1996) Domain structure of the ribozyme from eubacterial ribonuclease P. RNA **2:** 551–563

93 Harris M. E., Nolan J. M., Malhotra A., Brown J. W., Harvey S. C. and Pace N. R. (1994) Use of photoaffinity crosslinking and molecular modeling to analyze the global architecture of ribonuclease P RNA. EMBO J. **13:** 3953–3963

94 Kramer F. R. and Mills D. R. (1981) Secondary structure formation during RNA synthesis. Nucleic Acids Res. **9:** 5109–5124

95 Monforte J. A., Kahn J. D. and Hearst J. E. (1990) RNA folding during transcription by Escherichia coli RNA polymerase analyzed by RNA self-cleavage. Biochemistry **29:** 7882–7890

96 Komissarova N. and Kashlev M. (1998) Functional topography of nascent RNA in elongation intermediates of RNA polymerase. Proc. Natl. Acad. Sci. USA **95:** 14699–14704

97 Pan T., Artsimovitch I., Fang X. W., Landick R. and Sosnick T. R. (1999) Folding of a large ribozyme during transcription and the effect of the elongation factor NusA. Proc. Natl. Acad. Sci. USA **96:** 9545–9550

98 Golomb M. and Chamberlin M. (1974) Characterization of T7-specific ribonucleic acid polymerase. IV. Resolution of the major in vitro transcripts by gel electrophoresis. J. Biol. Chem. **249:** 2855–2863

99 Kadesch T. R. and Chamberlin M. J. (1982) Studies of in vitro transcription by calf thymus RNA polymerase II using a novel duplex DNA template. J. Biol. Chem. **257:** 5286–5295

100 Lewicki B. T. U., Margus T., Remme J. and Nierhaus K. H. (1993) Coupling of rRNA transcription and ribosomal assembly in vivo – formation of active ribosomal subunits in *Escherichia coli* requires transcription of rRNA genes by host RNA polymerase which cannot be replaced by bacteriophage T7 RNA polymerase. J. Mol. Biol. **231:** 581–593

101 Chao M. Y., Kan M. C. and Lin-Chao S. (1995) RNAII transcribed by IPTG-induced T7 RNA polymerase is nonfunctional as a replication primer for ColE1-type plasmids in *Escherichia coli*. Nucleic Acids Res **23:** 1691–1695

102 Repsilber D., Wiese S., Rachen M., Schroder A. W., Riesner D. and Steger G. (1999) Formation of metastable RNA structures by sequential folding during transcription: time-resolved structural analysis of potato spindle tuber viroid (−)-stranded RNA by temperature-gradient gel electrophoresis. RNA **5:** 574–584

103 Lambowitz A. M. and Perlman P. S. (1990) Involvement of aminoacyl-tRNA synthetases and other proteins in group I and group II intron splicing. Trends Biochem. Sci. **15:** 440–444

104 Mohr G., Zhang A., Gianelos J. A., Belfort M. and Lambowitz A. M. (1992) The neurospora CYT-18 protein suppresses defects in the phage T4 td intron by stabilizing the catalytically active structure of the intron core. Cell **69:** 483–494

105 Weeks K. M. and Cech T. R. (1995) Protein facilitation of group I intron splicing by assembly of the catalytic core and the 5′ splice site domain. Cell **82:** 221–230

106 Ho Y. and Waring R. B. (1999) The maturase encoded by a group I intron from *Aspergillus nidulans* stabilizes RNA tertiary structure and promotes rapid splicing. J. Mol. Biol. **292:** 987–1001

107 Lewin A. S., Thomas J. Jr and Tirupati H. K. (1995) Cotranscriptional splicing of a group I intron is facilitated by the Cbp2 protein. Mol. Cell. Biol. **15:** 6971–6978

108 Caprara M. G., Mohr G. and Lambowitz A. M. (1996) A tyrosyl-tRNA synthetase protein induces tertiary folding of the group I intron catalytic core. J. Mol. Biol. **257:** 512–531

109 Weeks K. M. and Cech T. R. (1996) Assembly of a ribonucleoprotein catalyst by tertiary structure capture. Science **271:** 345–348

110 Brehm S. L. and Cech T. R. (1983) Fate of an intervening sequence ribonucleic acid: excision and cyclization of the *Tetrahymena* ribosomal ribonucleic acid intervening sequence in vivo. Biochemistry **22:** 2390–2397

111 Bass B. L. and Cech T. R. (1984) Specific interaction between the self-splicing RNA of *Tetrahymena* and its guanosine substrate: implications for biological catalysis by RNA. Nature **308:** 820–826

112 Zhang F., Ramsay E. S. and Woodson S. A. (1995) In vivo facilitation of *Tetrahymena* group I intron splicing in *Escherichia coli* pre-ribosomal RNA. RNA **1:** 284–292

113 Nikolcheva T. and Woodson S. A. (1999) Facilitation of group I splicing in vivo: misfolding of the *Tetrahymena* IVS and the role of ribosomal RNA exons. J. Mol. Biol. **292:** 557–567

114 Karpel R. L., Miller N. S. and Fresco J. R. (1982) Mechanistic studies of ribonucleic acid renaturation by a helix-destabilizing protein. Biochemistry **21:** 2102–2108

115 Herschlag D. (1995) RNA chaperones and the RNA folding problem. J. Biol. Chem. **270:** 20871–20874

116 Herschlag D., Khosla M., Tsuchihashi Z. and Karpel R. L. (1994) An RNA chaperone activity of nonspecific RNA-binding proteins in hammerhead ribozyme catalysis. EMBO J. **13:** 2913–2924
117 Coetzee T., Herschlag D. and Belfort M. (1994) *Escherichia coli* proteins, including ribosomal protein S12, facilitate in vitro splicing of phage T4 introns by acting as RNA chaperones. Genes Dev. **8:** 1575–1588
118 Zhang A., Derbyshire V., Salvo J. L. and Belfort M. (1995) *Escherichia coli* protein StpA stimulates self-splicing by promoting RNA assembly in vitro. RNA **1:** 783–793
119 Jiang W. N., Hou Y. and Inouye M. (1997) CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J. Biol. Chem. **272:** 196–202
120 Pannone B. K., Xue D. H. and Wolin S. L. (1998) A role for the yeast La protein in U6 snRNP assembly: evidence that the La protein is a molecular chaperone for RNA polymerase III transcripts. EMBO J. **17:** 7442–7453
121 Staley J. P. and Guthrie C. (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell **92:** 315–326
122 Hamm J. and Lamond A. I. (1998) Spliceosome assembly: the unwinding role of DEAD-box proteins. Curr. Biol. **8:** R532–534
123 Cruz J. de la, Kressler D. and Linder P. (1999) Unwinding RNA in *Saccharomyces cerevisiae*: DEAD-box proteins and related families. Trends Biochem. Sci. **24:** 192–198
124 Nomura M. and Held W. A. (1974) Reconstitution of ribosomes: studies of ribosome structure, function, and assembly. In: Ribosomes, p. 193–223, Nomura M., Tissieres A. and Lengyel P. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
125 Bachellerie J. P., Nicoloso M., Qu L. H., Michot B., Caizergues-Ferrer M., Cavaille J. et al. (1995) Novel intron-encoded small nucleolar RNAs with long sequence complementarities to mature rRNAs involved in ribosome biogenesis. Biochem. Cell Biol. **73:** 835–843
126 Peculis B. A. (1997) The sequence of the 5′ end of the U8 small nucleolar RNA is critical for 5.8S and 28S rRNA maturation. Mol. Cell. Biol. **17:** 3702–3713
127 Chiaruttini C., Milet M. and Springer M. (1997) Translational coupling by modulation of feedback repression in the IF3 operon of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:** 9208–9213
128 Licis N., Duin J. van, Balklava Z. and Berzins V. (1998) Long-range translational coupling in single-stranded RNA bacteriophages: an evolutionary analysis. Nucleic Acids Res. **26:** 3242–3246
129 Brion P., Schroede r. R., Michel F. and Westhof E. (1999) Influence of specific mutations on the thermal stability of the td group I intron in vitro and on its splicing efficiency in vivo: a comparative study. RNA **5:** 947–958
130 Clodi E., Semrad K. and Schroeder R. (1999) Assaying RNA chaperone activity in vivo using a novel RNA folding trap. EMBO J. **18:** 3776–3782
131 Yanofsky C. (1981) Attenuation in the control of expression of bacterial operons. Nature **289:** 751–758
132 Gollnick P. (1994) Regulation of the *Bacillus subtilis* trp operon by an RNA-binding protein. Mol. Microbiol. **11:** 991–997
133 Du H. and Babitzke P. (1998) trp RNA-binding attenuation protein-mediated long distance RNA refolding regulates translation of trpE in *Bacillus subtilis*. J. Biol. Chem. **273:** 20494–20503
134 Switzer R. L., Turner R. J. and Lu Y. (1999) Regulation of the *Bacillus subtilis* pyrimidine biosynthetic operon by transcriptional attenuation: control of gene expression by an mRNA- binding protein. Prog. Nucleic Acid Res. Mol. Biol. **62:** 329–367
135 Altuvia S., Kornitzer D., Teff D. and Oppenheim A. B. (1989) Alternative mRNA structures of the cIII gene of bacteriophage lambda determine the rate of its translation initiation. J. Mol. Biol. **210:** 265–280
136 Ma C. K., Kolesnikow T., Rayner J. C., Simons E. L., Yim H. and Simons R. W. (1994) Control of translation by mRNA secondary structure: the importance of the kinetics of structure formation. Mol. Microbiol. **14:** 1033–1047
137 Gultyaev A. P., Batenburg F. H. van and Pleij C. W. (1995) The influence of a metastable structure in plasmid primer RNA on antisense RNA binding kinetics. Nucleic Acids Res. **23:** 3718–3725
138 Groeneveld H., Thimon K. and Duin J. van (1995) Translational control of maturation-protein synthesis in phage MS2: a role for the kinetics of RNA folding? RNA **1:** 79–88
139 Poot R. A., Tsareva N. V., Boni I. V. and Duin J. van (1997) RNA folding kinetics regulates translation of phage MS2 maturation gene. Proc. Natl. Acad. Sci. USA **94:** 10110–10115
140 Gerdes K., Gultyaev A. P., Franch T., Pedersen K. and Mikkelsen N. D. (1997) Antisense RNA-regulated programmed cell death. Annu. Rev. Genet. **31:** 1–31
141 Gultyaev A. P., Franch T. and Gerdes K. (1997) Programmed cell death by hok/sok of plasmid R1: coupled nucleotide covariations reveal a phylogenetically conserved folding pathway in the hok family of mRNAs. J. Mol. Biol. **273:** 26–37
142 Libri D., Stutz F., McCarthy T. and Rosbash M. (1995) RNA structural patterns and splicing: molecular basis for an RNA-based enhancer. RNA **1:** 425–436
143 Blanchette M. and Chabot B. (1997) A highly stable duplex structure sequesters the 5′ splice site region of hnRNP A1 alternative exon 7B. RNA **3:** 405–419
144 Cote J. and Chabot B. (1997) Natural base-pairing interactions between 5′ splice site and branch site sequences affect mammalian 5′ splice site selection. RNA **3:** 1248–1261
145 Eng F. J. and Warner J. R. (1991) Structural basis for the regulation of splicing of a yeast messenger RNA. Cell **65:** 797–804
146 Vilardell J. and Warner J. R. (1994) Regulation of splicing at an intermediate step in the formation of the spliceosome. Genes Dev. **8:** 211–220
147 Blanchette M. and Chabot B. (1999) Modulation of exon skipping by high-affinity hnRNP A1-binding sites and by intron elements that repress splice site utilization. EMBO J. **18:** 1939–1952
148 Howe K. J. and Ares M. Jr (1997) Intron self-complementarity enforces exon inclusion in a yeast pre-mRNA. Proc. Natl. Acad. Sci. USA **94:** 12467–12472
149 Hertel K. J., Lynch K. W., Hsiao E. C., Liu E. H. and Maniatis T. (1996) Structural and functional conservation of the *Drosophila* doublesex splicing enhancer repeat elements. RNA **2:** 969–981
150 Corden J. L. and Patturajan M. (1997) A CTD function linking transcription to splicing. Trends Biochem. Sci. **22:** 413–416
151 Eperon L. P., Graham I. R., Griffiths A. D. and Eperon I. C. (1988) Effects of RNA secondary structure on alternative splicing of pre-mRNA: is folding limited to a region behind the transcribing RNA polymerase? Cell **54:** 393–401
152 Lee R. C., Feinbaum R. L. and Ambros V. (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell **75:** 843–854
153 Matsumoto K. and Wolffe A. P. (1998) Gene regulation by Y-box proteins: coupling control of transcription and translation. Trends Cell Biol. **8:** 318–323
154 Wolffe A. P. (1995) The cold-shock response in bacteria. Sci. Prog. **78:** 301–310
155 Cech T. R., Damberger S. H. and Gutell R. R. (1994) Representation of the secondary and tertiary structure of group I introns. Nat. Struct. Biol. **1:** 273–280