Effects of polyvalent cations on the folding of an rRNA three-way junction and binding of ribosomal protein S15

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ABSTRACT

The *Bacillus stearothermophilus* ribosomal protein S15 binds to a phylogenetically conserved three-way junction formed by the intersection of helices 20, 21, and 22 of eubacterial 16S ribosomal RNA, inducing a large conformational change in the RNA. Like many RNA structures, this three-way junction can also be folded by the addition of polyvalent cations such as magnesium, as demonstrated by comparing the mobilities of the wild-type and mutant junctions in the absence and presence of polyvalent cations in nondenaturing polyacrylamide gels. Using a modification interference assay, critical nucleotides for folding have been identified as the phylogenetically conserved nucleotides in the three-way junction. NMR spectroscopy of the junction reveals that the conformations induced by the addition of magnesium or S15 are extremely similar. Thus, the folding of the junction is determined entirely by RNA elements within the phylogenetically conserved junction core, and the role of Mg²⁺ and S15 is to stabilize this intrinsically unstable structure. The organization of the junction by Mg²⁺ significantly enhances the bimolecular association rate (k_{on}) of S15 binding, suggesting that S15 binds specifically to the folded form of the three-way junction via a tertiary structure capture mechanism.

Keywords: *Bacillus stearothermophilus*; magnesium-dependent RNA folding; modification interference analysis; NMR; ribosome; 16S RNA

INTRODUCTION

The architecture of large RNAs is frequently determined by the combination of smaller RNA structural elements, such as hairpins, internal loops, junctions, and pseudoknots that serve as building blocks (Chastain & Tinoco, 1991). The formation of these simple elements serves as an important first step in the hierarchical view of the folding of highly structured RNAs (Zarrinkar & Williamson, 1996). Helical junctions have been shown to be an important motif for establishing global RNA structure, such as in the 5S rRNA (Westhof et al., 1989; Brunel et al., 1991) and the P4-P6 domain of the *Tetrahymena* group I self-splicing intron (Cate et al., 1996, 1997), protein binding sites (Ryan & Draper, 1989; Ryan et al., 1991; Kooi et al., 1993), and the active site of the hammerhead ribozyme (Long & Uhlenbeck, 1993). Interactions with polyvalent cations, coax-

ial stacking of helices, and noncanonical base pairing are common features shared by a number of nucleic acid junction motifs.

The importance of these features in establishing the structure and function of junction motifs is exemplified by the hammerhead ribozyme. The conserved nucleotides critical for catalytic activity are localized at the intersection of three helices (Uhlenbeck, 1987), and in the presence of a required magnesium cofactor (Dahm & Uhlenbeck, 1991), the hammerhead ribozyme specifically cleaves a substrate RNA oligonucleotide. A number of biochemical, biophysical, and structural studies of the hammerhead ribozyme have lead to a detailed picture of this three-way junction motif. Using native PAGE, it has been demonstrated that the three helices of the hammerhead reorient upon the addition of polyvalent ions (Bassi et al., 1995). Mutations in the junction core disrupt the ability of the RNA to adopt the folded conformation, even in the presence of high divalent metal ion concentrations (Bassi et al., 1996). Thus, the proper folding of the junction is critically dependent upon both the nucleotide sequence in the junction element and the presence of metal ions. The X-ray crystal structure of this ribozyme shows extensive ter-

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tiary interactions in the junction core, consistent with the sequence requirements for hammerhead function (Pley et al., 1994; Scott et al., 1995). This structure clearly illustrates the importance of coaxial stacking, metal ion binding, and noncanonical base pairing in dictating RNA junction fold and function.

The eubacterial ribosomal protein S15 interacts directly with a three-way junction element within the central domain of the 16S rRNA (Powers & Noller, 1995; Batey & Williamson, 1996a; Serganov et al., 1996). Mutagenesis, modification interference assays, and chemical probing of this RNA–protein interaction highlight the central importance of phylogenetically conserved nucleotides in helix 22 of the junction, and it has been proposed that noncanonical base pairs in the junction are important for protein recognition (Batey & Williamson, 1996b; Serganov et al., 1996). In the absence of divalent ions, the binding of S15 to this junction induces a significant conformational change in the orientation of helices 20, 21, and 22 (Batey & Williamson, 1996b), in which helices 21 and 22 become collinear and helix 20 forms an acute angle with respect to helix 22 (Orr et al., 1998). Magnesium ions also induce a conformational change about the homologous three-way junction from *Thermus thermophilus* 16S rRNA (Serganov et al., 1996). Using transient electric birefringence (TEB), a powerful physical technique for measuring interhelical angles, the global geometry of this three-way junction has been examined (Orr et al., 1998). This study demonstrated that in the presence of either 1 mM magnesium chloride or a stoichiometric amount of S15 protein, helix 21 coaxially stacks with helix 22, and helix 20 forms an acute angle with respect to helix 22. These studies have established that the global change in interhelical geometry induced in this three-way junction independently by magnesium binding and S15 binding is virtually identical.

The TEB experiments, however, do not establish whether magnesium binds to the junction in a nonspecific fashion to promote helical stacking, as is observed in DNA and RNA four-way junctions, or specifically binds in a way that requires active participation of elements with the RNA to establish a fold, as is observed in the hammerhead ribozyme. Characterization of S15 binding to the three-way junction has been performed in both the presence of magnesium (Serganov et al., 1996, 1997) and in its absence (Batey & Williamson, 1996b; Orr et al., 1998). Although either S15 or magnesium can induce a conformational change in the junction (Orr et al., 1998), the nature of the thermodynamic coupling between protein and ion binding has not yet been established. Therefore, in this study, we have investigated the nature of the magnesium-dependent conformational change about the three-way junction, its relationship to the S15-dependent conformational change, and how magnesium-dependent folding affects RNA recognition by ribosomal protein S15. Using

a combination of native gel electrophoretic methods, chemical modification interference analysis, and NMR spectroscopy, we have shown that the conformation of the three-way junction element is responsive to various polyvalent ions and, in the case of magnesium, folding is dependent upon specific phylogenetically conserved nucleotides at the junction. Furthermore, magnesium and S15 stabilize the same conformation of the three-way junction, indicating that the folding is largely determined by the RNA rather than by S15. By stabilizing the junction fold with magnesium, the bimolecular rate of association of S15 with its RNA binding site is significantly accelerated, which implies that this protein specifically recognizes the folded junction via a tertiary structure capture mechanism.

RESULTS

Folding of Fr1 RNA and mutants in the presence of polyvalent ions

Binding of S15 to Fr1 RNA (Fig. 1), an RNA fragment derived from the central domain of *Bacillus stearothermophilus* 16S rRNA, enhances its electrophoretic mobility in a native polyacrylamide gel, which has been interpreted to be the result of a conformational change in the RNA upon protein binding (Batey & Williamson, 1996b). Other studies of the S15–rRNA complex (Serganov et al., 1996; Orr et al., 1998) have revealed that the presence of magnesium alters the mobility of the protein–RNA complex in a native polyacrylamide gel with respect to the free RNA. Fr1 RNA demonstrates the same pattern of behavior in native gels. In the absence of magnesium, the wild-type BS15–Fr1 RNA complex migrates more rapidly than free RNA (Fig. 2, EDTA/wt), whereas, in the presence of magnesium, the complex migrates more slowly than the free RNA (Fig. 2, Mg^{2+} /wt).

To test if this conformational change specifically involved the three-way junction element that is recognized by S15, wild-type Fr1 and three point mutants were subjected to native PAGE in the presence and absence of magnesium chloride. Two of the point mutants, Fr1(U653Δ) and Fr1(G752C) (Fig. 1), are located in the phylogenetically conserved three-way junction element, whereas the third, Fr1(A748Δ), lies in helix 22. All of the RNAs comigrate in the absence of magnesium or BS15 (Fig. 2, EDTA), indicating that they adopt similar conformations under these conditions. As observed previously, Fr1 and Fr1(U653Δ) are capable of specifically binding BS15, yielding a characteristic mobility enhancement (Batey & Williamson, 1996b). The observed mobility enhancement of the BS15–Fr1(U653Δ) complex was less than that of the wild-type complex, suggesting that this mutation introduces a small perturbation in the RNA conformation relative to the wild-type RNA. The same series of RNAs

FIGURE 2. Electrophoresis of Fr1 RNA and mutants in 8% non-denaturing polyacrylamide gel containing (a) 1 mM EDTA, (b) 1 mM magnesium chloride, (c) 25 μ M cobalt (III) hexammine, or (d) 200 μ M spermidine chloride in the gel and running buffer, in the absence and presence of 100 nM BS15.

demonstrates a markedly different pattern of mobilities when subjected to electrophoresis in the presence of 1 mM magnesium chloride (Fig. 2, Mg^{2+}). In the absence of S15, Fr1 and Fr1(A748 Δ), both of which contain a wild-type three-way junction element, display equivalent mobilities in the gel, whereas the two RNAs containing mutant three-way junctions, Fr1(U653 Δ) and Fr1(G752C), display decreased mobility relative to wild-type RNA. The mobility retardation of Fr1(U653 Δ) is not as severe as Fr1(G752C), suggesting that this RNA is capable of a distorted conformational change around the three-way junction, consistent with its diminished mobility enhancement upon BS15 binding. The presence of magnesium does not alter the ability of Fr1 or the mutants to bind BS15; mutants that were incapable of binding S15 in the absence of divalent ions are not rescued by the addition of magnesium. This suggests that magnesium is capable of organizing the rRNA three-way junction in a specific fashion similar to that induced by S15 binding.

Other polyvalent cations demonstrate similar effects on the mobility of the free and bound RNAs. Calcium chloride, which is chemically very similar to magnesium chloride (Pan et al., 1993), induces exactly the same effect on the mobility patterns of the wild-type and mutant three-way junctions (data not shown). Cobalt(III) also forms hexacoordinate complexes with ligands such as H_2O or NH_3 , but undergoes ligand exchange extremely slowly (Pan et al., 1993) and has particular affinity for nitrogen donors such as $-\text{NH}_2$ and the N7 of purines (Cotton et al., 1987). Despite the

kinetically inert coordination sphere, hexammine cobalt induces the same conformational change in the RNA (Fig. 2, Co^{3+}), suggesting that the conformational change does not require inner-sphere metal coordination and can be accommodated by a variety of hexa-coordinate metal ions. The organic polyamine spermidine is also capable of inducing a moderate conformational change about the three-way junction; at concentrations of 200 μ M and 1 mM, spermidine causes a mobility enhancement in the wild-type RNA that is similar to that observed for the metal ions, but to a lesser degree (Fig. 2, spermidine). Thus, in the presence of spermidine, the BS15–Fr1 RNA complex has a slightly greater mobility than that of free Fr1, although not nearly as great as that observed in EDTA. This strongly indicates that the three-way junction does not contain a specific metal-binding pocket. Instead, folding of the three-way junction is facilitated by diverse ligands such as metal ions, organic polyamines, and the protein S15.

Modification interference of the RNA conformation change

Because mutations in the three-way junction are capable of affecting the mobility of Fr1 RNA in a native polyacrylamide gel, the modification interference assay can be utilized to elucidate functional groups in the RNA that are important for the divalent ion-induced conformational change. The experimental details here differ slightly from the S15 modification interference experiments (Batey & Williamson, 1996b), because no single magnesium concentration in the gel yields separate “unfolded” and “folded” bands that are analogous to the “free” and “bound” bands observed in experiments using protein. Instead, the modified RNA was subjected to electrophoresis on two separate polyacrylamide gels, one containing EDTA and one containing 1 mM magnesium chloride, where the EDTA gel serves as a control for modifications that do not specifically interfere with the cation-induced RNA conformational change. Because there is no distinct “unfolded” band generated in the gel, RNA modifications that do not appear in the magnesium “folded” band represent modifications that cause the RNA to migrate anomalously, but not necessarily as unfolded. Thus, in this assay, modified RNAs are selected for their ability to adopt a specific folded conformation in the presence of magnesium. From both the EDTA and magnesium gels, the major RNA band was excised and subjected to strand scission and sequencing, as described previously (Batey & Williamson, 1996b).

Modification interference experiments were performed on 3' end-labeled Fr4 RNA (Batey & Williamson, 1996a) using diethylpyrocarbonate (DEPC) and aqueous hydrazine. Fr4 (Fig. 1) was chosen for this experiment because it contains the three-way junction element and

FIGURE 3. Modification interference assays of Mg^{2+} binding to 3' end-labeled Fr4 RNA using (a) DEPC and (b) aqueous hydrazine as modification agents. Lane 1, modified RNA not subjected to gel electrophoresis; lane 2, RNA subjected to gel electrophoresis in the presence of EDTA; lane 3, RNA subjected to gel electrophoresis in the presence of 1 mM magnesium chloride. Arrows denote nucleotide positions whose modification interferes with magnesium-dependent folding; asterisks denote sites of nonspecific RNA hydrolysis.

displays an enhanced mobility upon organization of the RNA by specific recognition of BS15 (Batey & Williamson, 1996a), and, because it lacks helix 23, reading the sequence of the entire RNA is facilitated. Modifications of Fr4 with DEPC (Fig. 3a) and aqueous hydrazine (Fig. 3b) reveal four nucleotides that are important for the folding of the RNA about the three-way junction motif. DEPC modifications of A655 and A753, both located at the junction, strongly interfere with magnesium-dependent RNA folding and these adenosines are also implicated in recognition of the RNA by BS15 (Batey & Williamson, 1996b; Serganov et al., 1996). However, modifications at A663 and A668, which are located in the middle of helix 22 near the internal loop and also are implicated in protein binding (Batey & Williamson, 1996b), do not interfere with RNA folding. The hydrazine-induced depyrimidinations at U652 and U751, which are located near the three-way junction, are important for both protein binding (Batey & Williamson, 1996b) and magnesium-dependent folding. Modification of U740, another important determinant for BS15 binding that is located well outside the junction element in helix 22, does not interfere with the ability of the RNA to fold. Thus, nucleotides important for the magnesium-dependent folding of the RNA are localized to the three-way junction element and constitute a subset of the nucleotides that are highly phylogenetically conserved and important for protein binding.

Magnesium- and S15-induced local conformation of the three-way junction

To begin to understand the structural basis for the magnesium- and protein-induced conformational change about the three-way junction and BS15–RNA recognition, a minimal junction RNA (Fr18) was designed (Fig. 1). Fr18 is a minimal RNA three-way junction that contains all of the elements of specificity for magnesium-induced folding, although this RNA is incapable of binding BS15. This RNA differs from Fr15 (Fig. 1), a minimal RNA element that can specifically bind BS15, in that most of helix 22 is deleted. The magnesium-dependent folding of Fr18 was tested biochemically using a T_1 nuclease footprinting assay by probing the accessibility of the guanosine nucleotides as a function of magnesium. In the absence of magnesium, G654 and G752 within the three-way junction element are significantly more susceptible to attack by nuclease T_1 than the other guanoses (Fig. 4). Upon addition of increasing amounts of magnesium up to 10 mM, these two guanoses become protected from cleavage, whereas the cleavage of other guanoses remains relatively constant. This indicates that magnesium induces folding about the three-way junction in Fr18, protecting two guanoses critical for formation of the folded structure (Batey & Williamson, 1996b). The folding transition can also be readily observed using NMR spectroscopy by

FIGURE 4. T_1 nuclease protection assay of magnesium-induced folding of 3' end-labeled Fr18 RNA. Lane 1, RNA in 1 mM EDTA not subjected to T_1 nuclease; lane 2, RNA in 100 mM $MgCl_2$ not subjected to T_1 nuclease; RNA subjected to T_1 nuclease in the presence of lane 3, 1 μM ; lane 4, 10 μM ; lane 5, 100 μM ; lane 6, 1 mM; lane 7, 10 mM; lane 8, 100 mM $MgCl_2$; lane 9, 1 mM EDTA, along with lane 10, an alkaline hydrolysis ladder.

titration of a 1.5-mM sample of Fr18 RNA with magnesium chloride (Fig. 5). Upon addition of 15 mM magnesium chloride, the imino proton spectrum of Fr18 exhibits generally sharper lines and a number of new resonances, suggesting that a more kinetically stable RNA structure has been formed.

Proton chemical shifts observed by NMR are sensitive reporters of the local environment. The conformational change upon addition of magnesium chloride to Fr18 RNA is readily apparent from a number of shifted crosspeaks in DQF-COSY spectra acquired at 35°C (Fig. 6a,b). In this region of the spectrum, each crosspeak is due to the through-bond scalar coupling between the H5 and H6 protons in pyrimidines, and thus serves as a good fingerprint for the conformational change in the RNA. In the magnesium-folded form of Fr18, a number of new H5–H6 crosspeaks appear (three representative crosspeaks are circled in Fig. 6), indicative of the conformational change. These new

FIGURE 5. One-dimensional spectra of 1.5 mM Fr18 RNA in 90%/10% H₂O/D₂O showing a titration with magnesium chloride at 25 °C. A number of new imino proton peaks are evident in the RNA spectrum upon addition of 15 mM MgCl₂, evidence that this RNA undergoes a conformational change in the presence of divalent ions. Additional magnesium causes no further changes in the imino spectrum.

crosspeaks were not observed in NMR spectra of Fr18(U653Δ) titrated with the same amount of magnesium, indicating that the magnesium-dependent changes observed in the DQF-COSY spectrum of Fr18 were due to the conformational change about the three-way junction (data not shown). These same crosspeaks appear upon titration of a 1.0-mM sample of Fr15 RNA with magnesium (Fig. 6c,d), suggesting that these crosspeaks are likely to represent pyrimidines in or near the three-way junction element. When Fr15 RNA is titrated with one equivalent of BS15 in the absence of magnesium (Fig. 6e), these same crosspeaks appear in the DQF-COSY spectrum. Because the chemical shift of these crosspeaks is nearly identical in the magnesium- and protein-bound forms of the RNA, this strongly suggests that the conformation of the three-way junction element induced by the presence of BS15 or magnesium is extremely similar, if not nearly identical.

Close inspection of the DQF-COSY spectrum of the magnesium-bound form of Fr18 RNA indicates that this RNA is undergoing conformational exchange on the intermediate time scale. In the region of the spectrum shown in Figure 6b, there should be 18 crosspeaks, one for each pyrimidine base. If there is not internal dynamic motion or conformational exchange, each of the H5-H6 crosspeaks should be observable with the same intensity. Although there are more observed crosspeaks in the DQF-COSY spectrum of Fr18 RNA in the presence than in the absence of magnesium, even in the presence of magnesium, there are clearly not 18

FIGURE 6. DQF-COSY spectra of Fr18 and Fr15 RNA highlighting the region containing the H5-H6 crosspeaks of the pyrimidine nucleotides. Spectra of Fr18 RNA acquired at 35 °C in (a) the absence and (b) the presence of 15 mM magnesium chloride. The free RNA, which should display 18 pyrimidine H5-H6 crosspeaks, displays only a small number of strong crosspeaks, indicating that the RNA is exhibiting conformational exchange. DQF-COSY spectra of Fr15 RNA taken at 35 °C (c) in the absence of BS15 or magnesium, (d) in the presence of 15 mM magnesium chloride, and (e) titrated with BS15 to form a 0.95:1 BS15:Fr15 RNA complex. Circled resonances are H5-H6 crosspeaks that appear in the magnesium form of Fr18 RNA and are also observed to appear when Fr15 RNA is titrated with BS15.

peaks and those observed vary widely in their intensities. Thus, the RNA is exchanging between multiple forms (likely the folded and unfolded forms of the three-way junction) on a time scale that causes line broadening (Wüthrich, 1986). Similar results were obtained by titrating Fr18 RNA with hexammine cobalt. This line broadening problem makes Fr18 an unsuitable model for detailed structural analysis of the magnesium-dependent folding of this three-way junction element by NMR.

Magnesium-facilitated binding of S15 to ribosomal RNA

Although magnesium is not a required cofactor for the specific interaction of S15 with ribosomal RNA, be-

FIGURE 7. Electrophoresis of Fr1 RNA and several mutants in 8% nondenaturing polyacrylamide gels in the presence of either 1 mM EDTA or 10 mM magnesium. RNA was incubated with 100 nM BS15 in those reactions containing protein.

cause it can stabilize the folded form of the junction, magnesium might be expected to enhance S15 binding. The behavior of several Fr1 RNA mutants and their complexes with BS15 in native gels in the presence and absence of magnesium chloride (Fig. 7) supports this hypothesis. In the absence of magnesium, Fr1 RNA and two mutants in the three-way junction element, U653Δ and A753G, all migrate equivalently, but the BS15–RNA complexes have different mobilities. This indicates that BS15 alone cannot fully fold these mutant junctions into the wild-type conformation. Upon addition of 10 mM magnesium, the mobility of the three uncomplexed RNAs are enhanced to varying degrees that correlate with the degree to which BS15 alone can enhance their mobilities. However, in the presence of magnesium and BS15, these three RNAs have equivalent mobilities. This suggests that magnesium and BS15 may act cooperatively, and that pre-folding of the

TABLE 1. Apparent equilibrium dissociation constants for BS15 binding to RNA Fr1 and select mutants.^a

	Fr1 (nM)	Fr1(U653Δ) (nM)	Fr1(A753G) (nM)
0 mM Mg ²⁺	13.0 ± 7.0	18.0 ± 6.0	90.0 ± 30.0
10 mM Mg ²⁺	0.240 ± 0.070	0.290 ± 0.090	0.480 ± 0.160
$K_{d,Mg}/K_{d,EDTA}$	54	62	187

^aAll values are the product of at least three independent experiments.

RNA by the addition of magnesium may stimulate protein binding.

To test this hypothesis, the apparent equilibrium dissociation constant (K_d) and the apparent bimolecular association rate (k_{on}) for S15 binding were measured in the absence and presence of 10 mM magnesium using the native gel mobility shift assay. For Fr1 RNA (Fig. 8a) and both mutants, there was a moderate decrease in the apparent K_d in the presence of magnesium ions (Table 1). In contrast, the apparent bimolecular association rates were markedly different for the wild-type and mutant RNAs. In the case of Fr1 RNA, the presence of magnesium enhances the bimolecular association rate by more than 200-fold (Fig. 8b), indicating that stabilizing the folded form of Fr1 RNA promotes specific binding of BS15. However, in the case of both mutants, which are less stably folded in the presence of magnesium, the association rate is stimulated by only 10-fold (Table 2). This clearly indicates that BS15 is specifically recognizing the folded form of the three-way junction, rather than inducing the conformational change after binding.

a

b

FIGURE 8. Equilibrium and kinetic analysis of BS15 binding to Fr1 RNA in the absence and presence of 10 mM MgCl₂. **a:** Equilibrium binding of BS15 to Fr1 RNA in the presence of either 1 mM EDTA or 10 mM MgCl₂. **b:** Bimolecular association of BS15 and Fr1 RNA in the presence of either 1 mM EDTA or 10 mM MgCl₂.

TABLE 2. Apparent bimolecular association rate constants for BS15 binding to RNA Fr1 and select mutants.^a

	Fr1 (M ⁻¹ s ⁻¹)	Fr1(U653Δ) (M ⁻¹ s ⁻¹)	Fr1(A753G) (M ⁻¹ s ⁻¹)
0 mM Mg ²⁺	2.12 × 10 ³	3.19 × 10 ³	2.76 × 10 ³
10 mM Mg ²⁺	4.37 × 10 ⁵	2.39 × 10 ⁴	3.44 × 10 ⁴
<i>k</i> _{on,Mg} / <i>k</i> _{on,EDTA}	206	7.5	12.5

^aAll experiments are the product of the measurement of the pseudo-first-order rate constant at least five different protein concentrations.

DISCUSSION

Magnesium-facilitated folding of the three-way junction

It has been demonstrated previously that determinants for recognition of 16S rRNA by the primary ribosomal binding protein S15 from *B. stearotherophilus* lie in

two portions of helix 22, near an internal loop motif and a three-way junction (Fig. 9b) (Batey & Williamson, 1996b). The junction, a site containing four extremely highly phylogenetically conserved nucleotides in eubacterial 16S ribosomal RNAs (Fig. 9a), has been proposed to undergo a conformational change upon protein binding, including the formation of several noncanonical base pairs (Batey & Williamson, 1996b). One question that arose from those studies was whether folding of this junction motif requires specific functional groups supplied by S15 or if the conserved nucleotides in the junction were the principle participants in the conformational change.

Native gel electrophoresis of Fr1 RNA and several point mutants in the presence of chemically diverse polyvalent ions clearly indicates that the RNA is capable of a conformational change about the three-way junction without the involvement of S15. The ability of hexamine cobalt to fold this RNA demonstrates that the metal ion does not coordinate directly to functional

FIGURE 9. Comparison of nucleotides that are (a) phylogenetically conserved in eubacterial 16S rRNA, (b) important for binding of ribosomal protein S15, and (c) required for folding of the three-way junction between helices 20, 21, and 22. a: Nucleotides in capital letters are greater than 95% conserved across eubacteria, lowercase letters represent nucleotides that are 90–95% conserved, filled dots represent nucleotides that are 80–90% conserved, and open dots represent nucleotides that exist relative to *E. coli* 95% of the time (i.e., the existence of a nucleotide is conserved at this position, but the identity of the nucleotide is not conserved) [adapted from the 16S ribosomal database (Gutell, 1994)]. b: Nucleotides in capital letters are essential for BS15 recognition as determined by mutagenesis and modification interference, nucleotides in lowercase show some strong modification interferences, filled dots represent nucleotides that show medium/weak modification interferences, and open dots represent positions whose identities are not important for the BS15–rRNA interaction (Batey & Williamson, 1996b). c: Nucleotides in capital letters are important for folding of the three-way junction as determined by mutagenesis and modification interference analysis.

groups of the RNA. Previous studies of metal ion binding sites in RNA have identified a class of sites in which ions preferentially interact with regions of high charge density created by placing helices in close proximity, such as at a helical junction (Laing et al., 1994). A hallmark of these ion binding sites is that they are relatively insensitive to the coordination properties of the cation, and thus can accommodate magnesium and hexamine cobalt stabilization of RNA structure.

In the hammerhead ribozyme (Pley et al., 1994; Scott et al., 1995) and tRNA^{Phe} (Jack et al., 1976), a similar magnesium binding site is defined through its interaction with adjacent N7 positions at a GpA step in the major groove of the RNA. Magnesium may be interacting with this RNA three-way junction in a similar fashion. Modification interference analysis of S15 recognition of 16S rRNA has suggested that the phylogenetically conserved nucleotides in the three-way junction form two unusual base pairs that are critical for binding (Batey & Williamson, 1996b). This same experiment, applied to metal binding, implicates the same nucleotides as critical for metal-dependent folding the junction (Fig. 9c). Of particular note, these data strongly support participation of U652 in the formation of the folded junction structure and not U653 (Serganov et al., 1996). Because the junction forms the same local structure in the presence of magnesium as in the presence of S15, we believe that the same noncanonical base pairs are present. This model of the junction allows for these nucleotides to present a number of electronegative functional groups within the major groove, including the N7 positions of G654 and A655. Fe²⁺-EDTA probing of this junction shows that G654 and A655 become significantly protected upon binding of magnesium (Serganov et al., 1996), further supporting a direct role of these nucleotides in the formation of a site at which metals preferentially bind to stabilize the RNA fold.

The ability of divalent ions to stabilize the same conformation of the three-way junction as S15 suggests that this protein may act by providing appropriately placed arginine or lysine residues to relieve high negative charge density of the phosphate backbone in the folded conformation at the junction. Native gels have also suggested that magnesium and S15 are capable of simultaneously binding to their respective sites. In the case of both the U653Δ and the A753G mutants, magnesium and S15 could not produce an RNA with the same mobility as its wild-type counterpart. However, magnesium and S15 are capable of rescuing a wild-type mobility shift in concert. Because it has been established that S15 binds along the minor groove face of the junction (Batey & Williamson, 1996b), it may be that divalent ions bind within the major groove of the junction. Hexamine cobalt has been shown to preferentially bind in the major groove of RNA in the P4-P6 domain of a group I intron by interacting with a site of local high negative charge density formed by two tan-

dem G-U base pairs (Cate & Doudna, 1996; Kieft & Tinoco, 1997).

Despite the significant amount of evidence for the magnesium-induced folding of this three-way junction element, the structure of this form of the junction could not be addressed using NMR spectroscopy. This RNA does not adopt a single conformation under a number of temperature and divalent ion concentrations, as demonstrated by the lack of uniform peak intensities in DQF-COSY experiments on Fr18 and other similar RNA variants (R.T. Batey & J.R. Williamson, unpubl. obs.). Conformational heterogeneity was also observed about the three-way junction in NMR studies of a catalytically active hammerhead ribozyme (Kieft, 1997). Although the nucleotides within the helices could be assigned readily, indicating that the helices around the junction were formed, DQF-COSY spectra of the ribozyme suggest that the catalytic core does not adopt a single conformation. This suggests that the dynamic behavior of these naturally occurring three-way junctions may be an intrinsic property of this RNA motif and that, in large structured RNAs, they are locked into a single conformation by the binding of proteins (as in the case of S15) or by RNA-RNA contacts (as may be the case of the three-way junction in the P5abc subdomain of the P4-P6 domain of the *Tetrahymena* group I intron).

The relationship between RNA folding and protein binding

The conformational state of the three-way junction has a profound affect upon its recognition by S15. The bimolecular association rate constant for the wild-type RNA-protein interaction in the presence of magnesium is improved 20-fold relative to the rates for association with either junction mutant RNA. Thus, magnesium exerts its action by enhancing the rate of protein binding by shifting the equilibrium between the unfolded and folded forms of the three-way junction. Stabilization of the folded form of the three-way junction specifically promotes S15 binding, indicating that this protein recognizes its RNA target through a tertiary structure capture mechanism. In this mechanism, which also has been demonstrated for the interaction between the protein CBP2 and the group I intron bI5 (Weeks & Cech, 1996), the protein specifically recognizes its RNA target only after some degree of higher-order structure has been achieved. In both cases, inherently unstable RNA structures are stabilized via the binding of the protein component of the RNP.

The stabilization of RNA structure by protein binding has also been demonstrated to suppress mutations that cause structural defects. CYT-18, a protein that directly interacts with the catalytic core of the *Neurospora crassa* mitochondrial LSU group I intron to promote splicing (Mohr et al., 1994), is capable of suppressing structural defects throughout the catalytic core, but not else-

where in the RNA (Mohr et al., 1992). CBP2 has a similar ability to suppress RNA structural defects in the b15 intron (Weeks & Cech, 1995). Thus, it is not entirely surprising that structural defects in the three-way junction caused by the U653 Δ and A753G mutations can be overcome through the concerted binding of S15 and magnesium, as evidenced by moderate improvements in the apparent K_d and k_{on} for the BS15–mutant interaction in the presence of magnesium and by a wild-type-like mobility shift in a native gel. However, it is difficult to interpret the mechanism by which this enhancement occurs because these mutations are likely to include both RNA structural and protein-binding defects, and therefore this may be a different effect from the magnesium-dependent enhancement of the wild-type S15–RNA interaction.

These data suggest a model for the mechanism of S15 binding to the wild-type 16S rRNA as shown in Figure 10. In the absence of magnesium or S15, the three-way junction is in equilibrium between the unfolded (R) and folded (R^*) states, with the equilibrium strongly favoring the unfolded state. In the absence of magnesium, S15 specifically recognizes the folded state (R^*) to form a complex ($R^* \cdot S15$), which has been observed directly using native gel electrophoresis and NMR spectroscopy. State R^* is implied to exist because S15 appears to only recognize the folded junction, and all of the necessary elements required to fold the junction are in the RNA. In this case, protein bind-

ing is observed to be exceedingly slow because S15 only recognizes the unstable, and therefore transient, R^* state. In the presence of magnesium, the equilibrium between the R and R^* is shifted toward the folded state (R^*) because this state is stabilized by the presence of magnesium ($R^*(Mg^{2+})$). This state has been observed directly using native gels and NMR. In the presence of magnesium, S15 is observed to bind RNA rapidly because of the prevalence of the stabilized folded form of the junction. Although not observed directly, we believe that, in the presence of both magnesium and S15, the three-way junction can bind both (state $R^*(Mg^{2+}) \cdot S15$) via a minor groove protein binding site and a major groove ion binding site.

During ribosome assembly *in vivo*, it is unlikely that the ($R^* \cdot S15$) state is populated, and it is more likely that the ($R^*(Mg^{2+})$) state is formed prior to S15 binding. However, it has been demonstrated that binding of S6 and S18 to 16S rRNA in the presence of magnesium requires prior binding of S15 (Held et al., 1974), implying that assembly proceeds through the intermediate corresponding to ($R^*(Mg^{2+}) \cdot S15$). Therefore, either the conformation of the 16S rRNA corresponding to the ($R^*(Mg^{2+})$) and the ($R^*(Mg^{2+}) \cdot S15$) states are not equivalent, or binding of S6 and S18 requires additional interactions with S15.

Studies of S15 recognition of its mRNA further support the idea that it recognizes RNA using a tertiary structure capture mechanism. S15 also recognizes a

FIGURE 10. Thermodynamic model for BS15 binding to 16S rRNA. The RNA is in an equilibrium between unfolded (R) and folded (R^*) conformations. The R^* conformation is stabilized by either S15 ($R^* \cdot S15$) or magnesium ($R^* \cdot Mg^{2+}$) binding or both ($R^* \cdot S15 \cdot Mg^{2+}$).

structure in the 5' untranslated region of its own mRNA that is in a dynamic equilibrium between two tandem stem-loops and a pseudoknot (Portier et al., 1990a, 1990b), and S15 only binds specifically to the pseudoknot structure (Phillipe et al., 1993). Previously, we had suggested that S15 binds to the rRNA three-way junction and the mRNA pseudoknot in a similar fashion, interacting along the minor groove along an extended face of one helix along with recognition of a G-U pair common to both RNAs (Batey & Williamson, 1996b). In the case of the mRNA, the pseudoknot state is specifically recognized and stabilized by S15 binding, apparently through a tertiary structure capture mechanism (Weeks & Cech, 1996). Thus, it appears that S15 can recognize two apparently different RNA motifs using a common recognition mode and binding mechanism.

MATERIALS AND METHODS

Preparation RNA and BS15

Fr1 RNA, the mutants Fr1(U653Δ), Fr1(A748Δ), and Fr1(G752C), Fr4 RNA, and Fr15 RNA were transcribed in vitro using T7 RNA polymerase and purified as described previously (Batey & Williamson, 1996a). The mutant Fr1(A753G) RNA was prepared from a double-stranded DNA template that was generated by standard PCR reaction using pB16SFr1 as a template and a 3' primer incorporating the A753G mutation. The template was recovered from the PCR reaction by ethanol precipitation and was prepared for in vitro transcription as described previously for the plasmid templates (Batey & Williamson, 1996a). Fr18 RNA was prepared by transcription from single-stranded DNA oligonucleotides reactions under the following conditions: 40 mM Tris-Cl, pH 8.1, 1 mM spermidine, 5 mM DTT, 0.01% Triton X-100, 50 mM MgCl₂, 80 mg/mL PEG 8000, 4 mM each ribonucleotide 5' triphosphate, 300 nM each DNA strand, 0.5 μg/mL inorganic pyrophosphatase, and 3,000 U/mL T7 RNA polymerase (Milligan et al., 1987). The RNA was purified using established methods (Wyatt et al., 1991). *B. stearothermophilus* S15 was overexpressed and purified as described (Batey & Williamson, 1996a).

Native gel electrophoresis of RNA and RNA-protein complexes

Native polyacrylamide gel mobility assays of Fr1 RNA and BS15-Fr1 RNA complexes were performed in the presence of EDTA or various polyvalent ions. Fr1 RNA (approximately 0.1 pmol) labeled internally with ³²P was resuspended in binding buffer (10 mM K-Hepes, pH 7.5, 50 mM potassium acetate, 0.1 mM EDTA, 0.1 mg/mL tRNA, and 0.01% Nonidet P40) and renatured by heating to 90 °C for 1 min and chilling on ice for 3 min. Reactions were supplemented with either EDTA or polyvalent ions, as indicated in the text, and 1 pmol of BS15 for reactions containing protein, and brought to a 20-μL reaction volume. The reactions were incubated for 2 h on ice before adding 2 μL type III loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) (Sam-

brook et al., 1989). Samples were immediately loaded onto an 8% 29:1 acrylamide:bisacrylamide gel and subjected to electrophoresis using a running buffer containing 45 mM Tris-Cl, pH 8.1, 50 mM potassium acetate, and EDTA or polyvalent ions that were circulated at > 2 L/h. Gels (15-cm wide, 45-cm long, and 0.07-cm thick) were pre-run for 1 h at 100 V, constant voltage, at 4 °C. Samples (8 μL) were loaded onto the gel with the current on and the gel was subjected to electrophoresis for 16 h.

Modification interference analysis

Approximately 100 ng (5 × 10⁶ d.p.m.) of 3' end-labeled Fr4 RNA was modified with DEPC or hydrazine as described previously (Batey & Williamson, 1996b). The modified RNA was resuspended in either EDTA-binding buffer (10 mM K-Hepes, pH 7.5, 50 mM potassium acetate, 0.1 mM EDTA) or magnesium-binding buffer (10 mM K-Hepes, pH 7.5, 50 mM potassium acetate, 1 mM magnesium acetate) and renatured by heating to 90 °C for 1 min and incubated on ice for 1 h. RNA samples were loaded onto an 8% 29:1 acrylamide:bisacrylamide gels for electrophoresis at 12.5 V/cm at 4 °C for 4 h using a 45 mM Tris-borate, pH 8.3, running buffer that contained 1 mM EDTA or 1 mM magnesium acetate. The RNA band was visualized by exposing the gel to autoradiographic film for 1 h, excised, and eluted from the gel by soaking in elution buffer (0.3 M sodium acetate, 0.1 mM EDTA, 0.1% SDS). The eluted RNAs were purified, cleaved at the sites of modification, and analyzed on 8% denaturing polyacrylamide gels as described previously (Batey & Williamson, 1996b).

T₁ nuclease assay

3' End-labeled Fr18 RNA was incubated in a buffer containing 10 mM K-Hepes, pH 7.5, 50 mM potassium acetate, 0.1 mg/mL tRNA, and 0.01% Nonidet P-40, along with varying concentrations of magnesium acetate. The 100-μL reaction mixtures were incubated for 1 h at 4 °C prior to adding 5 μL of 100 U/mL T₁ nuclease (United States Biochemical) and incubating for 2 min. Reactions were quenched with 100 μL of 1:1 phenol:chloroform and precipitated with ethanol. Precipitated RNA was resuspended in 20 mL denaturing load buffer (9.8 M urea, 0.25% xylene cyanol, 0.1 mM Na-EDTA, pH 8.0) and subjected to electrophoresis on a denaturing 20% 29:1 acrylamide:bisacrylamide gel.

NMR spectroscopy

NMR spectra were collected on a Varian Unity 600 MHz spectrometer equipped with an HCN triple-resonance probe with z-gradients, and the data were analyzed using nmrPipe (Delaglio et al., 1995) on a Silicon Graphics Indy workstation. One-dimensional spectra were recorded in a buffer containing 1.5 mM Fr18 RNA and 50 mM potassium chloride in 90% ¹H₂O/10% ²H₂O to observe the exchangeable protons. These samples did not contain sodium phosphate buffer because the addition of magnesium would result in the precipitation of magnesium phosphate and the pH of the sample was adjusted directly. These spectra were acquired using 1331 binomial solvent suppression (Hore, 1983) with a sweep width

of 12,000 Hz, 16K complex data points, and 128 scans with the excitation maximum set to 3,500 Hz. DQF-COSY experiments were recorded in 99.996% $^2\text{H}_2\text{O}$ at 35 °C. Data sets were acquired with 2,048 complex points in the t_2 dimension and 1,024 complex points in the t_1 dimension with 32 scans per slice and a sweep width of 6,000 Hz in each dimension. Samples of Fr15 were all 1 mM concentration in a buffer containing 10 mM potassium phosphate, pH 6.5, and 50 mM potassium chloride except for the Fr15-magnesium chloride sample, which did not contain phosphate buffer.

Equilibrium and kinetic measurements of S15 binding

The apparent equilibrium dissociation constant (K_d) and bimolecular association rate (k_{on}) were measured using the mobility shift assay as described previously (Batey & Williamson, 1996a), with several differences. The binding buffer utilized for these studies was 10 mM K-Hepes, pH 7.5, 50 mM potassium acetate, 0.1 mg/mL tRNA, and 0.01% Nonidet P-40. After the RNA was renatured in binding buffer, 1 mM EDTA or 10 mM magnesium chloride and BS15 [which was serially diluted in binding buffer, resulting in slightly lower measured apparent equilibrium binding constants than previously observed (Batey & Williamson, 1996a)] were added to reactions, and the 20- μL reactions were incubated for 2 h on ice to ensure that the reactions were equilibrated fully. All samples were subjected to electrophoresis on nondenaturing 8% 29:1 acrylamide:bisacrylamide gels in $0.5\times$ TBE buffer at 4 °C. Gel mobility shifts were visualized, quantified, and analyzed using techniques described elsewhere (Batey & Williamson, 1996a).

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REFERENCES

Bassi GS, Mollegaard NE, Murchie AIH, von Kitzing E, Lilley DMJ. 1995. Ionic interactions and the global conformations of the hammerhead ribozyme. *Struct Biol* 2:45–55.

Bassi GS, Murchie AIH, Lilley DMJ. 1996. The ion-induced folding of the hammerhead ribozyme: Core sequence changes that perturb folding into the active conformation. *RNA* 2:756–768.

Batey RT, Williamson JR. 1996a. Interaction of the *Bacillus stearothermophilus* ribosomal protein S15 with 16 S rRNA: I. Defining the minimal RNA site. *J Mol Biol* 261:536–549.

Batey RT, Williamson JR. 1996b. Interaction of the *Bacillus stearothermophilus* ribosomal protein S15 with 16 S rRNA: II. Specificity determinants of RNA–protein recognition. *J Mol Biol* 261:550–567.

Brunel C, Romby P, Westhof E, Ehresmann C, Ehresmann B. 1991. Three-dimensional model of *Escherichia coli* ribosomal 5 S RNA as deduced from structure probing in solution and computer modeling. *J Mol Biol* 221:293–308.

Cate JH, Doudna JA. 1996. Metal-binding sites in the major groove of a large ribozyme domain. *Structure* 4:1221–1229.

Cate JH, Gooding AR, Podell E, Zhou K, Golden BL, Kundrot CE, Cech TR, Doudna JA. 1996. Crystal structure of a group I ribozyme domain: Principles of RNA packing. *Science* 273:1678–1685.

Cate JH, Hanna RL, Doudna JA. 1997. A magnesium ion core at the heart of a ribozyme domain. *Nature Struct Biol* 4:553–558.

Chastain M, Tinoco I Jr. 1991. Structural elements in RNA. *Prog Nucleic Acids Res Mol Biol* 41:131–177.

Cotton FA, Wilkinson G, Gaus PL. 1987. *Basic inorganic chemistry*. New York: John Wiley & Sons.

Dahm SC, Uhlenbeck OC. 1991. Role of divalent metal ions in the hammerhead RNA cleavage reaction. *Biochemistry* 30:9464–9469.

Delaglio F, Grzesiek S, Vuister G, Zu G, Pfeiffer J, Bax A. 1995. NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J Biol NMR* 6:277–293.

Gutell RR. 1994. Collection of small subunit (16S- and 16S-like) ribosomal RNA structures: 1994. *Nucleic Acids Res* 22:3502–3507.

Held WA, Mizushima S, Nomura M. 1974. Assembly mapping of 30 S ribosomal proteins from *Escherichia coli*. Further studies. *J Biol Chem* 249:3103–3111.

Hore PJ. 1983. Solvent suppression in fourier transform nuclear magnetic resonance. *J Magn Reson* 55:283–300.

Jack A, Lander JE, Klug A. 1976. Crystallographic refinement of yeast phenylalanine transfer RNA at 2.5 Å resolution. *J Mol Biol* 108:619–649.

Kieft JS. 1997. Structure and thermodynamics of a metal ion binding site in the RNA major groove: Cobalt (III) hexammine as a probe [dissertation]. Berkeley, California: University of California at Berkeley.

Kieft JS, Tinoco IJ. 1997. Solution structure of a metal-binding site in the major groove of RNA complexed with cobalt (III) hexammine. *Structure* 5:713–721.

Kooi EA, Rutgers CA, Mulder A, Riet JVT, Venema J, Raue HA. 1993. The phylogenetically conserved doublet tertiary interaction in domain III of the large subunit rRNA is crucial for ribosomal protein binding. *Proc Natl Acad Sci USA* 90:213–216.

Laing LG, Gluck TC, Draper DE. 1994. Stabilization of RNA structure by Mg ions: Specific and non-specific effects. *J Mol Biol* 237:577–587.

Long DM, Uhlenbeck OC. 1993. Self-cleaving catalytic RNA. *FASEB J* 7:25–30.

Milligan JF, Groebe DR, Witherell GW, Uhlenbeck OC. 1987. Oligoribonucleotide synthesis using T7 RNA-polymerase and synthetic DNA templates. *Nucleic Acids Res* 15:8783–8798.

Mohr G, Caprara MG, Guo Q, Lambowitz AM. 1994. A tyrosyl-tRNA synthetase can function similarly to an RNA structure in the *Tetrahymena* ribozyme. *Nature* 370:147–150.

Mohr G, Zhang A, Gianelos JA, Belfort M, Lambowitz AM. 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.

Orr JW, Hagerman PJ, Williamson JR. 1998. Protein and Mg^{2+} -induced conformational changes in the S15 binding site of 16 S ribosomal RNA. *J Mol Biol* 275:453–464.

Pan T, Long DM, Uhlenbeck OC. 1993. Divalent metal ions in RNA folding and catalysis. In: Gesteland R, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 271–302.

Phillippe C, Eyermann F, Benard L, Portier C, Ehresmann B, Ehresmann C. 1993. Ribosomal protein S15 from *Escherichia coli* modulates its own translation by trapping the ribosome on the mRNA initiation loading site. *Proc Natl Acad Sci USA* 90:4394–4398.

Pley HW, Flaherty KM, McKay DB. 1994. Three-dimensional structure of a hammerhead ribozyme. *Nature* 372:68–74.

Portier C, Dondon L, Grunberg-Manago M. 1990a. Translational autocontrol of the *Escherichia coli* ribosomal protein S15. *J Mol Biol* 211:407–414.

Portier C, Philippe E, Dondon L, Grunberg-Manago M, Ebel JP, Ehresmann B, Ehresmann C. 1990b. Translational control of ribosomal protein S15. *Biochim Biophys Acta* 1050:328–336.

Powers T, Noller HF. 1995. Hydroxyl radical footprinting of ribosomal proteins on 16S rRNA. *RNA* 1:194–209.

- Ryan PC, Draper DE. 1989. Thermodynamics of protein-RNA recognition in a highly conserved region of the large-subunit ribosomal RNA. *Biochemistry* 28:9949-9956.
- Ryan PC, Lu M, Draper DE. 1991. Recognition of the highly conserved GTPase center of 23 S ribosomal RNA by ribosomal protein L11 and the antibiotic thiostrepton. *J Mol Biol* 221:1257-1268.
- Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Scott WG, Finch JT, Klug A. 1995. The crystal structure of an all-RNA hammerhead ribozyme: A proposed mechanism for RNA catalytic cleavage. *Cell* 81:991-1002.
- Serganov A, Rak A, Garber M, Reinbolt J, Ehresmann B, Ehresmann C, Grunberg-Manago M, Portier C. 1997. Ribosomal protein S15 from *Thermus thermophilus*: Cloning, sequencing, overexpression of the gene and RNA-binding properties of the protein. *Eur J Biochem* 246:291-300.
- Serganov AA, Masquida B, Westhof E, Cachia C, Portier C, Garber M, Ehresmann B, Ehresmann C. 1996. The 16S rRNA binding site of *Thermus thermophilus* ribosomal protein S15: Comparison with *Escherichia coli* S15, minimum site and structure. *RNA* 2:1124-1138.
- Uhlenbeck OC. 1987. A small catalytic oligoribonucleotide. *Nature* 328:596-600.
- Weeks KM, Cech TR. 1995. Protein facilitation of group I intron splicing by assembly of the catalytic core and the 5' splice site domain. *Cell* 82:221-230.
- Weeks KM, Cech TR. 1996. Assembly of a ribonucleoprotein catalyst by tertiary structure capture. *Science* 271:345-348.
- Westhof E, Romby P, Romaniuk PJ, Ebel JP, Ehresmann C, Ehresmann B. 1989. Computer modeling from solution data of spinach chloroplast and of *Xenopus laevis* somatic and oocyte 5 S rRNAs. *J Mol Biol* 207:417-431.
- Wüthrich K. 1986. *NMR of proteins and nucleic acids*. New York: John Wiley & Sons.
- Wyatt JR, Chastain M, Puglisi JD. 1991. Synthesis and purification of large amounts of RNA oligonucleotides. *Biotechniques* 11:764-769.
- Zarrinkar PP, Williamson JR. 1996. The kinetic folding pathway of the *Tetrahymena* ribozyme reveals possible similarities between RNA and protein folding. *Nature Struct Biol* 3:432-438.