Biochemistry

 Volume 31, Number 24 June 23, 1992

Accelerated Publications

Recognition of G-U Mismatches by Tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III)†

Christine S. Chow and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received March 3, 1992; Revised Manuscript Received April 15, 1992

ABSTRACT: The coordination complex tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III) [$Rh(DIP)_3^{3+}$], which promotes RNA cleavage upon photoactivation, has been shown to target specifically guanine–uracil (G-U) mismatches in double-helical regions of folded RNAs. Photoactivated cleavage by $Rh(DIP)_3^{3+}$ has been examined on a series of RNAs that contain G-U mismatches, yeast $tRNA^{Phe}$ and yeast $tRNA^{Asp}$, as well as on 5S rRNAs from *Xenopus* oocytes and *Escherichia coli*. In addition, a "microhelix" was synthesized, which consists of seven base pairs of the acceptor stem of yeast $tRNA^{Phe}$ connected by a six-nucleotide loop and contains a mismatch involving residues G4 and U69. A U4·G69 variant of this sequence was also constructed, and cleavage by $Rh(DIP)_3^{3+}$ was examined. In each of these cases, specific cleavage is observed at the residue which lies to the 3′-side of the wobble-paired U; some cleavage by the rhodium complex is also evident in several structured RNA loops. The remarkable site selectivity for G-U mismatches within double-helical regions is attributed to *shape-selective* binding by the rhodium complex. This binding furthermore depends upon the orientation of the G-U mismatch, which produces different stacking interactions between the G-U base pair with the Watson–Crick base pair following it on the 5′-side of U compared to the Watson–Crick pair preceding it on the 3′-side of U. $Rh(DIP)_3^{3+}$ therefore serves as a unique probe of G-U mismatches and may be useful both as a model and in probing RNA–protein interactions as well as in identifying G-U mismatches within double-helical regions of folded RNAs.

As revealed through crystallography and NMR, the one-dimensional nucleotide sequence of RNA can fold into an abundance of three-dimensional structures (Kim et al., 1974; Quigley & Rich, 1976; Westhof et al., 1985; Patel et al., 1987; Cheong et al., 1990; Varani et al., 1991; Puglisi et al., 1990). Increasing evidence indicates that proteins may take advantage of the conformational polymorphism in the RNA backbone in recognizing specific binding sites on the macromolecule (Weeks & Crothers, 1991; Ruff et al., 1991; Calnan et al., 1991; Rould et al., 1989). RNA-binding proteins may distinguish their targets not only through specific hydrogen-bonding interactions with the RNA bases and phosphate backbone but also through specific electrostatic and van der Waals interactions. Recent studies, which involve the use of transition metal complexes as probes for RNA tertiary structure, have revealed an interesting correlation between sites on the RNA that are recognized by the metal complexes, regions of the RNA that deviate from canonical A-form RNA, and regions of the RNA that are important for interactions with RNA-binding proteins (Chow et al., 1992a).

Octahedral metal complexes of rhodium(III) are well suited for examining RNA structure. These complexes are coordinatively saturated, inert to substitution, and rigid and well-defined in structure. Furthermore, there is no direct coordination of the metal center to the RNA. Instead, binding to RNA by these complexes is based purely upon an ensemble of noncovalent interactions between the metal complex and the nucleic acid. Attention in our laboratory has been focused primarily upon octahedral metal complexes which contain no hydrogen-bonding functionalities. With these complexes, binding to an RNA site is based upon *shape selection*, the recognition of a site based upon matching the shape and symmetry of the complex to the nucleic acid binding site.

† We are grateful to the National Institutes of Health (Grant GM33309) for their financial support. In addition, C.S.C. thanks the Ralph M. Parsons Foundation for a graduate fellowship.

* To whom correspondence should be addressed.

0006-2960/92/0431-5423$03.00/0

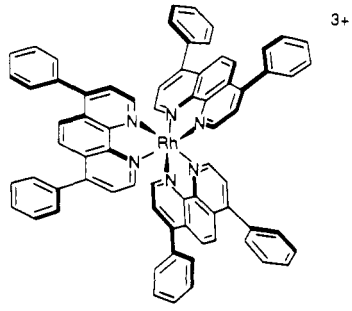

FIGURE 1: Schematic illustration of $Rh(DIP)_3^{3+}$.

Binding to an individual RNA site therefore involves van der Waals and electrostatic interactions rather than hydrogen bonding. Complexes of rhodium, then, have been useful probes, since, upon irradiation with ultraviolet light, these complexes induce strand cleavage at their binding sites.

In our studies of the photocleavage of tRNA with a series of transition metal complexes (Chow & Barton, 1990), we discovered that cleavage by tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III), $[Rh(DIP)_3]^{3+}$ (Figure 1), is remarkably site-selective. In particular, two distinct sites of metal-activated cleavage are observed on yeast $tRNA^{Phe}$. One of these sites, Ψ55, is also recognized by related intercalating transition metal complexes (Chow & Barton, 1990) and is marked by tertiary interactions between the D and TΨC loops of the folded tRNA. The second strong cleavage site occurs at C70. Interestingly, this residue lies to the 3′-side of U69, which is involved in a mismatched base pair with G4. No other reagent has been shown to target the C70 site on $tRNA^{Phe}$ with such high selectivity. It was unclear whether recognition of this site by the rhodium complex was particular to yeast $tRNA^{Phe}$ or if recognition of G-U mismatches was a general feature of $Rh(DIP)_3^{3+}$.

Presently, we have tested cleavage by $Rh(DIP)_3^{3+}$ on a series of small RNAs that contain G-U mismatches. In particular, we have examined cleavage by the rhodium complex on yeast $tRNA^{Asp}$, which contains four G-U mismatches, as well as on 5S rRNAs from *Xenopus* oocytes and *Escherichia coli* with proposed G-U mismatches in helices I, II, and IV. In addition, a "microhelix" was synthesized, which consists of seven base pairs of the acceptor stem of yeast $tRNA^{Phe}$ connected by a six-nucleotide loop. This microhelix contains a G-U mismatch involving residues G4 and U69. A U4·G69 variant of this sequence was also constructed, and cleavage by $Rh(DIP)_3^{3+}$ was examined. We find that $Rh(DIP)_3^{3+}$ promotes RNA cleavage with high site selectivity and in particular targets folded loop structures and G-U mismatches within regions of double-helical RNA.

Experimental Procedures

RNAs. Native $tRNA^{Phe}$ from brewers' yeast was obtained from Boehringer Mannheim (Indianapolis, IN); $tRNA^{Asp}$ from yeast was a gift from D. Moras (Institut de Biologie Moleculaire et Cellulaire du CNRS, Strasbourg, France); purified 5S rRNAs from *Xenopus* oocytes and *E. coli* were generously provided by P. W. Huber (Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN); unmodified $tRNA^{Phe}$ and $tRNA^{Phe}$ mutants were provided by O. C. Uhlenbeck (Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO). The oligoribonucleotides, based on the acceptor stem of $tRNA^{Phe}$ (24-mers), were chemically synthesized on an Applied Biosystems 392 DNA/RNA synthesizer using the phosphoramidite method and deprotected using the method described by Usman et al. (1987). The oligoribonucleotides were purified by the OPC desalting method (Applied Biosystem, Inc.) followed by polyacrylamide gel electrophoresis (12%, nondenaturing).

Preparation of Labeled RNAs. Yeast $tRNA^{Phe}$, yeast $tRNA^{Asp}$, *Xenopus* oocyte 5S rRNA, *E. coli* 5S rRNA, and the synthetic oligoribonucleotides were 3′-end labeled with cytidine 3′,5′-[5′-^{32}P]bisphosphate using T4 RNA ligase (England & Uhlenbeck, 1978) or 5′-end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The labeled RNAs were gel purified on a 20%, 8 M denaturing polyacrylamide gel (40 cm long, 0.8 mm thick), located by autoradiography, excised, and eluted from the gel in 45 mM Tris-HCl, 45 mM boric acid, and 1.25 mM EDTA, pH 8.0. The eluted RNAs were precipitated twice with ethanol and stored in 10 mM Tris-HCl, pH 7.5.

Cleavage of RNA by $Rh(DIP)_3^{3+}$. $[Rh(DIP)_3]Cl_3$ was prepared in our laboratory by M. R. Kirshenbaum (Kirshenbaum et al., 1988). $Rh(DIP)_3^{3+}$ stock solutions (1 mM) were freshly prepared in ethanol. The end-labeled tRNAs and oligoribonucleotides were renatured by heating to 70 °C for 10 min in 10 mM Tris-HCl, pH 7.5, and slowly cooling to room temperature prior to use. Similarly, the end-labeled 5S rRNAs were renatured by heating to 65 °C for 10 min in 10 mM Tris-HCl, 10 mM $MgCl_2$, and 300 mM KCl, pH 7.5, and slowly cooling to room temperature. The 20-μL cleavage mixtures contained labeled RNA, 2.5 μM $Rh(DIP)_3^{3+}$ (freshly diluted in H_2O), and Tris-HCl buffer (5 mM Tris, 50 mM NaCl, pH 7.0) and were brought to a final concentration of 100 μM in nucleotides with carrier $tRNA^{Phe}$. Irradiation for 6 min at 313 nm at ambient temperature using a 1000-W Hg/Xe lamp and monochromator yielded site-specific cleavage of the RNA samples only in the presence of the rhodium complex. The reaction mixtures were precipitated with ethanol, washed at least three times with 70% ethanol to remove buffer salts, and analyzed on either 15% or 20% polyacrylamide/8 M urea gels. The full-length RNAs and cleavage products were identified by coelectrophoresing with diethyl pyrocarbonate (DEPC) (A-specific), dimethyl sulfate (DMS) followed by sodium borohydride treatment (G-specific), and hydrazine (U-specific) reactions (Peattie, 1979) and viewed by autoradiography.

Results

Cleavage of Yeast $tRNA^{Phe}$. The $Rh(DIP)_3^{3+}$ cleavage sites have been assigned by comparison with end-labeled products of DEPC, DMS, and hydrazine reactions, which lead to specific cuts at A, G, and U residues, respectively. These base-specific reactions followed by aniline-catalyzed eliminations give 5′- and 3′-phosphate termini (Peattie, 1979). Similarly, strand scission induced by the rhodium complex leads to the production of 5′- and 3′-termini that comigrate exactly with the phosphate termini generated by DEPC, DMS, and hydrazine. The cleavage mechanism is likely related to that of $Rh(phen)_2phi^{3+}$, in which no diffusible species mediates the reaction (Sitlani et al., 1992). Therefore, the site of cleavage indicates directly the site of binding by the metal complex.

As can be seen in Figure 2 (panel A, lane 3), two specific sites of cleavage by the rhodium complex are evident on 3′-end-labeled $tRNA^{Phe}$. Strong cleavage occurs at residues Ψ55 and C70. Identical sites of cleavage are observed in experiments conducted on 5′-end-labeled tRNA (data not shown). Cleavage at Ψ55 occurs in the TΨC loop and may resemble structurally specific cleavage observed by $Rh(DIP)_3^{3+}$ on DNA cruciforms (Kirshenbaum et al., 1988). This site is also recognized by related transition metal complexes, $Rh(phen)_2phi^{3+}$ and $Ru(phen)_3^{2+}$ (phen = 1,10-phenanthroline; phi = 9,10-phenanthrenequinone diimine), both of which may

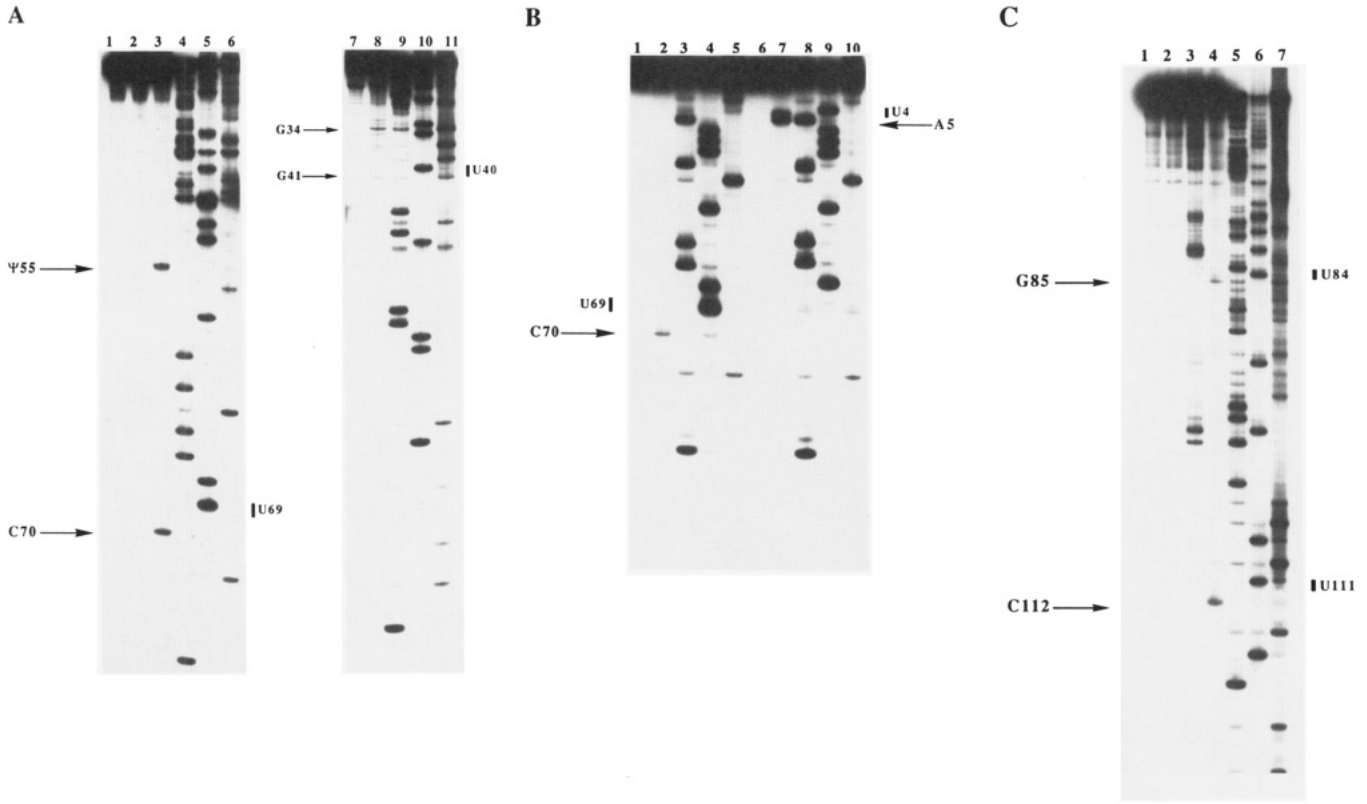

FIGURE 2: (A) Cleavage of ^{32}P 3′-end-labeled yeast tRNA^{Phe} and yeast tRNA^{Asp} by Rh(DIP)$_3^{3+}$. Cleavage was performed in 5 mM Tris and 50 mM NaCl, pH 7.0. Lanes 1 and 7: tRNA^{Phe} and tRNA^{Asp} controls; RNA without metal or irradiation. Lane 2: light control; tRNA^{Phe} irradiated in the absence of metal. Lanes 3 and 8: specific cleavage by Rh(DIP)$_3^{3+}$ on tRNA^{Phe} and tRNA^{Asp}. Lanes 4–6: sequencing reactions on tRNA^{Phe}; A-, U-, and G-specific reactions, respectively. Lanes 9–11: sequencing reactions on tRNA^{Asp}; A-, U-, and G-specific reactions, respectively. Arrows indicate the major Rh(DIP)$_3^{3+}$ cleavage sites, and bars indicate the neighboring U residues to the 3′-side, which are involved in G-U mismatches. (B) Cleavage of ^{32}P 3′-end-labeled G4·U69 microhelix^{Phe} and U4·G69 microhelix^{Phe} by Rh(DIP)$_3^{3+}$. Cleavage was performed in 5 mM Tris-HCl and NaCl, pH 7.0. Lanes 1 and 6: G4·U69 and U4·G69 microhelix controls; RNA without metal or irradiation. Lanes 2 and 7: specific cleavage by Rh(DIP)$_3^{3+}$ on G4·U69 and U4·G69 microhelices. Lanes 3–5: sequencing reactions on G4·U69 microhelix; A-, U-, and G-specific reactions, respectively. Lanes 8–10: sequencing reactions on U4·G69 microhelix; A-, U-, and G-specific reactions, respectively. Arrows indicate the major Rh(DIP)$_3^{3+}$ cleavage sites, and bars indicate the neighboring U residues to the 3′-side, which are involved in G-U mismatches. (C) Cleavage of ^{32}P 3′-end-labeled 5S rRNA from *Xenopus* oocytes by Rh(DIP)$_3^{3+}$. Cleavage was performed in 5 mM Tris-HCl and 50 mM NaCl, pH 7.0. Lane 1: 5S rRNA control; RNA without metal or irradiation. Lane 2: light control; 5S rRNA irradiated in the absence of metal. Lane 3: specific cleavage by Rh(phen)$_2$phi^{3+} on 5S rRNA. Lane 4: specific cleavage by Rh(DIP)$_3^{3+}$ on 5S rRNA. Lanes 5–7: sequencing reactions on 5S rRNA; A-, U-, and G-specific reactions, respectively. Arrows indicate the major Rh(DIP)$_3^{3+}$ cleavage sites, and bars indicate the neighboring U residues to the 3′-side, which are involved in G-U mismatches.

intercalate (Chow & Barton, 1990). Cleavage at C70 is adjacent to a G-U mismatch on the 3′- side of U69. This cleavage site is different from those observed using other transition metal complexes (Chow & Barton, 1990; Brown et al., 1985) as well as being different from sites recognized by other structure-mapping reagents (Behlen et al., 1990; Latham & Cech, 1989; Murakawa et al., 1989; Garrett-Wheeler et al., 1984). The fact that cleavage by Rh(DIP)$_3^{3+}$ differs from that by other rhodium complexes, such as Rh(phen)$_2$phi^{3+}, supports the notion that the specificity is derived from the recognition characteristics of the metal complex rather than being a function of hyperreactivity or cleavage chemistry associated with the site. Cleavage at C70 could depend on the neighboring G-U wobble pair, which causes structural distortions in the secondary structure of the acceptor stem of tRNA (Kim et al., 1974; Quigley & Rich, 1976).

Cleavage of Yeast tRNA^{Asp}. The sites of Rh(DIP)$_3^{3+}$ cleavage on yeast tRNA^{Asp} have been determined using 3′-end-labeled RNA. In contrast to cleavage on tRNA^{Phe}, Figure 2 (panel A, lane 8) reveals only weak cleavage by the rhodium complex on tRNA^{Asp} at residues G41 in the anticodon stem and G34 in the anticodon loop. No additional sites are revealed when 5′-end-labeled RNAs are employed for cleavage by Rh(DIP)$_3^{3+}$. Cleavage induced by Rh(DIP)$_3^{3+}$ at G34 may be related to cleavage by Rh(phen)$_2$phi^{3+} at Ψ32 in the anticodon loop of this tRNA (Chow et al., 1992b). As revealed by its crystal structure, the anticodon loop in tRNA^{Asp} is single stranded, yet structured through continued stacking interactions with the anticodon stem (Westhof et al., 1985). The stacked residues in the anticodon loop may provide a favorable structure for interaction with both rhodium complexes. However, the two different complexes, Rh(phen)$_2$phi^{3+} and Rh(DIP)$_3^{3+}$, appear to recognize different stacked structures in the anticodon loops of the tRNAs.

Importantly, cleavage at residue G41 occurs adjacent to a G-U mismatch. As with the C70 cleavage site on tRNA^{Phe}, G41 lies to the 3′-side of the wobble-paired U residue. Apparently, Rh(DIP)$_3^{3+}$ can recognize a similar distortion which is created by the G30-U40 mismatch tRNA^{Asp} and the G4-U69 mismatch in tRNA^{Phe}.

Cleavage of Microhelix RNAs. In order to define further the recognition by Rh(DIP)$_3^{3+}$ and test whether the G4-U69 base pair retains its strong influence on rhodium cleavage in a smaller RNA, we have examined cleavage on synthetic RNAs representing the acceptor stem of tRNA^{Phe}. A microhelix was synthesized chemically on a solid support using the phosphoramidite method developed for RNA (Usman et al., 1987). The microhelix is related to microhelix^{Ala}, which was designed by Francklyn and Schimmel (1989) to study the influence of the G-U mismatch on recognition of tRNA by

aminoacyl tRNA synthetase. The helix in this study (microhelix^{Phe}) consists of the seven base pairs of the acceptor stem of tRNA^{Phe} from yeast connected by a six-nucleotide loop. In the sequence of G4·U69 microhelix^{Phe}, the sequence of the loop starts at U8 and continues into the 5′-side of the D stem, such that C13 is joined to A66 of the acceptor helix. A U4·G69 variant of this microhelix sequence has also been synthesized to test whether or not the directionality of the G-U mismatch is important for recognition by $Rh(DIP)_3^{3+}$.

As shown in Figure 2 (panel B, lane 2), cleavage by $Rh(DIP)_3^{3+}$ on G4·U69 microhelix^{Phe} is nearly identical to cleavage observed on native tRNA^{Phe}. On 3′-end-labeled RNA, strong cleavage is apparent at C70, with minor cleavage occurring at G10. Once again, cleavage occurs at C70, which lies to the 3′-side of U69 which is wobble-paired with G4. Similarly, strong cleavage on U4·G69 microhelix^{Phe} occurs at A5; minor cleavage is apparent at G10 (Figure 2, panel B, lane 7). Residue A5 is also adjacent to a G-U mismatch. Consistent with cleavage on tRNA^{Phe}, tRNA^{Asp}, and G4·U69 microhelix^{Phe}, this site lies to the 3′-side of U4 which is wobble-paired with G69. It is also noteworthy that cleavage at this site yields two bands, only one of which appears to comigrate with the 5′-phosphate, suggestive of a secondary reaction mechanism. Nonetheless, the A5 site is the strongest of the many observed on different RNAs and is also the one observed at highest resolution. Moreover, cleavage by $Rh(DIP)_3^{3+}$ at G10 on both microhelices is consistent with the cleavage at G34 on tRNA^{Asp}, in which the single-stranded loops following a helical stem are structured and interact favorably with the metal complex. Cleavage on both microhelices at G10 suggests that, despite differences in sequence at residues 4 and 69, the overall folded structures of the two helices are the same.

Cleavage of 5S rRNAs. Models have suggested the existence of G-U wobble pairs in helix I and helix IV of *Xenopus* ooctye 5S rRNA (Fox & Woese, 1975; Luehrsen et al., 1981). Similarly, a secondary structure model proposed for *E. coli* 5S rRNA pointed to the existence of a G-U mismatch in helix I, two in helix II, and two in helix IV (Pieler & Erdmann, 1982). We have tested the recognition of $Rh(DIP)_3^{3+}$ on 5S rRNAs from *Xenopus* and *E. coli*. As shown in Figure 2 (panel C, lane 4), strong, site-selective cleavage by $Rh(DIP)_3^{3+}$ occurs on 3′-end-labeled *Xenopus* oocyte 5S rRNA at residues C112 and G85. These sites are different from those obtained with cleavage by the metal complex $Rh(phen)_2phi^{3+}$ (Chow et al., 1992a) (Figure 2, panel C, lane 3). Again as predicted, both C112 and G85 are located on the 3′-side of a U involved in G-U base pairing. Residue C112 is located in helix I adjacent to U111-G8, while G85 resides in helix IV next to U84-G93. Similarly, the proposed G-U mismatches within double-helical regions in *E. coli* 5S rRNA are recognized by $Rh(DIP)_3^{3+}$ (data not shown). In particular, strong cleavage is apparent at G112; weaker cleavage is observed at G81 in helix IV. The proposed secondary structure for *E. coli* 5S rRNA reveals that G112 is located on the 3′-side of U111, which base pairs with G9; G81 lies on the 3′-side of U80-G96. It is also interesting to note that while G81 is involved in a G-U wobble pair with U95, no cleavage is apparent on the 3′-side at G96. This result indicates that the adjacent G-U mismatches are not symmetric. In addition, no cleavage is apparent at the proposed G-U mismatches in helix II of *E. coli* 5S rRNA.

Discussion

The results for the cleavage by $Rh(DIP)_3^{3+}$ on several tRNAs, 5S rRNAs, and microhelix RNAs are summarized in Figure 3. As with $Rh(phen)_2phi^{3+}$ (Chow et al., 1992a,b), the cleavage by $Rh(DIP)_3^{3+}$ does not occur in standard double-helical or single-stranded regions of the RNAs that we have examined. Instead, cleavage is apparent at two families of sites: (i) structured loop regions, such as the TΨC loop of yeast tRNA^{Phe}, the anticodon loop of yeast tRNA^{Asp}, or the loop region of the microhelices; and (ii) the nucleotide adjacent to a G-U mismatch on yeast tRNA^{Phe}, yeast tRNA^{Asp}, G4·U69 microhelix^{Phe}, U4·G69 microhelix^{Phe}, *Xenopus* oocyte 5S rRNA, and *E. coli* 5S rRNA.

Recognition of Loop Structures in RNA by $Rh(DIP)_3^{3+}$. Cleavage by $Rh(DIP)_3^{3+}$ occurs at specific sites within the loop regions of RNA. In particular, strand scission is evident at Ψ55 in the TΨC loop of yeast tRNA^{Phe}, G34 in the anticodon loop of yeast tRNA^{Asp}, and G10 in the center of the six-nucleotide loop of both microhelix sequences. The crystal structures of the two tRNAs reveal that the anticodon and TΨC loops are single stranded, yet the bases in the anticodon loop continue to stack in an A-like helical manner and the nucleotides in the TΨC loop may be involved in long-range interactions with the D loop (Westhof et al., 1985; Kim et al., 1974; Quigley & Rich, 1976). The nucleotides in the microhelix loop may stack in a manner similar to that in the anticodon loop residues in tRNA^{Asp}. Since not all loop residues are cleaved by the metal complex, the recognition by $Rh(DIP)_3^{3+}$ is likely governed by specific tertiary structures that arise within the RNA loops.

Additional observations have been made regarding cleavage at Ψ55 on yeast tRNA^{Phe} by $Rh(DIP)_3^{3+}$. No cleavage at this site is apparent on a tRNA^{Phe} transcript which lacks modified bases (data not shown). The cleavage reaction is not Ψ-specific, since other positions containing Ψ on tRNA^{Phe} and tRNA^{Asp} are not cleaved. The unmodified transcript has been shown by NMR studies to be structurally less stable than the native tRNA^{Phe}, even in the presence of high concentrations of magnesium ion (Sampson & Uhlenbeck, 1988; Hall et al., 1989). Similarly, no cleavage was apparent by $Rh(DIP)_3^{3+}$ at U55 on a series of tRNA^{Phe} mutants designed by Sampson et al. (1990), even when the mutation is far from the TΨC loop (data not shown). Thus, cleavage at this site must depend on the precise three-dimensional folding of the D and TΨC loops. These results are consistent with cleavage by the rhodium complex on DNA cruciforms (Kirshenbaum et al., 1988) and other unusual DNA structures (I. Lee, unpublished results). The complex, a hydrophobic trication, likely binds in a hydrophobic pocket between charged helices of the DNA or RNA.

Recognition of G-U Mismatches in RNA by $Rh(DIP)_3^{3+}$. Perhaps most interesting is the cleavage which we observe by $Rh(DIP)_3^{3+}$ at the nucleotide adjacent to a G-U mismatch on six different RNAs. As summarized in schematic diagrams in Figure 3, C70 in tRNA^{Phe}, C70 in G4·U69 microhelix^{Phe}, A5 in U4·G69 microhelix^{Phe}, C112 and G85 in 5S rRNA from *Xenopus* oocytes, and G112 in 5S rRNA from *E. coli* are strong cleavage sites; weak cleavage at G41 of tRNA^{Asp} is also apparent. Importantly, however, not all G-U mismatches in these RNAs are recognized by the metal complex. No cleavage is apparent at the proposed G-U mismatches which neighbor loops in helix II of *E. coli* 5S rRNA or in tRNA^{Asp}. It appears that the rhodium complex preferentially targets G-U mismatches within double-helical regions of an RNA.

Cleavage always occurs at the residue located on the 3′-side of U, regardless of the nucleotide composition of that site or the flanking sites. Recognition of these sites is not identical, however. Different relative intensities of the bands are ap-

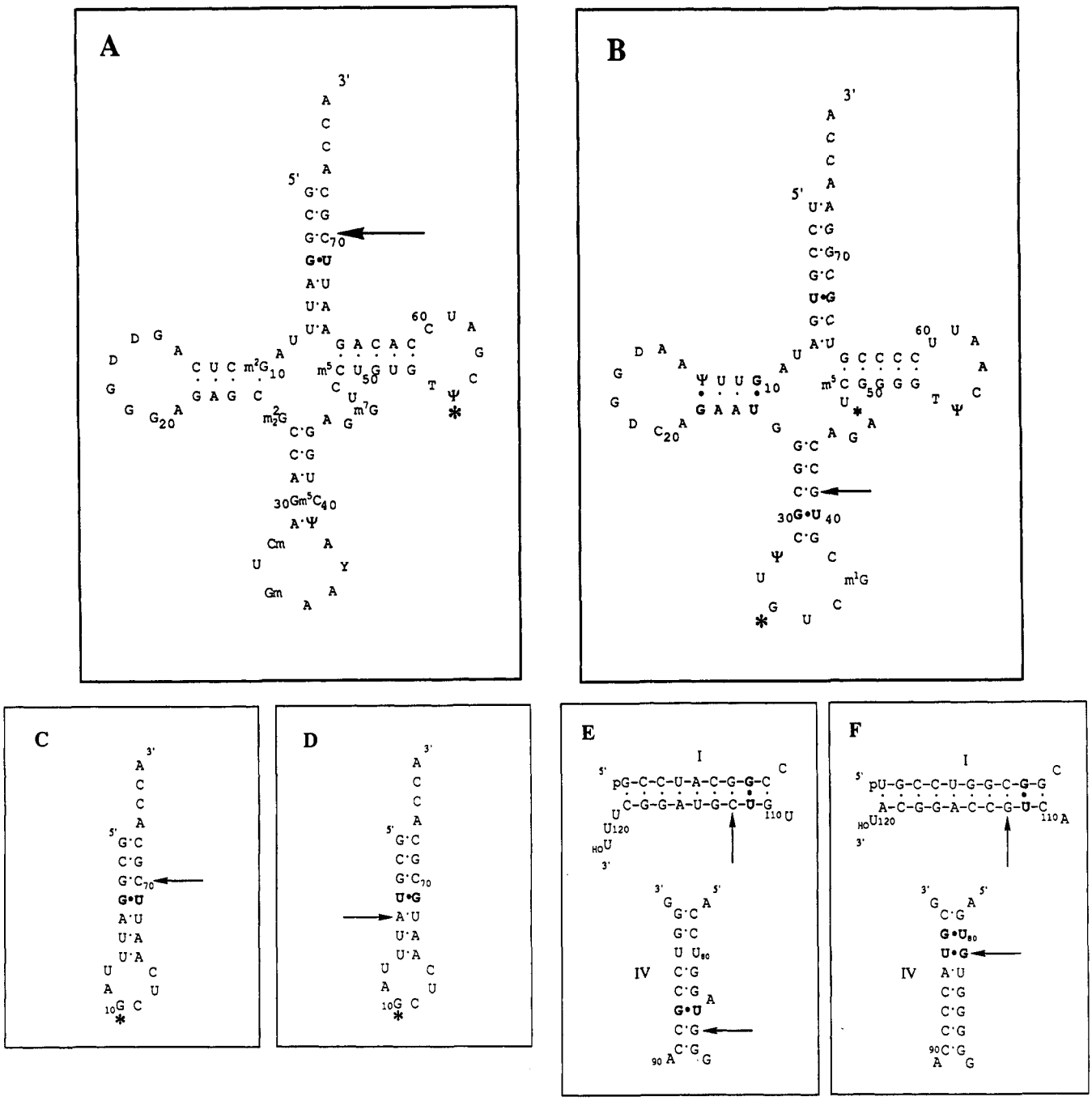

FIGURE 3: Schematic illustrations of yeast $tRNA^{Phe}$ (A), yeast $tRNA^{Asp}$ (B), G4·U69 microhelix^{Phe} (C), U4·G69 microhelix^{Phe} (D), helix I and helix IV of *Xenopus* oocyte 5S rRNA (E), and helix I and helix IV of *E. coli* 5S rRNA (F) with designations of $Rh(DIP)_3^{3+}$ cleavage sites. The arrows indicate the positions of $Rh(DIP)_3^{3+}$-promoted strand scission that lie adjacent to G-U mismatches. The large black dots between residues in bold face represent G-U wobble pairs (and one G-Ψ mismatch in $tRNA^{Asp}$). The large asterisks represent cleavage by $Rh(DIP)_3^{3+}$ in loop regions. The small asterisk (B) between residues A46 and U48 represents residue 47, which is present in $tRNA^{Phe}$ but absent in $tRNA^{Asp}$.

parent at the observed cleavage sites. This may be a reflection of the subtle changes in structure that are associated with different bases at or directly flanking these sites. A double band is in fact apparent at A5 of U4·G69 microhelix^{Phe}, the strongest site of cleavage.

Our results indicate that the recognition of the G-U mismatch depends only upon the structure local to the site. The interaction of $Rh(DIP)_3^{3+}$ with $tRNA^{Phe}$, for example, is dependent solely on the structure of the acceptor helix. RNA hairpin microhelices have been designed to correspond to this region of the molecule, and identical cleavage with $Rh(DIP)_3^{3+}$ is observed. In addition, specific cleavage is observed at C70 on an unmodified $tRNA^{Phe}$ transcript, and the identical result is obtained on a series of $tRNA^{Phe}$ mutants (data not shown). Apparently, mutations in the D stem, D loop, or the TΨC loop of $tRNA^{Phe}$ have no effect on cleavage at the G-U site in the acceptor stem.

Furthermore, the recognition depends specifically upon the orientation of the G-U mismatch. When the G4-U69 mismatch is switched to a U4-G69 mismatch, the cleavage occurs on the opposite side of the helix, just two nucleotides away from cleavage observed on the G4·U69 microhelix, and consistently at the 3′-side of U. As a consequence, no cleavage is apparent neighboring some G-U mismatches. In $tRNA^{Asp}$, G10-U25 and Ψ13-G22 directly neighbor loops (to the 3′-side of the U or Ψ). The U5-G68 mismatch is two bases away from the central fold; here no substantive binding site may exist for the rhodium complex at the 3′-side of U. In the case of helix II

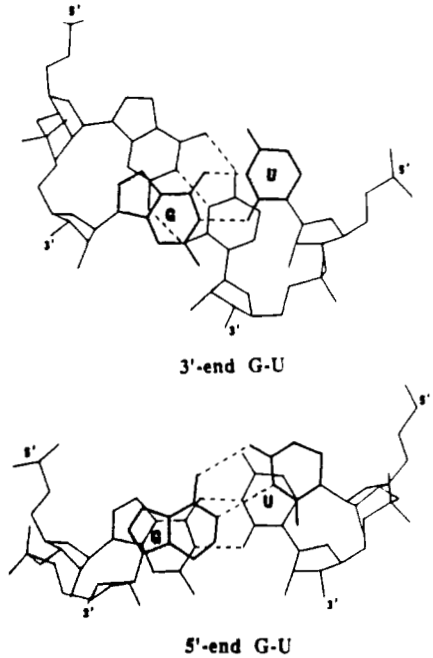

FIGURE 4: Stacking of the G4-U69 wobble pair in the middle of the acceptor stem of yeast $tRNA^{Phe}$ with the G3-C70 base pair and with the A5-U68 base pair. The former corresponds to the "3'-end G-U" base pair type; the latter corresponds to the "5'-end G-U" base pair type. This figure is adapted from Mizuno and Sundaralingam (1978).

of *E. coli* 5S RNA, the proposed G61-U22 mismatch also directly neighbors loop B.

Can we understand this recognition on the basis of the specific structure of a G-U mismatch? The occurrence of a single G-U wobble pair in the amino acid acceptor stem of $tRNA^{Phe}$ has been confirmed by the three-dimensional X-ray structure (Kim et al., 1974). Similarly, the elucidation of the crystal structure of yeast $tRNA^{Asp}$ revealed the existence of one G-Ψ and three G-U base pairs in three of the four helical stems (Westhof et al., 1985). The observed differences in the recognition of these sites by $Rh(DIP)_3^{3+}$ may be rationalized because of the remarkably different base-stacking interactions with the Watson–Crick base pairs situated on either side of the G-U mismatch. Upon examination of the tRNA crystal data, Mizuno and Sundaralingam (1978) noticed that the geometry of the G-U wobble pair results in unusual stacking of its bases with the neighboring bases. As shown in Figure 4, the G-U base pair exhibits greater stacking interactions with the Watson–Crick base pair following it on the 5'-side of U than the Watson–Crick pair preceding it on the 3'-side of U. The former corresponds to the "5'-end G-U" base pair; the latter corresponds to the "3'-end G-U" base pair. The 5'-end G-U exhibits stacking interactions similar to the stacking of two normal Watson–Crick base pairs. In contrast, the 3'-end G-U base pair does not stack well with the flanking base pair, and the wobble-paired U residue is pushed away from the helix interior into the major groove of the RNA. The $Rh(DIP)_3^{3+}$ cleavage sites adjacent to the G-U mismatches all exhibit stacking of the 3'-end G-U base pair type, in which the G-U pair is offset from the adjacent Watson–Crick pair. This stacking displacement in the major groove of the helix appears critical to the unique recognition by $Rh(DIP)_3^{3+}$.

It is interesting that, in the case of $tRNA^{Asp}$, no cleavage is observed with $Rh(DIP)_3^{3+}$ adjacent to any of the mismatch sites, U5-G68, G10-U25, or Ψ13-G22. For the latter two mismatches, the base pairs are located at either end of the D stem. Mizuno and Sundaralingam (1978) have shown that the unusual stacking properties of G-U mismatches have special repercussions at the ends of RNA helices. At a helix with a U-G pair stacked in a 5'-end G-U fashion with its neighboring base pair, the last two base pairs are stacked well. Both mismatches, Ψ13-G22 and G10-U25, are oriented as in a 5'-end G-U, and therefore no interaction with the metal complex is expected (Westhof et al., 1985). For the U5-G68 pair in the acceptor stem of $tRNA^{Asp}$, we might expect cleavage at G6; perhaps this particular site is hindered from interaction with $Rh(DIP)_3^{3+}$, since it lies only two base pairs away from the D and TΨC stems. The folding of these two arms may create a structure that prohibits binding by $Rh(DIP)_3^{3+}$ at G6.

Finally, unusual cleavage by $Rh(DIP)_3^{3+}$ is observed at G81 in helix IV of *E. coli* 5S rRNA. This residue lies to the 3'-side of U80-G96; however, G81 itself is involved in a G-U mismatch. No cleavage is apparent at G96, which lies to the 3'-side of U95-G81. Apparently, the stacking for adjacent mismatched base pairs is not the same as the stacking between a G-U mismatch and a Watson–Crick base pair. Our results indicate an asymmetry at this site since cleavage is observed at only one of the two G-U mismatches.

Implications for Protein Binding. The occurrence of the noncomplementary G-U base pair was initially envisioned by Crick (1966) in his wobble hypothesis for RNA codon–anticodon interactions. The existence of the wobble base pair has since been established by X-ray crystal structures and NMR studies (Kim et al., 1974; Quigley & Rich, 1976; Westhof et al., 1985; Patel et al., 1987). Internal G-U mismatches are quite common features that occur in tRNA and in the proposed secondary structures of other RNAs. Thus, the presence of G-U base pairs at conserved positions in RNA suggests that these mismatches may play a defined structural or functional role. Studies of RNA–protein interactions have indicated that G-U mismatches are important recognition elements. For example, two independent groups have discovered that a single G3-U70 base pair within the amino acid acceptor helix is a major determinant of the identity of *E. coli* $tRNA^{Ala}$ (Hou & Schimmel, 1988, 1989; McClain & Foss, 1988; Francklyn & Schimmel, 1989). However, in the absence of crystal data for *E. coli* $tRNA^{Ala}$ bound to its cognate synthetase, it is not clear how the G-U mismatch plays a role in the amino acid acceptor identity of this tRNA. A G-U wobble may influence the acceptor identity of certain tRNAs by introducing an irregularity in the acceptor helix of the molecule. In the case of $tRNA^{Asp}$, the G30-U40 mismatch serves as a hinge to orient the tRNA against the synthetase (Ruff et al., 1991).

The specific recognition of G-U mismatches by $Rh(DIP)_3^{3+}$ may provide some basis for considering the recognition of similar sites by proteins. The stacking interactions on either side of the G-U mismatch differ greatly, with the base pair on the 3'-side of the U exhibiting highly destacked structures with the U extended into the major groove of the RNA. It appears that $Rh(DIP)_3^{3+}$ is able to detect these differences in base stacking with the flanking Watson–Crick base pairs. $Rh(DIP)_3^{3+}$ recognizes G-U mixmatches solely on the basis of shape considerations. The rhodium complex contains no hydrogen-bonding groups, ruling out a hydrogen-bonding interaction with O-4 of the U that protrudes into the major groove; such specific hydrogen bonding is therefore more generally not required. It is possible that G-U mismatches may locally destabilize helical regions, which are important for protein recognition and similarly for recognition by $Rh(DIP)_3^{3+}$. However, related metal complexes do not significantly target these sites. Perhaps proteins also target G-U

matches primarily through shape considerations; a complementarity of a molecule to the site based upon shape certainly appears sufficient to distinguish both the G-U site and its orientation. The local variation in the secondary structure of RNA that is present in the G-U mismatch clearly provides the basis for recognition by the simple coordination complex and may similarly be critical in defining specific RNA–protein contacts. NMR studies of the rhodium complex bound to a G-U mismatch could be extremely useful in specifying these distinguishing contacts.

$Rh(DIP)_3^{3+}$ therefore serves as a unique probe of G-U mismatches. The complex may be applied (i) as a model to examine and define recognition elements of the mismatched structure, (ii) to probe and inhibit RNA–protein contacts so as to establish whether given G-U mismatches play a functional role, and (iii) to identify G-U mismatches within double-helical regions of folded RNAs. It will be interesting, further, to establish whether $Rh(DIP)_3^{3+}$ specifically targets G-T mismatches in double-helical DNA.

Acknowledgments

We thank D. Moras for yeast $tRNA^{Asp}$, P. W. Huber, K. M. Hartmann, and S. L. Rawlings for *E. coli* and *Xenopus* oocyte 5S rRNA, and O. C. Uhlenbeck and L. S. Behlen for unmodified $tRNA^{Phe}$ and $tRNA^{Phe}$ mutants.

Registry No. Tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III), 94552-81-5; guanine, 73-40-5; uracil, 66-22-8.

References

Behlen, L. S., Sampson, J. R., DiRenzo, A. B., & Uhlenbeck, O. C. (1990) *Biochemistry 29*, 2515–2523.

Brown, R. S., Dewan, J. C., & Klug, A. (1985) *Biochemistry 24*, 4785–4801.

Calnan, B. J., Tidor, B., Biancalana, S., Hudson, D., & Frankel, A. D. (1991) *Science 252*, 1167–1171.

Cheong, C., Varani, G., & Tinoco, I., Jr. (1990) *Nature 346*, 680–682.

Chow, C. S., & Barton, J. K. (1990) *J. Am. Chem. Soc. 112*, 2839–2841.

Chow, C. S., Hartmann, K. M., Rawlings, S. L., Huber, P. W., & Barton, J. K. (1992a) *Biochemistry 31*, 3534–3542.

Chow, C. S., Behlen, L. S., Uhlenbeck, O. C., & Barton, J. K. (1992b) *Biochemistry 31*, 972–982.

Crick, F. H. C. (1966) *J. Mol. Biol. 19*, 548–555.

England, T. E., & Uhlenbeck, O. C. (1978) *Nature 275*, 560–561.

Fox, G. E., & Woese, C. R. (1975) *Nature 256*, 505–507.

Francklyn, C., & Schimmel, P. (1989) *Nature 337*, 478–481.

Garrett-Wheeler, E., Lockard, R. E., & Kumar, A. (1984) *Nucleic Acids Res. 12*, 3405–3423.

Hall, K. B., Sampson, J. R., Uhlenbeck, O. C., & Redfield, A. G. (1989) *Biochemistry 28*, 5794–5801.

Hou, Y.-M., & Schimmel, P. (1988) *Nature 333*, 140–145.

Hou, Y.-M., & Schimmel, P. (1989) *Biochemistry 28*, 6800–6804.

Kim, S.-H., Sussman, J. L., Suddath, F. L., Quigley, G, J., McPherson, A., Wang, A. H. J., Seeman, N. C., & Rich, A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4970–4974.

Kirshenbaum, M. R., Tribolet, R., & Barton, J. K. (1988) *Nucleic Acids Res. 16*, 7943–7960.

Latham, J. A., & Cech, T. R. (1989) *Science 245*, 276–282.

Luehrsen, K. R., & Fox, G. E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2150–2154.

McClain, W. H., & Foss, K. (1988) *Science 240*, 793–796.

Mizuno, H., & Sundaralingam, M. (1978) *Nucleic Acids Res. 5*, 4451–4461.

Murakawa, G. J., Chen, C. B., Kuwabara, M. D., Nierlich, D. P., & Sigman, D. S. (1989) *Nucleic Acids Res. 17*, 5361–5375.

Patel, D. J., Shapiro, L., & Hare, D. (1987) *Q. Rev. Biophys. 20*, 78–90.

Peattie, D. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1760–1764.

Pieler, T., & Erdmann, V. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4599–4603.

Puglisi, J. D., Wyatt, J. R., & Tinoco, I., Jr. (1990) *J. Mol. Biol. 214*, 437–453.

Quigley, G. J., & Rich, A. (1976) *Science 194*, 796–806.

Rould, M. A., Perona, J. J., Söll, D., & Steitz, T. A. (1989) *Science 246*, 1135–1142.

Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C., & Moras, D. (1991) *Science 252*, 1682–1689.

Sampson, J. R., & Uhlenbeck, O. C. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1033–1037.

Sampson, J. R., DiRenzo, A. B., Behlen, L. S., & Uhlenbeck, O. C. (1990) *Biochemistry 29*, 2523–2532.

Sitlani, A., Long, E. C., Pyle, A. M., & Barton, J. K. (1992) *J. Am. Chem. Soc. 114*, 2303–2312.

Usman, N., Ogilvie, K. K., Jiang, M.-Y., & Cedergren, R. J. (1987) *J. Am. Chem. Soc. 109*, 7845–7854.

Varani, G., Cheong, C., & Tinoco, I., Jr. (1991) *Biochemistry 30*, 3280–3289.

Weeks, K. M., & Crothers, D. M. (1991) *Cell 66*, 577–588.

Westhof, E., Dumas, P., & Moras, D. (1985) *J. Mol. Biol. 184*, 119–145.