ingly, the orbitals are labeled as symmetric (S) or antisymmetric (A) with respect to this C_2 operation, and it is clear that the ^2E state of $\mathbf{1}^{\bullet+}$ correlates adiabatically with the $^2B_2$ ground state of $\mathbf{2}^{\bullet+}$. Similarly, it is readily deduced that since the $^2A_1$ ground state of $\mathbf{1}^{\bullet+}$ correlates with a $^2A_1$ highly excited state of $\mathbf{2}^{\bullet+}$, the ground-state reaction is state-symmetry forbidden if C_2 symmetry is maintained. Although no thermal isomerization was observed in the accessible matrix temperature range up to 150 K, a ground-state reaction at higher temperature obviously cannot be ruled out, especially since the C_2 symmetry restriction could well be relaxed in the course of such a reaction. However, AM1-UHF calculations for the ground-state radical cations indicate that $\mathbf{1}^{\bullet+}$ is more stable than $\mathbf{2}^{\bullet+}$ by ca. 10 kcal/mol.

Finally, we note that while the general applicability of electrocyclic processes to the photochemical isomerization of radical cations has recently been quite properly questioned,[16] the discovery of this novel reaction confirms that at least a subset of photoisomerizations can display true electrocyclic character. Specifically, the title reaction clearly accords with a concerted synchronous pathway from the *lowest* reactant excited state to the product ground state with conservation of orbital symmetry.

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Shape-Selective Cleavage of tRNA^{Phe} by Transition-Metal Complexes

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In order to determine the biological and chemical functions of RNA molecules, an understanding of the tertiary folding patterns of these molecules is required. At present, there is a considerable amount of information on the three-dimensional conformations of small transfer RNA molecules based upon crystallographic[1-4] and NMR[5] characterizations. In contrast, little is known about the folding patterns of other RNA molecules. Enzymatic and chemical probes are becoming increasingly more important to detect structural variations, yet the number of structure-specific reagents for mapping RNA is limited.[6-8] In our laboratory, there has been considerable effort in the design of shape-selective probes of DNA structure.[9] In particular, the complexes tris(1,10-phenanthroline)ruthenium(II) [Ru(phen)$_3^{2+}$], tris(3,4,7,8-tetramethylphenanthroline)ruthenium(II) [Ru(TMP)$_3^{2+}$],[10] tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III) [Rh(DIP)$_3^{3+}$],[11] and bis(phenanthroline)(9,10-phenanthrenequinone diimine)rhodium(III) [Rh(phen)$_2$phi^{3+}][12] have been shown to target local variations in conformation along the DNA helix and, upon photoactivation, to induce DNA strand scission, thereby marking sites of alternate structure. We report here that these transition-metal complexes are capable also of shape-selective cleavage of natural, structured RNA. This cleavage involves the same chemical schemes as found with DNA and follows the same patterns of recognition, matching the shape of the metal complex to the nucleic acid structure.

Cleavage by the metal complexes was assayed on yeast tRNA^{Phe}, a well-characterized[1-3] RNA. Fragmentation patterns are shown in Figure 1. At added ruthenium concentrations of 2.5 μM and irradiation in the MLCT band for 20 min, the ruthenium complexes efficiently cleave RNA, but only after treatment with aniline. Reactions with Ru(TMP)$_3^{2+}$ and Ru(phen)$_3^{2+}$ (Figure 1A) reveal cutting preferentially at guanine residues. HPLC analysis[12] does not show the liberation of free bases after aniline treatment, and high-resolution electrophoresis points to the production of 5'-phosphate and 3'- or 2'-phosphate termini.[13] These cleavage results are equivalent to those obtained on DNA[10] and are consistent, as with DNA, with attack on the nucleic acid base (with guanine most reactive) in a reaction mediated by singlet oxygen, generated by photoexcitation of the ruthenium complex. The cleavage chemistry differs considerably for the rhodium complexes. As shown in Figure 1B, at a 2.5 μM Rh(DIP)$_3^{3+}$ concentration, cleavage is observed after 2 min of irradiation at 313 nm; with 10 μM Rh(phen)$_2$phi^{3+}, cleavage is found after 4 min of irradiation at 365 nm. No preferred base composition is apparent in cleavage; aniline is not required for fragmentation; and HPLC analysis[12] shows the release of free nucleic acid bases. After cleavage with these complexes, high-resolution experiments also indicate both 5'-phosphate and 3'- or 2'-phosphate termini.[13] Also, specificity in cleavage on the RNA molecule is evident. Based upon these data, photoinduced cleavage by the rhodium complexes, therefore, appears to occur through a direct oxidative path, and the target is the nucleic acid sugar, also consistent with results found earlier with DNA.[9,12] In all cases, no RNA cleavage was observed in the presence of light or metal alone. Competition experiments also indicate that cleavage on tRNA by the metal complexes is comparable in efficiency to cleavage on double-stranded DNA.

The site selectivities associated with this cleavage chemistry can be understood by superimposing the results onto the three-dimensional structure of the tRNA.[1] Figure 2A displays cleavage results for the ruthenium complexes. Somewhat different patterns of cleavage are observed for the two ruthenium complexes, despite the fact that they share a $^1O_2$-mediated reactivity.[14] The different site selectivities must then necessarily be determined by their different binding characteristics, which are governed only by their different molecular shapes.[15] All guanine residues except G24[16] are cleaved upon photolysis with Ru(phen)$_3^{2+}$. Ru(phen)$_3^{2+}$ also

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(13) The presence of 5'- and 3'- or 2'-phosphate termini is based upon the comigration of cleaved fragments with products of chemical sequencing reactions (diethylpyrocarbonate or hydrazine followed by aniline treatment) and the lack of correspondence to fragment mobilities following alkaline hydrolysis.
(14) Ru(TMP)$_3^{2+}$ and Ru(phen)$_3^{2+}$, at higher concentrations ($\geq$10 μM), cleave all guanine residues. Understandably, under such conditions, the local singlet-oxygen concentration becomes greater, increasing the rate of reaction at all guanine sites.

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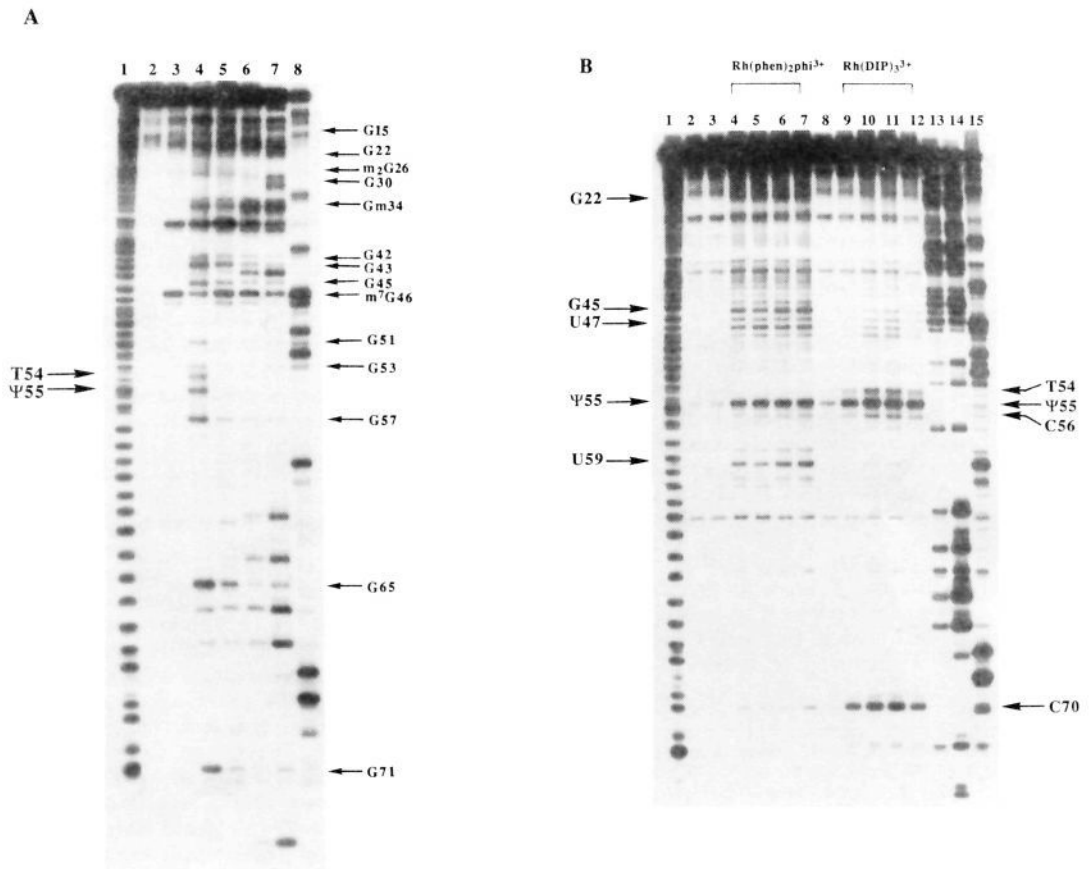

Figure 1. Cleavage of $tRNA^{Phe}$ by transition-metal complexes. Autoradiograms of 8 M urea denaturing 20% polyacrylamide sequencing gels of yeast $tRNA^{Phe}$ ^{32}P-labeled at the 3′-end.[23] The RNA samples were brought to a concentration of 100 μM in nucleotides with carrier tRNA. The concentration of metal complex used was 2.5 μM except for experiments with $Rh(phen)_2phi^{3+}$, in which a concentration of 10 μM was employed. Ruthenium samples were treated with weak base to induce cleavage. To determine the position of cleavage generated by the complexes, the tRNA was electrophoresed with neighboring sequencing reactions.[24] Buffer conditions for the $Rh(DIP)_3^{3+}$ and ruthenium samples were 5 mM Tris, 50 mM NaCl, pH 7.0, and for experiments with $Rh(phen)_2phi^{3+}$ were 50 mM Tris, 20 mM sodium acetate, 18 mM NaCl, pH 7.0. Other buffer conditions may be used; with 10 mM Mg^{2+} and higher concentrations of metals, similar results were obtained. (A) Lane 1: Alkaline hydrolysis. Lane 2: RNA control. Lane 3: Light control. Bands Y37 and m^7G46 appear due to aniline treatment. Lanes 4 and 5: $Ru(phen)_3^{2+}$ and $Ru(TMP)_3^{2+}$ photocleavage products, respectively. Irradiations were at 442 nm for 20 min at ambient temperature using a He/Cd laser, 20 mW, followed by aniline treatment.[24] Singlet-oxygen-mediated photocleavage by the ruthenium complexes yields a reaction at guanine residues, as indicated by the arrows at the right. Only G24 is protected from cleavage by both complexes. Guanines between residues 1 and 14 have not been indicated due to the resolution of this gel. Sites of preferential cleavage by $Ru(phen)_3^{2+}$ that are not guanines are indicated by the arrows at left. $Ru(TMP)_3^{2+}$ (lane 5) promotes cleavage at a subset of guanine residues; note that G22, G24, G30, G51, G53, G57, and G71 are not cleaved. Lanes 6–8: Sequencing reactions; A at 37 °C, A at 90 °C, and U, respectively. Weak depurination at G's is evident in the A reaction. (B) Lane 1: Alkaline hydrolysis. Lane 2: RNA control. Lanes 3 and 8: Light controls; irradiations at 365 nm for 10 min and 313 nm for 8 min, respectively. Lanes 4–7: Specific cleavage by $Rh(phen)_2phi^{3+}$ at irradiation times of 4, 6, 8, and 10 min, at 365 nm. Lanes 9–12: Specific cleavage by $Rh(DIP)_3^{3+}$ at irradiation times of 2, 4, 6, and 8 min at 313 nm. Irradiations were performed at ambient temperature with a 1000-W Hg/Xe lamp and monochromator. Lanes 13–15: Sequencing reactions; A at 37 °C, A at 90 °C, and U, respectively. Arrows on the left indicate $Rh(phen)_2phi^{3+}$ cleavage sites, and arrows on the right show $Rh(DIP)_3^{3+}$ cleavage sites.

promotes cleavage at T54 and Ψ55, and likely $Ru(phen)_3^{2+}$ more closely associates with this region. For comparison, $Fe(EDTA)^{2-}$, which does not itself bind to the polymer, cleaves at all solvent-accessible residues,[17] and in contrast, $Cu(phen)_2^{+}$ preferentially cleaves single-stranded segments.[18] $Ru(TMP)_3^{2+}$ cleaves at a subset of sites cleaved by $Ru(phen)_3^{2+}$ and with somewhat different relative intensities. Compared to $Ru(phen)_3^{2+}$, guanine residues 22, 30, 51, 53, 57, and 71 are protected from cleavage by $Ru(TMP)_3^{2+}$. These sites appear to mark the edges of the double-helical regions. $Ru(TMP)_3^{2+}$ is matched in shape to binding against an A-form double helix,[10] but perhaps the shortness of these helical stems in tRNA and the bulkiness of $Ru(TMP)_3^{2+}$ lead to the protection of the helical borders.

The cleavage patterns with the rhodium complexes (Figure 2B) illustrate their particular utility as structural probes. Again the cleavage chemistry is the same for the rhodium complexes, but different sites are cleaved, indicating that the site targeted is governed by the shape and binding characteristics rather than by the reactivity. $Rh(DIP)_3^{3+}$ induces strong cleavage at residues Ψ55 and C70 with other weaker sites present at T54 and C56 (still weaker cleavage is evident at m^7G46, U47, and C48). The cleavage centered at Ψ55 in a pocket between two infolded loops resembles the DNA cleavage sites by $Rh(DIP)_3^{+}$ found adjacent to cruciforms.[11] This site is also recognized, but not with high selectivity, by the smaller $Ru(phen)_3^{2+}$ and $Rh(phen)_2phi^{3+}$, both of which may intercalate. Most interestingly, $Rh(phen)_2phi^{3+}$ induces strong cleavage at residues G22, G45, U47, and U59.

(15) $Ru(phen)_3^{2+}$ appears to bind through two modes, the Δ isomer being favored at low concentration ratios and with no enantioselective preference evident at higher concentration ratios. Luminescence results are consistent with a surface-bound model for $Ru(TMP)_3^{2+}$. Equilibrium dialysis experiments show an enantiomeric selectivity favoring the Λ isomer, which is also consistent with surface binding, and overall greater binding of $Ru(TMP)_3^{2+}$ compared to $Ru(phen)_3^{2+}$ to tRNA.

(16) The absence of cleavage at G24 by both $Ru(phen)_3^{2+}$ and $Ru(TMP)_3^{2+}$ must reflect the relative accessibility of G24 to attack by $^1O_2$.

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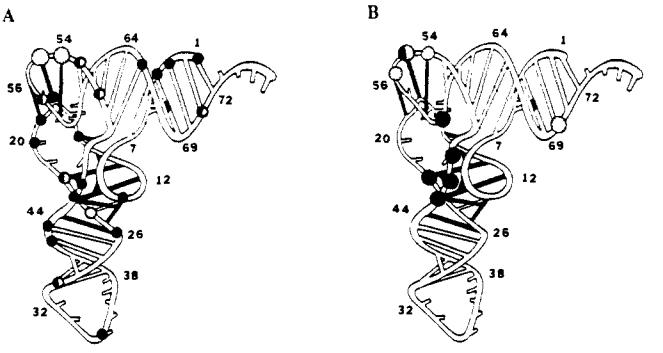

Figure 2. Cleavage data for the metal complexes mapped onto the three-dimensional structure[1] of $tRNA^{Phe}$. All bases involved in non-Watson-Crick base pairing, indicative of tertiary interactions, are shown by black lines. (A) Cleavage by $Ru(phen)_3^{2+}$ and $Ru(TMP)_3^{2+}$. Guanine residues cut by both $Ru(phen)_3^{2+}$ and $Ru(TMP)_3^{2+}$ are indicated by black circles. Guanine residues cleaved only by $Ru(phen)_3^{2+}$ are indicated by half black/white circles. Stippled circles indicate other (non-guanine) residues cleaved by $Ru(phen)_3^{2+}$. G24, protected from cleavage by both $Ru(phen)_3^{2+}$ and $Ru(TMP)_3^{2+}$, in indicated by the white circle. (B) Specific cleavage by $Rh(phen)_2phi^{3+}$ (black circles) and $Rh(DIP)_3^{3+}$ (shaded circles). Note the correspondence between shaded regions where tertiary interactions are found and the $Rh(phen)_2phi^{3+}$ cleavage sites.

Under denaturing conditions,[19] no cleavage is observed at these sites, indicating that the native structure is required for interaction. These sites do not correspond to regions that are purely helical or single-stranded. Instead, the major cleavage sites are located in the D and T loops and within the variable loop, a uniquely organized and structured region of the molecule. In particular, cutting is found at G22, G45, and m^7G46 (weaker), bases that are involved in triple interactions, in which normal Watson-Crick base pairs interact with a third base in the major groove. Cutting at U47, directly above these sites, and U59 is likely also a function of binding at these same positions. *No other reagents specifically target these regions of tRNA.* The selective targeting of these positions by $Rh(phen)_2phi^{3+}$ is in fact consistent with its recognition pattern on DNA, where cleavage is seen at sites that are open in the major groove to permit intercalation by the bulky complex.[12] In double-helical regions of RNA, the A-like conformation limits access to its deep major groove. As is evident from the crystal structure,[1] however, the addition of a third base distorts the groove from the usual A-like helix, now facilitating intercalative stacking with this third base by the rhodium complex.[20] $Rh(phen)_2phi^{3+}$ may more generally target such triply bonded positions in folded RNA molecules.[21]

In conclusion, transition-metal complexes developed in our laboratory show a distinctive diversity in site-selective cleavage of tRNA. Photocleavage by the complexes parallels reactions on DNA, both in terms of products formed and patterns of recognition. In particular, $Rh(phen)_2phi^{3+}$, an important shape-selective probe of DNA, appears to target triply bonded sites in tRNA. Given the uniqueness of sites cleaved, these probes should be valuable in assessing the structural integrity of new tRNAs.[22] More generally, these complexes may become extremely useful in deducing the major secondary and even tertiary structural features of other RNA molecules.

Acknowledgment. We are grateful to the NIH for their financial support of this research.

(19) Conditions for the denaturation experiment were identical with those described in Figure 1 except that irradiations were conducted at 80 °C.

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(21) Of the three triply bonded sites in the tRNA (G45-m^2G10-C25, A9-A23-U12, and m^7G46-G22-C13, which are adjacent to one another), only the central A9-A23-U12 site shows no cleavage, but here, owing to the folding of the strand, the sugars are not accessible to attack from the major groove.

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(1-Oxidoalkylidene)pentacarbonylchromium Anion $[R(O^-)C{=}Cr(CO)_5]$ ↔ Acylpentacarbonylchromate $[Acyl{-}Cr^-(CO)_5]$ Chemistry: In Situ Preparation and Reactions with Alkynes and Enynes

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Some of our recent studies have shown that differences in heteroatom "X" donor ability in the neutral chromium carbene complexes $(CO)_5Cr{=}C(X)R$ can significantly alter their reactivity patterns.[2] Addition of carbanions to metal carbonyls produces acyl metallates (e.g., **1'**), which can also be viewed as carbene complexes bearing a particularly electron rich donor atom (e.g., **1**). The robust nature of some of these anions [lithium salts (e.g., **1** ↔ **1'a**) are stable in aqueous solution,[3a] and the tetramethylammonium salt **1** ↔ **1'b** can be purchased[3b]] led us to examine their reactivity with some substrates typically used as reaction partners with carbene complexes.

Whereas simple alkynes react with the neutral carbenes $(CO)_5Cr{=}C(X)R$ to give various annulated products,[4] reaction of the *anion* **1** ↔ **1'a** with hexyne gave butenolide **2a** as the sole isolable product (Table I, entry 1, notes *a* and *b*). Although butenolide formation is unprecedented in group-VI metal carbene chemistry, such a process has been observed for isoelectronic acylpentacarbonyl manganese species.[5]

Possible mechanistic interpretations for this outcome (Scheme I, brackets) include an intramolecular addition of the anionic oxygen in the vinylcarbene intermediate **3** (or vinylogous acylate **3'**) to a CO ligand to generate the anionic bis(carbene) complex[6] **4** (or carbene-"acylate" **4'**) which might collapse[7] to an anionic furan complex like **5** (or the related η^4-species). Alternatively,

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