no 15-kb DNA (mitochondrial size). Therefore, it is very unlikely that the transient single-standed DNA represents mitochondrial DNA.

Earlier when we examined cells treated with aphidicolin, we interpreted the incorporation of label as due to events occurring during the joining of preformed large DNA replication intermediates and the joining of adjacent replicons (Lönn & Lönn, 1983). This may still be one explanation, but the present data open also another possibility, i.e., the occurrence of the strand separation in human cells (Gaudette & Benbow, 1986).

There exist high levels of single-stranded DNA during DNA synthesis of *Xenopus laevis* embryos. In these cells the number of replication forks is underrepresented whereas single-stranded DNA is abundant. This had led Gaudette and Benbow (1986) to propose an alternative mechanism of DNA replication which does not involve formation of replication forks (the strand-separation hypothesis). According to this hypothesis, the DNA strands separate, and the resulting single-stranded DNA chains serve as substrate for attachment of enzymes necessary for DNA synthesis. The attachment does not necessarily occur at the fork at each end of the region that has separated but anywhere along the single-stranded DNA. This mechanism would allow rapid DNA synthesis. This hypothesis is supported by the finding that replication forks are underrepresented in *X. laevis* embryos. Instead, single-stranded DNA is abundant. The amount of single-stranded DNA was inversely correlated with the length of S phase during embryogenesis.

In this paper we have shown that in cells treated with aphidicolin one can detect single-stranded DNA. This supports the strand-separation hypothesis for DNA synthesis, indicating that this mechanism may also exist in human cells. Also, a mechanism for strand separation must exist that is independent of functional DNA polymerase α.

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Structure of M1 RNA As Determined by Psoralen Cross-Linking†

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ABSTRACT: The RNA moiety of ribonuclease P from *Escherichia coli* (M1 RNA) has been photoreacted with 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) and long-wave UV light (320–380 nm) in a buffer containing 60 mM Mg^{2+}, where the RNA moiety acts as a true catalyst of tRNA processing. Limited specific digestion and two-dimensional gel electrophoresis yield fragments cross-linked by HMT. By photoreversal of the isolated cross-linked fragments and enzymatic sequencing of the fragments, the positions of the cross-links have been elucidated. This method allows us to locate the cross-link to ±15 nucleotides. Further assignments of the exact locations of the cross-links have been made on the basis of the known photoreactivity of the psoralen with different bases. Nine unique cross-links have been isolated in the M1 RNA including four long-range interactions. The short-range interactions are discussed here in detail.

Maturation of biologically active tRNA[1] in both eucaryotes and procaryotes involves the processing of a long RNA transcript. This processing includes both the cleavage of the RNA transcript at specific sites and chemical modification of specific bases. RNase P from *Escherichia coli* is a ribonucleoprotein complex that cleaves tRNA transcripts specifically at the 5′ end of the tRNA molecule. The structure of the RNA of this ribonucleoprotein complex (M1 RNA) is of great interest

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[1] Abbreviations: A, adenine; ATP, adenosine 5′-triphosphate; C, cytidine; EDTA, ethylenediaminetetraacetic acid; G, guanidine; HMT, 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen; RNA, ribonucleic acid; RNase, ribonuclease; Tris, tris(hydroxymethyl)aminomethane; tRNA, transfer ribonucleic acid; U, uracil.

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because this RNA moiety is catalytic (Guerrier-Takada et al., 1983). Previous structure predictions have been postulated on the basis of its known primary structure and thermodynamic parameters of double-strand helix formation (Reed et al., 1982) and on the basis of single strand and double strand specific nuclease data (Guerrier-Takada & Altman, 1984). Several structures have been postulated, but unfortunately, with one exception, the conditions under which these structures were determined are not those under which the RNA is optimally catalytic.

Psoralens are bifunctional furocoumarins that intercalate between the base pairs of a double-stranded nucleic acid and are able to photoreact with pyrimidine bases to form monoadducts and cross-links [for review see Cimino et al. (1985)]. The psoralen cross-link occurs only when the psoralen adds to adjacent and opposite pyrimidine bases in the double helix. The reaction is primarily with uracil in native RNAs, but reaction with cytidine has also been reported (Turner & Noller, 1983; Garrett-Wheeler et al., 1985). Psoralens react specifically with double-stranded nucleic acids. Psoralen photochemistry does not require specific ionic strengths for reactivity (Hyde & Hearst, 1978; Thompson et al., 1981), so it is ideal for secondary and tertiary structure determination of moderately sized RNA molecules. The conditions under which the M1 RNA moiety is catalytic are appropriate for the intercalation and photoreaction of psoralens with RNA.

The methods of Thompson and Hearst (1983) are used here to analyze the secondary and tertiary structure of the M1 RNA under the conditions where catalytic activity of the RNA alone has been observed.

Materials and Methods

Isolation and Psoralen Cross-Linking of M1 RNA. M1 RNA was isolated and purified as previously described (Reed et al., 1982) and was the generous gift of Dr. Cecilia Guerrier-Takada. Ethanol-precipitated M1 RNA (20–50 μg) was suspended in 50 mM Tris-HCl, pH 7.5, and 100 mM NH_4Cl at an RNA concentration of 200 μg/mL. [^{3}H]-4′-(Hydroxymethyl)-4,5′,8-trimethylpsoralen ([^{3}H]HMT, 12.8 Ci/mmol, HRI Associates, Emeryville, CA) was added to 40 μg/mL. The solution was allowed to dark bind overnight at 4 °C. $MgCl_2$ was added to 60 mM, and the solution was allowed to equilibrate for 1 h at 4 °C. The sample was then irradiated for 5 min in a 1.5-mL Eppendorf centrifuge tube at 4 °C with a focused beam from a 2500-W Hg–Xe lamp (Canrad-Hanovia) which was filtered through a 100-mm quartz windowed water filter, followed by a 10-mm Pyrex filter and a 100-mm quartz windowed 1.5% $Co(NO_3)_2$–2% NaCl in water filter. The light at the sample chamber was composed of wavelengths between 320 and 400 nm with an intensity of 1 W/cm^2 (Cimino et al., 1986). HMT was then added again to a concentration of 20 μg/mL followed by a 20-min incubation period and another 5-min irradiation.

Digestion of Photoreacted M1 RNA. Irradiated M1 RNA was extracted 3 times with an equal volume of chloroform–isoamyl alcohol (24:1) followed by two ether extractions and then three ethanol precipitations to remove unreacted psoralen and other photoproducts. The M1 RNA was suspended in T_1 buffer (20 mM sodium citrate, pH 5.0, 1 mM EDTA) and reacted with 0.25 unit of T_1 RNase (P-L Biochemicals)/μg of M1 RNA for 20 min at 37 °C. The reaction was stopped by adding proteinase K (Boehringer Mannheim) to a concentration of 0.66 μg of proteinase K/unit of T_1 and incubated at 37 °C for 5 min. The solution was extracted with chloroform–isoamyl alcohol (24:1), ether extracted, and ethanol precipitated as described above.

Labeling of Digested M1 RNA. The digested M1 RNA was 5′ end labeled by use of [γ-^{32}P]ATP (>7000 Ci/mmol, 200 mCi/mL, ICN, Irvine, CA) and 10 units of polynucleotide kinase (New England Biolabs) overnight at 37 °C in 30 μL of kinase buffer (50 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 14 mM β-mercaptoethanol). Cold ATP (Boehringer Mannheim) was added to 10 mM, an additional 10 units of kinase was added, and the reaction was allowed to proceed for another 4 h at 37 °C. The solution was then made 2.5 M in ammonium acetate and ethanol precipitated.

Isolation and Identification of Cross-Linked Fragments by Two-Dimensional Polyacrylamide Gel Electrophoresis. Cross-linked fragments were isolated and identified by a modification of the Thompson and Hearst procedure (1983). In brief, the precipitated RNA was fractionated on a two-dimensional polyacrylamide gel. For the first dimension, the RNA was dissolved in 20 μL of a nondenaturing loading buffer (50 mM Tris–borate, pH 8.3, 1 mM EDTA, 10% glycerol, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol). The RNA solution was heated to 90 °C for 1 min, quick cooled in an ice/water bath, and then loaded into a 1 cm wide lane of a native gel (12% polyacrylamide, 15 cm × 40 cm × 0.7 mm). The gel was run at 500 V until the bromophenol blue indicator had migrated to the bottom of the gel. The running buffer was TBE (50 mM Tris–borate, pH 8.3, 1 mM EDTA). After removal of the top glass plate, the bottom 30 cm of the gel lane was excised with a razor blade and a straight edge. The excised lane was incorporated into a second denaturing gel. The gel slice (1 cm × 30 cm) was placed horizontally between two new glass plates (34 cm × 43 cm). A 20% polyacrylamide–7 M urea gel was poured around the gel slice. After about 2 h of polymerization, a denaturing gel was run at 40 W (~50 °C) until the xylene cyanol dye had migrated to the bottom of the gel. RNA fragments were located by autoradiography for 1 h at room temperature with Kodak XAR-5 film. Psoralen-cross-linked RNA fragments were localized as spots above a main diagonal. These spots were excised from the gel, and each spot was eluted into 350 μL of LES solution (100 mM LiCl, 10 mM EDTA, 0.1% w/v sodium dodecyl sulfate) overnight in 1.5-mL Eppendorf tubes. After the elution solution was removed, each gel slice was washed twice with 100 μL of additional LES solution. The RNA in the combined eluant and washes was ethanol precipitated in the presence of 10 μg of unlabeled carrier tRNA. To ensure that each spot contained only a single, cross-linked RNA fragment, each sample was subjected to electrophoresis on another 20% polyacrylamide–7 M urea gel. Electrophoresis was performed as described above. Single bands were excised from the gel and eluted as above. The fragments, still in the Eppendorf tubes (left open but covered with Reynolds film), were photoreversed from the top under a 40-W germicidal lamp (Sylvania 254 nm) at a distance of 10 cm for 2 h. tRNA was added as above to each sample, and the samples were precipitated. The photoreversed fragments were again run down a 20% polyacrylamide–7 M urea gel as before. After autoradiography the fragments of interest were eluted from the gel and precipitated.

Sequencing of Isolated Fragments. Fragments were sequenced enzymatically (Donis-Keller et al., 1977). Three-lane sequencing was performed by using RNase T_1 (G specific), RNase U_2 (A specific), and alkaline digestion (all enzymes from Pharmacia). The digestions were all for 20 min; T_1 was in 10 mM sodium citrate, pH 5.5, 1 mM EDTA, and 7 M urea, at 55 °C; U_2 was in 10 mM sodium citrate, pH 3.5, 1 mM EDTA, and 7 M urea, at 55 °C; alkaline digest was in

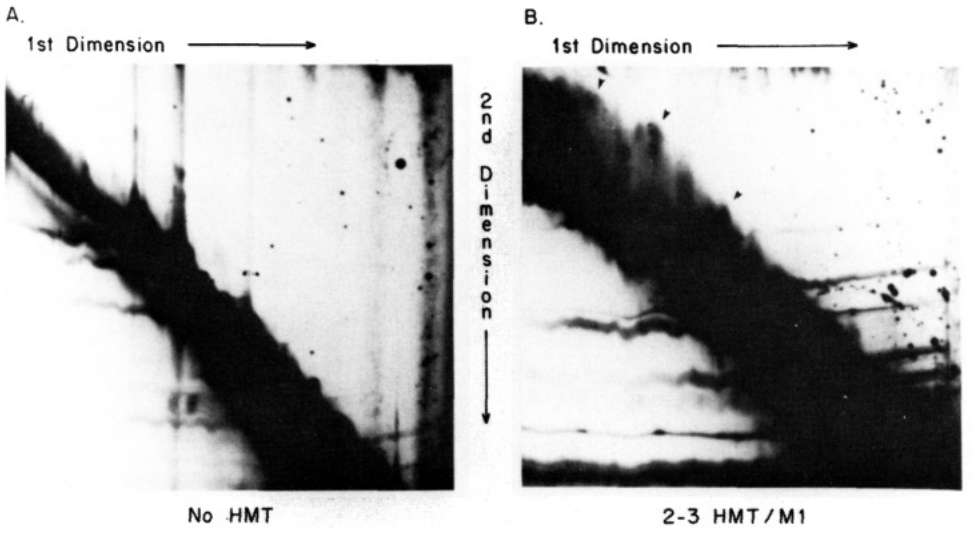

FIGURE 1: Two-dimensional gel analysis of M1 RNA. Panel A shows a 2D gel of the unphotoreacted M1 RNA used as a control. Panel B shows a 2D gel of psoralen-photoreacted M1 RNA as described under Materials and Methods. The native first dimension runs from left to right, and the second dimension from top to bottom, for each panel. Xylene cyanol was run to the bottom of the second dimension. Examples of off-diagonal spots are indicated with arrows.

20 mM sodium carbonate buffered with sodium bicarbonate to pH 9.0 at 90 °C. After the digestion the samples were run down a 20% polyacrylamide–7 M urea gel and autoradiographed with Kodak XAR-5 film and Du Pont Lightning Plus intensifying screens for several weeks at −70 °C.

Results

The intercalation and photoreaction of 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) with the M1 RNA were carried out in activity buffer (50 mM Tris-HCl, pH 7.5, 60 mM $MgCl_2$, 100 mM NH_4Cl) which renders M1 RNA biologically active (Guerrier-Takada et al., 1983). HMT was photoadducted to a level of 2–3 HMT per M1 RNA as determined by scintillation counting of tritiated photoadducted HMT and UV spectroscopy of the RNA. Low levels of psoralen addition do not change the gross conformation of 5S rRNA from *Drosophila melanogaster* (Thompson et al., 1981) or 16S rRNA from *E. coli* (Thompson & Hearst, 1983), and we assumed the same for M1 RNA.

The photoreacted RNA was subjected to partial RNase T_1 digestion to generage fragments that were long enough to sequence and to unambiguously identify within the M1 RNA sequence. RNA fragments 15–60 bases in length were generated, as shown by the length of the off-diagonal fragments isolated on the two-dimensional (2D) gel (Figure 1) and length determinations compared to dye markers on the purification gel (Figure 2) To ensure that the above diagonal spots contain only psoralen-cross-linked RNA fragments, it was necessary to melt out double-stranded RNA before applying it to the 2D gel procedure. This was accomplished by heating the RNA briefly to 90 °C, followed by quick cooling. Following this procedure, only HMT-cross-linked fragments and snap back hairpins contain secondary structure prior to loading on the native polyacrylamide gel. The second dimension was then run under denaturing conditions and high temperature (~50 °C). Most of the RNA migrates with the same relative mobility in both dimensions and forms the diagonal. The material that migrates above this diagonal is material that contains HMT cross-links, as the HMT-modified RNA will migrate spread out as an "X" in the denaturing dimension of the gel while it runs as a double-stranded molecule in the native dimension. The X-shaped molecule has a larger effective radius and will run slower than a comparable helical molecule.

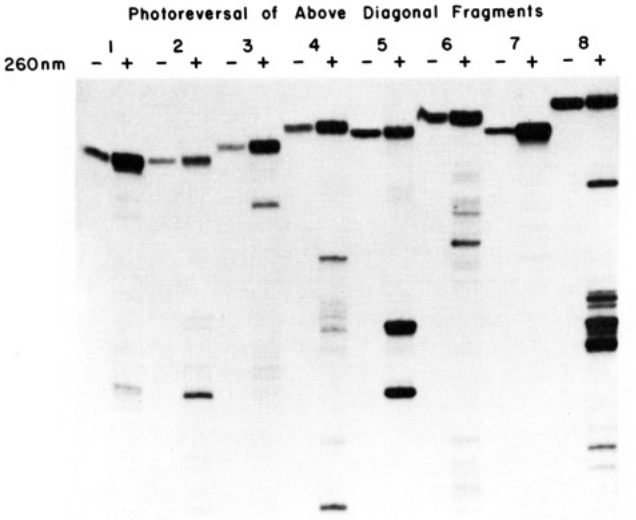

FIGURE 2: Photoreversal of off-diagonal fragments. Each fragment is separated into two lanes; the left lane contains 10% of the fragment and is not photoreversed, and the right lane is photoreversed as described under Materials and Methods. Fragment 7 shows no photoreversal (an example of material running just above the diagonal but having no psoralen cross-link). The single bands in fragments 1–3 change migration upon photoreversal. These fragments are interpreted to have been cross-linked hairpins. Fragment 5 photoreverses to two fragments, indicating and interaction involving two separate RNA fragments. Fragments 4, 6, and 8 are photoreversed to more than two bands. Fragments resulting from multiple-band photoreversal were sequenced, but no useful information was obtained.

The same is true for cross-linked hairpins. Un-cross-linked RNA will run as single-stranded material in both dimensions with no change in its hydrodynamic shape since we denatured the RNA prior to running the first dimension gel. Short RNA fragments that form strong hairpins in the absence of a psoralen cross-link will run below the diagonal because in the denaturing dimension the effective radius of these fragments will be smaller than their effective radius in the native dimension. A purification of the above diagonal fragments cross-linked is essential because the 2D gel does not resolve all of the off-diagonal fragments from each other. Off-diagonal spots are spread out and difficult to resolve completely.

The psoralen cross-links in the purified fragments were photoreversed under a low-pressure Hg lamp (254 nm) for 2

Table I: Cross-Links Identified by Psoralen Mapping

hairpins	possible cross-links	hairpins	possible cross-links
17–82	C-26 × C-54	277–291	C-279 × C-290
	U-28 × C-54		U-281 × C-290
	C-29 × C-50	337–377	17–82 × 337–377
	U-31 × C-50	(long-range	165–194 × 337–377
136–170	U-149 × C-168	interactions)	197–250 × 337–377
	C-152 × U-167		277–306 × 337–377
	C-154 × C-162		
	C-156 × C-162		
199–250	C-221 × C-228		
	U-223 × C-228		
	U-223 × C-226		

h. The photoreversed samples were then run down another 20% polyacrylamide–7 M urea gel. A change in the mobility of a fragment in the gel after the photoreversal irradiation implies the reversal of a psoralen cross-link. Specifically, if the result is one band with a different mobility, then a hairpin was cross-linked (Figure 2). If there are two resultant bands, two strands of RNA were cross-linked to each other through the psoralen. In about 40% of the photoreversals more than two bands were seen. These multiple-band photoreversals are explained by either multiple cross-linked fragments migrating together on the 2D gels or photodamage to the RNA during photoreversal. Since one cannot gauge the pairing of bands by their intensity on an autoradiogram, due to possible uneven labeling of the two 5′ ends of the cross-link, those lanes that photoreversed into more than two bands were discarded. We have sequenced some of the bands from these multiple-band photoreversals and found that some of the shorter bands are subfragments of the longer fragments. These subfragments could be due to damage during photoreversal. Some of the photoreversal gels indicated families of cross-links (cross-links that differ in one strand by one or more bases). These families usually lie next to each other on the 2D gel, running parallel to the diagonal. We found one such family (hairpin 136–170, 138–170) in this analysis.

Each band that was identified as a photoreversal product was subjected to enzymatic sequencing (Donis-Keller et al., 1977). The partial sequence was read directly off the autoradiogram. Due to the number of counts in each band prepared for sequencing we only used RNase T_1, RNase U_2, and alkaline digestion for sequencing. We were able to determine the guanine residues, adenine residues, and pyrimidines. Since the sequence of the M1 RNA is known, and our terminal cuts on these fragments were made by T_1 (G specific), we were able to reconstruct the first and last bases in a fragment, the correct sequence for each of the fragments, and the position of each fragment within the M1 RNA sequence. Table I is a summary of the fragments found to be cross-linked.

Discussion

RNase P catalyzes the cleavage of the 5′ terminus of precursor tRNA molecules to maturity and is composed of an RNA 377 nucleotides in length (M1 RNA in *E. coli*) and a protein (C5-protein in *E. coli*). The RNA molecule alone can catalyze the same reaction as RNase P under specific conditions including 60 mM Mg^{2+} and 100 mM NH_4^+ (Guerrier-Takada et al., 1983). Mapping of single-stranded and double-stranded regions in the molecule has been performed (Guerrier-Takada & Altman, 1984), yielding a model for the secondary structure of the M1 RNA. These analyses were performed under conditions where the nucleases and chemical reactants are most active. Unfortunately, these conditions do not correspond to those under which the M1 RNA shows optimal catalytic activity with the exception of RNase A and RNase T_1. We have investigated the structure of M1 RNA with psoralen cross-linking under conditions where the M1 RNA is optimally active. Our data confirm two interactions proposed by the nuclease digestion and chemical mapping of the M1 RNA and contradict two other interactions. Our data also show five different interactions with the 3′ terminus of the M1 RNA. The kinetics of the catalytic reaction mediated by M1 RNA is second order in M1 RNA concentration (Guerrier-Takada et al., 1986), implying the M1 RNA might act as a dimer. This might explain our multitude of interactions with the 3′ terminus of the molecule. Preliminary experiments in our laboratory have identified a band electrophoresing in a polyacryamide gel which migrates at the expected position of a dimer of M1 RNA only when M1 RNA is irradiated in the presence of HMT (S. Lipson, unpublished observations). We have isolated these cross-linked molecules and are attempting to elucidate the interactions involved. The following sections discuss the short-range, intramolecular interactions we have found in our cross-linking of the M1 RNA.

UC or GC Cross-Links. Although the predominant reaction of psoralens is with T in DNA, UC cross-links have been observed (Turner & Noller, 1983; Garret-Wheeler et al., 1985) in RNA, and if this is the case, one would expect that CC cross-links can also exist. Although U still appears to be the major reactive base, C is much more reactive in RNA than it is in DNA. Because of these observations, we do not hesitate to assign psoralen reactions with C when evidence indicates that it is probably correct.

Hairpin 17–82. This hairpin is consistent with that proposed by Reich et al. (1986). Our data confirm this element. The cross-link may occur between U-31 and C-50, or C-26 and C-54, in this structure. There is a mismatch adjacent to each of these sites, making it favorable for intercalation and photoreaction.

Hairpin 136–170. The model proposed by Guerrier-Takada and Altman (1984; referred to as the 1984 model) does not explain the hairpin that we have isolated. Therefore, we propose a new secondary structure for this region that fits both the nuclease data and our psoralen data. Figure 3 shows the predicted structure alongside that of the 1984 model. We propose that the psoralen cross-link may occur either between C-154 and C-162 or between C-157 and C-162.

Hairpin 199–250. This hairpin confirms the 1984 model secondary structure as there are two possible psoralen cross-link sites in the hairpin at bases 220–230. The cross-link may occur between U-223 and C-226 or U-223 and C-228 or C-221 and C-228.

Hairpin 277–291. The hairpin fragment 277–291 is not consistent with the secondary structure proposed in the 1984 model. There is little doubt about the existence of this hairpin since it is clearly indicated by the psoralen cross-linkage data. We propose a different secondary structure model for this region. It is calculated to be comparable in energy to the 1984 model, but it fits our psoralen cross-link data, with the cross-link between C-279 and C-290 (Figure 4). The respective energies are −4.5 kcal/mol for the 1984 model and −4.3 kcal/mol for our model (Cech et al., 1983; Salser, 1978). This structure is also proposed by Lawrence and Altman (1986) as one of four different possibilities.

There are some mutagenesis results [Lawrence and Altman (1986), Table II] that imply that the region between 282 and 292 is essential for activity of the M1 RNA. Within this region a fragment that is complementary to the T-Ψ-C loop

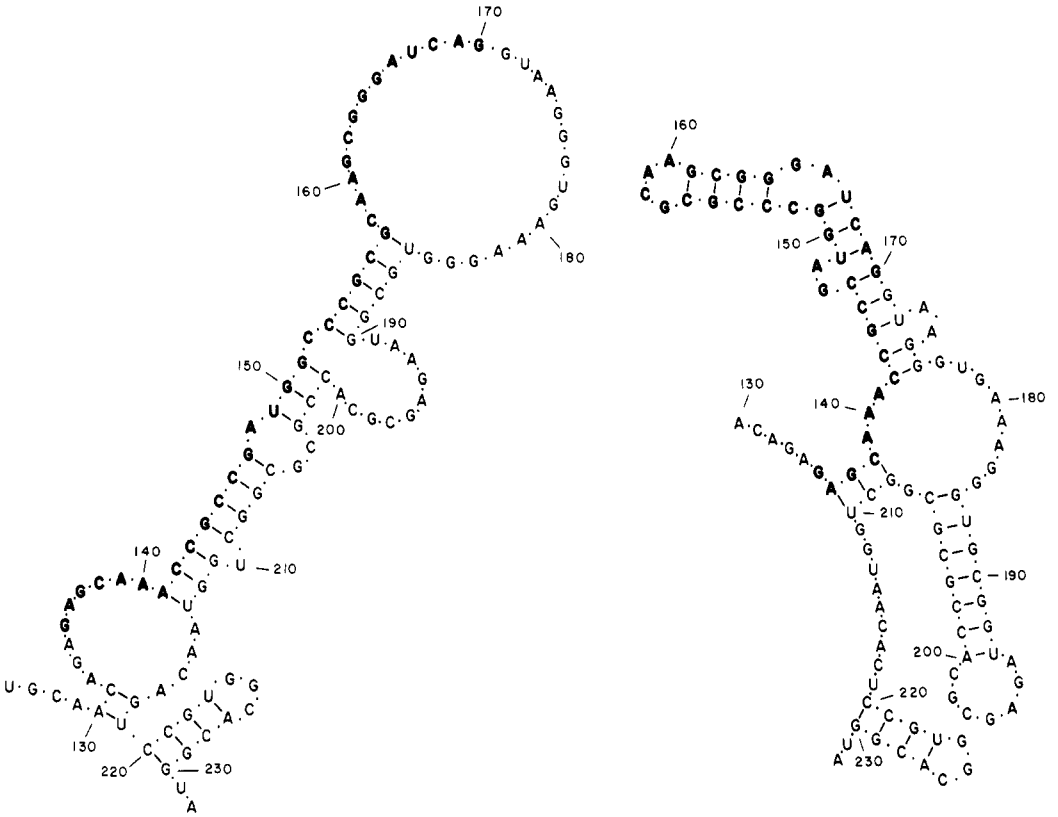

FIGURE 3: Secondary structure of M1 RNA from nucleotides 130 to 232. The structure depicted on the left shows the previous secondary structure model (Guerrier-Takada & Altman, 1984). The structure on the right shows our new prediction based on the hairpin isolated. The fragment isolated as a hairpin is in bold face in both panels. We propose that the psoralen cross-link occurs either between C-154 and C-162 or between C-156 and C-162.

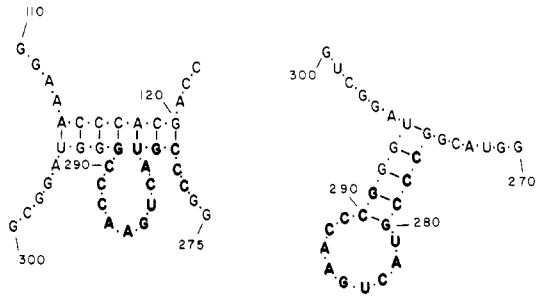

FIGURE 4: Secondary structure of M1 RNA from nucleotides 277 to 291. The left structure shows the previous secondary structure model. The right structure shows our new prediction based on the hairpin isolated. The fragment isolated as a hairpin is in bold face in both panels. We propose that the psoralen cross-link occurs between C-279 and C-290.

Table II: Mutation Data from Lawrence and Altman (1986)

type	location	bases	activity (%)[a]
insert	290, 291	GGCC	75
delete	290	C	87
delete	289, 290	CC	52
delete	289–292	CCGG	11
delete	283–290	CUGAACCC	6
delete	291–296	GGGUAG	6
delete	291, 292	GG	16

[a] 100% = M1.

in most tRNA molecules (5′-UGAAC-3′, 285–290) can be found. We believe that our new structure fits the mutation data, as well as the psoralen cross-linking data, assuming that the exposure of the T-Ψ-C loop complementarity is essential for M1 activity.

Each mutation will now be discussed in detail in relation to our model; the mutations that still show activity will be presented first, and then those that show greatly reduced activity will be presented. The first active mutant is an insertion of a 4-mer (GGCC) between bases 290 and 291. This mutation preserves the hairpin stem and the T-Ψ-C complement still accessible in the hairpin loop. The second active mutant is a deletion of base 290 (C). This mutation still maintains the hairpin stem pairing C-289 with G-280, and the T-Ψ-C region is still accessible for base pairing. The last active deletion removes bases 289 and 290 (CC), it contains a hairpin stem of only four base pairs but still allows a stable hairpin stem to form, and it maintains the accessibility of the T-Ψ-C complement. The low-activity deletion mutation which deletes CGG at 290–292 would break $^3/_5$ of the hairpin stem and might not allow it to form. This could cause the T-Ψ-C complement to be inaccessible for base pairing and make the molecule less active. Deletion of 289–292 both removes $^2/_5$ of the T-Ψ-C complement and breaks $^3/_5$ of the base pairs in the hairpin stem and is found to remove substantial activity. Deletion of the region between 283 and 290 removes the complete hairpin loop, and so there is no T-Ψ-C complement. Finally, deletion of the region 291–296 breaks the hairpin stem completely, and again the mutant is relatively inactive. These data along with our psoralen cross-linked hairpin strongly imply that the structure we present is correct.

Guerrier-Takada and Altman (1986) have shown that M1 RNA with large terminal deletions shows minimal activity (ca. <1% native M1 RNA). One such molecule includes only the first 272 bases of the M1 RNA. This molecule does not have the hairpin at 277–291 but does have minimal activity. The direct interaction of this hairpin may not be essential to activity but rather may greatly enhance the ability of M1 RNA to interact with its substrate.

Interactions with the 3′ Terminus. We have found five different interactions with the 3′ end of the molecule. We feel that this area of the molecule is important to normal activity even though minimal activity has been found with large 3′ end deletions (Guerrier-Takada & Altman, 1986). Since we have found five different interactions occuring between 337–377 and either itself or other parts of the molecule, we do not feel confident in predicting "switches" or other possible intramolecular interactions at this time. This is especially true since all of the nonhairpin cross-links occur with the 3′ end of the molecule. Any or all of the nonhairpin interactions may be intermolecular (Guerrier-Takada et al., 1986). This was not addressed in this series of experiments. Before we make a statement on these cross-links, we will have to identify which of the cross-links between the 3′ end of the M1 RNA are inter- and intramolecular in nature. We are assured that all of the hairpin cross-links observed are intramolecular, but we cannot make any conclusions on the two-strand cross-links observed. Since the idea of the dimer was not present at the onset of this project, we did not separate cross-linked monomers of M1 RNA from cross-linked dimers of M1 RNA. This makes the structural interpretation of the nonhairpin interactions difficult. It is possible that an active site which binds substrate is created by the intermolecular interaction occuring in a dimer of M1 RNA.

Our data allow us to make modifications to the predicted secondary structure of the M1 RNA that we feel might be important in determining what portions of the molecule are necessary for biological activity. Although we cannot make any predictions about tertiary interactions within M1 RNA, we feel very confident with our predictions describing the hairpins around 136–170 and 277–291. These modifications to the predicted secondary structure may prove helpful in designing experiments to more accurately probe the structure of M1 RNA under catalytic conditions.

Work currently in progress is attempting to isolate intermolecular cross-links to resolve the observation of five unique interactions with the 3′ terminus of the M1 RNA.

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