Secondary Structure of the RNA Component of a Nuclear/Mitochondrial Ribonucleoprotein*

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RNAse mitochondrial RNA processing (MRP) is a site-specific endoribonuclease located in both the nucleus and mitochondria of vertebrate cells. The enzyme is a ribonucleoprotein whose RNA component has been shown to be encoded by a nuclear gene. Because RNAse MRP is particular in its substrate requirement, RNA-RNA interaction has been proposed as important for the cleavage reaction. A secondary structure of this RNA from mouse cells has been derived by chemical modification of in vitro MRP RNA in ribonucleoprotein form, as isolated free RNA, and as RNA synthesized in vitro. Full-length MRP RNA appears to adopt a conformation containing a significant number of single-stranded residues and may form a pseudoknot. The data are consistent with both the RNA within the ribonucleoprotein and the free RNA possessing comparable secondary structures and suggest a possible site of interaction between enzyme and substrate. The human MRP RNA can be folded into a conformation very similar to that predicted for the mouse MRP RNA. A more limited analysis of human MRP RNA is consistent with the structure proposed for the mouse species.

MRP RNA (for mitochondrial RNA processing) is a ribonucleoprotein (RNP) present in the nucleus and mitochondria of vertebrate cells. Within mitochondria, this RNP is thought to be involved in the endonucleolytic processing of RNA primers at the origin of leading strand mtDNA synthesis. In vitro, RNAse MRP isolated from mitochondria is capable of processing RNA derived from this origin in a manner consistent with this role (Chang and Clayton, 1987a, 1987b). RNAse MRP RNA exists in the nucleus of vertebrate cells at a level approximately 1% that of U1 small nuclear RNA. The nuclear form of RNAse MRP, which represents the majority of this RNP in the cell, is also capable of cleaving mitochondrial primer RNA in vitro; however, its role within the nuclear compartment is unknown (Chang and Clayton, 1987b; Topper and Clayton, 1990).

Both forms of the RNP, nuclear and mitochondrial, contain an RNA (MRP RNA) essential for endoribonucleolytic function. This RNA has been characterized in mouse and human cells where it exists as a 275- and 265-267-nucleotide species, respectively (Chang and Clayton, 1987b, 1989; Topper and Clayton, 1990). In both cases it is the product of a single copy nuclear gene. Recently, we have demonstrated that the MRP RNA of human cells is identical to the Th or 7-2 RNA originally described as being precipitable with some autoimmune sera (Gold et al., 1989). Since this species (MRP/Th) is known to be present in the nucleolus (Hashimoto and Steitz, 1983; Reddy et al., 1983), it has been suggested to play a role in the metabolism of rRNA (Gold et al., 1989; Yuan et al., 1989; Topper and Clayton, 1990).

The MRP RNAs of human and mouse cells are 84% identical at the primary sequence level (Topper and Clayton, 1990). In vitro, the RNPs containing the MRP RNAs from the two species are quite analogous in their biochemical properties. They display essentially identical purification profiles over ion-exchange resins such as DEAE-Sephadex and phosphocellulose, in addition to copurifying similarly during glycerol gradient sedimentation (Topper and Clayton, 1990). Enzymatically, the two species’ RNPs appear identical in their ability to cleave both wild-type and mutant substrates derived from the origin of mtDNA replication (Bennett and Clayton, 1990; Bennett and Clayton, unpublished data). In the light of this apparent conservation of form and function, the RNAs from the two species may adopt similar structures that mediate these characteristics. Knowledge of these structures should provide insight into the potential role(s) of this RNA species within the cell.

Standard phylogenetic comparison of the sequences of the two RNAs failed to yield significant information on their structures because of the lack of sufficient sequence variation between the two species. In addition, predictions of optimal secondary structures based purely on energy considerations were inadequate because the algorithm employed (Zuker and Stiegler, 1981; Zuker, 1989) predicted completely different structures for the two RNAs (data not shown). In order to determine the structure of these RNAs within the cell, we undertook a direct analysis using chemical probes. Utilizing two reagents capable of identifying nucleotides not involved in base pairing interactions, we were able to investigate the secondary structure of the mouse MRP RNA as a complex with protein within the RNP and as a free molecule in solution. The human MRP RNA can be folded into a structure closely resembling the experimentally derived mouse model, and limited chemical probing of the human MRP RNP revealed a pattern entirely consistent with the predicted structure, thus confirming the validity of the model.

MATERIALS AND METHODS
Reagents and Buffers—Modification buffers were CMK (80 mM potassium cacodylate, 100 mM KCl, 5 mM MgCl₂, pH 7.2) for the DMS reactions and BMK (80 mM potassium borate, 100 mM KCl, 5 mM MgCl₂, pH 8.1) for the CMCT reactions. Oligonucleotides were
briefly, the extract or free RNA was preincubated at 25 °C for 15 min and centrifuged in an SW41Ti rotor for 15 min at 10,000 rpm. The nuclear pellet was resuspended in the appropriate modification buffer and sonicated at setting 2 with a Branson microtip. 

The chromatin was sonicated at setting 2 with a Branson microtip. The obtained chromatin was sonicated at setting 2 with a Branson microtip. The chromatin was isolated by phenol extraction and ethanol precipitation. The RNA was isolated by phenol extraction and ethanol precipitation. The RNA was then isolated and labeled with DCD

RESULTS

Nuclear extracts from mouse LA9 cells were treated with the water-soluble modification reagents DMS, which reacts with the N1 position of unpaired adenines and the N3 position of unpaired cytosines, or CMCT, which reacts with the N3 position of unpaired uridines and the N1 position of unpaired guanines (Ehresmann et al., 1987). The modifications induced by these reagents within the RNA prevent the elongation of a complementary strand by reverse transcriptase and as a result are detectable by the presence of stops during a primer extension reaction. Since the modified nucleotide cannot base pair correctly, reverse transcriptase will arrest one nucleotide before the modified base, thus generating a distinct primer extension product. This method was chosen because the use of specific oligonucleotide primers allows the structure of the RNA, as it exists within a crude nuclear extract or within a mixture of RNAs, to be probed specifically. In theory, this method exposes every accessible nucleotide of the RNA to potential modification by the chemical reagents (Inoue and Cech, 1985); in practice, a number of factors limit the resolution of the technique. The first is the need to use oligonucleotide primers to detect the modifications induced; this fails to yield information about the extreme 3' end of the RNA. The second is the presence of naturally occurring or modification-independent reverse transcriptase blocks. These result in primer extension stops in both the unmodified and modified RNA reactions, obscuring information regarding possible base pairing of those particular nucleotides. Despite these limitations, this technique has proved powerful in predicting secondary structures of biologically active RNAs (Noller, 1984).

Probing Mouse Nuclear Extracts Containing the MRP/Th RNP—Nuclear extracts containing the MRP/Th RNP were isolated as described under “Materials and Methods.” These extracts contain MRP/Th RNA that is largely in its RNP form as evidenced by the fact that essentially all of the detectable MRP/Th RNA sediments at 15 S in a glycerol gradient (free RNA sediments at about 7 S) and is precipitable by the antiserum (data not shown). In addition, by Northern analysis, these extracts contain predominantly the full-length MRP/Th RNA of 275 nucleotides and less than 1% of the smaller forms that have been observed, as well as only a very small amount of mitochondrial tRNA (Chang and Clayton, 1987b; Topper and Clayton, 1990). Consequently, we consider them to be free of significant mitochondrial contamination.

The extracts were treated with DMS or CMCT such that on the average less than one modification was introduced in each molecule. The RNA was then isolated and primer extended with specific 5'-end-labeled oligonucleotide primers to detect the modifications. Six primers (detailed under “Materials and Methods”) complementary to various regions of the 275-nucleotide mouse MRP/Th RNA were used for all experiments described. Figs. 1 and 2 are representative examples of primer extension reactions on RNA isolated from nuclear extracts treated with DMS and CMCT, respectively. In each case a control was performed which consisted of treating the extract with the highest amount of modification reagent after addition of the quenching buffer. This acts as a null modification standard as well as a control for any modification that may occur during the isolation of the RNA for subsequent primer extension (Parker and Steitz, 1987).

The existence of bands in the modified lanes that are absent in the stop controls indicates the presence of modified nucleotides (Figs. 1 and 2). These nucleotides, which are presumably in a single-stranded conformation within the RNP, can be mapped by comparison with the sequence ladders generated with the same primers on single-stranded templates containing the gene for mouse MRP/Th RNA. Note that the control lanes in all of the experiments contain reverse transcriptase.
Fig. 1. Examples of DMS modification of mouse nuclear extracts. A–D are examples of primer extension analysis of RNA isolated from DMS-treated nuclear extracts. A, primer extension with oligo 1. Lane 1, stop control; lane 2, 1 μl of DMS; lane 3, 2 μl of DMS. B, primer extension with oligo 2. Lane 1, stop control; lane 2, 1 μl of DMS; lane 3, 2 μl of DMS. C, primer extension with oligo 4. Lanes 1 and 4, stop control; lane 2, 1 μl of DMS; lane 3, 2 μl of DMS. D, primer extension with oligo 6. Lanes 1 and 5, stop control; lane 2, 0.5 μl of DMS; lane 3, 1 μl of DMS; lane 4, 2 μl of DMS. In all cases the lanes marked C and A represent dideoxy sequence ladders generated with the corresponding oligonucleotide primer on single-stranded DNA templates. Reference nucleotide numbers appear on the left of the panels and correspond to the numbering system of Fig. 5.

Fig. 2. Examples of CMCT modification of mouse nuclear extracts. A–D are examples of primer extensions of RNA isolated from CMCT-treated nuclear extracts. A, primer extension with oligo 1. Lane 1, stop control; lane 2, 10.5 mg/ml CMCT; lane 3, 21 mg/ml CMCT. B, primer extension with oligo 2. Lane 1, stop control; lane 2, 21 mg/ml CMCT; lane 3, 21 mg/ml CMCT. C, primer extension with oligo 4. Lanes 1 and 4, stop control; lane 2, 10.5 mg/ml CMCT; lane 3, 21 mg/ml CMCT. D, primer extension with oligo 5. Lane 1, stop control; lane 2, 10.5 mg/ml CMCT; lane 3, 21 mg/ml CMCT. Lanes C and A are sequence ladders as in Fig. 1.

stops. These stops probably arise for one of the following reasons: nicks in the RNA induced during modification, naturally occurring modified bases, structures forming within the RNA during the primer extension reaction which interfere with elongation by reverse transcriptase, or incomplete quenching of the chemical probes. As a result, a nucleotide was considered to be single stranded in the RNP when a band corresponding to modification of that nucleotide was present in the modified lanes (but absent in the unmodified lanes) or was significantly enriched in the modified lanes with respect to the controls. For example, in the region from positions C60 to A35 of Figs. 1C and 2C, the sequence (with nucleotides assigned as modified in parentheses) is (C)(U)(U)(U)(C)(C) (U)(A)(U)UCCU(U)(G)(U)(A)CAGGAG(C)(A). Nucleotide C60 is designated as modified because the band present in the experimental lanes is significantly enriched with respect to those of the controls. In contrast, nucleotides C50 and C43 are not assigned because of the presence of bands in all of the
Structure of MRP/Th RNA

Modification of Protein-free MRP RNA—In addition to probing the structure of the RNA as an RNA-protein complex, we investigated the conformation of the molecule as a free entity in solution. This was accomplished in two ways. First, we used the standard approach of deproteinizing nuclear extracts containing the MRP RNP and then subjecting these to chemical modification. However, because of the availability of the gene for this RNA, a second approach, consisting of in vitro synthesis of the RNA and subsequent chemical probing, was also undertaken by constructing the clones schematized in Fig. 3A and described under "Materials and Methods." This afforded the opportunity to determine whether any posttranscriptional modification in vivo was critical for MRP RNA conformation. These clones consist of the coding region of the mouse MRP/Th RNA fused to a T7 RNA polymerase promoter, which supports the synthesis of MRP/Th RNA in vitro. The clone designated T7-275 has the entire coding sequence from nucleotides 1-275 fused to the promoter; the RNA produced by transcription of this clone contains the wild-type mouse sequence with the addition of three guanosine residues on the 5' end and one extra nucleotide on the 3' end (designated T7-275 RNA). The clone T7-136 produces the 3'-half of the MRP/Th RNA, from nucleotides 140-275, again with the addition of three 5' guanosines and one nucleotide on the 3' end (designated T7-136 RNA). After the transcription reactions, the RNA was isolated and subjected to chemical modification and primer extension. Fig. 3B is an example of an aliquot of the in vitro synthesized RNA that was subjected to a stop control reaction with DMS and subsequently end labeled with pCp and analyzed by denaturing gel electrophoresis. This experiment indicates that greater than 99% of the RNA is full length even after the modification procedure.

The results of the free RNA analysis were the same regardless of the source of the RNA (i.e. isolated from nuclei or synthesized in vitro). Fig. 4, A-C, shows examples of primer extensions of T7-275 RNA modified with either CMCT or DMS and Fig. 4, D-F, shows examples of similar analyses performed on the T7-136 RNA. The fact that the modification pattern of the deproteinized nuclear RNA was identical to that of the T7-275 RNA produced in vitro suggests that the MRP/Th RNA synthesized in vitro is capable of forming a structure similar to that of its in vivo counterpart. However, the pattern of modification observed for these free RNAs displayed some differences from that of RNA within the RNP; these are noted in the legend of Fig. 5.

Analysis of the Human MRP/Th RNA—As an additional test of the validity of the experimentally derived secondary structure pictured in Fig. 5, human MRP RNA was examined for its ability to form a similar structure. As shown in Fig. 6C, human MRP RNA can be drawn in a structure closely resembling that of the mouse. In order to determine that this structure was representative of the in vivo situation, we attempted to confirm it by limited chemical modification. The data of Fig. 6, A and B, represent examples of chemical

was then 3'-end-labeled with pCp and analyzed by gel electrophoresis. Lane M, HpaII-cleaved pBR322; lane 1, RNA derived from T7-275; lane 2, RNA derived from T7-136.
DISCUSSION

Secondary Structure of Mouse MRP/Th RNA within the Nuclear RNP—Fig. 5 presents a secondary structure derived from the data obtained from DMS and CMCT modification of mouse nuclear extracts. The arrows indicate sites of modification of MRP/Th RNA by either DMS or CMCT. We believe these data reflect the structure of the RNA as present within the RNP for the following reasons. First, it appears that most, if not all, of the MRP/Th RNA found in these nuclear extracts is in the form of a specific RNP. Second, a number of differences in the pattern of chemical modification exist between the RNA present in the nuclear extracts and the deproteinized RNA. This implies that the presence of proteins in the nuclear extract had some specific effect on the susceptibility of these nucleotides to the reagents and likely reflects the fact that the RNA was present within an RNP. Note that nucleotides 239–275 were not assessed because this is the region complementary to the 3′-most primer. The brackets connecting nucleotides 186–191 with nucleotides 123–128 denote a proposed base-pairing interaction between these regions (see below).

Existing models of the secondary structure of MRP/Th RNA are based on energy considerations with little or no experimental support (Chang and Clayton, 1989; Yuan et al., 1989). Given the well characterized limitations of the algorithms used to generate these proposals (Zuker, 1989), they must be interpreted with caution. The structure derived here is different and is based on direct experimental information. Although it appears to resemble the conformation proposed by Chang and Clayton (1989), some regions predicted to be base-paired in that model are found to be modified and are thus assigned as single stranded in the current structure. This is most evident in the region from nucleotides 192–224, which is in this model is completely single stranded, but was extensively base-paired in previous models. In addition, the pseudoknot proposed to form between nucleotides 186–191 and nucleotides 123–128 was not part of the previous model; it should be noted that the computer algorithm used to generate the earlier model does not consider these types of interactions when finding an optimal folding (Zuker, 1989). There are unmodified bases which do not appear to have obvious complements and are thus not base paired in this model (this includes the nucleotides that could not be assessed due to the presence of primer extension stops in the controls). This may reflect the influence of bound proteins or be a result of some higher order structure of the RNA which protects these nucleotides from the chemicals (Moazed et al., 1986).

A number of lines of evidence, in addition to the chemical modification data presented, supports the structure shown. First, it has one of the lowest energy conformations consistent with the data; other possible conformations were energetically less favorable. We estimate that the difference in free energy between the structure proposed and that of the next most stable structure consistent with these data is approximately 30 Kcal at 37 °C (Freier et al., 1986). Second, the structural analysis of the free T7–136 RNA is consistent with the proposed structure. The 3′-half of the full-length MRP/Th molecule (nucleotides 140–275) is proposed to base pair entirely with itself, thus removal of the 5′-half of the molecule would not require any major rearrangement. This appears to be the case based on the analysis of the T7–136 RNA, which showed an almost identical pattern of modification to that of the corresponding region of the full-length MRP RNA (see below). The exception would be the interaction proposed to occur between nucleotides 186–191 and nucleotides 123–128, which would not be possible in the smaller species alone.

probing of human nuclear extracts. The nucleotides which are modified within the human RNP are marked by the arrows in Fig. 6C. These data are consistent with the structure proposed for mouse MRP/Th RNA and suggest that the two molecules adopt very similar secondary structures.

Fig. 4. Examples of chemical modification of RNA synthesized in vitro. A–C are examples of modification experiments of the T7–275 RNA and D–F are examples of experiments with T7–136 RNA. A, primer extension of CMCT-treated T7–275 RNA with oligo 1. Lane 1, stop control; lane 2, 1.05 mg/ml CMCT; lane 3, 2.1 mg/ml CMCT; lane 4, 4.2 mg/ml CMCT. B, primer extension of DMS-treated T7–275 RNA with oligo 6. Lane 1, stop control; lane 2, 1.05 mg/ml CMCT; lane 3, 2.1 mg/ml CMCT; lane 4, 4.2 mg/ml CMCT. C, primer extension of CMCT-treated T7–275 RNA with oligo 6. Lane 1, stop control; lane 2, 1.05 mg/ml CMCT; lane 3, 2.1 mg/ml CMCT; lane 4, 4.2 mg/ml CMCT. D, primer extension of DMS-treated T7–136 RNA with oligo 1. Lane 1, stop control; lane 2, 0.2 μl of DMS; lane 3, 0.5 μl of DMS. E, primer extension of CMCT-treated T7–136 RNA with oligo 2. Lane 1, stop control; lane 2, 1.05 mg/ml CMCT; lane 3, 2.1 mg/ml CMCT. F, primer extension of DMS-treated T7–136 RNA with oligo 2. Lane 1, stop control; lane 2, 0.2 μl of DMS; lane 3, 0.5 μl of DMS; lane 4, 1 μl of DMS. Sequence ladders are as in Fig. 1.
Indeed, some indirect evidence suggests that this area is in a different conformation in the T7-136 RNA. In Figs. 1, A and B, and 2, A and B, a very strong primer extension stop is visible at approximately nucleotide C192. This stop is observed consistently and may be due to a significant G + C stem forming in this region during the reverse transcription reaction (note its occurrence in an analogous position with human MRP RNA (see Fig. 2B of Topper and Clayton, 1990). Reverse transcriptase is known to have difficulty negotiating the Cl92 stop in the control lanes of the free RNA analysis prevented some of the bases from being analyzed. Those nucleotides which appear susceptible to chemical attack in the RNA, but not in the RNP, may represent positions that are protected from chemical attack by the interaction of protein with the RNA molecule within the RNP. This phenomenon has been observed for many of the RNPs for which this kind of analysis has been carried out (Noller, 1984; Ehresmann et al., 1987). Conversely, there is also a significant number of nucleotides, designated by asterisks, that are modified in the RNP but not in the free RNA (C55, C56, A90, U115, C122, A134, U139, C139, U201, C202, C207, A208, C209, U211, A224, C231). Again, this is an observation that has been made in other systems and attributed to the influence of proteins. In this case nine of the 17 nucleotides that are modified in the RNP, but not the RNA, are present in one of the major single-stranded regions spanning nucleotides 192–224. Assignment data for nucleotides 85–140 were obtained from experiments using oligo 3 as primer.

Third, one prediction of this structural model is that oligonucleotides complementary to regions shown to be susceptible to attack by the chemical probes, and thus accessible to exogenous reagents, would be potential inhibitors of function. Indeed, two oligonucleotides that have been shown to inhibit the endoribonucleolytic activity of the MRP RNP in vitro are complementary to nucleotides 41–55 and nucleotides 197–216, regions that are largely single stranded in this conformation (Chang and Clayton, 1987b; Topper and Clayton, 1990). Fourth, MRP/Th RNA from human cells, which is 84% identical to mouse MRP/Th RNA at the primary sequence level, but for which energy considerations alone predict a distinctly different conformation, can be folded into a very similar structure and the limited modification data on this RNA are consistent with it (Fig. 6). Finally, in a number of systems where the structure of the RNA has been investigated by multiple methods in addition to chemical probing, for example, some tRNAs, 16S rRNA, and the U3 small nuclear RNA, the agreement between the methods is quite reasonable (Noller, 1984; Parker and Steitz, 1987; Stern et al., 1989). Analysis of MRP/Th RNAs from multiple species, containing sufficient sequence variation, should allow us to confirm and refine this model (James et al., 1988).

Comparison of the RNA Structure within the RNP Versus the Free RNA—The structure of the MRP/Th RNA was also probed in the absence of associated proteins. This was accomplished by deproteinizing the RNA isolated from mouse cells or by synthesizing the RNA in vitro using the gene as a template; the source of the free RNA did not affect the pattern of chemical modification observed. This indicates that in vivo modifications do not appear to play a significant role in determining the structure of this molecule in solution. The major difference between the patterns of the free RNA and the RNA within the RNP is in the region spanning nucleotides 192–224, which contains nine nucleotides that are modified in the RNP but not in the RNA. It may be that this region is maintained in a single-stranded conformation by the influence of proteins in the RNP, but upon removal of these proteins or synthesis of the RNA in their absence (in the case of MRP RNA made in vitro) it adopts an alternate structure that protects these nucleotides from chemical attack. Despite these differences, it is clear that the gross structure of the MRP/Th RNA free in solution is quite similar to that of the RNA present in the RNP.

The modification pattern of the T7–136 RNA is nearly
Chemical probing of nuclear extracts containing human MRP RNP. Nuclear extracts containing MRP RNP were treated with DMS or CMCT as described under "Materials and Methods." Primer extensions were performed using primers complementary to nucleotides 70-83, 90-139, and 229-265 of human MRP RNA (Topper and Clayton, 1990). Approximately $5 \times 10^7$ cell equivalents of RNA were analyzed per lane in the experiments shown. Sequence ladders were generated on M13 templates containing the MRP RNA gene. A. example of DMS-induced modification of the MRP RNA detected by primer extension with an oligonucleotide complementary to nucleotides 90-139 of the MRP RNA. The nucleotides which were determined to be modified in this particular experiment are indicated on the right. Lanes 1 and 4, no modification controls; lane 2, 1.0% DMS; lane 3, 2.0% DMS. B, example of CMCT-induced modification detected by primer extension with an oligonucleotide complementary to nucleotide positions 70-83 of the human MRP RNA. Lane 1, no modification control; lane 2, 5.25 mg/ml CMCT; lane 3, 10.5 mg/ml CMCT; lane 4, 21 mg/ml CMCT. C, secondary structure of human MRP RNA. The conformation shown is consistent with the chemical modification data of the human MRP RNA as isolated from nuclear extracts; this model is also consistent with the structural data available for mouse MRP RNA. Arrows indicate sites of modification by either DMS or CMCT. The putative interaction between nucleotides 123-128 and 178-183 is denoted by the brackets and line connecting these regions.

Role of MRP/Th RNA—Previous studies have indicated the importance of MRP RNA in the cleavage of mtRNA in vitro. The ability to cleave an RNA substrate is eliminated by treatment with RNases or specific complementary oligonucleotides (Chang and Clayton, 1987b; Topper and Clayton, 1990). One possibility is that the RNA acts as a scaffold upon which the protein component(s) of the RNP can assemble into an active unit. The data presented here indicate that free MRP RNA maintains a conformation similar to that of the RNA present in the RNP. Thus any structural features within MRP RNA that are recognized by proteins would likely be present in free RNA and the binding of these proteins appears not to distort this structure significantly. MRP/Th RNA may also be mediating the cleavage reactions by some direct interaction with the substrate, such as base pairing. This type of interaction occurs in processing of premessenger RNA by U1, U2, and U7 small nuclear RNPs (Parker et al., 1987; Birnstiel, 1988). Examination of the structure proposed here reveals multiple stretches of unpaired nucleotides that are readily accessible to the chemical probes. These regions are candidates for sites of base-pairing interactions with the substrates of RNase MRP/Th. One such region, nucleotides 115-142 of
Also, human MRP RNA and H1 RNA (the RNA component similar or identical proteins in the two RNPs (Gold et al., 1989). These regions have been hypothesized to interact with the mouse RNA (Fig. 5) and nucleotides 116-144 of the human RNA (Fig. 6C), contains a sequence capable of base pairing with a region of its mtRNA substrate (Fig. 7). These potential interactions, which are largely conserved between the two species' RNAs, may account for the ability of the human or mouse enzyme to process the heterologous species' mtRNA with approximately equal efficiency (Topper et al., 1988). In addition, analysis of substrates containing point mutations has yielded results consistent with this interaction (Bennett and Clayton, 1990).

Examination of the secondary structures shown reveals that the stretch of MRP/Th RNA proposed to interact with its substrate is involved in an intramolecular base-pairing interaction (Figs. 5 and 6C). This raises the possibility that recognition and/or cleavage of the mtRNA substrate is regulated by this interaction. It may be that this potential substrate binding domain is usually occupied by a "substrate-like" feature of the MRP RNA molecule itself until some regulatory event, such as passage through the mitochondrial membrane or nicking of the MRP RNA molecule, allows its release and interaction with exogenous substrates.

A region of complementarity between nucleotides 154-163 of mouse MRP/Th RNA and a conserved region of its mtRNA substrate was noted previously (Chang and Clayton, 1989). The chemical modification pattern of this region of the MRP/Th RNA indicates that seven of the 10 nucleotides are not modified and thus may not be available for base pairing without a change in conformation at this site. Additionally, analysis of RNase MRP cleavage of mutant substrates does not support this region as a primary site of potential interaction (Bennett and Clayton, 1990).

The observation by Gold et al. (1989) that MRP/Th and RNase P RNAs of human cells are consistently coprecipitated by a class of autoimmune sera suggests some relationship between these two species. These RNAs, or their corresponding RNPs, share a number of properties, the most notable of which is that they are both site-specific endoribonucleases. Also, human MRP RNA and H1 RNA (the RNA component of human RNase P) contain four regions of sequence similarity. These regions have been hypothesized to interact with similar or identical proteins in the two RNPs (Gold et al., 1989). It is therefore interesting that these sequence elements appear to reside in similar stems within the proposed structures of the two RNAs (Barkklewicz et al., 1989 and Fig. 6C).

RNase P RNA from bacteria can function as an endoribonuclease in the absence of protein. Previous data have demonstrated the importance of associated protein in RNase MRP/Th cleavage by showing that the activity observed in vitro could be abolished by protease or N-ethylmaleimide treatment (Chang and Clayton, 1987a). We have extended this work by using the cloned templates described here to produce large amounts of the free RNA in vitro and then testing for any evidence of catalytic function. The validity of this approach is supported by the structural analysis which demonstrates that the pattern of chemical modification of the RNA made in vitro is the same as that of the free RNA isolated from cells. All attempts to demonstrate endoribonucleolytic activity of the MRP/Th RNA alone, under a variety of conditions, have been unsuccessful. Although it is possible that we have not duplicated conditions necessary for RNA catalysis in this particular system, a more likely explanation is that the proteins are playing a crucial role in the catalytic process. At least part of this function may be the stabilization of a particular conformation of MRP/Th RNA responsible for mediating substrate cleavage. From this analysis it is clear that the proteins have effects on the susceptibility of some nucleotides to chemical attack and specifically enhance this reactivity in a number of cases. It may be that one of the functions of the proteins present in the RNP is to maintain stretches of MRP/Th RNA in a conformation available to interact with exogenous substrates.

It is important to note that since considerable RNase MRP is present in the nucleus, the existence of one or more substrates within the nuclear compartment is quite likely. Thus other regions of the RNA may also be involved in substrate interaction. Definitive proof of these types of interactions may come from reconstitution experiments with wild-type and altered MRP RNAs, and the structures proposed here suggest areas of the MRP RNA that are good candidates for an initial mutational analysis.

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