Characterization of human MRP/Th RNA and its nuclear gene: full length MRP/Th RNA is an active endoribonuclease when assembled as an RNP

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Received November 15, 1989; Revised and Accepted January 20, 1990

ABSTRACT

Vertebrate cells contain a site-specific endoribonuclease (RNase MRP) that cleaves mitochondrial RNA transcribed from the origin of leading-strand mitochondrial DNA replication. This report presents the characterization of the human enzyme and its essential RNA component. Human RNase MRP is a ribonucleoprotein with a nucleus-encoded RNA of 265 nucleotides. As expected, the single-copy RNA coding region is homologous (84%) to the corresponding mouse gene; surprisingly, at least 700 nucleotides of the immediate 5′-flanking region are conserved. The 265-nucleotide MRP RNA and an MRP RNA cleavage product representing the 3′-terminal 108 nucleotides exist in nuclear and mitochondrial RNA isolates; the larger MRP RNA is present in greatest abundance in the nucleus. The putative processing site within the 265-nucleotide MRP RNA is offset from that of mouse MRP RNA, but in each case cleavage is precise and occurs at the sequence ANCCCGC. Oligonucleotide-mediated inhibition experiments reveal that both the 5′ and 3′ portions of the MRP RNA are involved in cleavage by RNase MRP; this implies that full length MRP RNA complexed with proteins is an active species in vertebrate cells.

INTRODUCTION

Ribonucleoproteins (RNPs) have been implicated in an array of biological processes within the cell. These include established roles in RNA processing and translation, as well as more recently discovered functions relevant to synthesis of chromosomal telomeres (1) and organellar DNA replication (2). RNase MRP is an RNP present in both the nucleus and mitochondria of vertebrate cells that can act as a site-specific endonuclease in vitro. This endonuclease has been implicated in the metabolism of RNA primers at one of the origins of DNA replication in the vertebrate mitochondrial genome (3); the RNA component of this RNP (MRP RNA) is encoded by a nuclear gene (4,5).

The original description of mouse MRP RNA species revealed a 275-nucleotide (nt) transcript present in both nuclear RNA and mitochondrial RNA (mtRNA) populations and a smaller RNA, corresponding to the 3′ half of this molecule, which was preferentially isolated with mitochondrial RNase MRP isolates (4). This smaller form of MRP RNA appeared to copurify with the endonucleolytic activity from mouse mitochondria and was thus designated as the active species within the endonuclease. Recently it has become clear that full length MRP RNA is identical to the previously described 7-2 or Th RNA (see ref. 6). This species is precipitable by a unique class of autoimmune sera and is believed to be localized to the granular region of the nucleolus, prompting speculation on its possible role in ribosomal biogenesis. RNase MRP also appears to be related antigenically to RNase P in mammalian cells. This situation raises a number of questions regarding the biology of this RNA and the enzymatic RNP. Is the RNP containing the full length MRP RNA capable of acting as a site-specific endonuclease? What are the respective roles of the full length MRP RNA and its smaller cleavage product within the cell?

One approach to these questions is to examine MRP RNPs from distinct species to identify aspects of the system that are conserved and thus likely important in understanding the role of this RNP within the cell. RNase MRP was originally identified in mouse cells, and the murine system has been the only one for which any information regarding the nature of this enzyme was available. This report details the characterization of human MRP RNA and its nuclear gene. Comparison of the human gene with its counterpart from mouse reveals a significant amount of sequence similarity within both the RNA coding sequence and the upstream transcriptional control region. Although the human MRP RNA also is subject to a cleavage event generating a smaller form of the RNA, the site of cleavage is offset relative to that of mouse. Analysis of RNase MRP endonucleolytic activity from human cells reveals that the nuclear form of this enzyme is present in greater abundance than determined previously (4). Furthermore, oligonucleotide-mediated inhibition studies of both mouse and human enzymes show that the RNP containing the full length MRP RNA is active as a site-specific endonuclease.

MATERIALS AND METHODS

Isolation of the MRP RNA gene and RNA analysis

A human placental DNA genomic library in Charon 4A (kindly provided by M.L. Cleary, Stanford) was screened as described (7). The 1.04 kb *Pst*I fragment encompassing the region coding

for the MRP RNA was sequenced in its entirety on both strands. 5′ and 3′ probes for S1 analysis were prepared by labeling the 1.04-kb *Pst*I fragment at an internal *Acc*I site. Hybridization and S1 digestion conditions were as in Maniatis et al. (7). RNA termini were determined by electrophoresis of the S1 digestion products adjacent to Maxam-Gilbert sequence ladders. RNA sequencing with complementary oligonucleotides and reverse transcriptase was performed as described in Parker and Steitz (8). The oligonucleotides used were complementary to nucleotides 90−129 and 229−265 of the human MRP RNA. Primer extensions were performed without the addition of dideoxynucleotides. For the primer extension analyses of Figure 5, primers complementary to nucleotides 70−83 of human MRP RNA and nucleotides 90−139 of mouse MRP RNA were used. Northern analysis was performed as in Chang and Clayton (4). Hybridization conditions were 4× SSPE, 40% formamide, 5× Denhardt's solution, 200 μg/ml tRNA, 1.0 mM pyrophosphate, 0.1% SDS at 42°C. The probes were generated by 5′-end labeling the oligonucleotide or nick translating the MRP RNA gene as described (7). The blot was washed in 0.1× SSPE, 0.1% SDS at 60°C.

Oligonucleotide inhibition of RNase MRP

Extracts from human KB cell or mouse LA9 cell mitochondria were fractionated by glycerol gradient sedimentation as described (4). Fractions containing RNase MRP activity as assayed on the mouse mtRNA substrate (3) were pooled. For the oligonucleotide-inhibition experiments involving Oligo 2C (Fig. 4A), enzyme fractions were preincubated with either 25 or 50 μM of the oligonucleotide for 3 hr at 4°C. Aliquots were then removed and assayed as described (4). For the experiments involving Oligos H and M (Fig. 4B,C) this protocol was modified; enzyme fractions were incubated with 20 μM of the appropriate oligonucleotide for 3 hr at 4°C and the samples were then incubated at 37°C for 20 min and RNase H was added to the appropriate samples. Aliquots were then removed for assay as above. RNase H was purchased from Bethesda Research Laboratories. Cleavage of the substrate was assayed by electrophoresis on denaturing gels as described. The sequences of the oligonucleotides used were: Oligo 2C, 5′-TCTCTGTGT-GAGCTGACAAA-3′; Oligo H, 5′-GGGAGGAACAGAGTC-3′; Oligo M, 5′-GATAAGGAACATGTC-3′; and, 33-mer, 5′-CCTTCAGAGCGAGCTCTATAGTGAGTCGTATTA-3′.

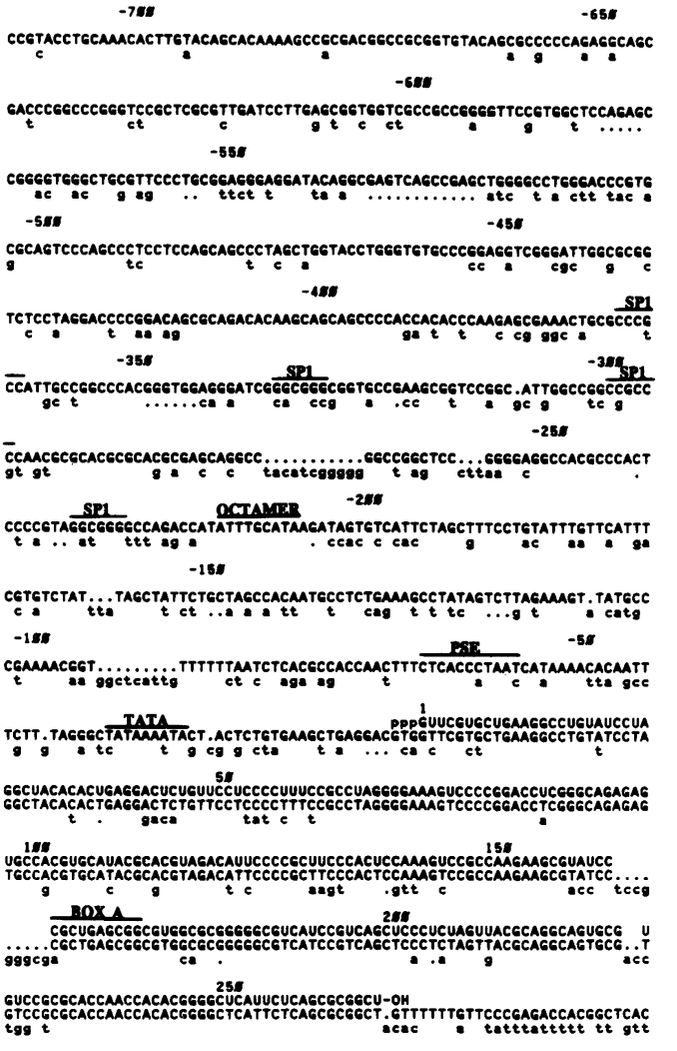

Fig. 1. Sequence of the human MRP RNA gene. The sequence of the noncoding strand is shown from nucleotides −715 to +293. The sequence of the MRP RNA shown is derived from the DNA sequence and has been confirmed by direct RNA sequencing (6). Nucleotides in lower case below the sequence are those in which the mouse MRP RNA gene differs from that of human. The alignment of the two sequences was done with the program BESTFIT. This algorithm introduces gaps to maximize the similarities and these are denoted by dots. The ends of the RNA were determined by the analyses of Figure 2.

RESULTS

Significant features of RNase MRP RNA genes and their putative control regions

The human MRP RNA gene was cloned by screening a human genomic library in Charon 4A with both the mouse MRP RNA gene and an oligonucleotide complementary to nucleotides 90 through 139 of the mouse MRP RNA (5). Five individual phage comprising three classes were isolated and each was found to contain the same 1.04-kb *Pst*I fragment that hybridized with the oligonucleotide. This fragment was sequenced and contained the coding region for the human MRP RNA gene (see below). Figure 1 contains the sequence of the noncoding strand from nucleotides −715 to +293 with respect to the start site of transcription; the sequence has been aligned with that of mouse with gaps introduced in both sequences (indicated by dots) in order to maximize the proposed similarities. Nucleotides below the human sequence represent positions in which the mouse sequence differs from that of human; the sequence of the human MRP RNA is shown above the DNA sequence.

Inspection reveals a high degree of conservation between the human and mouse loci. The coding regions of the two RNAs are approximately 84% identical; this is slightly less than the 90−95% conservation between human and mouse observed for members of the U series of small nuclear RNAs (snRNAs) (9). The MRP RNA genes of the two species are also very similar as far as 715 bp upstream; they are approximately 70% conserved in the 5′-flanking sequences shown. This conservation is greatest in the regions from −390 to −510 and −580 to −715 where the degree of similarity is approximately 85%, comparable to the level found in the coding regions of the two genes. The extent of conservation between the upstream regions of the human and mouse MRP RNA genes is significantly greater than that found in the upstream regions of the genes for the human and mouse

U series of snRNAs. The 5′-flanking regions of the latter genes are only about 50% conserved and this conservation is limited to a few hundred base pairs upstream of the start sites. Indeed, even the genes for the different U-series snRNA families within humans display only a limited amount of 5′-sequence conservation that is confined to within a few hundred nucleotides of the start sites (9). Thus the genes for the MRP RNA appear to be unique among the genes for small RNAs in possessing highly conserved 5′-flanking regions.

A variety of putative transcriptional regulatory elements are present in the 5′-flanking sequence of the human MRP RNA gene. Among these are a number of sequences resembling transcriptional control elements thought to be important in the expression of the U-series snRNAs, specifically U6 snRNA (10,11). These include a TATA sequence at about −30, a proximal sequence element at −65, and a putative distal sequence element, which may consist of the octamer motif, situated 215 bp upstream, and one or more of the four possible Sp1 interaction sites situated from −220 to −400. The coding region contains a good match for a Box A internal promoter sequence, the nucleotides TGGT at the start site of transcription and ends just upstream of a run of thymidine residues. All of these sequence elements resemble those found in most U6 genes identified thus far and, with the possible exception of the internal Box A, they have been shown to be important for accurate expression in vivo or in vitro (9,12). The presence of these features in both the MRP and U6 RNA genes is consistent with the MRP RNA gene being an RNA polymerase III transcription unit (5).

These U-series-like transcriptional elements are situated relatively near the start of transcription; this raises the likelihood that the highly conserved regions present upstream of these elements contain additional regulatory sequences. One candidate is a sequence closely resembling the nuclear respiratory factor 1 (NRF-1) binding site, located between −280 and −290. NRF-1 is a factor that binds to an element upstream of the gene for the nucleus-encoded mitochondrial protein cytochrome *c* and possibly within the first exon of the cytochrome *c* oxidase subunit VIc gene (13).

Little or no homology exists downstream of the genes. This is due at least in part to the presence of a B1 repetitive element at the 3′ end of the mouse MRP gene (5,14). The presence of this element, which probably resulted from a transposition event since the splitting of the human and mouse lineages, argues against immediately downstream sequences playing a critical role in the expression or function of these genes.

The gene for the human MRP RNA is present as a single copy. The evidence for this consists of Southern hybridization analysis, which demonstrates a single species in digests of human genomic DNA with a variety of restriction enzymes, as well as chromosomal localization studies involving in situ hybridization and analysis of somatic cell hybrids (15).

Properties of the human RNase MRP RNA

Figure 2 presents primer extension and S1 nuclease protection analyses of human MRP RNA. These data, together with information derived from direct RNA sequencing of the human MRP RNA and hybrid selection of MRP RNA with the genomic clone (data not shown), demonstrate that this gene is expressed in human cells and is not a pseudogene, which are known to exist for other small RNA species (16). The major species of human MRP RNA is 265-nt long; however, some 3′-end heterogeneity

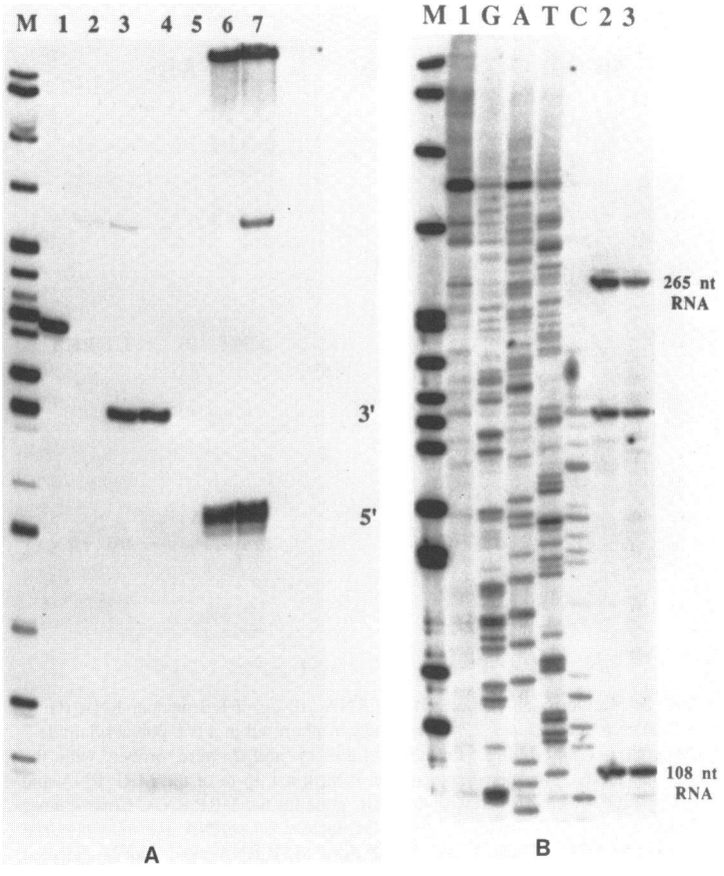

Fig. 2. Analysis of the human MRP RNA. (A) S1 nuclease protection analysis of human KB cell RNA. The 1.04-kb *Pst*I fragment containing the gene was labeled at an internal *Acc*I site to obtain 3′ (lanes 3,4) and 5′ (lanes 6,7) probes and hybridized with 20 μg KB RNA (lanes 3,4,6,7) or *E. coli* tRNA (lanes 2,5) and digested with S1 nuclease. Lane 1 is the undigested 3′ probe and lane M is pBR322 DNA digested with *Hpa*II. The fragments protected by hybridization with the MRP RNA are indicated as 5′ and 3′. The larger species in lanes 2,3,7 is the size of the renatured probe. (B) Primer extension of human KB cell RNA. 10 μg of RNA (lanes 2,3) were hybridized with a 5′-end labeled oligonucleotide complementary to the 3′-terminal 36 nt of human MRP RNA and extended with reverse transcriptase. The sequence ladder was generated by hybridizing the same oligonucleotide with the genomic clone and elongating with reverse transcriptase in the presence of dideoxynucleotides. Lane 1 is a primer extension of the genomic clone with no dideoxynucleotides added. The extension products corresponding to the full length RNA and the smaller 3′ fragment are indicated. The species at approximately 190 nt is a strong primer extension stop observed consistently.

appears to be present. S1 analysis assigned the major 3′ nucleotide as U at position 265, but evidence of slightly longer species exists (6). Many of the U-series snRNAs are synthesized as precursors with additional nucleotides at the 3′ terminus which are then removed (see ref. 9); a similar phenomenon may be occurring in this instance. As in the case of mouse MRP RNA, the human 5′ end is cappable with GTP and guanylyl transferase (data not shown).

Figure 3 reveals the subcellular distribution of human MRP RNA as judged by northern blot analysis. Nuclear RNA and mtRNA were isolated from human KB cells and probed with either the entire coding sequence (lanes 3 and 4) or an oligonucleotide complementary to the 3′-terminal 36 nt (lanes 1 and 2) of the human MRP RNA. Three major species are observed. The largest species represents full length MRP RNA of 265 nt; this 265-nt RNA is also found in the mitochondrial

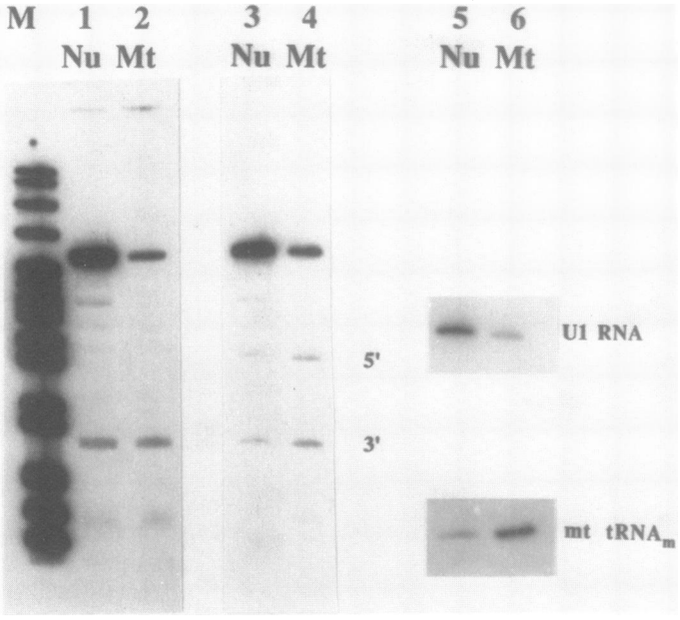

Fig. 3. Northern analysis of KB cell RNA. 10 μg of KB nuclear RNA (lanes 1,3,5) or mtRNA (lanes 2,4,6) were electrophoresed in a 6% polyacrylamide-7 M urea gel and blotted to nylon. Lanes 1 and 2 were probed with the oligonucleotide complementary to the 3'-terminal 36 nt of the MRP RNA and lanes 3 and 4 were probed with the entire gene for the MRP RNA. Control lanes 5 and 6 were probed with the genes for nuclear U1 snRNA and mitochondrial methionyl-tRNA. These 5' and 3' halves of MRP RNA would not be detected in the analysis of Figure 2A due to the configuration of the S1 probes.

fraction. In addition to full length MRP RNA, a smaller species, designated 3', is present in significant amounts in the mitochondrial fraction. As the name implies, this species represents the 3'-108 nt of MRP RNA, resulting from a cleavage event between residues 157 and 158 (see Figs. 1 and 2B). This 3' 108-nt species is reproducibly isolated from mitochondria at the level present in lane 2 of Figure 3, whereas the relative amount present in nuclear MRP isolates ranges from below detection to the level in lane 1 of Figure 3. The 5' half is also present as indicated by its ability to hybridize with the entire gene, but not with the 3'-specific probe (Fig. 3). Two control probes, complementary to nuclear U1 snRNA and mitochondrial methionyl-tRNA (lanes 5 and 6), were used to assess the degree of cross-contamination of the nuclear and mitochondrial RNA isolates. The result was similar to that of mouse cells (4) in that >90% of each human species was localized to the appropriate fraction. Thus the majority of the 265-nt MRP RNA was in the nuclear RNA isolate and it appears that the ratio of the 265-nt to 108-nt species differs in the two cellular compartments.

The presence of multiple RNA species is reminiscent of the mouse situation, where a cleavage event generates a small MRP RNA that co-isolates with mitochondria (4). The human system differs in two respects. First, cleavage does not occur at the same position (Fig. 2B), despite the good conservation of RNA sequence in this area (using the alignment of Fig. 1). Human MRP RNA is hydrolyzed 17 bases downstream relative to its mouse counterpart, although in both cases cleavage is exact and occurs at the sequence ANCCCGC. The second difference is the presence of the 5' half of the RNA molecule; this species was not detected in RNA isolates from mouse cells (4).

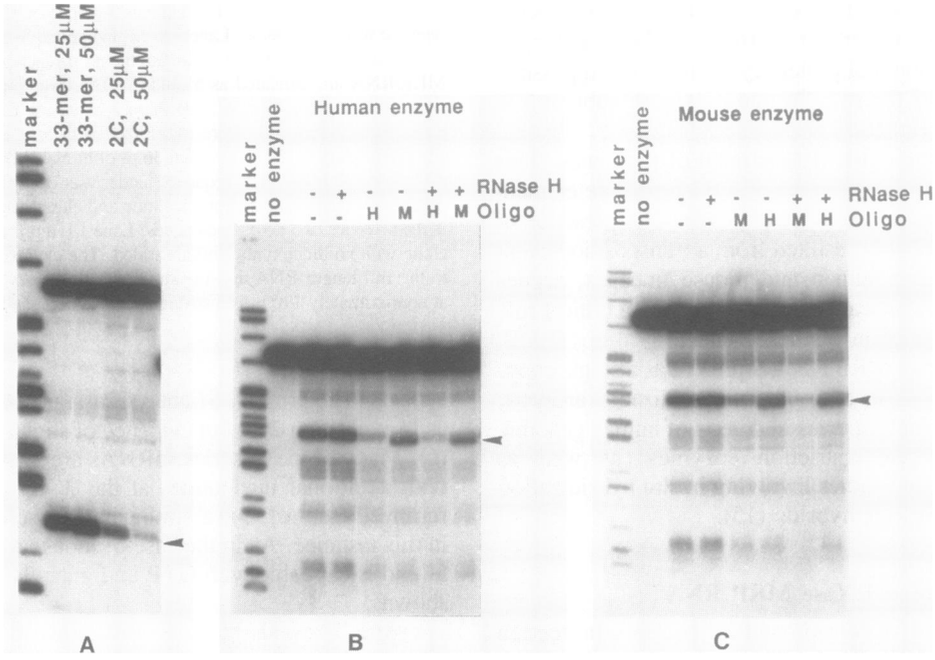

Fig. 4. Oligonucleotide-mediated inhibition of the endonucleolytic activity of RNase MRP. (A) Human MRP RNase activity was assayed after preincubation with the indicated concentration of an unrelated 33-nt oligonucleotide (33-mer) sequence; or, Oligo 2C, complementary to a region in the 3' half of human MRP RNA. The arrowhead indicates the product resulting from RNase MRP cleavage. (B) Human RNase MRP activity isolated by glycerol gradient sedimentation of KB cell mitochondrial extracts was preincubated with the appropriate oligonucleotide as described under MATERIALS AND METHODS. The samples were then removed for assay with mouse mtRNA substrate. The oligonucleotides used were: Oligo H, complementary to residues 40 through 54 of the human MRP RNA; and Oligo M, complementary to residues 41 through 55 of the mouse MRP RNA. Where indicated by a +, RNase H was added to a concentration of 0.1 units/μl during the last 20 min of preincubation. Marker lane, *Hpa*II-cleaved pBR322 DNA. The cleavage product is denoted by the arrowhead. (C) Mouse RNase MRP activity was isolated and assayed in the same manner as that of human.

Specific oligonucleotide-mediated inhibition of RNase MRP

Within the mitochondrion RNase MRP is involved in the processing of RNA primers at the origin of leading-strand DNA replication. In vitro, the partially purified MRP RNP will cleave specifically a single-stranded RNA molecule derived from this origin in a manner consistent with this function (3). The RNase MRP activity isolated from human cells appears to be very similar to the mouse enzyme. Biochemical analysis of this activity reveals that the behavior of the human MRP RNP over a number of ion exchange columns (including DEAE, phosphocellulose and FPLC Mono Q) is the same as that of mouse (data not shown). In addition, overall substrate specificity is similar for human and mouse enzymes (17). This close similarity in biochemical properties suggests that the various components of the two species' RNPs are themselves conserved. However, it is clear that the generation of the smaller form of the MRP RNA is not a strictly conserved process since the site of cleavage in the human system is offset from that of mouse, thereby generating a 3′ product almost 30 bases shorter.

In order to identify more definitively which RNAs were essential for enzymatic activity, oligonucleotide-mediated inhibition studies were performed. Previous work on the murine system showed that an oligonucleotide (designated 2C) complementary to a region in the 3′ half of the MRP RNA was inhibitory (4). This oligonucleotide is also complementary to the human MRP RNA and its ability to inhibit the human enzyme activity was tested. Oligonucleotide 2C was able to inhibit the human enzyme, while a 33-mer of unrelated sequence was without effect (Fig. 4A). Thus in humans, as for mouse, the 3′ half of the molecule is essential for activity.

To determine whether the 5′ half of the molecule is also required for activity, both human and mouse RNases MRP were incubated with oligonucleotides specific for a region in the 5′ half of either human or mouse MRP RNA (Fig. 4B,C). Oligonucleotide H, complementary to nucleotides 40 through 54 of the human MRP RNA, inhibits the human enzyme (Fig. 4B). This inhibition occured both in the presence or absence of RNase H, but was more pronounced when exogenous RNase H was added (it should be noted that the presence of endogenous RNase H activities in the partially pure MRP fraction is not excluded). As a control, oligonucleotide M (complementary to nucleotides 41 through 55 of mouse MRP RNA) was tested for its ability to inhibit the human enzyme; it had significantly less effect than did oligonucleotide H when tested against the human RNase MRP. The converse is observed when these oligonucleotides are tested against enzyme derived from mouse cells (Fig. 4C); here only oligonucleotide M was strongly inhibitory. In both cases some decrease in activity is seen on treatment with the heterologous oligonucleotides; this is not unexpected. Since oligonucleotides H and M each contain a stretch of seven identical nucleotides and these experiments involved a prolonged incubation at low temperature (see MATERIALS AND METHODS), some interaction of the oligonucleotides with the heterologous RNA should be occurring. Thus the modest reduction in activity is likely due to either endogenous RNase H activity acting during the preincubation or incomplete melting of the heterologous RNA-DNA hybrids during the subsequent 37°C incubation prior to assay. Despite these effects, it is apparent from these analyses that specific oligonucleotides are able to inhibit selectively the homologous RNase MRP activity. The experiments shown were performed with extracts from mitochondria and similar results were found with enzyme isolated from nuclear extracts (data not shown).

To confirm that these oligonucleotides were directly targeting the MRP RNA for degradation by RNase H, we analyzed MRP RNA isolated from enzyme fractions that had been treated with oligonucleotides complementary to the 5′ half of MRP RNA. This was accomplished by reverse transcription of RNA isolated from oligonucleotide- and RNase H-treated enzyme fractions. Specific primers complementary to the MRP RNA downstream of the target region of the inhibitory oligonucleotides were used. Cleavages resulting from oligonucleotide-mediated RNase H hydrolysis should be detectable by the synthesis of specific run-off transcripts during the primer extension reaction (Fig. 5).

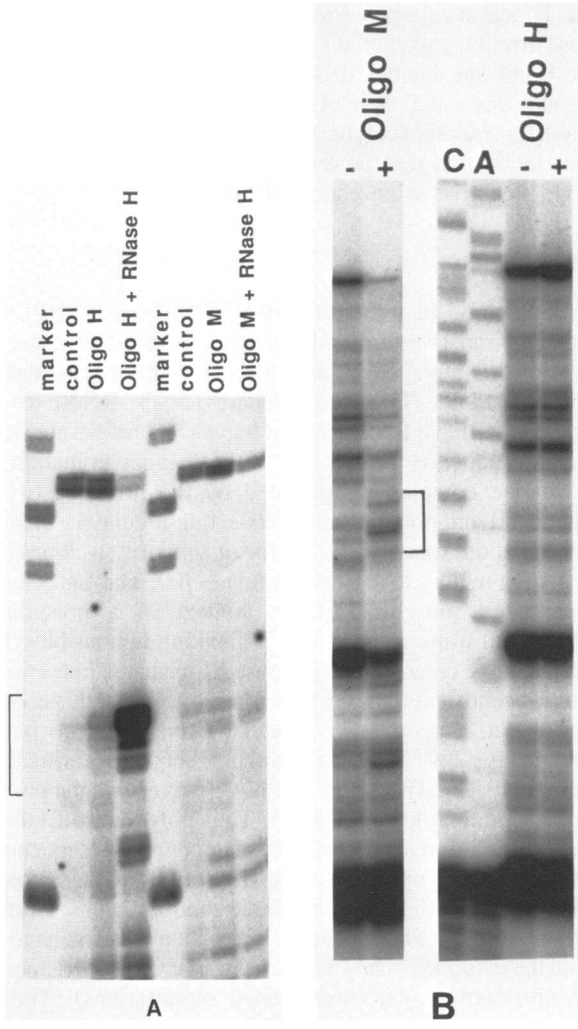

Fig. 5. Primer extension analysis of RNA derived from enzyme fractions treated with oligonucleotides complementary to the 5′ half of MRP RNA. RNA was isolated from fractions containing RNase MRP from human cells (A) or mouse cells (B) after treatment with complementary oligonucleotides and RNase H as in Figure 4. Primer extensions were performed with a primer that hybridized downstream of the region of complementarity of Oligos H and M. (A) Human RNase MRP RNA treated with Oligo H (complementary to human MRP RNA) or Oligo M (complementary to mouse MRP RNA). Control lanes were preincubated without the addition of any oligonucleotide. Where indicated, RNase H was added to 0.1 units/μl. The brackets denote the approximate region of complementarity of Oligo H. Marker lanes, *Hpa*II-cleaved pBR322 DNA. The increased intensity of primer extension products in lane Oligo H + RNase H may be due to increased efficiency of primer hybridization to the potentially more accessible cleaved MRP RNA. (B) Mouse RNase MRP-containing fractions were analyzed as above. In this case RNase H was added to all fractions and the + or − denotes the presence or absence of the indicated oligonucleotide. The sequence ladders were generated with the same primer used to extend the MRP RNA. The bracket denotes the approximate region of complementarity to Oligo M.

Primer extensions of human and mouse MRP RNAs after treatment with the oligonucleotides used in the inhibition experiments of Figure 4 showed that in each case MRP RNA is cleaved specifically only after treatment with the homologous oligonucleotide (compare lanes Oligo H + RNase H and Oligo M + RNase H of Fig. 5A for human and lanes Oligo M,+ and Oligo H,+ of Fig. 5B for mouse). Furthermore, as expected, these cleavages occur in the regions complementary to the respective oligonucleotides (brackets). The other species present in the lanes of Figure 5 represent primer extension stops that occur independently of any pretreatment with oligonucleotides and RNase H; the exception is the additional species present in the Oligo M lanes of Figure 5A, which may be due to endogenous RNase H action as posited above.

These results, together with the cleavage inhibition data of Figure 4, indicate that the MRP RNA is an integral part of RNase MRP, and that the 5′ half of the molecule, like the 3′ half, is required for endonucleolytic activity. This constitutes the first demonstration that the full length MRP RNA, assembled into an RNP, is active as an endonuclease.

DISCUSSION

Determination of the basic properties of the human MRP RNA gene and its transcription products reveals some of the essential features of this novel RNP, which has recently been shown to be identical to the Th autoantigen (MRP/Th RNA) (6). As anticipated, MRP/Th RNA genes of human and mouse are similar in size and sequence. Expression of these genes in mammalian cells may be complex as indicated by the presence of both polymerase II and polymerase III *cis*-acting regulatory elements as well as the unexpected high degree of similarity well upstream of the coding region, to at least position −700. This latter feature is even more intriguing in the context of a presumptive polymerase III transcription unit. 5′-flanking regions have been implicated in the control of polymerase III-mediated transcription, but only recently have these been shown to be both necessary and sufficient; e.g., the U6 gene has been shown to possess approximately 200 bp of 5′-flanking sequence critical for expression (10,11). The MRP/Th RNA genes extend the potential control region to at least −700 bp. Thus polymerase III and its associated factors may be capable of recognizing sequences a substantial distance upstream of the transcriptional initiation site in addition to the well known intragenic control regions. Northern blot hybridization and nuclease protection assays have not revealed the existence of any additional transcripts complementary to the upstream region (unpublished observations). The full significance of this unique finding for the regulation, maintenance and organization of this genetic unit remains to be established.

The human MRP/Th RNA is 84% identical in sequence to that of mouse. It possesses a 5′-triphosphate terminus and is subject to a cleavage event that generates a smaller RNA. The RNase MRP activity from human cells is essentially identical to that of mouse in its biochemical properties. Thus it seems reasonable to assume that the two RNPs are very similar in both their constituent proteins and their overall structure. Interestingly, computer analysis of the structure of human MRP/Th RNA using the programs of Zuker and Stiegler (18) predicts a structure completely different from that for the mouse MRP/Th RNA despite the conservation of sequence (5). Because the sequences are so similar, analysis of compensatory changes in nucleotides that could reveal the presence of conserved secondary structure elements is limited by the small number of changes in relevant regions. Knowledge of the secondary structure of these RNAs will require additional sequences and direct examination of the structure by experimental means (19).

The results of the oligonucleotide-mediated inhibition experiments support the idea that the human MRP/Th RNA is an integral part of the MRP endonucleolytic activity present in human cells. These results also imply that the 5′ portion of the 265-nt MRP/Th RNA molecule, which contains the region complementary to these inhibitory oligonucleotides, is important for enzymatic activity. Earlier work established the requirement for the 3′ region of the molecule (4); taken together, these results support the concept that the full length 265-nt MRP/Th RNA (275-nt in mouse) is assembled into a catalytically active RNP. These data do not bear definitively on whether a liberated 3′ portion of full size MRP/Th RNA can support an endonucleolytic event when assembled with protein, as has been suggested for mouse enzyme based on co-purification arguments (4). A more complete resolution of the two RNAs in RNP form (or a genetic approach) should decide the issue of whether the 3′ portion of MRP/Th RNA (108 nt in human, 136 nt in mouse) is sufficient to support any step of the cleavage reaction.

From this work it is now clear that the RNP containing the full length MRP/Th RNA is enzymatically active as a site-specific endonuclease. Furthermore, the nuclear compartment of the cell contains a significant amount of this RNP that is active in vitro. These facts raise the importance of developing strategies for assessing its possible function in the nuclear organelle. Based on the demonstrated ability of RNase MRP to act as a site-specific endonuclease, at least two possible functions can be proposed. The first is some role in pre-ribosomal RNA processing, an idea supported by the observation that MRP/Th RNP appears to be preferentially localized to the nucleolus (6,20,21). However, our current knowledge of the substrate requirements of this endonuclease do not point to any obvious candidates for efficient processing sites within the currently available ribosomal RNA sequences (Bennett and Clayton, submitted).

Given its assigned role in mitochondrial primer RNA metabolism, a second hypothesis for a function of RNase MRP within the nucleus is some participation in nuclear DNA replication. As in the mitochondrion, it may be involved in the processing of RNA primers at origins of DNA replication. Resolution of these issues, and an understanding of the central intracellular targeting phenomenon that permits localization of this RNP in both the nucleus and mitochondria, will be aided by a more complete knowledge of the essentials of substrate requirement, the identity of RNase MRP protein components and the development of an in vitro reconstitution system.

ACKNOWLEDGMENTS

We thank J.L. Bennett for helpful comments on the manuscript. J.N.T. is a Medical Scientist Training Program Trainee of the National Institute of General Medical Sciences (GM07365-12). This investigation was supported by grant GM33088-19 from the National Institute of General Medical Sciences and grant NP-9O from the American Cancer Society, Inc.

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