Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4615–4617, May 1994

Commentary

A nuclear function for RNase MRP

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The site-specific endoribonuclease RNase MRP was first identified 7 years ago as an RNA-processing activity in mammalian cells (1, 2). In the original description of this ribonucleoprotein, as isolated from either mouse or human cells, it was shown that the enzyme had the capacity to cleave RNA in a sequence-specific manner that matched one of the transition sites from RNA to DNA synthesis at the origin of leading-strand replication in mitochondrial DNA (mtDNA). It was soon learned that the RNA portion of this ribonucleoprotein entity was encoded by a nuclear gene. Further work identified the sequences of the mouse (3) and human (4) single-copy genes for the RNA component of the respective RNase MRP enzymes. In the last few years, both RNase MRP enzymatic activity and the identity of the RNase MRP RNA species (MRP RNA) and its respective nuclear gene have been obtained and characterized for *Bos taurus* (5), *Xenopus laevis* (6), and the yeast *Saccharomyces cerevisiae* (7). In all of these cases, the activities appear capable of processing mitochondrial RNA (mtRNA) sequences from both the homologous leading-strand mtDNA origin as well as across species boundaries.

However, in the initial and subsequent analyses of intracellular distribution of MRP RNA and enzymatic activity (2, 4, 8), it has been clear that the majority of both is located in the nucleus. Furthermore, the major autoantigen associated with this ribonucleoprotein particle (Th or To), for which patient sera are available for the detection of antigen inside cells, is preferentially located in the nucleolus (9, 10). For these reasons it has been logical to assume that RNase MRP might have an important function in the nucleus, and its apparent predominant localization to the nucleolus has invited continuing speculation that RNase MRP might somehow be involved in nuclear rRNA processing. The possibility of a critical role for RNase MRP in the nucleus was underscored by the finding that the RNA component of this enzyme in *S. cerevisiae* was essential for viability (7).

With the availability of the yeast gene for MRP RNA, the stage was set for a hunt for a nuclear function for this activity. Success came from two routes. In a recent article in the *Proceedings*, Lindahl and coworkers (11) reported their results of searching for genes which might complement a previously characterized mutant of *S. cerevisiae* that displayed an abnormal rRNA processing pattern. Their earlier studies with this mutant (*rrp2-2*) demonstrated that an altered pattern of rRNA processing was present that exhibited itself as both an increased amount of some precursor rRNA forms and an alteration in the stoichiometry of 5.8S rRNAs, the formation of which occurs at mid to late stages in the overall rRNA processing pathway (12). In searching for the basis of the altered processing pattern (11), it was shown that the genetic defect responsible for the faulty processing phenotype was in the *NME1* gene, previously reported to encode *S. cerevisiae* MRP RNA (7). Once implicated, the *NME1* gene was subjected to site-directed mutational analysis by deletion of bases within the gene from a midpoint restriction site. Small deletions from this site had no effect on rescue of rRNA processing or growth patterns, whereas larger deletions affected cell viability at the restrictive temperature while also failing to rescue abnormal processing. These data were taken to confirm the initial conclusion that the *rrp2-2* mutation was indeed complemented by the *NME1* gene. The mutation was further characterized by sequencing the *NME1* gene in both wild-type and mutant strains. Wild-type sequence was as reported (7), whereas the *NME1* sequence in the *rrp2-2* mutant contained a single G-to-A transition at nucleotide position 122. The authors tested for the relative stability of the wild-type MRP RNA versus that with a single base change and found that there was no difference in the relative abundance of full-length transcripts that could be isolated from each of the two strains. It was therefore concluded that the most likely explanation for the problem resulting from a single base change in MRP RNA was at the level of catalytic activity of the enzyme and that the defect could not be explained on the basis of differential MRP RNA stability.

All of these results are in excellent agreement with the report of Schmitt and Clayton (13) in which the ramifications of altered levels of expression of wild-type MRP RNA or the expression of altered forms of MRP RNA were examined in order to search for a nuclear phenotypic consequence. As the *NME1* gene is essential for cellular viability (7), the experimental strategy was to examine the effects of regulated expression in a strain in which the chromosomal copy of *NME1* had been inactivated and the viability of the cell was rescued by maintenance of a plasmid-borne copy of *NME1*. When *NME1* was down-regulated by glucose repression of expression from the *GAL1* promoter, there was a significant depletion of the amount of the *NME1* gene product, wild-type intracellular MRP RNA. Concomitant with the decrease in MRP RNA it was found that there was a reversal in the stoichiometry of the two mature forms of 5.8S rRNA; in the MRP RNA-depleted condition, the 7-nucleotide-longer version of 5.8S rRNA was ≈10 times more abundant than the shorter species lacking this 7-nucleotide sequence at the 5′ end. These results were in contrast to the normal stoichiometry in which the shorter version of 5.8S rRNA is ≈10-fold more abundant than the slightly longer version.

Two temperature-conditional mutant strains were examined in which the *NME1* gene had suffered either an internal deletion or a point mutation consisting of an A-to-G transition at position 122 of the RNA sequence. Indeed, this single base mutation, which was selected as a temperature-conditional mutation for growth and is a tight mutation for this phenotype, is identical to the *rrp2* mutations isolated independently by Lindahl and coworkers (11, 12) and by Shuai and Warner (14) (*rrp2-2* and *rrp2-1*, respectively). Thus it is likely that this particular position defines a critical location within the MRP RNA sequence with regard to its functional capacity. With both of their conditional mutants, Schmitt and Clayton (13) found a similar phenotype—namely, the altered stoichiometry of 5.8S rRNA forms. This was most pronounced with the single-base mutation (termed P6; ref. 13); in this case there was no obvious accumulation of larger precursor rRNAs to the extent observed by others (12, 14). However, the clear alteration in the processing pattern of mature 5.8S rRNAs is a result that is consistent between two studies (11, 13), and in each case (11, 13, 14) an accumulation of a slightly larger

aberrant precursor to mature 5.8S rRNAs was observed.

What then is the role of MRP RNA (more specifically, RNase MRP) in the nucleus and what are the consequences of its failure to function there? Finding an alteration in nuclear rRNA processing is a satisfying result, since RNase MRP clearly has the ability to process RNA and its preferential nucleolar location in the nucleus is consistent with a role in processing rRNA. Therefore the early speculations in this regard have now received experimental support. But, as noted by the authors, the results of Schmitt and Clayton (13) and Chu *et al.* (11), although in agreement, do not exclude other phenotypic consequences of RNase MRP or MRP RNA dysfunction, nor is it established that altered 5.8S rRNA metabolism is the basis for the essential requirement for cell viability. One would anticipate that a systematic and complete genetic analysis that characterizes suppressor mutations of RNase MRP-specific functional defects should reveal the full spectrum of processes that require or utilize RNase MRP action.

With regard to nuclear function, the simplest possibility consistent with the data in these two works (11, 13) and all earlier studies would be that RNase MRP acts directly as an RNA-processing activity on a precursor of fully mature 5.8S rRNA. Interestingly, a conserved region of MRP RNA (positions 80–88 for *S. cerevisiae* MRP RNA) can, in principle, form a 9-bp duplex with 5.8S rRNA (positions 3–11). This region of complementarity is thus directly adjacent to a potential processing site for production of the most abundant, smaller 5.8S rRNA species. Attempts to achieve cleavage with nuclear rRNA substrates *in vitro* have failed to demonstrate such a direct event, but these reactions have only been performed with short RNA substrates (13). It could well be that RNase MRP recognizes a more complicated structure formed by the association of RNA-binding proteins with rRNA precursors or is also involved with other ribonucleoprotein entities (small nucleolar ribonucleoproteins, snoRNPs) that define a larger and more complicated processing complex in this system. In either case, or if both situations are involved, it will be necessary to learn of any direct cleavage role for RNase MRP through a more sophisticated *in vitro* assay or by further identification of interactive components. Of course, it is also possible that RNase MRP functions at a point upstream in a pathway that eventually results in maturation of 5.8S rRNA. RNase MRP might process an RNA molecule that, in turn, becomes part of a processing complex which interacts with a 5.8S rRNA precursor.

One of the more obvious functional parameters that one might invoke after a failure to process mature forms of rRNA would be a translation defect. Schmitt and Clayton (13) obtained information which suggested that the overall capacity for translation was not impaired under restrictive conditions when MRP RNA was present only in the mutated P6 form. But it was noted that the overall crude profile of polypeptides synthesized was subtly different in abundance and form, dependent upon whether the cells were grown under permissive or nonpermissive conditions. This observation affords an opportunity for future investigations aimed at determining whether or not ribosomes containing one or the other form of 5.8S rRNA are functionally distinct, so that their ability to translate different mRNAs is somehow selective. If so, this would reveal a purpose for the cellular maintenance of different forms of 5.8S rRNA, a condition that is pervasive in eukaryotic cells.

None of the experiments in these works (11, 13) bear directly on the issue of the requirement for RNase MRP in mtRNA processing. The data and arguments that support a role for RNase MRP in mitochondrial primer RNA metabolism have been summarized (15). However, it has been concluded from a subcellular and suborganellar fractionation evaluation of the localization of MRP RNA that there is too little MRP RNA associated with mitochondrial fractions to rationalize its *in vivo* existence in the organelle (16). Although the nucleic acid controls in these experiments were mtRNA sequences (16), it would have been informative to monitor the presence of the most relevant components of the mtDNA replication machinery, such as mitochondrial transcription or replication proteins or mtDNA itself.

A more recent analysis of RNase MRP RNA distribution, using direct *in situ* hybridization, has provided data indicating that a definable portion of the intracellular RNase MRP RNA is localized in mitochondria of mouse myogenic cells in culture (17). In addition, as one of their control experiments for intracellular localization of RNase P RNA in human HeLa cells, R. S. Puranam and G. Attardi found a small and reproducible amount of RNase MRP RNA in rigorously defined mitochondrial fractions (G. Attardi, personal communication). There is also evidence that after injection of rhodamine-tagged MRP RNA in human tissue culture cell nuclei, there is an early accumulation of molecules in nucleoli with a subsequent appearance of a limited amount of MRP RNA in cytoplasmic structures that are most likely mitochondria (M. R. Jacobson, L. G. Cao, Y.-l. Wang, and T. Pederson, personal communication). Thus the available data are most consistent with the view that a small portion of the intracellular RNase MRP RNA, and presumably enzymatic activity as well, is localized to mitochondria.

It has been proposed that the potent endodeoxyribonuclease G (18) may play a role in mammalian mtDNA primer formation. Endonuclease G has recently been localized predominantly to mitochondria (19), a result consistent with the earlier work of Low *et al.* (20). Endonuclease G has been concluded to have a limited ability to cleave RNA alone or in the form of an RNA·DNA hybrid (19). The positions of RNA cleavage when bovine endonuclease G is tested with a heterologous mouse RNA·DNA hybrid origin sequence include one of the minor sites of possible transition from RNA to DNA synthesis. Based on this result, Côté and Ruiz-Carrillo (19) have suggested that endonuclease G action provides primers for mtDNA replication.

Although endonuclease G clearly cleaves mtDNA with some sequence preference and is a mitochondrial activity, any conclusions as to a role in mitochondrial primer RNA metabolism must be viewed as premature. First, it will be important to assay the integrity of the DNA strand of any RNA·DNA hybrid after endonuclease G cleavage, as endonuclease G is able to digest DNA in general, whereas an RNase H-like enzyme should not hydrolyze DNA. Second, the enzyme has not been purified to homogeneity and it is possible that other activities are responsible for the limited RNase action observed. Third, the positions of RNA cleavage fall both within and outside the mtDNA origin sequence, not in alignment with predicted transition sites, and there is no obvious cleavage seen at the major 5′-end DNA map positions. Finally, in the case of yeast mtDNA replication, a null mutant of the yeast endonuclease G homologue (*nuc1*) maintains a normal mtDNA phenotype (21).

If we assume at least a major nuclear function of RNase MRP is in late-stage processing of rRNA and that a second cellular function involves primer RNA metabolism in mitochondria, is there any logical linkage between these two phenomena? When one considers ribosomal and mitochondrial biogenesis, there are certain similarities that are intriguing. One of these is the long-studied correlation between ribosome synthesis and the overall rate of cellular metabolism and growth. A similar situation is true for mitochondria. At the nucleic acid level, the nature of promoter organization, transcription, and specificity of interaction of trans-activating transcription factors is similar between RNA polymerase I, responsible for nuclear rRNA production, and mtRNA polymerase. These features include the general observation of species-specific transcription, location of the transcription initiation site within a

core promoter sequence, production of polycistronic transcripts that require processing, and existence of high-mobility-group-box-containing DNA-binding proteins that are active in transcriptional activation in both of these systems.

RNase MRP and RNase P (which has as a principal function the maturation of the 5′ end of tRNAs) are related at the level of sequence homology of the RNA components of the two enzymes (10). In addition, these two RNAs appear to have structural similarities (22, 23) and the enzymes share a common antigenic determinant, as evidenced by the ability of patient autoantisera to coprecipate mammalian RNase MRP and RNase P (10). Thus, some of the same evidence taken to indicate the nucleolar localization of RNase MRP, such as intracellular antigen detection (9), could be applied equally well to RNase P, leading to speculation about alternative catalytic roles of this enzyme.

Could RNase P also be involved in rRNA processing? Perhaps so, given the fact that RNase P exhibits cleavage activity on various substrates, one being the *in vivo* precursor to 4.5S RNA of *Escherichia coli*, an RNA functionally related to the RNA component of the eukaryotic signal-recognition particle (24, 25). Furthermore, the RNA component of human RNase P (H1 RNA) is most likely also functional in mitochondria (26), perhaps even in concert with RNase MRP. In this regard, one of the published cleavage sites of RNase MRP on mtRNA substrates (8) can also be cleaved by *E. coli* RNase P (27). Consider the fact that rRNA processing in vertebrate mitochondria may be regarded as a problem of tRNA excision with the resultant products being mature rRNAs and mature tRNAs, due to the direct juxtaposition of rRNA genes and tRNA genes in this system. Therefore, an RNase P-like activity, responsible for tRNA excision, would also be, by definition, an rRNA-processing activity. The potential overlap of cleavage capacity between the related RNase MRP and RNase P activities may suggest a regulatory interplay critical to mitochondrial nucleic acid metabolism and/or perhaps a redundancy of function that is able to serve different needs under different conditions. Thus, the bold prediction is that both RNase MRP and RNase P are involved in rRNA processing in the nucleus as well as in a more global role in RNA processing in mitochondria. Further work in both fungal and mammalian systems will be required to establish the degree to which the foregoing concept is valid.

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