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Letters to the Editor

Difference between Mitochondrial RNase P and Nuclear RNase P

We previously demonstrated that mammalian cells contain at least two distinct RNase P activities, one nuclear and one mitochondrial (5, 7). These enzymes were shown to have different substrate specificities and distinct molecular and enzymatic properties (5–7). Puranam and Attardi recently reported the purification of "nuclear" RNase P from mitochondrial preparations (2). However, in the discussion of their findings they made incorrect and misleading reference to previous work on mitochondrial RNase P, necessitating a reevaluation of their conclusions.

The presence of low levels of nuclear RNase P or other nuclear RNA processing factors in purified mitochondrial preparations is not a new observation, and digitonin treatment is well-known to drastically reduce the levels of these contaminants (1, 3, 7). Mitochondrial RNase P activity, however, is definitely independent of the presence or absence of nuclear RNase P or its RNA component (H1 RNA) (7). Reduction of contaminating nuclear RNase P by digitonin pretreatment of mitochondria or its complete removal from mitoplast extracts by either immunoprecipitation or purification did not result in any change of mitochondrial RNase P activity in these preparations (7).

Yet, inevitably, Puranam and Attardi (2) failed in the identification of this RNase P activity independent of H1 RNA: the use of *Escherichia coli* pre-tRNA$^{Tyr}su_3^+$ as a substrate throughout their purification procedure made the assay of mitochondrial RNase P activity naturally impossible. Mitochondrial RNase P as identified in 1995, which faithfully cleaves mitochondrial tRNA precursors (3–7), does not process *E. coli* pre-tRNA$^{Tyr}su_3^+$ (7). On the other hand, nuclear RNase P, which is capable of cleaving pre-tRNA$^{Tyr}su_3^+$, does not cleave mitochondrial pre-tRNA^{Tyr} (7). Given the considerable structural differences between cytoplasmic and mitochondrial tRNAs, this particularly useful distinction of mitochondrial and nuclear RNase P by their substrate specificity is not surprising but apparently is the result of the coevolution of enzymes and substrates. Substrate recognition of both enzymes nevertheless overlaps as long as the precursors fulfill the respective structural requirements (7).

Thus, is it possible that mammalian mitochondria contain two forms of RNase P, one distinct from and one identical to the nuclear enzyme? Such a scenario of two RNase P enzymes within one cellular compartment would be without precedent and therefore requires careful evaluation. Of course, the relative and absolute levels of both enzymes in mitochondria are critical in this argument. Even in small amounts of crude mitochondrial extracts, detection of mitochondrial RNase P activity appears to be straightforward, thereby indicating a high level of enzyme (7). In contrast, nuclear RNase P activity could not be detected in mitochondrial extracts (7) but required enrichment by biochemical purification to allow assay of its activity (2). Puranam and Attardi nevertheless provided an estimate of the quantities of nuclear RNase P (H1) RNA as well as other snRNAs present in purified mitochondria (2). Taking their numbers for granted, (i) the actual levels of U snRNAs (nuclear splicing factors) and H1 RNA are in an astonishingly similar range and (ii) the estimated number of H1 RNA molecules in the mitochondrial matrix (digitonin/micrococcal nuclease resistant and "corrected for losses of mitochondrial markers") of cells in the G_2 phase of the cell cycle is only one-third of the number of mitochondria (2). The latter implies that the mitochondria of cells during other phases of the cell cycle do not contain any nuclear RNase P, which would only be possible by cell cycle stage-dependent import of nuclear RNase P followed by rapid intramitochondrial degradation or expulsion. Moreover, assuming that this scenario is correct, the numbers given by Puranam and Attardi would actually be overestimates of RNase P amounts, as their preparations were "enriched in heavy mitochondria" (2). Thus we feel that although it is impossible to formally exclude the possibility that mitochondria contain trace amounts of nuclear RNA processing factors, contamination of mitochondrial preparations during subcellular fractionation still appears to be the more plausible interpretation.

REFERENCES

1. **Kiss, T., and W. Filipowicz.** 1992. Evidence against a mitochondrial location of the 7-2/MRP RNA in mammalian cells. Cell **70:**11–16.
2. **Puranam, R. S., and G. Attardi.** 2001. The RNase P associated with HeLa cell mitochondria contains an essential RNA component identical in sequence to that of the nuclear RNase P. Mol. Cell. Biol. **21:**548–561.
3. **Rossmanith, W.** 1996. Ph.D. thesis. University of Vienna, Vienna, Austria.
4. **Rossmanith, W.** 1997. Processing of human mitochondrial tRNA$^{Ser(AGY)}$: a novel pathway in tRNA biosynthesis. J. Mol. Biol. **265:**365–371.
5. **Rossmanith, W., and R. M. Karwan.** 1998. Characterization of human mitochondrial RNase P: novel aspects in tRNA processing. Biochem. Biophys. Res. Commun. **247:**234–241.
6. **Rossmanith, W., and R. M. Karwan.** 1998. Impairment of tRNA processing by point mutations in mitochondrial tRNA$^{Leu(UUR)}$ associated with mitochondrial diseases. FEBS Lett. **433:**269–274.
7. **Rossmanith, W., A. Tullo, T. Potuschak, R. Karwan, and E. Sbisà.** 1995. Human mitochondrial tRNA processing. J. Biol. Chem. **270:**12885–12891.

Walter Rossmanith*
Institute of Anatomy
University of Vienna
Währinger Str. 13
1090 Vienna, Austria

Thomas Potuschak
ICMB
University of Edinburgh
Edinburgh EH9 3JR, Scotland, United Kingdom

*Phone: 43 1 4277 61187
Fax: 43 1 4277 61198
E-mail: walter.rossmanith@univie.ac.at

Authors' Reply

In earlier work (1), we showed that HeLa cell mitochondria carry a micrococcal nuclease-sensitive RNase P activity representing a fraction of a percent of the total activity in the postmitochondrial cytosol fraction (consisting mostly of the leaked-out nuclear activity). In agreement with this result, we recently demonstrated that highly purified HeLa cell mtRNase P contains an intact RNA identical to H1 RNA of nuclear RNase P, representing ~0.3% of that associated with the nucleocytosolic compartment; crucially, this RNA can be removed from mitochondria only by treatments destroying the integrity of the organelles (6). That mitochondria carry such a small amount of RNase P is not surprising, knowing that they

contain only <1% of the cell tRNAs (2). Most significantly, an analysis of the expected functional requirements for tRNA processing in HeLa cell mitochondria showed that the detected level of mtRNase P fully satisfies those requirements (6).

Our conclusions are in apparent contrast with the results of Rossmanith and colleagues, who described an abundant reportedly RNA-free mtRNase P (7, 8). Our failure to detect such enzyme was not due to using *E. coli* $ptRNA^{Tyr}$ as a substrate. In fact, contrary to a statement in their letter, this substrate was clearly cleaved by their mtRNase P, although incorrectly, i.e., four nucleotides upstream of the mature 5′ end (7). No such processing activity was observed at any stage during purification of our mtRNase P, and we would have definitely been able to see it (6).

In answer to another statement in the letter, the proportions of total cell- and washed mitochondrion-associated U snRNAs (which is a more appropriate parameter than "levels") that we found in purified mitochondria were, respectively, 1 and 2 orders of magnitude lower than the corresponding proportions of H1 RNA. Also, our most conservative estimate of mitochondrial H1 RNA amount per cell in unsynchronized cells, which quantifies only intact H1 RNA (~175 molecules), when referred to transcriptionally active cells (5), would be about twice the number of mitochondria per cell.

There are two obvious differences in the experiments by Rossmanith et al., as compared to ours. (i) The first difference is the quality of their mitochondrial preparations, which exhibited a massive degradation of their abundant nuRNase P contaminant, as well as of their mitochondrial RNAs (as exemplified by $tRNA^{Glu}$). This degradation, which resulted presumably from those authors' use of frozen cells for cell fractionation and from the expected consequent molecular damage, contrasts dramatically with our patterns of mostly intact RNA. This damage could account for the differences in enzymatic and physical properties and in RNA content of their RNase P. (ii) The second difference is the lack of any clearly interpretable quantification of RNase P activity and RNA content. In particular, in their most recent paper (8), Rossmanith et al. acknowledged the presence of degraded RNA in their partially purified mtRNase P, but no quantification was presented. It is well established that yeast mtRNase P retains partial activity after extensive degradation of its mtDNA-encoded RNA (3).

As to the physiological significance for HeLa cells of the presence of a limiting, though adequate, amount of RNase P in mitochondria, which we determined by two independent approaches, it is important to point out that the RNase P tRNA processing sites are the sites of polyadenylation, and resulting stabilization, of the upstream encoded RNAs (4, 6). Thus, limiting the amount of RNase P in mitochondria to the minimum required for tRNA synthesis would not allow stabilization of the 10- to 15-fold excess of fast turning-over nascent L-strand transcripts and prevent both a disproportionately high rate of synthesis of the eight L-strand-encoded tRNAs relative to the H-strand-encoded tRNAs, which could negatively affect translation (2), and an accumulation of abundant antisense RNA.

REFERENCES

1. **Doersen, C.-J., C. Guerrier-Takada, S. Altman, and G. Attardi.** 1985. Characterization of an RNase P activity from HeLa cell mitochondria. Comparison with the cytosol RNase P activity. J. Biol. Chem. **260:**5942–5949.
2. **King, M. P., and G. Attardi.** 1993. Post-translational regulation of the steady-state levels of mitochondrial tRNAs in HeLa cells. J. Biol. Chem. **268:**10228–10237.
3. **Morales, M. J., C. A. Wise, M. J. Hollingsworth, and N. C. Martin.** 1989. Characterization of yeast mitochondrial RNase P: an intact RNA subunit is not essential for activity *in vitro*. Nucleic Acids Res. **17:**6865–6881.
4. **Ojala, D., J. Montoya, and G. Attardi.** 1981. tRNA punctuation model of tRNA processing in human mitochondria. Nature **290:**470–474.
5. **Pica-Mattoccia, L., and G. Attardi.** 1972. Expression of the mitochondrial genome in HeLa cells. IX. Transcription of mitochondrial DNA in relationship to the cell cycle. J. Mol. Biol. **57:**615–621.
6. **Puranam, R. S., and G. Attardi.** 2001. The RNase P associated with HeLa cell mitochondria contains an essential RNA component identical in sequence to that of the nuclear RNase P. Mol. Cell. Biol. **21:**548–561.
7. **Rossmanith, W., A. Tullo, T. Potuschak, R. Karwan, and E. Sbisà.** 1995. Human mitochondrial tRNA processing. J. Biol. Chem. **270:**12885–12891.
8. **Rossmanith, W., and R. M. Karwan.** 1998. Characterization of human mitochondrial RNase P: novel aspects in tRNA processing. Biochem. Biophys. Res. Commun. **247:**234–241.

Giuseppe Attardi*
Division of Biology
California Institute of Technology
Pasadena, CA 91125

Ram S. Puranam
Department of Medicine (Neurology)
Duke University Medical Center
Durham, NC 27710

*Phone: (626) 395-4930
Fax: (626) 449-0756
E-mail: attardi@caltech.edu