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The Evolution of the RNase P– and RNase MRP–Associated RNAs: Phylogenetic Analysis and Nucleotide Substitution Rate

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Abstract. We report a detailed evolutionary study of the RNase P– and RNase MRP– associated RNAs. The analyses were performed on all the available complete sequences of RNase MRP (vertebrates, yeast, plant), nuclear RNase P (vertebrates, yeast), and mitochondrial RNase P (yeast) RNAs. For the first time the phylogenetic distance between these sequences and the nucleotide substitution rates have been quantitatively measured.

The analyses were performed by considering the optimal multiple alignments obtained mostly by maximizing similarity between primary sequences. RNase P RNA and MRP RNA display evolutionary dynamics following the molecular clock. Both have similar rates and evolve about one order of magnitude faster than the corresponding small rRNA sequences which have been, so far, the most common gene markers used for phylogeny. However, small rRNAs evolve too slowly to solve close phylogenetic relationships such as those between mammals. The quicker rate of RNase P and MRP RNA allowed us to assess phylogenetic relationships between mammals and other vertebrate species and yeast strains. The phylogenetic data obtained with yeasts perfectly agree with those obtained by functional assays, thus demonstrating the potential offered by this approach for laboratory experiments.

Key words: RNase P — RNase MRP — Ribonucleoproteins — Multialignment — Molecular clock — Phylogenetic tree — Quantitative evolutionary rate — Yeasts — Vertebrates — Mitochondria

Introduction

RNA is a fascinating molecule because, in addition to informational role (mRNA), it is entrusted, both in the nucleus and in the cytoplasm, with multiple functions: protein synthesis (rRNA, tRNA, mRNA) and RNA catalysis, e.g., mRNA splicing (splicesomal snRNA), processing of noncoding protein transcripts, ribosome biogenesis (RNase P, RNase MRP, snoRNAs), and editing (guide RNA).

Recently it has been unexpectedly discovered that a number of RNA species have catalytic function either in association with protein components (ribonucleoproteins) or on their own (ribozymes). These data gave rise to the hypothesis that an RNA world preceded (and remnants still exist) the familiar DNA and protein contemporary biological world, based on the distinct dichotomy between informational and catalytic roles. Evolutionary analysis may be a powerful approach to the study of genes in terms of structure-function relationships and also useful in defining the taxonomy of organisms.

In this paper we have studied the evolution of the RNA components of two ribonucleoproteins: RNase P (nuclear and mitochondrial) and RNase MRP. These are site-specific endonucleases which have been identified in bacteria, animals, plants, and yeasts. It has been suggested that these enzymes are related. Indeed, the RNA components of both can be folded into a similar secondary structure (Forster and Altman 1990); and, in verte-

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Table 1. List of the sequences used in the phylogenetic analysis

Organism	Acc. number	Range	Reference
	RNase MRP RNAs		
Vertebrates			
Homo sapiens[a]	X51867	716..980	(Topper and Clayton 1990)
Homo sapiens	M29212	1..268	(Gold et al. 1989)
Homo sapiens	M29916	745..1009	Topper and Clayton (unpublished)
Bos taurus	Z18244	712..988	(Dairaghi and Clayton 1993)
Rattus norvegicus	J05014	1..274	(Yuan et al. 1989)
Mus musculus	J03151	845..1119	(Chang and Clayton 1989)
Xenopus laevis	Z11844	491..767	(Bennett et al. 1992)
Plant			
Arabidopsis thaliana	X65942	195..454	(Kiss et al. 1992)
Yeast			
Saccharomyces cerevisiae	Z14231	273..612	(Schmitt and Clayton 1992)
	Nuclear RNase P RNAs		
Vertebrates			
Homo sapiens[a]	X15624	1..340	(Bartkiewicz et al. 1989)
Homo sapiens	S68670	98..438	(Hannon et al. 1991)
Homo sapiens	X16612	375..715	(Baer et al. 1990)
Pongo pigmaeus	L08688	1..341	(Altman et al. 1993b)
Pan troglodites	L08686	1..341	(Altman et al. 1993b)
Gorilla gorilla	L08685	1..341	(Altman et al. 1993b)
Macaca mulatta	L08687	1..341	(Altman et al. 1993b)
Tarsius syrichta	L08801	1..336	(Altman et al. 1993b)
Rattus norvegicus	L08800	1..306	(Altman et al. 1993b)
Mus musculus	L08802	1..310	(Altman et al. 1993b)
Xenopus laevis	X56558	1..320	(Doria et al. 1991)
Yeasts			
Saccharomyces cerevisiae	M27035	408..776	(Lee et al. 1991)
Saccharomyces bayanus	L12745	408..776	(Tranguch and Engelke 1993)
Saccharomyces carlsbergensis	L12746	286..644	(Tranguch and Engelke 1993)
Saccharomyces diastaticus	L12747	408..776	(Tranguch and Engelke 1993)
Saccharomyces globosus	L12748	275..613	(Tranguch and Engelke 1993)
Saccharomyces kluyveri	L12750	268..603	(Tranguch and Engelke 1993)
Saccharomyces uvarum	L12749	751..1119	(Tranguch and Engelke 1993)
Schizosaccharomyces pombe	X04013	192..477	(Krupp et al. 1986)
Schizosaccharomyces octosporus	X52531	35..319	(Zimmerly et al. 1990)
Schizosaccharomyces pombe	X52530	537..822	(Zimmerly et al. 1990)
Schizosaccharomyces malidevorans			(Zimmerly et al. 1990)
Schizosaccharomyces japonicus			(Zimmerly et al. 1990)
Schizosaccharomyces versatilis			(Zimmerly et al. 1990)
	Mitochondrial RNase P RNAs		
Yeasts			
Saccharomyces exiguus	M69218	117..393	(Wise and Martin 1991)
Saccharomycopsis fibuligera	M69219	12..175	(Wise and Martin 1991)
Saccharomyces cerevisiae	K01432	247..699	(Miller and Martin 1983)
Saccharomyces douglasii	X53519	340..695	(Ragnini et al. 1991)
Torulopsis glabrata	X02168	149..375	(Shu et al. 1991)
Saccharomyces chevalieri			Miller D.L. (personal communication)
Saccharomyces ellipsoideus			Miller D.L. (personal communication)
Saccharomyces diastaticus			Miller D.L. (personal communication)

[a] Sequence which has been used in the multiple alignment

brates, the enzymes share antigenic determinants: they are immunoprecipitated by a group of sera from patients with certain autoimmune diseases (Gold et al. 1989; Rossmanith and Karwan 1993).

RNase MRP (for mitochondrial RNA processing) was found in eukaryotes only and has been reported to function in two different compartments: the mitochondrion and the nucleus. In vertebrates it processes in vitro in a site-specific way the mitochondrial (mt) RNAs that correspond to the primers for mtDNA replication (Chang and Clayton 1987; Tullo et al. 1995) (references in Table 1), and no nuclear function has been reported. In the yeast *Saccharomyces cerevisiae* a role in nuclear 5.8S rRNA processing has been demonstrated (Schmitt and Clayton 1992). In plants an snRNA gene has been suggested to be the equivalent of the vertebrate RNase MRP RNA and a role in pre-rRNA processing has been proposed. The plant protein components differ from those of

vertebrates since they do not share the same antigenic epitopes (Kiss et al. 1992).

RNase P is a ubiquitous endoribonuclease that cleaves precursor tRNA molecules, generating mature 5′ termini. RNA species copurify with RNase P activity in eubacteria, archaebacteria, fungi, vertebrates, and fungal mitochondria (for review see Darr et al. 1992; Altman et al. 1993a). However, while in eubacterial RNase P the RNA component has catalytic activity in vitro in the absence of its protein subunit, in all other organisms the RNA components, even if essential to the function, have been shown to have no catalytic activity on their own.

Although fundamental differences (RNA component, size, protein content) distinguish the eubacterial and eukaryotic RNase P, the enzyme is functionally well conserved. There are no striking similarities between the RNase P RNAs of eukaryotes and prokaryotes, and it is not established to what extent RNase P diverges across species and what the essential structural elements are which need to be conserved.

We report here the first quantitative evolutionary analysis of the RNA components of RNase MRP and RNase P RNA, both of which share evolutionary dynamics following the molecular clock.

The analyzed sample includes all the complete available sequences in the databases (Table 1) with the exclusion of bacterial RNase P, for which exhaustive reviews (Gopolan et al. 1995) and a specialized database are available (Brown et al. 1994).

Our phylogenetic inference agrees very well with individual species functional relatedness, which has been experimentally determined.

The results provide new insight in the phylogenetic relationships of the organisms for which sequences are available.

Materials and Methods

Multiple Alignments. Nucleotide sequences were extracted from Genbank database (release 89) by using the retrieval program ACNUC (Gouy et al. 1985). Accession numbers and references are reported in Table 1.

Preliminary multiple alignments were obtained by using the PILEUP program (GCG 1993) and then carefully revised manually in order to maximize sequence similarity. In this way, we obtained multiple alignments showing an overall degree of similarity significantly higher than that provided by the computer program.

The PRETTYPLOT program was used to display boxed sequence alignments and to calculate consensus sequences. The output produced by PRETTYPLOT allows one to single out visually the regions common to all the compared sequences and those with a similarity common only to some of the compared sequences (GCG 1993).

Evolutionary Analyses. We have carried out evolutionary analyses on the aligned sequences by using the Stationary Markov Clock (SMC) model (Saccone et al. 1990), which allowed us to calculate pairwise genetic distances for the construction of phylogenetic trees and to estimate nucleotide substitution rates. The only basic prerequisite for the application of SMC is the stationarity in base composition—i.e., the sequences to be analyzed should have the same base composition at homologous sites within statistical fluctuations.

Only the multiple alignment gap-free sites were used in the evolutionary analysis.

The phylogenetic trees were obtained by using the genetic distances calculated by the SMC method and the programs NEIGHBOR (UPGMA method) and DRAWGRAM of the Phylip package (Felsenstein 1993).

Statistical significance of phylogenetic trees was calculated using the bootstrap resampling technique (100 replicates) with the program CONSENSE of the Phylip package (Felsenstein 1993).

Results

Yeast Nuclear RNase P RNAs

In yeast, the RNA has been demonstrated to be an essential component of the RNase P by using defective alleles. In Saccharomycetes the gene (RPR1) is organized into upstream, leader, mature, and downstream regions. It is a single-copy gene which is transcribed by RNA polymerase III in vivo starting from canonical promoters but located in the leader region upstream of the mature RNA structural domain (Tranguch and Engelke 1993). In Schizosaccharomycetes the nuclear RNase P copurifies with two RNA species (K1 and K2) differing only in length (285–270) and transcribed by the same single-copy gene (Zimmerly et al. 1990). The most recent hypothesis predicts that only one RNA, rather than one molecule of each RNA, is present per holoenzyme.

Figure 1 shows the multiple alignment of *Saccharomyces uvarum* (369 nt), *Saccharomyces diastaticus* (369 nt), *Saccharomyces cerevisiae* (369 nt), *Saccharomyces carlsbergensis* (359 nt), *Saccharomyces kluyveri* (336 nt), *Saccharomyces globosus* (339 nt), *Schizosaccharomyces pombe* (286 nt), and *Schizosaccharomyces octosporus* (285 nt) mature RNA domains (Table 1).

Among the Saccharomycetes, *Saccharomyces uvarum* and *Saccharomyces bayanus* (369 nt, not reported in the multiple alignment) sequences are identical and almost identical to those of *Saccharomyces diastaticus* and *Saccharomyces cerevisiae* (Fig. 2) whereas the *Saccharomyces globosus* sequence is the most divergent.

The genes from *Schizosaccharomyces malidevorans, Schizosaccharomyces japonicus,* and *Schizosaccharomyces versatilis* are identical to those of *Schizosaccharomyces pombe* (Zimmerly et al. 1990) and are not reported in the multiple alignment. *Schizosaccharomyces octosporus* differs from other *Schizosaccharomycetes;* the RNAs from Schizosaccharomycetes are shorter than those from Saccharomycetes and show limited similarity.

The phylogenetic tree in Fig. 2, constructed by using the UPGMA method on pairwise distances calculated with the Markov method, shows the Schizosaccharomycetes and the Saccharomycetes clustered into two different groups; thus, both are monophyletic. It is striking that the relationships represented in the tree coincide exactly

Fig. 1. Yeast nuclear RNase P RNAs. Multiple alignment of *Saccharomyces uvarum* (369 nt), *Saccharomyces diastaticus* (369 nt), *Saccharomyces cerevisiae* (369 nt), *Saccharomyces carlsbergensis* (359 nt), *Saccharomyces kluyveri* (336 nt), *Saccharomyces globosus* (339 nt) *Schizosaccharomyces pombe* (286 nt), and *Schizosaccharomces octosporus* (285 nt) mature RNA domains. *Saccharomyces bayanus,* which has identical sequences to *Saccharomyces uvarum,* and *Schizosaccharomyces malidevorans, Schizosaccharomyces japonicus,* and *Schizosaccharomyces versatilis,* identical to *Schizosaccharomyces pombe,* are not reported in the multiple alignment.

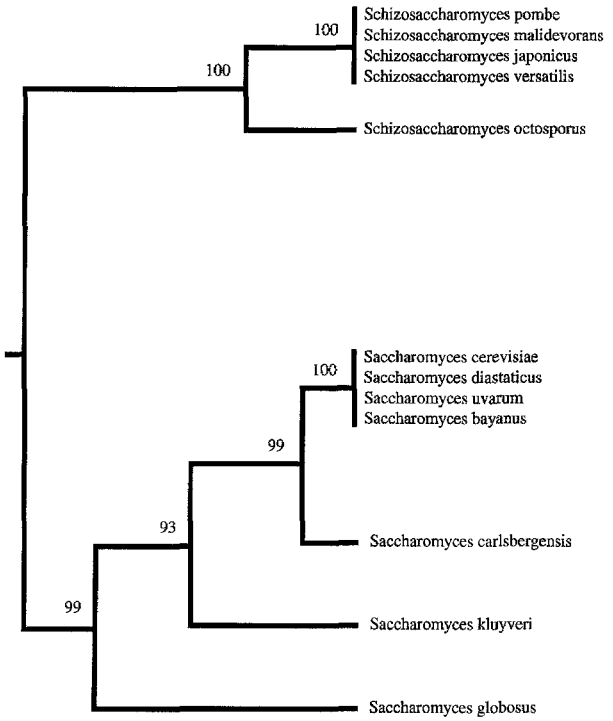

Fig. 2. Phylogenetic tree of yeast nuclear RNase P RNA. Bootstrap values are reported for each node of the tree.

with experimental data reported in other works. In particular, the different level of cross-hybridization strength (specificity) in Northern experiments between the RNA of *Saccharomyces cervisiae* and the RNA of other Saccharomycetes (Tranguch and Engelke 1993) reflects the distances of the species reported in our tree. Similarly in in vivo experiments, the differential ability of yeast heterologous RNase P RNA to complement disrupted *Saccharomyces cerevisiae* RNA genes (Pagàn-Ramos et al. 1994) correlates with our estimate of genetic distance.

Phylogenetic relationships in *Saccharomyces* and in *Schizosaccharomyces* have been previously inferred from comparison of RNase P RNA secondary structures, taking into account whether specific stem/loop elements are shared or not by sequences from different organisms. Indeed, it has been reported that, despite size heterogeneity, the RNA of *Schizosaccharomyces* and *Saccharomyces* can be folded into similar overall secondary structures (Tranguch and Engelke 1993).

Neither approach, however—the one based on hybridization and complementation tests or the other on the assessment of secondary structures—can give a measurement of the genetic distance between sequences. Our analysis reliably assesses quantitative phylogenetic relationships between species.

Yeast Mitochondrial RNase P RNAs

In yeasts, a second kind of RNase P activity was characterized in mitochondria. The RNA component (9S RNA) is coded for the organellar genome by a gene called "tRNA synthesis locus" (Miller and Martin 1983). The peculiar features of these RNAs are a high A + U content and extremely variable sequence size (from 144 to 477 nucleotides). It has been hypothesized that a minimal core structure shared by all homologous RNAs accounts for the activity (Wise and Martin 1991).

Figure 3 shows the best alignment of *Saccharomyces chevalieri* (398 nt), *Saccharomyces ellipsoideus* (477 nt), *Saccharomyces diastaticus* (416 nt), *Saccharomyces cerevisiae* (453 nt), *Saccharomyces douglasii* (356 nt), *Saccharomyces exiguus* (277 nt), *Torulopsis glabrata* (227 nt), and *Saccharomycopsis fibuligera* (144 nt) sequences (Table 1).

The variable length and the peculiar A + U–rich base composition make the alignments quite difficult. Two regions of similarity clearly evident and common to all yeasts and to *E. coli* RNase P RNAs represent the blocks involved in the long-range interaction (Forster and Altman 1990).

In the phylogenetic tree all *Saccharomyces* sequences cluster together and are outgrouped by *Torulopsis glabrata* (Fig. 4). *Saccharomyces chevalieri, Saccharomyces ellipsoideus, Saccharomyces diastaticus, Saccharomyces cerevisiae,* and *Saccharomyces douglasii* are very closely related.

A remarkable finding is the extreme variability in the nucleotide size of these RNAs even in closely related species. For example, *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus,* which have 98% nucleotide similarity, differ for the presence of insertions/deletions for a total of about 60 nucleotides. These data suggest that deletion and insertion of DNA sequences may occur very quickly, relatively speaking, in terms of evolutionary events.

It is peculiar that the insertions are GC rich. A GC-rich insertion is present in *Saccharomyces ellipsoideus* (83 nt), *Saccharomyces diastaticus* (30 nt), and *Saccharomyces cerevisiae* (38 nt), which are also very closely related in the phylogenetic tree. The GC stretch is flanked by a short repeated sequence AGUC also present in *Saccharomyces douglasii,* which seems to indicate that the insertion was gained prior to the ancestor of *Saccharomyces douglasii.*

Other RNase P activities have been identified in the organelles of higher eukaryotes. In spinach chloroplasts (Wang et al. 1988) and mammalian mitochondria (Doersen et al. 1985; Manam and Van Tuyle 1987; Rossmanith et al. 1995) no RNA component has been purified and no conclusive proof of RNA presence has been obtained; in potato mitochondria a small RNA fragment has been sequenced and suggested to be the RNA moiety of the plant mt RNase P (Marchfelder and Brennicke 1994).

Vertebrate Nuclear RNase P RNAs

In higher eukaryotes, RNA polymerase III is responsible for the transcription of the RNA component of the RNase

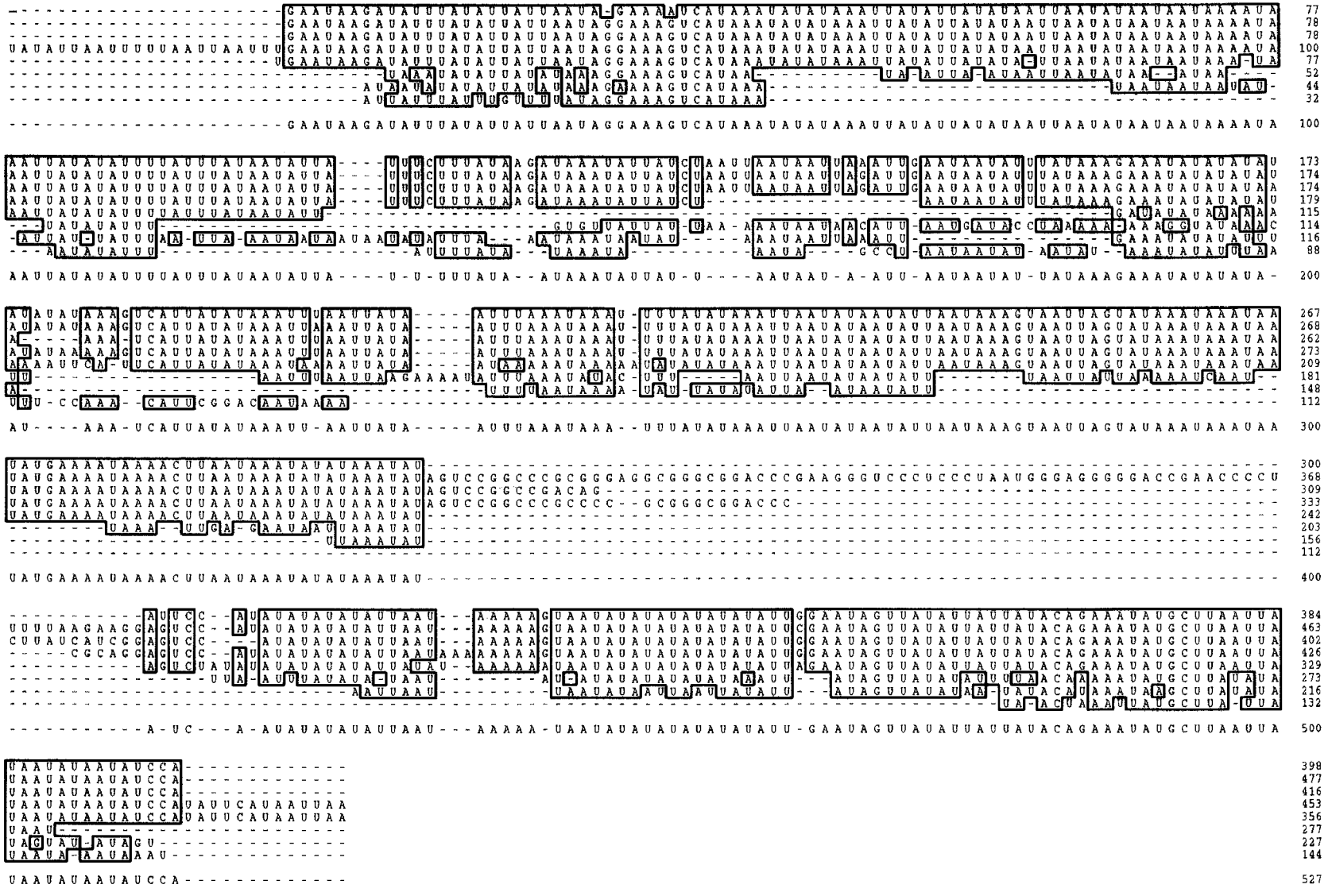

Fig. 3. Yeast mitochondrial RNase P RNAs. Multialignment of *Saccharomyces chevalieri* (398 nt). *Saccharomyces ellipsoideus* (477 nt), *Saccharomyces diastaticus* (416 nt), *Saccharomyces cerevisiae* (453 nt), *Saccharomyces douglasii* (356 nt), *Saccharomyces exiguus* (277 nt), *Torulopsis glabrata* (227 nt), and *Saccharomycopsis fibuligera* (144 nt) sequences. The GC-rich insertion, flanked by the AGUC repeat, is at position 302–384 in *Saccharomyces ellipsoideus.*

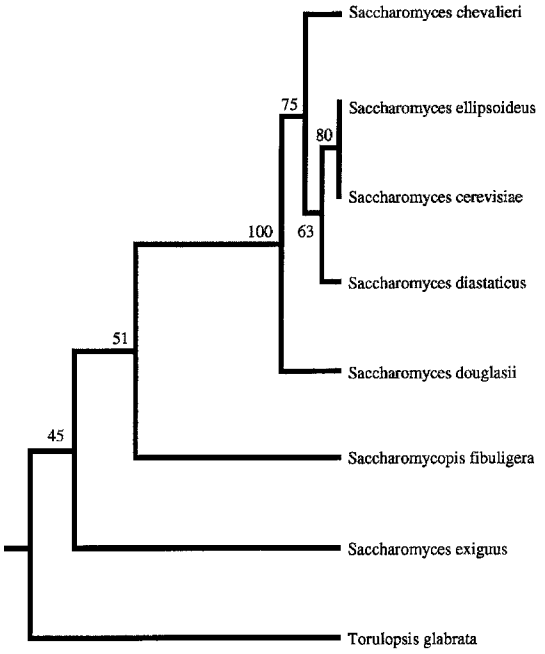

Fig. 4. Phylogenetic tree of yeast mitochondrial RNase P RNA. Bootstrap values are reported for each node of the tree.

P as well as for that of a number of small stable RNAs. The RNA polymerase III transcription unit appears to have different requirements with respect to their counterparts in yeasts. In human it has been reported that RNase P RNA (H1 RNA) synthesis does not depend upon internal sequence elements (Hannon et al. 1991).

Figure 5 reports the best alignment of rat (*Rattus norvegicus,* 257 nt), mouse (*Mus musculus,* 288 nt), gorilla (*Gorilla gorilla,* 320 nt), orangutan (*Pongo pigmaeus,* 320 nt), chimpanzee (*Pan troglodytes,* 320 nt), human (*Homo sapiens,* 340 nt), rhesus (*Macaca mulatta,* 320 nt), tarsier (*Tarsius syrichta,* 286 nt) and xenopus (*Xenopus laevis,* 319 nt) sequences (Table 1).

It is striking to note that the apes and rhesus (primates) have a higher sequence similarity with rodents (90% similarity) than with Tarsiidae primates (86% similarity). In addition, the tarsier sequence is not stationary in base composition with all other RNase P RNA sequences and thus has not been considered in the evolutionary analysis. Probably this gene is under a peculiar evolutionary pressure in this family.

The phylogenetic tree obtained by analyzing vertebrate RNase P RNAs fits well with the data obtained with other molecular markers (Benton 1990) (Fig. 6). Rodents and primates appear clustered into two groups with xenopus as the outgroup. The relationship between apes and rhesus cannot be solved as their sequences are almost identical. In this figure we also present a timescale calculated by fixing the divergence between primates and rodents at 80 million years (Mya)—the most faithful estimate obtained with fossil records (Benton 1990). The divergence time between mammals and amphibia is in the range 200–300 Mya and that between mouse and rat is in the range 20–30 Mya. These estimates agree well with other molecular and nonmolecular data (Janke et al. 1994).

RNase MRP RNAs

MRP RNA, also termed 7-2 RNA, is an uncapped RNA transcribed by RNA polymerase III both in vertebrates and in plant. The gene contains upstream promoter elements characteristic of gene encoding U-snRNAs.

The vertebrate (mouse, 275 nt; rat, 274 nt; cow, 277 nt; human, 265 nt; xenopus, 277 nt), the plant *Arabidopsis thaliana* (260 nt), and the yeast *Saccharomyces cerevisiae* (340 nt) sequences were compared (Table 1, Fig. 7).

Alignment of the vertebrate RNase MRP RNAs is straightforward, due to the high degree of similarity, whereas the plant and yeast sequences are highly divergent, thus making the alignment more difficult. The regions involved in the predicted long-range interaction are the most conserved.

In the alignment reported previously, the analysis is performed by maximizing the comparison of homologous structural elements (Schmitt et al. 1993).

In vertebrates and yeast the secondary structure is similar to that proposed for prokaryotic RNase P RNA. Plant RNA cannot be folded into a similar structure except for the cage-shaped model (Kiss et al. 1992).

The phylogenetic tree obtained by analyzing the vertebrate RNase MRP RNAs shows a close relationship between rat and mouse, and human and cow, with xenopus as the outgroup (Fig. 8). The tree has been calibrated by fixing the divergence between primates and rodents at 80 Mya. This tree agrees well with the most supported phylogeny for the triplet primates-artiodactyls-rodents, with primates more closely related to artiodactyls than to rodents (Janke et al. 1994). The distance measurements give results comparable to those obtained on vertebrate RNase P RNA (Fig. 6), showing once again a reliable clock-like behavior of these molecules.

Due to the high sequence divergence and to the differences in base composition, the sequences of *Arabidopsis thaliana* and *Saccharomyces cerevisiae* are not considered in the phylogenetic analysis.

The MRP and RNase P RNA nucleotide substitution rate has been compared to that of the nuclear and mitochondrial small ribosomal RNA subunit (SSU rRNA) (Table 2). RNase P and MRP RNAs evolve at a comparable rate, one order of magnitude faster than nuclear SSU rRNA and only half as fast as mitochondrial SSU rRNA. Due to their small size the nucleotide substitution rates have rather large fluctuations. However, the two RNA molecules display enough nucleotide variability to be used as phylogenetic markers (see Figs. 6, 8) within relatively short time spans when nuclear rRNAs, remaining practically invariant, are useless.

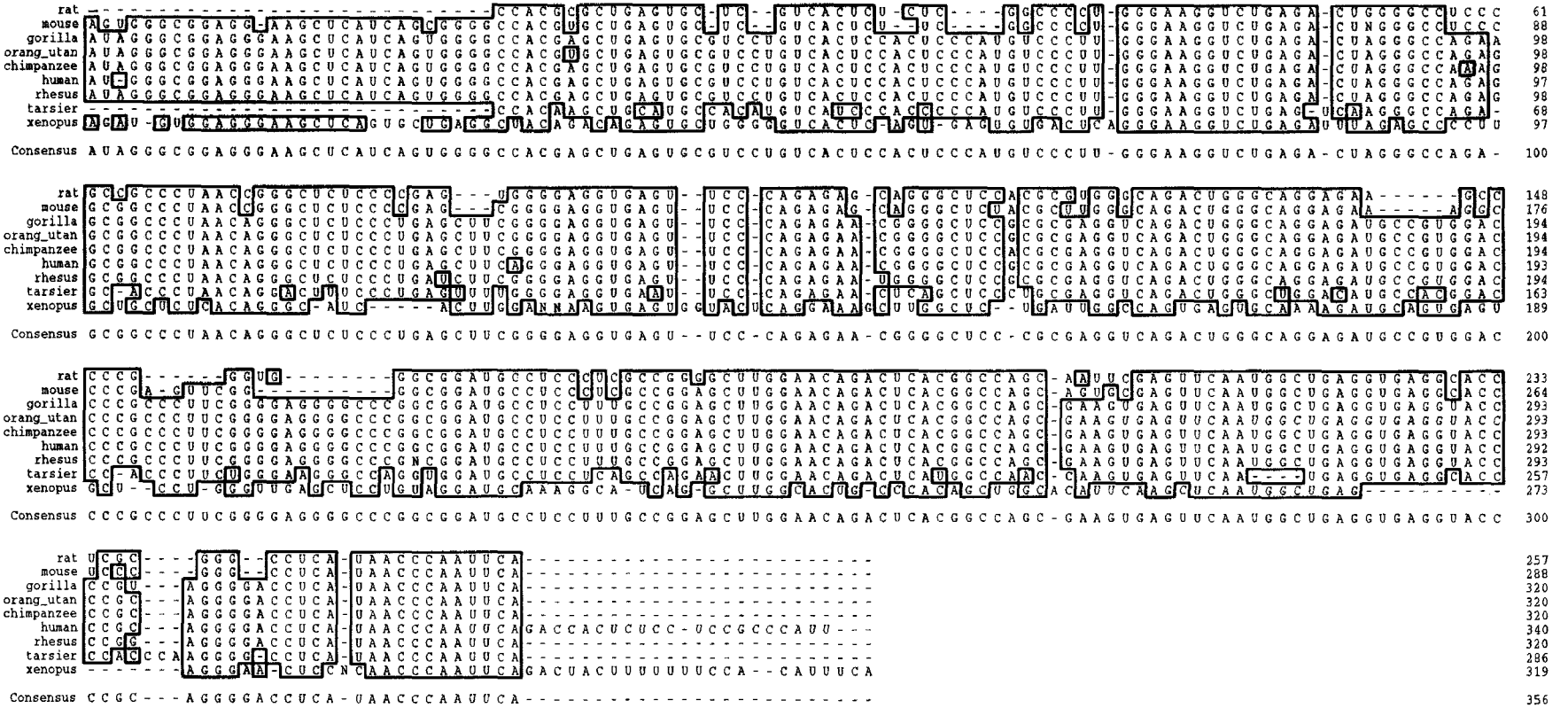

Fig. 5. Vertebrate nuclear RNase P RNAs. Multialignment of rat (257 nt), mouse (288 nt), gorilla (320 nt), orangutan (320 nt), chimpanzee (320 nt), human (340 nt), rhesus (320 nt), tarsier (286 nt), and xenopus (319 nt) sequences.

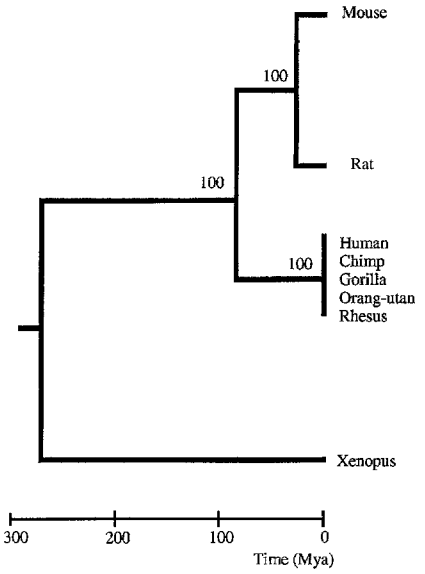

Fig. 6. Phylogenetic tree of vertebrate nuclear RNase P RNAs. Bootstrap values are reported for each node of the tree.

Discussion

The evolutionary studies on such ancient enzymes have focused the interest of several scientists for years. The first attempts at a phylogenetic analysis were based on the conservation of the secondary structures. Later, complementation studies confirmed the observed similarities experimentally. Yet neither approach can provide a quantitative measurement for genetic distance between sequences.

In this paper, we report a quantitative evolutionary analysis of the RNA species that are essential components of RNase MRP and RNase P ribonucleoproteins. Quantitative analysis defines to what extent the RNA components diverge across species and to what extent primary structural features were conserved. Our results may thus provide valuable information on the invariant sites of these RNA species and might be particularly useful in guiding site-directed mutagenesis experiments aimed at determining structure-function relationships in these molecules. In addition, the evolutionary analysis may be of great help in the understanding of the phylogeny of the organisms under consideration.

For the first time an optimal multiple alignment of the RNA sequences on the basis of their primary structure is presented. The alignments reported in previous studies, based on the common secondary structures, lose some common stretches if they belong to different structural domains (e.g., homologous sites located in stem regions can be found in loop regions and vice versa). This is particularly true for these RNA species, which experienced multiple insertion/deletion events during their evolution.

We have calculated the nucleotide substitution rate of RNase P and MRP RNA components and the results have been compared with those of the corresponding small rRNA sequences which are the most commonly used markers for phylogenetic inferences (Olsen and Woese 1993).

We have calculated that RNase P and MRP RNAs evolve one order of magnitude faster than the small rRNAs. It is well known that rRNAs cannot be used to depict the phylogenetic relationship between closely related organisms, e.g., mammals, as they do not show enough sequence divergence. On the other hand, RNase P and MRP RNAs seem to be accurate markers for reconstructing the relationship between vertebrate species. In our case, given a calibration time of 80 Mya between humans and rodents we obtain a divergence time for rat-mouse of 20–30 Mya and for mammals-xenopus of 200–300 Mya. It is well known that these datings are still a matter of discussion; however, they are widely supported by other molecular and nonmolecular data (Benton 1990). In order to test whether rodents have a nucleotide substitution rate higher than primates we applied the method of Muse and Weir (1992) using xenopus as the outgroup. The chi-square test (data not shown) gave no statistical support (5% confidence level) of a faster substitution rate for rodents than for humans in the case of either RNase P or MRP RNAs, thus supporting the molecular clock hypothesis. We can thus consider these RNA species as good phylogenetic markers both within mammals and between mammals and vertebrates, which could shed light on some controversial phylogenetic issues.

In yeasts, as no divergence time between any pair of the analyzed species is known from other sources, the times of divergence cannot be calculated. Yet the evolutionary analysis provides reliable data on the phylogenetic relationship between species since our results are in good agreement with experimental data (Tranguch and Engelke 1993; Pagàn-Ramos et al. 1994).

The correlation between yeast and vertebrate RNase P RNAs as well as with MRP RNAs, suggested in previous reports on the basis of their similar folding, cannot be established by our analysis, as their primary structures are highly divergent, and no significant sequence similarity can be assessed with the exception of short conserved sequence blocks involved in the cage interaction.

It has also been reported that RNase MRP and RNase P enzymes might derive from a common ancestor since they share common antigenic determinants and substrates (Potuschak et al. 1993; Morrissey and Tollervey 1995), and their RNA components can be folded into similar cage-shaped structures (Forster and Altman 1990). Also in this case this evidence cannot be inferred from our analysis due to high sequence divergence. It should be considered that the close relationship between the two enzymes might also be due to homologous pro-

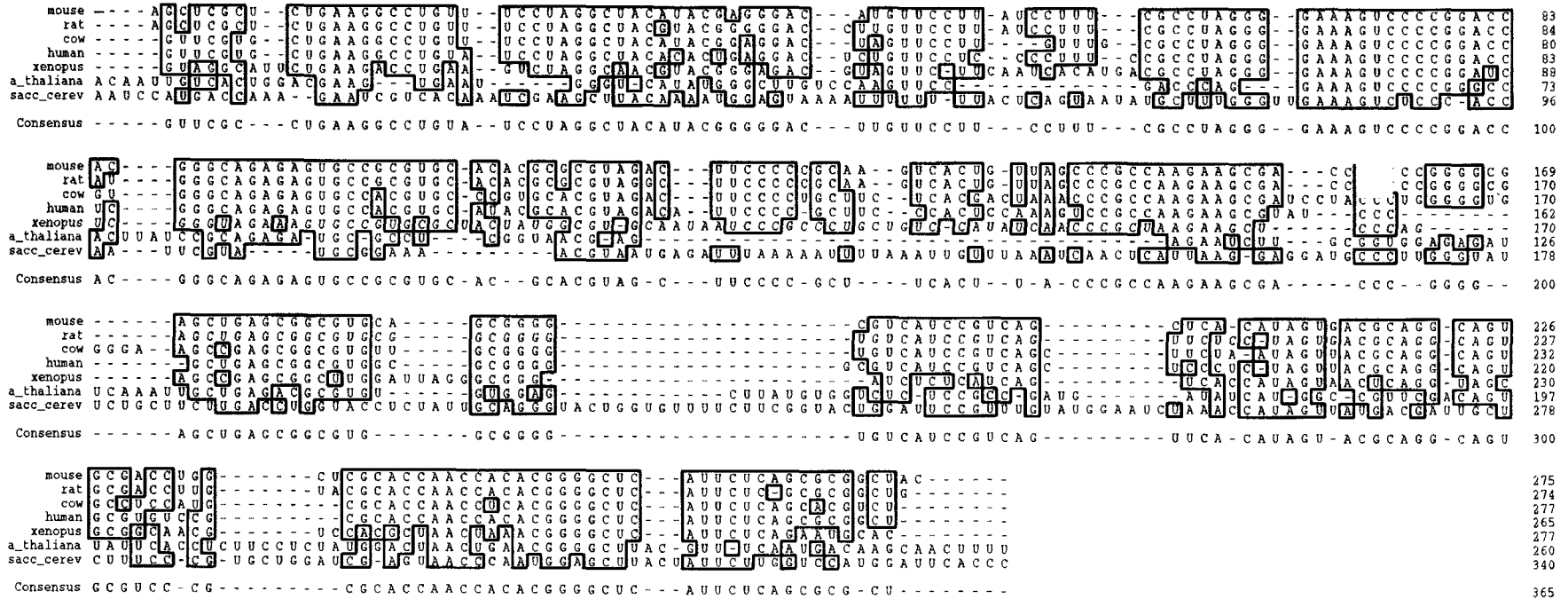

Fig. 7. RNase MRP RNAs. Multialignment of mouse (275 nt), rat (274 nt), cow (277 nt), human (265 nt), xenopus (277 nt), *Arabidopsis thaliana* (260 nt), and *Saccharomyces cerevisiae* (340 nt) sequences.

Table 2. Nucleotide substitution rate and percent nucleotide divergence of RNase P and MRP RNAs as compared to nuclear and mitochondrial (mt) SSU rRNA

Pair	Nucleotide substitution rate (substitutions/site) (% nucleotide divergence)			
	RNase P RNA	MRP RNA	mt SSU rRNA	SSU rRNA
Rat – mouse	0.037 ± 0.034 (2.8%)	0.039 ± 0.038 (3.5%)	0.076 ± 0.027 (7.1%)	0.003 ± 0.003 (0.3%)
Human – rodents	0.111 ± 0.075 (9.8%)	0.146 ± 0.098 (12.5%)	0.250 ± 0.065 (20.8%)	0.005 ± 0.003 (0.5%)
Mammals – xenopus	0.428 ± 0.215 (32.0%)	0.371 ± 0.233 (25.8%)	0.466 ± 0.114 (33.4%)	0.046 ± 0.014 (4.2%)
S. cerevisiae – S. pombe	0.373 ± 0.197 (27.3%)	—	0.730 ± 0.140 (44.0%)	0.098 ± 0.025 (7.4%)

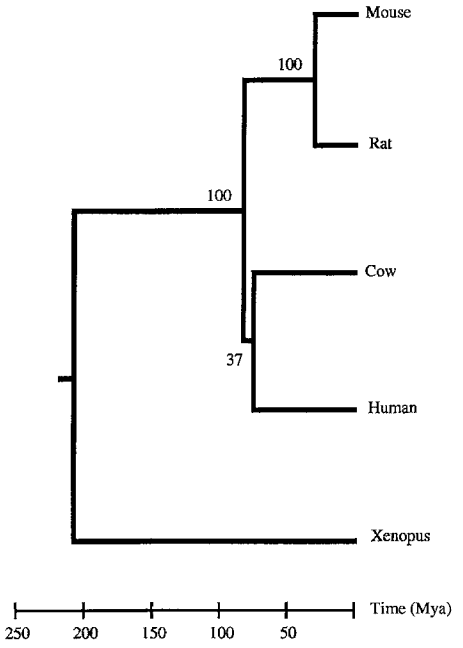

Fig. 8. Phylogenetic tree of RNase MRP RNAs. Bootstrap values are reported for each node of the tree.

tein components. Indeed, in yeast a gene has been characterized which encodes a protein component common to the RNase MRP and RNase P (Lygerou et al. 1994).

Additional RNA sequences will assist in the refinement of phylogenetic relationships. This will be beneficial to future investigations for defining elements critical to the functional role of these RNA species.

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