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SURVEY AND SUMMARY

Archaeal/Eukaryal RNase P: subunits, functions and RNA diversification

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ABSTRACT

RNase P, a catalytic ribonucleoprotein (RNP), is best known for its role in precursor tRNA processing. Recent discoveries have revealed that eukaryal RNase P is also required for transcription and processing of select non-coding RNAs, thus enmeshing RNase P in an intricate network of machineries required for gene expression. Moreover, the RNase P RNA seems to have been subject to gene duplication, selection and divergence to generate two new catalytic RNPs, RNase MRP and MRP-TERT, which perform novel functions encompassing cell cycle control and stem cell biology. We present new evidence and perspectives on the functional diversification of the RNase P RNA to highlight it as a paradigm for the evolutionary plasticity that underlies the extant broad repertoire of catalytic and unexpected regulatory roles played by RNA-driven RNPs.

ARCHAEAL VERSUS BACTERIAL RNase P: INCREASED PROTEIN COMPLEXITY IN PROKARYOTIC CATALYTIC RNPs

RNase P was first identified and characterized from bacteria as an endoribonuclease that cleaves the 5′-leader of precursor tRNAs (pre-tRNAs; 1,2). RNase P functions as a Mg^{2+}-dependent ribonucleoprotein (RNP) made up of an RNA (termed RPR) and a variable number of proteins (termed Rpps), depending on the organism. In both Gram-positive and -negative bacteria, RNase P is composed of an RPR and an Rpp (3–5). The RNA subunit of bacterial RNase P is the catalytic moiety of the enzyme (6), while the protein acts as an essential co-factor which enhances the RPR's affinity for substrate/catalytic metal ions, cleavage efficiency and fidelity (7–10). In addition to processing of precursor tRNAs, bacterial RNase P cleaves other RNA substrates, such as precursor 4.5S RNA, precursor tmRNA, polycistronic tRNA, mRNA transcripts and riboswitches (11–14). These processing activities highlight the ability of bacterial RNase P, a simple RNP, to recognize assorted RNA substrates and cleave them in a site-specific manner.

To appreciate the evolution of an RNA enzyme that accompanied recognition of seemingly unrelated substrates, it is instructive to discuss its structural attributes. Bacterial RPRs can be demarcated into two independently folded domains: a specificity domain with conserved nucleotides recognizing the T stem–loop of the pre-tRNA, and a catalytic domain that can cleave the pre-tRNA while binding to the 5′-leader sequence, acceptor stem and the 3′-RCCA sequence (15–19). The tertiary structure of two different bacterial RPRs, solved by X-ray crystallography, confirms this modular arrangement (20,21). Co-axially stacked helices, arranged in two one-helix-thick layers, are connected by local and long-range interactions to create a structure that includes a pre-tRNA-binding crevice (20,21). However, detailed insights into the catalytic mechanism of this RNA will have to await a high-resolution structure of the substrate-bound RPR with and without its protein co-factor.

The structure of the bacterial RPR allows speculation into the origin and evolution of this primordial biocatalyst. A hierarchical model for RPR evolution could be elaborated based on the structure and evolution of its substrates, and interactions with the protein co-factor. An *in vitro* selection study that simulated evolution of substrates for bacterial RPR in the presence and absence of its protein cofactor revealed that pre-tRNA-like substrates must have preceded precursor 4.5S RNA-like molecules, and that pre-tRNAs were likely substrates even when the RPR functioned independently of protein (11). If this were the case, then the most ancient RPR structural elements would be those implicated in recognition and cleavage of pre-tRNAs. Interestingly, tRNAs themselves are primordial molecules made up of two helical stacks that were presumably linked

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during evolution; the ancient helical module likely reflecting the self-replicating RNA from a putative RNA world and the anticodon-bearing module a subsequent addition coinciding with the advent of protein translation (22,23). Given the parallels mirrored in the modularity of the RPR enzyme and its pre-tRNA substrate, it is possible that the RPR's catalytic domain might have sufficed to recognize and cleave a simple mini/micro-helix as substrate (24,25), while its specificity domain was acquired later in evolution to accommodate the recognition of larger substrates with multiple functions, e.g. pre-tRNAs. Although there is support for this idea from a recent study that validated the antiquity of the C domain from an examination of rooted phylogenetic tress of RPR substructures (26), validation from high-resolution structures of the bacterial RPR bound to substrates of varying structural complexity would prove more persuasive. Overall, this notion is nevertheless consistent with the general theme that large catalytic RNAs are ensembles of RNA domains with specialized roles (27).

While acquisition of a new RNA module might have enhanced the versatility of an ancient but simpler RPR, association with a protein co-factor might not only have protected the RNA from degradation but also might have been necessary to overcome limitations imposed on the RPR's functional repertoire by the fundamental make-up of RNA. Given that tRNAs interact with the translational machinery and the ribosome, as well as various processing and modification enzymes, optimal recognition by such diverse catalysts would have helped fix various sequence and structure changes. Under such a scenario, the RPR's initial substrate recognition rules might have been violated at least in some instances. However, by virtue of new substrate–identity or enzyme–substrate complex determinants that are uniquely recognized by the protein co-factor, the RPR's substrate specificity would have been broadened since it would have become easier to maintain in a varied pool of RNA substrates a minimal suite of enzyme–substrate contacts required for efficient binding and cleavage by the holoenzyme (10,25,28,29).

In contrast to the single protein associated with bacterial RNase P, archaeal RNase P has five distinct protein subunits (30–32). These proteins are designated archaeal Rpp21, Rpp29, Rpp30, Pop5 and Rpp38/L7Ae (Figure 1). While some archaeal RPRs process pre-tRNAs *in vitro* in the absence of their cognate protein co-factors, others display such an activity only when the substrate is provided in *cis*, perhaps reflecting weakened substrate binding (33). These studies collectively demonstrate that the active site rests with the archaeal RPR, an observation also consistent with the fact that none of the recombinant archaeal Rpps (singly or in any combination) show any trace of activity (32,34,35). RNase P holoenzymes from different archaea have been successfully reconstituted using recombinant RPR and Rpps (31,32,34–37). In the case of *Pyrococcus furiosus* RNase P, there was no activation of the RPR when only one Rpp was added and among the six possible two-Rpp combinations, only two were active: Pop5+Rpp30 and Rpp21+Rpp29 (35). Structural and biochemical studies are beginning to furnish insights into the functional coordination between the RPR and Rpps (31,37–42). Footprinting studies reveal that Rpp21–Rpp29 contacts the specificity domain, while Pop5-Rpp30 recognizes the catalytic domain of their cognate RNA subunit (35,43). Consistent with this delineation of their respective RPR binding sites are the findings that Pop5-Rpp30 is solely responsible for increasing the RPR's rate of cleavage, while Rpp21–Rpp29 enhances affinity for the substrate (34,44).

The high protein:RNA molecular mass ratio (50:50) in archaeal RNase P, when compared with its bacterial counterpart (10:90), is quite intriguing in terms of subunit make-up given that we are dealing with unicellular prokaryotes in both cases. It is thus informative to examine possible reasons for this structural variation that might underlie functional disparity. First, the genome size and gene number in an archaeon can be smaller than that of a bacterium. Additionally, the overall organization of an archaeal genome is similar to that of bacteria, even though the fraction of the bacterial genes organized in operons can be higher (45). Second, genome-wide transcriptome analyses have revealed that gene expression in archaea is complex, albeit comparable to that of bacteria (44). While these data do not provide any reason *a priori* to suspect more substrates for archaeal RNase P compared to its bacterial cousin, it is possible that the protein-rich archaeal RNase P might either process new 'types' of RNA substrates with high fidelity or support non-processing functions. In this regard, identification of new substrates for archaeal (and eukaryal) RNase P will be instructive.

EUKARYAL RNase P: LARGE RNPs IN UNI- AND MULTI-CELLULAR HOSTS

Human nuclear RNase P possesses an RPR, termed H1 RNA, and at least 10 distinct Rpps (46–48). These protein subunits are designated Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38, Rpp40, Pop1 and Pop5 (Figure 1). It remains unknown if human RNase P in different cell types or tissues has either distinct combinations of these ten Rpps or even a diverse composition due to recruitment of new proteins (49). *Saccharomyces cerevisiae* nuclear RNase P also possesses nine distinct protein subunits, all of which, except one, share human homologs (50). Interestingly, despite significant variations in protein content, yeast nuclear and bacterial RNase P seem to be limited by product release and likely employ similar kinetic mechanisms (51). A high protein:RNA mass ratio has also been inferred for an RNase P partially purified from the slime mold *Dictyostelium discoideum* (52). The genome of this amoeba has at least eight candidate genes that code for Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp40, Pop1 and Pop5 (53). The conservation of eight Rpps even in unicellular organisms, i.e. yeast and amoeba, indicates that the increased protein content in eukaryal RNase P preceded the emergence of *bona fide* multi-cellular organisms.

The presence of Rpp21, Rpp29, Rpp30, Pop5 and Rpp38 from archaea to human (31,32,54,55) suggests

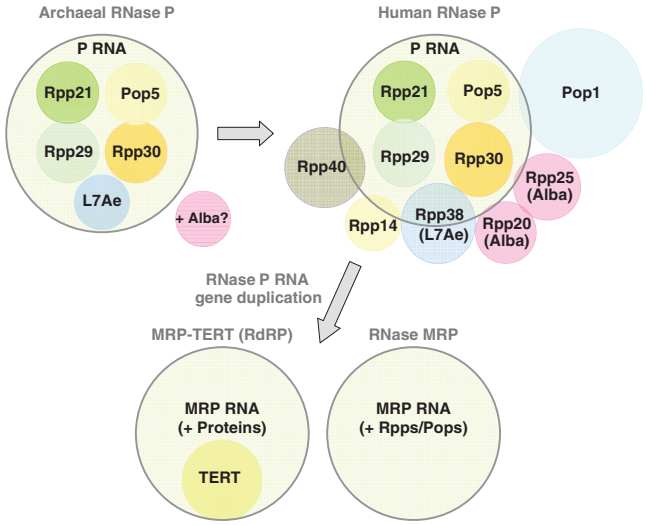

Figure 1. Conservation and evolution of the RNA and protein subunits of RNase P. Archaeal RNase P has five proteins: Rpp21, Rpp29, Rpp30, Pop5 and Rpp38 (L7Ae). The association of Alba with archaeal RNase P remains in question (see text). Human nuclear RNase P has ten distinct protein subunits, termed Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38, Rpp40, Pop1 and Pop5. Five of these subunits share archaeal homologs. Rpp20 and Rpp25 are homologous to Alba in archaea. In Eukarya, an ancestral RNase P RNA likely gave rise to RNase MRP RNA by a gene duplication mechanism; MRP RNA is part of RNase MRP and MRP-TERT (see text). Except for Rpp21, all the other proteins of human RNase P are shared by RNase MRP, but it remains unknown if these proteins are also associated with MRP-TERT. In *S. cerevisiae*, nucleolar RNase MRP possesses two additional proteins, termed RMP1 and SNM1 (see ref. 111 and references therein); it is unknown if these two specific subunits have homologs in human RNase MRP. The protein shapes are not drawn to scale.

that their respective RNase P holoenzymes share a common ancestor, consistent with the view that the first eukaryote evolved from archaea (56,57). This observation also supports the expectation that these proteins fulfill similar but essential functions. Indeed, human Rpp21 and Rpp29 are sufficient for reconstitution of the endonucleolytic, pre-tRNA processing activity of H1 RNA under reaction conditions of low divalent ion concentration and neutral pH (58; Reiner *et al.*, manuscript under revision); a similar finding was reported with the archaeal counterparts (34,35).

The subunits Rpp14, Rpp20, Rpp25, Rpp40 and Pop1 have no archaeal homologs as inferred from database mining and therefore appear to be unique to eukaryal RNase P (Figure 1; 54). Nonetheless, a native archaeal RNase P is yet to be characterized in terms of its full complement of Rpps and the absence of the five Eukarya-specific Rpps in archaeal RNase P does not imply their dispensability for eukaryal RNase P catalysis. By virtue of facilitating RNA folding (59), these accessory proteins might be vital for RNase P function in the cell. In fact, depletion of the homologs of these Rpps in *S. cerevisiae* caused inhibition of nuclear RNase P-mediated pre-tRNA processing and disappearance of the RPR, presumably due to structural destabilization (60).

The higher protein:RNA molecular mass ratio (70:30) in eukaryal RNase P compared to bacterial RNase P does not come at the expense of either the RPR's size or function. The lack of correlation between size and function of the telomerase RNA from different organisms has also been discussed while comparing the subunit composition of telomerase, another catalytic RNP (27,61). To illustrate for RNase P, the *S. cerevisiae* and human nuclear RPRs are larger than the 276-nt catalytic *Mycoplasma fermentans* RPR and are only moderately smaller than the 377-nt *Escherichia coli* RPR (62). In fact, the largest RPR described so far is that of *Candida glabrata*, which is 1149 nt in length (63). More importantly, despite the increased protein content in eukaryal RNase P, catalysis is retained by the RPR, which possesses a conserved catalytic core (64), recognizes pre-tRNAs (58,64) and can cleave tRNA substrates under certain *in vitro* conditions (65).

The increased number of Rpps implies a dynamic remodeling of the eukaryal RPR's structure to permit its assembly with these proteins. Consequently, the tertiary fold and function of the RPR might show an intimate dependence on the Rpps, not necessarily due to direct contributions of Rpps to RPR catalysis. Some bacterial RPR structural elements, essential for substrate binding, catalysis and global stability, were either never acquired or lost during evolution of archaeal/eukaryal RPRs accounting for their lower activity and stability in the absence of cognate Rpps; it is likely that RNA–protein interactions in archaeal and eukaryal RNase P have replaced the molecular struts comprised of RNA–RNA interactions in the bacterial RPR (66,67). On the other hand, the overall increase in the number of Rpps, particularly those unique to Eukarya, might not be necessarily related to catalytic roles but rather reflect the acquisition of new functions and coordination with other molecular machines. This new and broader perspective is motivated by studies that have revealed unforeseen functions of RNase P in the nucleus (see below).

NEW FUNCTIONS FOR EUKARYAL RNase P

Recent discoveries have unveiled an unexpected role for human RNase P in transcription of small non-coding RNA genes (ncRNA) by RNA polymerase III (Pol III) and in efficient transcription of rRNA genes by Pol I (68–70). NcRNA genes, such as those that code for rRNA, 5S rRNA, tRNA and 7SL RNA, are essential for protein translation and cell growth. Another surprising discovery relates to the observations that long ncRNAs, such as the metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) and multiple endocrine neoplasia β (MEN-β) transcript, are processed by human and mouse RNase P (71,72). These transcripts are cleaved at pre-tRNA-like structures located upstream of their 3′-end cleavage/polyadenylation signals (71,72). The 3′-end cleavages of mRNA and snRNA transcripts are typically carried out by specific endoribonucleases that exist in multiprotein complexes, i.e. CPSF/CstF and Integrator, respectively (73,74). These latter complexes associate with chromatin and operate cotranscriptionally through their recruitment by Pol II (73,74). Therefore, it

will be interesting to examine if RNase P, an endoribonuclease, acts as part of larger complexes to process the MALAT1 and MEN β transcripts, and if Pol II recruits RNase P and renders it a chromatin-associated RNP (see below).

Human RNase P exerts its role in transcription through association with chromatin of transcriptionally active rRNA, 5S rRNA and tRNA genes (68–70). This association is dependent on the cell cycle, in that cessation of transcription in mitosis results in disengagement of the Rpps from rRNA, tRNA and 5S rRNA genes (70). The reassociation of Rpps with these target genes after exit of cells from mitosis and entry to G1 is not concurrent, supporting the idea that RNase P is not recruited to chromatin as a fully pre-assembled complex (70).

Either catalytic or structural roles for RNase P in Pol III transcription could have important implications for understanding the coordination between transcription and processing. For instance, RNase P can act solely as a transcription factor that aids transcription of 5S rRNA and as both a transcription factor and endoribonuclease in the case of transcription and processing of pre-tRNAs (Figure 2). Furthermore, by binding to chromatin, RNase P marks its genetic locus and it has been proposed that this RNP may demarcate euchromatin boundaries (75). It has also been postulated that maintenance of heterochromatin loci might be related to RNase P processing a non-coding RNA and generating a product, which in turn is converted by the RNAi machinery to small RNAs that silence their corresponding loci (75). Such possibilities, if proven, will broaden the functional scope of RNase P to encompass epigenetic regulation.

The requirement for the human RPR (H1 RNA) in Pol I and Pol III transcription has been established in an *in vitro* transcription system (68,70; Dehtiar *et al.*, manuscript in preparation). This role evokes the question if H1 RNA has any element or domain specialized in transcription. Comparing the secondary structure of H1 RNA with its bacterial counterparts reveals that it has unique or 'expansion segments' in the paired regions P3 and P12 (76), which might have evolved to fulfill specific assignments

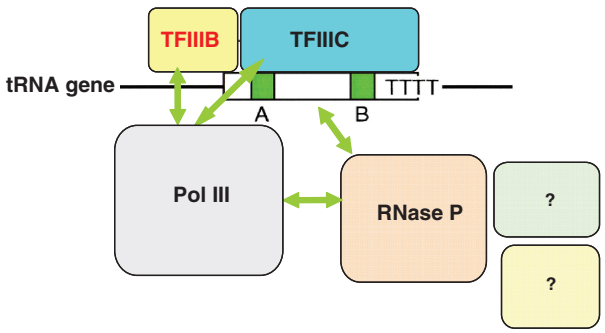

Figure 2. A model for association of human RNase P with Pol III and tRNA genes. Schematic of a tRNA gene with conserved internal boxes A and B regulatory elements bound by the transcription factors TFIIIC and TFIIIB. These two transcription factors recruit Pol III. Human nuclear RNase P associates with Pol III and with the tRNA gene (69). This may facilitate coordination of transcription and tRNA processing, and involve other tRNA processing and modifying activities (indicated by question marks).

other than pre-tRNA processing. The P3 domain in eukaryal RNase P RNAs differs from its prokaryotic relatives and it serves as a binding site for Rpp20 and Rpp25 (77,78), two proteins which associate with chromatin of target genes (70). In support of a possible role of the P12 domain in transcription, it has been shown that targeted cleavage of P12 in H1 RNA by RNase H abolished Pol III transcription in whole-cell extracts; this cleavage also altered RNase P activity, as manifested in the aberrant cleavage of a pre-tRNA (68).

Overexpression of the *S. cerevisiae* nuclear RNase P RNA, RPR1, suppresses a slow-growing strain with a deletion mutation in Bdp1, a subunit of the transcription factor TFIIIB of Pol III (79). Maturation of tRNA is aberrant in this mutant strain. Although RPR1 interacts with Bdp1p, as judged from co-immunoprecipitation and pull-down assays (79), it is unclear if the interaction of RNase P with Bdp1p (as part of the Pol III holoenzyme) is critical for the association of RNase P with the chromatin of target genes in yeast.

The participation of the RNA moiety of eukaryal RNase P in Pol I/Pol III transcription and in processing of short/long non-coding RNAs indicates that its original functions have been greatly expanded. Thus, the RPR is neither fading out nor are its functions being substituted by proteins. On the contrary, this RNA seems to have been selected by evolution for gene duplication to promote further functional diversification (see below).

RNase P, ALBA-LIKE PROTEINS AND THE CHROMATIN CONNECTION

As mentioned earlier, human RNase P associates with the chromatin of rRNA, 5S rRNA and tRNA genes (Figure 2). Two protein subunits, Rpp20 and Rpp25, are of special interest in this context. Rpp20 and Rpp25 form heterodimers and belong to the Alba-like superfamily of proteins which were predicted to bind to both DNA and RNA as part of RNPs (80). Alba was first identified in *Sulfolobus solfataricus*, an archaeon, which encodes two related Alba proteins, Alba 1 and Alba 2 (81). Like histones, Alba (or Sso10b) is one of the major proteins that forms chromatin in thermophiles and hyperthermophiles of archaea (81). Alba is not associated with RNase P partially purified from *Methanothermobacter thermautotrophicus* (82). In contrast, Alba was shown to interact weakly with the *P. horikoshii* OT3 RPR but had little or no influence on the pre-tRNA processing activity of reconstituted *P. horikoshii* RNase P (83). Perhaps, it is worth re-evaluating the possibility that Alba is part of the RNase P holoenzyme in some archaea, and that in such cases the RNP performs a distinct chromatin-associated function. Such a premise is underscored by the fact that eukaryal RNase P has the two Alba-like protein subunits Rpp20 and Rpp25 as permanent and functional subunits (Figure 1; see below).

Rpp20 and Rpp25 are associated with chromatin of transcriptionally active rRNA genes and small ncRNA genes, i.e. tRNA and 5S rRNA genes (68,70). Strikingly, while Rpp20 binds to rRNA genes at early G1 phase after

exit of cells from mitosis, Rpp25 occupies these gene repeats only at late G1 (or S phase), in which transcription by Pol I and Pol III is increasing, thus implicating the latter subunit in transcriptional activation (70). Hence, Rpp20 and Rpp25 can bind separately to chromatin of target genes, a finding that is inconsistent with their ability to bind to RNA only as heterodimers (67,77,84). Pop6 and Pop7, the yeast homologs of Rpp25 and Rpp20, also bind to the P3 domain of *S. cerevisiae* nuclear RNase P RNA only in the form of heterodimers (85). However, our recent findings show that recombinant Rpp20 and Rpp25 can bind to intact H1 RNA independently of each other but with moderate binding affinities (Reiner *et al*., under revision). The dual capability of Rpp20 and Rpp25 to bind to DNA and RNA suggests that these proteins might take part in directing RNase P to chromatin of target genes. Because the genes thus far identified as bound by RNase P have no common sequences, it seems likely that Rpp20 and Rpp25 (as part of RNase P) might recognize generic structural features in chromatin, an idea supported by the fact that their homolog Alba is a non-specific DNA-binding protein.

The above findings on the role of RNase P in transcription and its association with chromatin share an interesting parallel in the growing appreciation of a crosstalk between mRNA splicing, transcription and chromatin organization. Variability in the rate of transcription, nucleosome positioning and histone modifications have recently been identified as novel regulatory mechanisms for fine-tuning splicing (86). Whether chromatin (transcription)-related attributes influence the rate and scope of processing by RNase P remain to be determined.

FUNCTIONAL H1 RNA AND MRP RNAs IN THE CYTOPLASM?

H1 RNA (human RPR) is present at ~50 000 copies per cell, while there are ~30 000 copies of MRP RNA (87). These two transcripts can be detected in the nucleus as well as in the cytoplasm of cells, with the bulk of H1 RNA in the latter (88,89). Although at least one form of human mitochondrial RNase P activity includes an RNP comprising H1 RNA (87,90), the entire cytoplasmic H1 RNA is likely not reflecting the pool *en route* to mitochondria. It remains unknown if H1 RNA associates with its cognate protein subunits in the cytoplasm to form a RNP complex before being transported to the nucleus, as is the case with the spliceosomal U snRNPs. Therefore, the functional implications for the existence of H1 RNA in the cytoplasm remain unclear. Nevertheless, recent findings reveal that an active form of Pol III exists in the cytoplasm (91,92). Pol III senses foreign DNA in the cytoplasm and uses it as a template to synthesize 5′ppp-RNA that activates type I interferon production and mount an innate immune response (91,92). Since H1 RNA is required for Pol III transcription in the nucleus (68,70), it is worth testing if H1 RNA (free or as part of an RNP) is required for the biogenesis of cytoplasmic Pol III products generated using either poly dA-dT or viral DNA as templates.

Computer-aided comparative searches of databases for putative RPRs revealed a camelpox virus gene candidate, which exhibits ~98% homology with all other orthopoxviruses and encodes an RNA with some secondary structure similarity to bacterial/archaeal RPRs (93); but this RNase P-like viral RNA did not show any pre-tRNA processing activity when tested *in vitro*. While this viral transcript is expressed in infected HeLa cells, it reduced by only 20% the pre-tRNA processing activity of endogenous RNase P, thus ruling out its ability to sequester human Rpps *in vivo* (93). However, it is unclear if such a modest effect on RNase P activity might somehow translate into viral replication gains. Nonetheless, the camelpox virus RNase P-like RNA might be used to interfere with Pol III transcription. If Pol III is employed as a sensor of viral DNA, then it is possible that Pol III is targeted by viral RNase P-like RNA decoys to counter the antiviral response mechanism of the host. Therefore, an experiment to assess if the camelpox virus RNase P-like RNA can inhibit Pol III transcription in extracts or cells merits consideration.

EVOLUTION AND FUNCTIONAL DIVERSIFICATION VIA RNA

The eukaryotic genome is pervasively transcribed for the production of short and long ncRNAs (94,95). Genomic means for the invention of new regulatory and functional RNAs include DNA- and RNA-mediated mechanisms (96). Diversification of RNA function through gene duplication is one such DNA-mediated mechanism (96). Gene duplication is a key path to the evolution of new biological functions, a phenomenon called neo-functionalization, since one or more of the new gene copies would be liberated from selection placed on the original copy and would have the chance to develop a new function (56,97). It is widely accepted that an ancestral RNase P RNA gave rise to RNase MRP RNA (Figure 1) via gene duplication in eukaryotes (98,99), although some other less likely alternatives have been considered (98). The RNA moiety of RNase MRP is related in sequence and structure to the RNase P RNA (100). Like RNase P, RNase MRP acts as an endoribonuclease but with altered specificity in processing of rRNA (100,101). This nucleolar RNase MRP shares most of its protein subunits with RNase P (100), even though the specificity and function of its catalytic RNA moiety have diverged in evolution. In yeast, RNase MRP cleaves the 5′-UTR of the B-type cyclin (CLB2) mRNA, and triggers rapid degradation of this mRNA by the 5′ to 3′ exoribonuclease Xrn1p (102). Since ubiquitination-mediated CLB2 protein degradation by the anaphase promoting complex is critical for progression of cells to anaphase, cleavage of the CLB2 mRNA by RNase MRP, which occurs in cytoplasmic specialized TAM (temporal asymmetric MRP) bodies is vital for the completion of mitosis (102).

A recent discovery uncovered the association of human RNase MRP RNA with the telomerase reverse transcriptase (TERT) to form an RNA-dependent RNA polymerase (103). TERT utilizes the MRP RNA as template to

generate dsRNA, which is then processed in a Dicer-dependent manner to generate small interfering RNAs that silence the expression of the RMRP gene, which codes for the MRP RNA (103). RMRP-TERT does not affect the expression of the human RPPH gene, which codes for H1 RNA, but it remains unknown if this silencing complex exerts its influence on the expression of other genes and if it possesses non-TERT protein components, especially ones shared with RNase P/MRP (Figure 1).

The existence of the three RNP complexes with distinct functions (as described above) raises the possibility that evolution selected the RNA subunit of RNase P for gene duplication, thus providing further functional diversification. In support of this neo-functionalization mechanism, we report here that the RPPH and RMRP genes are duplicated in the human genome. Thus, alignment of the human genome sequence with itself using the UCSC genomic browser reveals that the RPPH gene, which is located in chromosome 14 (position chr14:19881035–19881410), has an RNase P RNA-like gene that resides in chromosome 4 (position chr4:158126939–158127302; reverse strand; Figure 3). In contrast to the RPPH gene that is highly conserved in the genomes of mammals and vertebrates (Figure 3A), the RNase P RNA-like gene could be detected in genomes of primates and some other mammals (e.g. cow and squirrel; Figure 3B; also, data not shown). This copy of the human RNase P RNA-like gene exhibits 76% sequence identity with the parental version (Supplementary Figure S1). Regulatory sequences, such as the TATA box and proximal sequence element (PSE), could be located in this duplicated gene (Supplementary Figure S1). Preliminary results from ChIP analysis revealed that Rpp21, Rpp25 and RPB8, a core protein subunit of Pol I/II/III, bind to the RNase P RNA-like gene in a specific human cell line, while the unique subunits of Pol III, e.g. RPC6 and RPC7, produced weaker binding signals (Serruya,R. and N.J., unpublished data).

Strikingly, the human RMRP gene, which is located in chromosome 9 (UCSC chr9:35647746–35648012), also has an RNase MRP RNA-like gene, positioned in the same chromosome but at a different genetic locus (UCSC chr9:24895469–24895738; Supplementary Figure S2). Alignment of the original RMRP gene with RNase MRP RNA-like gene reveals 70% sequence identity (Supplementary Figure S2) with an unambiguous conservation in the genomes of primates and other mammals (Supplementary Figure S2; also data not shown). The transcription of this gene would result in the generation of a putative transcript of 267 nt in length, which is similar to the bona fide 265-nt RNase MRP RNA. Of note, it has been shown that the mouse has three genes homologous to its RNase P RNA gene, RPPH1, but the predicted

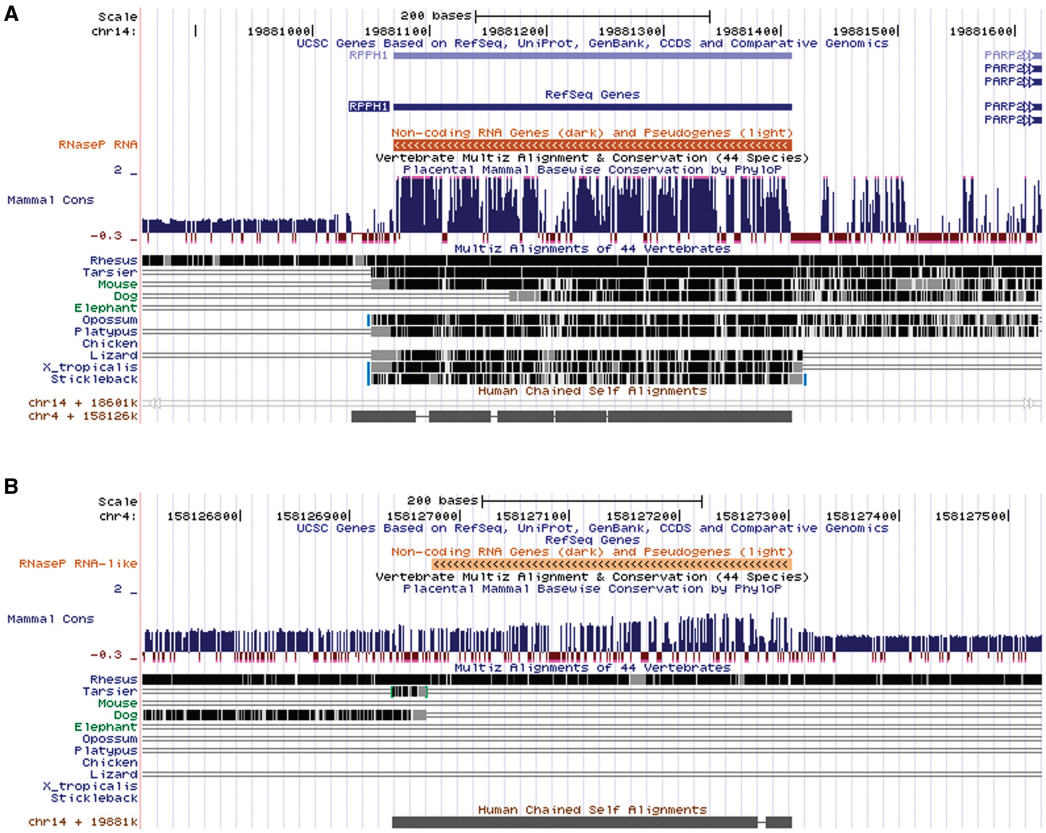

Figure 3. An RNase P RNA-like gene exists in the human genome. (**A**) Profile generated by the UCSC Genome Browser software showing the conservation from primates to fish (stickleback) of the human RPPH gene (located in chromosome 14). Human chained self-alignment reveals the existence of an RNase P RNA-like gene in chromosome 4 (last stripe). (**B**) In contrast to the RPPH gene, the RNase P RNA-like gene could be computationally detected only in Rhesus.

transcripts are almost identical in sequence with the RPPH1 transcript and are shorter as a result of premature transcriptional termination (104). Moreover, duplication of RNase P RNA is also found in zebrafish, and appears to be distinct from the whole genome duplication this event suspected to have taken place in the zebrafish lineage before the teleost radiation (105).

The preservation of duplicates for the RPPH and RMRP genes suggests that they might have exapted functions in select organisms. While our preliminary ChIP data suggest transcription of these genes in certain types of human cell lines, we are now investigating if Pol III recognizes these gene copies as independent transcription loci and generates corresponding transcripts. Since RNAs derived from duplicated genes can regulate gene expression (106), we will examine if such putative products from the RPPH- and RMRP RNA-like genes could affect expression of the parental copies. Moreover, co-variation and comparative structural analyses of these genes with their parental ones will be performed once we confirm the expression and size of their transcripts in cells.

While gene duplication might be partly instrumental for diversification of catalytic RNA functions, an equally important factor was likely the association of these RNAs with protein co-factors, whose origins sometimes reveal valuable insights including why they were selected. Rpp20 and Rpp25, two protein subunits of RNase P/MRP, are homologs of Alba, a DNA-binding protein, providing a functional and evolutionary link between eukaryal RNase P and chromatin organization. Similarly, although eukaryal RNase P does not have ribosomal protein L7Ae, recently validated as a subunit of archaeal RNase P (32), it has an L7Ae homolog called Rpp38. Replacement of L7Ae with a homolog has also occurred in eukaryotic snoRNPs. While the presence of L7Ae in the ribosome, snoRNPs and RNase P might present opportunities (yet to be deciphered) for coordinate regulation of different translation-associated machineries in archaea, the presence of distinct L7Ae homologs in orthologous eukaryal RNPs might reflect both the need for finer regulation and for specialized protein co-factors that helped broaden the functional repertoire of RNA catalysts.

PROSPECTS

RNase P is best known for its activity as an RNA enzyme with endoribonucleolytic activity. Recent findings, however, implicate this RNP complex in transcription and processing of short and long ncRNAs. Although one might argue that the scope of these recent assignments remain to be broadly investigated, we decided to emphasize these new facets of RNase P because these functional roles shed a different light on this primordial RNP. The exact contribution of the RNA and protein subunits of RNase P to each of these latter assignments remains to be determined. Nonetheless, the functions of the RNA moiety have been elaborated in the nucleus (68,70–72). Both the RPR and Rpps should therefore be seen from an inclusive perspective in which these subunits can fulfill one or more roles in large RNP complexes. For example, a form of nuclear RNase P is associated with the transcription machinery and with chromatin of target genes (Figure 2; 107). However, the RNase P-interacting partners relevant to this functional linkage remain to be identified. Moreover, the exact role of RNase P in the Pol III transcription cycle needs to be determined (Dehtiar *et al.*, manuscript submitted for publication).

Evolution has used the RNA moiety of RNase P for functional diversification; this is not surprising given that the active site resides in the RNA, even though the RNase P RNP includes ten proteins. Nevertheless, recent findings of a protein-only RNase P activity in human mitochondria and in *Arabidopsis* mitochondria and plastids (108,109) point to a far more complicated evolutionary scenario for RNase P activity and 5′-end processing of tRNA. Although Arabidopsis organellar RNase P activity can functionally substitute for its RNP cousin in *E. coli* (109), these organellar variants are unrelated to the proteins present in the RNP forms of bacterial, archaeal and eukaryotic (nuclear) RNase P. It remains to be determined if the ancestral RNA-containing RNase P has been replaced by a protein-based catalyst or whether the two forms evolved independently to fulfill different functions. Regardless, the existence of proteinaceous RNase P activity does not serve as a paradigm for the evolution of an RNP to a protein enzyme, a transition perhaps best exemplified by the signal recognition particle in chloroplasts (110). The functional diversity of RNase P, however, highlights the existence of multiple routes for generating similar active sites from different subunit compositions.

The emergence of RNase MRP and MRP-TERT from RNase P provides a unique paradigm for an RNA-based evolutionary mechanism that facilitated the recruitment of pre-existing protein cofactors to support new functions. One could argue that MRP-TERT is distinct from RNases P and MRP in that it is not an RNA-processing enzyme; nevertheless, it is an illustration of adapting a cellular RNA for a new function by virtue of altering protein partners. Another example is found in yeast nucleolar and mitochondrial RNase MRP, which share RNA but not protein subunits (111).

As RNase P, MRP and MRP-TERT are three catalytic RNPs which share sequence-related RNAs and/or protein subunits, one might expect that their expression, function and evolution are interconnected. In fact, Maida *et al.* (104) demonstrated an inverse correlation between MRP-TERT and RNase MRP, a relationship that is based on the role of MRP-TERT in silencing RNase MRP. Since TERT is activated by insertion of retroviruses in its promoter/enhancer (112), the down-regulation of MRP in the pathobiology of cancer merits scrutiny. Mutations in telomerase are found in patients with dyskeratosis congenita while mutations in the RNase MRP RNA are associated with cartilage hair hypoplasia (113); since both these diseases result from stem cell failure, it is worth investigating how RNase P, RNase MRP and/or MRP-TERT contribute to stem cell function. Moreover, a recent study points to a potential role for a regulated Rpp transcript in the neurobiology of autism, a developmental brain disorder (114).

Finally, the existence of RNase P RNA- and RNase MRP RNA-like genes in the human genome (Figures 3) adds a new layer to the regulation, function and evolution of RNase P and its related RNPs.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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