

# Human RNase P: a tRNA-processing enzyme and transcription factor

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## ABSTRACT

**Ribonuclease P (RNase P) has been hitherto well known as a catalytic ribonucleoprotein that processes the 5' leader sequence of precursor tRNA. Recent studies, however, reveal a new role for nuclear forms of RNase P in the transcription of tRNA genes by RNA polymerase (pol) III, thus linking transcription with processing in the regulation of tRNA gene expression. However, RNase P is also essential for the transcription of other small non-coding RNA genes, whose precursor transcripts are not recognized as substrates for this holoenzyme. Accordingly, RNase P can act solely as a transcription factor for pol III, a role that seems to be conserved in eukarya.**

## NUCLEAR RNase P: AN ENSEMBLE OF RNA WITH HIGHLY CONSERVED PROTEINS

Ribonuclease P (RNase P) was originally described as an endoribonuclease that processes the 5' leader sequence of precursor tRNA (1). In bacteria, RNase P is a small ribonucleoprotein complex (2), consisting of a catalytic RNA and a protein cofactor (3,4). The crystal structures of bacterial RNase P RNAs have recently been resolved, revealing the locations of the substrate-binding domains and the active sites in these RNA enzymes (5–7). By contrast, when compared with their bacterial counterparts, nuclear forms of eukaryal RNase P are large ribonucleoprotein complexes (8–10). Biochemical purification analyses have shown that a highly purified nuclear RNase P from HeLa cells has at least 10 distinct protein subunits associated with a single RNA species, H1 RNA (8,10). These protein subunits are termed Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38, Rpp40, hPop1 and hPop5 (Table 1) (8,10–13). Since this purified HeLa RNase P has been enzymatically defined by virtue of its ability to cleave precursor tRNA *in vitro* (8,10), the potential existence of other forms of RNase P complexes with different subunit compositions is not excluded.

Similarly, *Saccharomyces cerevisiae* nuclear RNase P possesses nine protein subunits, designated Pop1p, Pop3p, Pop4p, Pop5p, Pop6p, Pop7p/Rpp2p, Pop8p, Rpp1p and Rpr2p (14), most of which are homologous to protein subunits of human RNase P (Table 1) (14,15). In addition, these protein subunits are shared with RNase MRP (14–17), a mitochondrial and ribosomal RNA-processing ribonucleoprotein (18). However, it is not known if these protein subunits are shared with the mitochondrial form of human RNase P, a ribonucleoprotein particle shown to have an RNA moiety that is identical to H1 RNA (19).

Rpp21, Rpp29, Rpp30, Rpp38 and hPop5 are highly conserved proteins that have homologs in Archaea (Table 1) (20–23). Rpp21, Rpp29 and H1 RNA are sufficient for reconstitution of RNase P activity in tRNA processing *in vitro* (24). The archaeal Rpp21, Rpp29, Pop5, Rpp30 and Rpp38 proteins are required for efficient reconstitution of thermostable RNase P ribonucleoprotein (23,25–27). Nonetheless, recent findings reveal that protein subunits of *Pyrococcus horikoshii* OT3 RNase P are individually dispensable for enzyme activity *in vitro* (26). In the case of the *P. furiosus* RNase P, it has been shown that pairs of its protein subunits are sufficient for reconstitution of enzyme activity (27). Thus, archaeal Rpp21 with Rpp29 or Pop5 with Rpp30 are sufficient for RNase P RNA-based catalysis (27). These pairs of archaeal proteins interact with each other in two-hybrid system (28). The reconstitution studies described above underline the conserved role of archaeal and eukaryal RNase P RNAs in substrate recognition and cleavage. Recent progress in modeling the tertiary folding of eukaryal RNase P RNA uncovers that it has a conserved core structure similar to that of its bacterial counterpart (29). Mutations that disrupt the predicted tertiary folding of H1 RNA abolish catalysis *in vitro* (24). Remarkably, Kikovska et al., has shown that H1 RNA is active in tRNA processing *in vitro* in the absence of any protein (30), a finding that is consistent with previous observation that H1 RNA alone binds to precursor tRNAs *in vitro* and has a conserved catalytic core (24,29).

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**Table 1.** Subunits of human RNase P, functions and evolutionary conservation

| Subunit <sup>a</sup> | Function/interaction<br>(in tRNA processing) | Chromatin<br>binding <sup>b</sup> /pol<br>III transcription <sup>c</sup> | Homolog<br>yeast <sup>d</sup> /archaea <sup>e</sup> |
|----------------------|--|--|---|
| Rpp14                | RNA binding                                  | +/+  | Pop8p <sup>f</sup>                                  |
| Rpp20                | ATPase,<br>helicase/Hsp27,<br>SMN, Rpp25     | +/+  | Pop7p, Rpp2p  |
| Rpp21                | RNA binding,<br>activity <sup>g</sup> /Rpp29 | +/+  | Rpr2p/aRpp21  |
| Rpp25                | RNA binding/Rpp20                            | + <sup>h</sup> /ND   | Pop6p <sup>f</sup>                                  |
| Rpp29                | tRNA binding,<br>activity/Rpp21              | +/+  | Pop4p/aRpp29  |
| Rpp30                | RNA binding,<br>activity/Pop5                | +/+  | Rpp1p/aRpp30  |
| Rpp38                | RNA binding,<br>activity                     | +/+  | Pop3p/aRpp38  |
| Rpp40                |  | +/+  |   |
| hPop1                |  | ND/ND  | Pop1p   |
| hPop5                | RNA binding,<br>activity/Rpp30               | ND/+   | Pop5p/aPop5   |
| H1 RNA               | Activity/Rpp21,<br>Rpp29, Rpp30, Rpp38       | ND/+   | Rpr1/RPR RNA  |

<sup>a</sup>Jarrous and Altman (8).<sup>b</sup>Binding to chromatin of tRNA and 5S rRNA genes (33).<sup>c</sup>Required for pol III transcription in whole HeLa extracts and/or in cells (33).<sup>d</sup>Walker and Engelke (14).<sup>e</sup>Hall and Brown (20).<sup>f</sup>Rosenblad *et al.* (15).<sup>g</sup>Enzyme activity of reconstituted RNase P (24,30).<sup>h</sup>Guerrier-Takada *et al.* (13).

ND, not determined.

Since most of the protein subunits of nuclear RNase P are shared with RNase MRP, including those responsible for reconstitution of RNase P activity *in vitro*, it seems that the RNA subunits alone may be sufficient for diversification of function of these catalytic ribonucleoproteins as tRNA and rRNA endoribonucleases (24,29–31). Hence, the precise role of the protein subunits of RNase P and RNase MRP in hydrolyzing the phosphodiester bond in different RNA substrates remains unknown. Apparently, protein subunits may allow substrate recognition and catalysis by facilitating the proper folding of the RNA subunits of RNase P and RNase MRP. Protein subunits may also define some structural properties of RNase P and RNase MRP. For instance, Rpp20 and Rpp25 transiently associate with 12S mono-particles of RNase MRP, but these subunits dissociate from subsets of RNase MRP bound to 60–80S pre-ribosomal complexes (32). Additionally, protein subunits may permit the recognition of yet unknown RNA substrates for RNase P and RNase MRP or implicate these complexes in other biological settings, such as chromatin binding and transcription (33; see below), cell cycle progression (34) and RNA metabolism (35). Accordingly, studying the protein subunits of RNase P and RNase MRP could be useful in elucidating the diversification of functions of these two evolution-related ribonucleoprotein complexes (36).

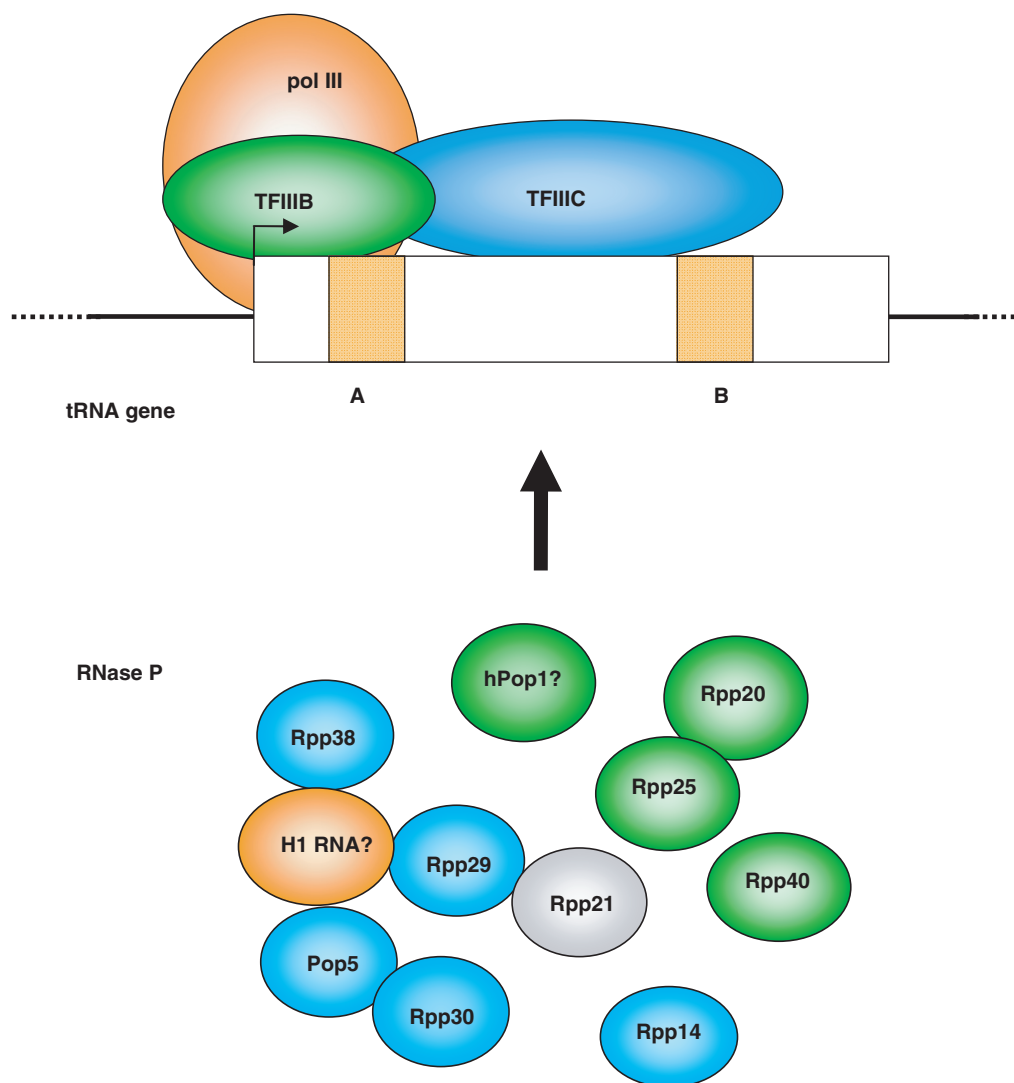
## SUBUNITS OF HUMAN RNase P DIFFERENTIALLY LOCALIZE IN INTRANUCLEAR COMPARTMENTS ASSOCIATED WITH GENE TRANSCRIPTION

Studies in cell biology reveal that H1 RNA and protein subunits of human RNase P are differentially concentrated in distinct intracellular compartments, including the nucleoplasm, nucleolus, Cajal bodies, perinucleolar compartment and cytoplasm (17,37–42). Rpp21 and Rpp29, two protein subunits that are sufficient for reconstitution of RNase P activity (24), localize mainly in the nucleoplasm and nucleolus, though the latter subunit rapidly shuttles between these compartments (43). Cajal bodies are sites of assembly of transcription and processing machines (44,45), while the nucleolus specializes in transcription and processing of rRNA and ribosome biogenesis (46). The nucleoplasm, which defines the interchromatin compartment, hosts transcription factories for pol II and pol III (47–49). The lack of a specific locale for the H1 RNA and protein subunits of human RNase P and the differential distribution of these subunits in intranuclear compartments specialized in gene transcription suggested the possibility that the assembly of RNase P is dynamic and linked to active gene transcription (50).

## A NOVEL ROLE FOR HUMAN RNase P IN POL III TRANSCRIPTION

A recent study has shown that human nuclear RNase P is required for transcription of tRNA and other small noncoding RNA genes by pol III in whole HeLa cells and cell extracts (33). RNase P exerts its role on transcription through association with chromatin of tRNA and 5S rRNA genes as determined by chromatin immunoprecipitation analysis (33). All the protein subunits of RNase P tested so far, i.e. Rpp14, Rpp20, Rpp21, Rpp29, Rpp30, Rpp38 and Rpp40, can be found associated with chromatin of tRNA and 5S rRNA genes in rapidly dividing cells (Figure 1) (33; also unpublished data), an indication that a multi-protein RNase P complex binds to the chromatin of these genes. Rpp25 has also been noted to be associated with nucleosomes (13). Binding of these protein subunits to chromatin is dynamic, in the sense that they associate with tRNA and 5S rRNA genes in dividing HeLa cells and dissociate from these genes when cells cease proliferating. Furthermore, chromatin occupancy by RNase P associates with active gene transcription in extracts and in cells, and coincides with that of pol III, which could be brought down with active RNase P ribonucleoprotein in coimmunoprecipitation experiments (33).

Knockdown of the essential protein Rpp29 of human RNase P by RNA interference did not affect the binding of RPB8 (a core component of pol III) to tRNA and 5S rRNA genes, even though this knockdown results in severe inhibition of RNase P activity and pol III transcription (33). Additionally, Rpp29 disengaged from target genes independently of RPB8 in cells that ceased proliferating (33). Hence, the possibility exists that



**Figure 1.** RNase P subunits bind to chromatin of active tRNA genes. Many protein subunits of human RNase P associate with chromatin of tRNA genes, as determined by chromatin immunoprecipitation (33). Subunits that their binding to chromatin was not verified are shown with a question mark. Rpp25 has also been reported to be associated with nucleosomes (13). Based on knockdown analysis (33), RNase P subunits seem not to be required for the recruitment of pol III subunits and hence its transcription factors TFIIIB and TFIIIC (51,52) to the tRNA gene.

recruitment of pol III to tRNA and 5S rRNA genes is independent of that of RNase P (or at least Rpp29).

The association of RNase P with chromatin of tRNA genes could be explained in terms of coordination of tRNA gene expression at transcription and processing steps. However, RNase P is also critical for transcription of 5S rRNA, 7SL RNA and U6 snRNA genes (33), whose precursor transcripts are not recognized as substrates for RNase P. Hence, RNase P acts solely as a transcription factor for pol III in transcribing these latter small RNA genes.

#### ROLE OF RNase P IN TRANSCRIPTION IS CONSERVED IN YEAST

Screening of a genomic library identified RPR1 RNA, the RNA subunit of *S. cerevisiae* RNase P, as the specific overexpression suppressor of very slow growth at 37°C

due to a small deletion of Bdp1, a subunit of the transcription factor TFIIIB complex (53). Processing of the 5' leader sequence of precursor tRNA<sup>Leu</sup> is defective in cells producing this mutant Bdp1p, called Bdp1 $\Delta$ 253–269. Transcription of the *RPR1* RNA gene is selectively diminished when recombinant Bdp1 $\Delta$ 253–269 replaced wild-type Bdp1p in an *in vitro* pol III transcription system. The physical interaction of RNase P with Bdp1p was demonstrated by coimmunoprecipitation and pull-down assays, implying a role for TFIIIB in 5' end processing of precursor tRNA (53).

In *S. cerevisiae*, early processing of precursor tRNAs occurs in the nucleolus, which is enriched with RNase P RNA (54). Additionally, tRNA gene families, which are dispersed in various chromosomes, colocalize with 5S rDNA genes at the nucleolus (55,56). This nucleolar clustering of yeast tRNA genes depends on transcription-complex formation and the existence of

proficient promoters. Clustering of tRNA and 5S rRNA genes in the nucleolus forms pol III transcription sites containing concentrations of pol III and its general transcription factors, TFIIB, TFIIC and TFIIIA. Such nucleolar sites may also initiate nucleolar organization of the tRNA-processing pathway that includes 5' end processing by RNase P (55,56). It should be noted that in contrast to the localization of pol III and RNase P in the nucleolus in *S. cerevisiae*, confocal immunofluorescence microscopy analyses of HeLa cells reveal that specific protein subunits of human pol III and RNase P primarily colocalize in the nucleoplasm, even though fluorescent signals are also visible in defined spots in large nucleoli (R.R. and J.N., unpublished data).

### RECRUITMENT OF RNase P TO TARGET GENES

How human RNase P is recruited to tRNA and 5S rRNA genes? One possibility could be that RNase P as a whole particle is recruited to these target genes. Nonetheless, protein subunits of human RNase P have been shown to exhibit differential patterns of binding to chromatin of tRNA and 5S rRNA genes (33). Moreover, siRNA knockdown of Rpp29, a key core component for the assembly of active RNase P, does not affect chromatin binding by other subunits, including Rpp20 and Rpp21 (33; R.R. and N.J., unpublished data). Hence, chromatin occupancy by Rpp29 is not a prerequisite for binding by other subunits. Stepwise assembly of catalytic ribonucleoproteins, including the spliceosome and H/ACA small nucleolar ribonucleoprotein, at transcriptionally active genes has been proposed (45,57–59).

### PROSPECTS

Archaeal and eukaryal RNase P are ensembles of structurally and functionally related RNAs with highly conserved protein subunits (8–10,14,17,20,50). Reconstitution experiments of archaeal and eukaryal RNase P activities reveal that two protein subunits, e.g. Rpp21 and Rpp29, with their corresponding RNA moieties are sufficient for having endonucleolytic cleavage of tRNA substrates neutral pH7 and 5–30 mM divalent ion (24,27). Notably, Rpp29 but not Rpp21 can activate a bacterial RNase P RNA (24,60). In addition, *P. furiosus* Pop5 has an  $\alpha$ - $\beta$  sandwich structure that bears structural similarity to the bacterial RNase P protein (25). Since archaeal and eucaryal RNase P RNAs are active under unphysiological conditions of high ion concentrations (30,61), it would be interesting to check if a single protein cofactor (Rpp29, Pop5 or other) can reconstitute some activity under physiological reaction conditions.

Previous studies which revealed ambiguous properties related to RNase P or its subunits that were not consistent with its activity as a tRNA-processing enzyme could be reexamined from the perspective of its transcriptional activity. For instance, Rpp20 that exhibits ATPase activity (62) and chromatin-binding capability (33) may enable RNase P (and/or RNase MRP) to use ATP as a cofactor for binding and modulating chromatin structure

and function. Rpp20, as well as Rpp25, belong to the Alba superfamily of proteins which seem to have originated as RNA-binding proteins, attaching to a variety of ribonucleoprotein complexes, including RNase P and RNase MRP (13,17) and then being recruited as chromatin-binding proteins (63). A dual role for Rpm2p, a component of yeast mitochondrial RNase P, in tRNA processing and transcription has also been described (64).

The molecular mechanisms by which human RNase P binds to chromatin of noncoding RNA genes and controls transcription by pol III are not known. Future work will reveal if RNase P acts at initiation, elongation and/or termination of transcription. In addition, the physical and functional links between pol III and RNase P during the cell cycle remains to be studied. Future studies will also unveil the significance of the effect of binding of RNase P (or its subunits) to hundreds of small noncoding RNA genes and others (R.R. and N.J., unpublished data) on chromatin structure, organization and function of the human genome. Whatever the outcome of these studies, the discovery that human RNase P is a chromatin-binding complex which is critical for normal gene transcription (33) expands the definition of this entity as an enzyme that hydrolyzes a phosphodiester bond in precursor tRNA.

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