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review

The final cut

The importance of tRNA 3′-processing

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To generate functional tRNA molecules, precursor RNAs must undergo several processing steps. While the enzyme that generates the mature tRNA 5′-end, RNase P, has been thoroughly investigated, the 3′-processing activity is, despite its importance, less understood. While nothing is known about tRNA 3′-processing in archaea, the phenomenon has been analysed in detail in bacteria and is known to be a multistep process involving several enzymes, including both exo- and endonucleases. tRNA 3′-end processing in the eukaryotic nucleus seems to be either exonucleolytic or endonucleolytic, depending on the organism analysed, whereas in organelles, 3′-end maturation occurs via a single endonucleolytic cut. An interesting feature of organellar tRNA 3′-processing is the occurrence of overlapping tRNA genes in metazoan mitochondria, which presents a unique challenge for the mitochondrial tRNA maturation enzymes, since it requires not only the removal but also the addition of nucleotides by an editing reaction.

Introduction

tRNA molecules play a central role not only in translation, but also in a number of other cellular processes such as amino acid transformations and porphyrin biosynthesis (Söll, 1993). To convert a precursor transcript into a functional tRNA molecule, several processing steps are required, including the removal of 5′- and 3′-extensions. The enzyme responsible for the generation of the mature 5′-end is RNase P, which has been found in all organisms studied hitherto (Frank and Pace, 1998). Much less is known about the RNase P counterparts that are active at the tRNA 3′-end, probably because removal of tRNA 3′-sequences occurs by different mechanisms in different organisms. Here, we review what is currently known about tRNA 3′-processing, a reaction that is essential for aminoacylation, and thus protein synthesis, in all organisms.

Bacteria: are there tRNA-specific RNases?

In bacteria, tRNA transcripts are generally part of a polycistronic RNA encoding several tRNAs, sometimes even including rRNAs and mRNAs (Deutscher, 1995). These tRNA precursors are transcribed with long 5′- and 3′-extensions. In most cases, the latter encode the 3′- terminal CCA triplet, a common feature of all mature tRNA 3′-ends. The processing pathway of tRNAs in *Escherichia coli* (Apirion and Miczak, 1993; Deutscher, 1995) is initiated by an endonucleolytic cleavage downstream of the CCA terminus (Sekiya *et al.*, 1979) followed by an exonucleolytic trimming reaction (Figure 1). After removal of the 5′-extension by RNase P, a second exonucleolytic trimming reaction generates the mature tRNA 3′-end. Two enzymes, RNase E (Ray and Apirion, 1981a,b) and RNase III (Apirion and Miczak, 1993; Deutscher, 1995), have so far been identified as being involved in the endonucleolytic cleavage. Both of these RNases (as well as RNase P) seem to be associated with the inner membrane (Miczak *et al.*, 1991) and cleave RNA in the context of small primary or secondary structural elements rather than being specific for a particular type of RNA. RNase III has been found to recognize double-stranded RNA structures as substrates (Nicholson, 1999), while RNase E cleaves single-stranded AU-rich sequences (McDowall *et al.*, 1994; Nicholson, 1999). The 3′-trimming reaction that follows the endonucleolytic cleavage can be catalysed by a variety of exonucleases: six different enzymes (RNases II, BN, D, PH, PNPase, T) are able to catalyse this reaction *in vitro* and *in vivo*, although there seems to be a hierarchy of preferences for the use of these exonucleases (RNase T and RNase PH seem to be most effective). As is the case for the endonucleolytic cleavage, none of these is specific for tRNA maturation (Deutscher, 1990). These data suggest that, at least in *E. coli*, there seems to be a set of endo- and exonucleases that are not specific for a certain class of RNA (rRNA, mRNA and tRNA), but instead recognize small structural

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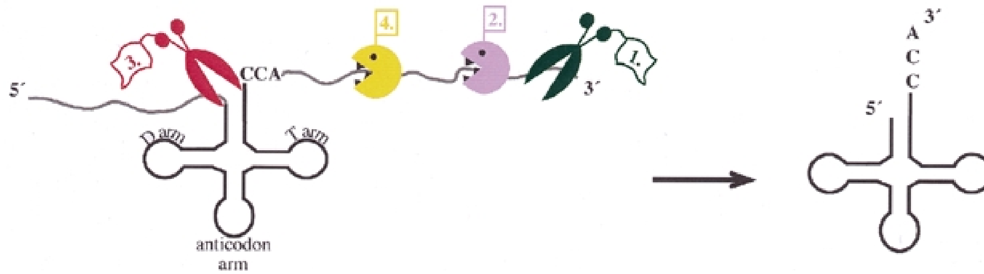

Fig. 1. tRNA processing in *E. coli*. The initial processing step for tRNA precursors in *E. coli* is an endonucleolytic cut occurring several nucleotides downstream of the tRNA 3′-end (1). An exonuclease removes some of the nucleotides at the 3′-end (2) before RNase P generates the mature 5′-end (3). The remaining nucleotides at the 3′-end are subsequently removed exonucleolytically (4). Since the CCA sequence is encoded in *E. coli* tRNA genes, the tRNA nucleotidyl transferase has only a repair function.

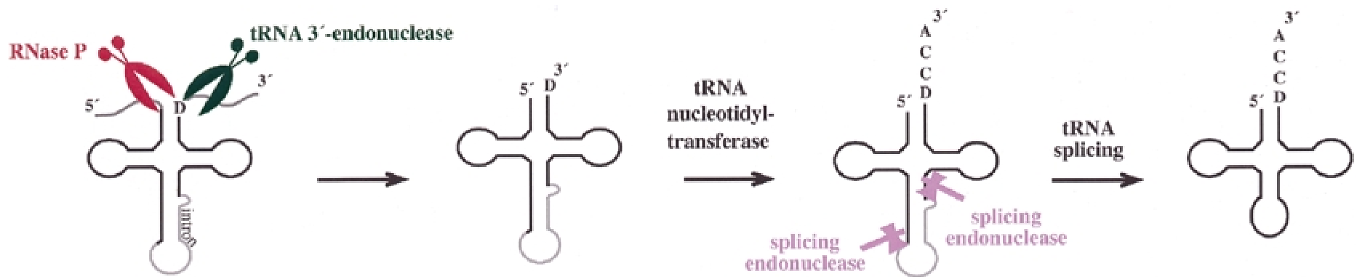

Fig. 2. Maturation of tRNAs in eukaryotes. The mature tRNA 5′-end is generated by an RNase P activity in all eukaryotes. Processing at the 3′-end is different from organism to organism, but the prevalent reaction seems to be an endonucleolytic cut close to or at the tRNA 3′-end. Endonucleases catalyzing this step have been termed 3′-tRNase (Mohan *et al.*, 1999) and RNase Z (Mayer *et al.*, 2000) by different investigators in different systems. The terminal CCA sequence is added by tRNA nucleotidyl transferase and if the precursor contains introns they are removed by a splicing endonuclease. The temporal order of the cleavage reactions at the 5′- (RNase P) and 3′-ends (RNase Z/3′-tRNase) of the tRNA is currently not known. The nucleotide 5′ to the CCA triplet is called discriminator (D) and serves as an identity element in many tRNAs.

elements that are common to several classes of RNA molecules. At least in the case of the exonucleases, these enzymes are redundant, thereby ensuring complete tRNA processing (Li and Deutscher, 1996). As most of the bacterial tRNA genes encode the 3′-terminal CCA triplet, the tRNA is ready for aminoacylation immediately after the final 3′-processing step. Since tRNAs probably fold into their typical structure during transcription, the processing enzymes may start cleaving the precursor RNA co-transcriptionally (Apirion, 1983). Since the described situation is based on data obtained from the *E. coli* system, one should be aware that things might be somewhat different in other prokaryotes like *Bacillus subtilis*.

Eukaryotes prefer endonucleases!

Precursor molecules for nuclear tRNAs contain short 5′- and 3′-extensions of ~5–15 nucleotides, some are interrupted by introns, and they generally do not encode the 3′-terminal CCA triplet (Deutscher, 1995). In yeast and other eukaryotes, a large complex (300–400 kDa) consisting of multiple 3′-to-5′-exonucleases, termed the exosome, was discovered and found to be responsible for the processing of a variety of snRNAs and snoRNAs. Some of the exonucleases in the exosome show similarity to *E. coli* RNase PH and might therefore also participate in tRNA 3′-end processing (van Hoof and Parker, 1999). However, analysis of yeast exosome mutants showed that tRNA processing seems not to be impeded, indicating that the exosome is not involved in tRNA 3′-end trimming (van Hoof *et al.*, 2000).

In *in vitro* systems, exo- as well as endonucleolytic 3′-maturation pathways have been observed, although the majority of the eukaryotic 3′-processing pathways seem to involve solely endonucleolytic cleavage exactly at the 3′-end of the tRNA (for references see Mayer *et al.*, 2000) (Figure 2). In *Saccharomyces cerevisiae*, the presence of Lhp1p (the yeast homologue of the human La protein, which binds to all polymerase III transcripts) is crucial for the endonucleolytic *in vivo* processing pathway. Binding of this protein probably stabilizes the tRNA conformation and thereby facilitates the cleavage reaction (Yoo and Wolin, 1997). Removal of the Lhp1p protein results in an exonucleolytic tRNA 3′-maturation pathway *in vivo*. These data suggest that under normal conditions eukaryotes use the endonucleolytic pathway for 3′-end maturation, but exonucleases can act as a back-up system. The order of the tRNA processing events seems to depend on precursor concentrations. Lund and Dahlberg (1998) showed that under physiological conditions splicing occurs before tRNA end maturation, but that at high precursor concentrations end maturation precedes splicing. In eukaryotes, tRNA 3′-processing occurs in the nucleus, and it might be possible that the tRNA 3′-processing enzymes are located in the nucleolus, as has been shown to be the case for RNase P (Bertrand *et al.*, 1998).

Organelles: loss of the original processing activities

In all mitochondria and chloroplasts, tRNAs are embedded in longer precursor molecules, which generally do not encode the 3′-CCA terminus, and very few organellar tRNA genes contain introns (Oda *et al.*, 1992; Maréchal-Drouard *et al.*, 1993). All tRNA 3′-processing activities from organelles have been reported to be endonucleases cleaving the precursor close to or at the discriminator (for references see Mayer *et al.*, 2000). In yeast, rat and plant mitochondria, an endonucleolytic activity cleaves the precursor immediately 3′ to the discriminator (Manam and Van Tuyle, 1987; Chen and Martin, 1988; Kunzmann *et al.*, 1998), while in chloroplasts tRNA 3′-maturation is catalysed by an endonuclease that leaves one extra nucleotide at the tRNA 3′-end. When this base is a C residue, it might be used as the first position of the CCA end; otherwise it must be removed to allow CCA addition (Oommen *et al.*, 1992).

Metazoan mitochondria contain very small compact genomes (generally ranging in size from 14 to 18 kb) with partially overlapping tRNA genes (Anderson *et al.*, 1981; Yokobori and Pääbo, 1995a; Tomita *et al.*, 1996). The maturation of tRNAs overlapping on the same strand is an especially intriguing process. In these cases, the downstream tRNA is released intact, leaving the upstream tRNA with 1–6 nucleotides missing at the 3′-end. These bases are then post-transcriptionally incorporated by an editing reaction (Yokobori and Pääbo, 1995a,b; Tomita *et al.*, 1996; Reichert *et al.*, 1998). Initial studies of the cleavage processes in human mitochondria showed that the endonuclease that catalyses this reaction at one set of overlapping tRNAs cannot cleave another set of overlapping tRNAs, and is thus tRNA precursor specific (Reichert *et al.*, 1998). In addition, metazoan mitochondria contain tRNAs with very unusual structures (missing the D or T arm), which also have to be recognized by the tRNA processing enzymes (Okimoto and Wolstenholme, 1990).

In the mitochondria of several organisms, some tRNAs are edited by base substitutions, which can influence the cleavage reactions at 5′- and 3′-ends: in *Physarum* and land plant mitochondria, only the edited molecules are processed by the 5′- and 3′-processing enzymes (Price and Gray, 1998). In summary, organellar 3′-processing resembles the prevalent eukaryotic single endonuclease step rather than the bacterial multistep pathway. This observation suggests that organelles have lost their original bacterial activity and either have acquired copies of the corresponding nuclear enzymes or have invented their own processing activities.

Archaea: still a mystery?

As in other organisms, tRNA precursors in archaea are transcribed with 5′- and 3′-extensions and sometimes contain several tRNA genes within one transcript (Frey *et al.*, 1990; Smith *et al.*, 1997). Currently, little is known about the fine details of archaeal tRNA processing, except that 5′-maturation precedes 3′-processing and both end-processing reactions can occur before splicing (Palmer *et al.*, 1994). Preliminary experiments on tRNA 3′-processing in *Haloferax volcanii* have implicated an endonuclease that cleaves the precursor close to or at the discriminator (R. Rupprecht and A. Marchfelder, in preparation), suggesting that the archaeal activity resembles the endonucleolytic eukaryotic pathway more than the multistep bacterial processing. This observation adds to the accumulating data supporting the idea that archaea share certain features of the eukaryotic nucleus, e.g. histones and fibrillarin (Reeve *et al.*, 1997). It will therefore be interesting to investigate whether a precursor to the nucleus, which serves to concentrate snoRNAs and RNA processing and modification enzymes (including the tRNA processing enzymes), exists in archaea.

Conclusion and outlook

While tRNA 5′-processing by RNase P is similar in all kingdoms, tRNA 3′-end maturation differs from one to another. While bacteria use a multistep process involving endo- and exonucleases, the nuclei and other organelles of eukaryotes appear to employ mainly endonucleases for this process, as do archaea.

The redundant bacterial processing system highlights the pitfalls of *in vitro* processing systems and the need to use *in vivo* analyses and mutants to identify back-up functions and specificities of individual enzymes. So, even after >20 years of research on tRNA processing, there are still many unresolved questions about this maturation step, which is so crucial for protein synthesis and cellular life.

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